Heat Shock Proteins 22
*Series Editors*: Alexzander A. A. Asea · Punit Kaur

Alexzander A. A. Asea
Punit Kaur *Editors*

# Heat Shock Proteins in Inflammatory Diseases

Springer

# Heat Shock Proteins

Volume 22

**Editor-in-Chief**

Alexzander A. A. Asea, Department of Medicine, University of Toledo, Toledo, OH, USA

**Associate Editor**

Punit Kaur, Department of Medicine, University of Toledo, Toledo, OH, USA

**Heat Shock Proteins** offers rapid publication of novel and outstanding research on all aspects of Heat Shock Protein research, the Heat Shock Response, and Cell Stress. In addition, as cells possess additional responses to Hypoxia, Genomic Stress, Oxidative Stress and related pathways that may involve molecular chaperones as well as unique molecular responses, these topics are welcomed. Degeneration of HSP expression and the multiple stress responses underlies many human, animal and plant diseases and this forms a major component of our content. This range of subjects have great topicality as the stresses associated with climate change advance rapidly in the earth's environment.In addition, the potential to escape from the eroding earthbound environment, such as with space travel will impose novel challenges to the human system that we will cover in the series.

The coverage of **Heat Shock Proteins** spans all areas from basic science, bioengineering, artificial intelligence, translational research, clinical research, drug and device development, environmental and climate change. Heat Shock Proteins welcomes Invited Book Chapters, Unsolicited Research Articles, Case Reports, Short Communications, Reviews and Letters to the Editor (Commentaries).

**Heat Shock Proteins** is committed to upholding the integrity of the scientific record and will follow the Committee on Publication Ethics (COPE) guidelines on how to deal with potential acts of misconduct and correcting the literature.

**Heat Shock Proteins** utilizes single-blind peer-review for all manuscripts. In addition, manuscripts are checked for plagiarism.

More information about this series at http://www.springer.com/series/7515

Alexzander A. A. Asea • Punit Kaur
Editors

# Heat Shock Proteins in Inflammatory Diseases

Springer

*Editors*
Alexzander A. A. Asea
Department of Medicine and Precision Therapeutics Proteogenomics Diagnostic Center
Eleanor N. Dana Cancer Center
University of Toledo College of Medicine and Life Sciences
Toledo, OH, USA

Punit Kaur
Department of Medicine and Precision Therapeutics Proteogenomics Diagnostic Center
Eleanor N. Dana Cancer Center
University of Toledo College of Medicine and Life Sciences
Toledo, OH, USA

NampEVA BioTherapeutics LLC
Dover, DE, USA

ISSN 1877-1246 ISSN 1877-1254 (electronic)
Heat Shock Proteins
ISBN 978-3-030-85167-5 ISBN 978-3-030-85168-2 (eBook)
https://doi.org/10.1007/978-3-030-85168-2



This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

# Preface

The heat shock protein (HSP) family consists of several proteins, which are synthesized in response to a variety of stressors including hyperthermia, ischemia, infarct, lesions, and seizures or following less drastic metabolic changes as those produced by physical exercise or psychological stress. HSP is present in all living organisms. HSP regulates the stability, activation, and degradation of a diverse array of proteins associated with growth, proliferation, and survival. Thus, it is core to regulation of protein stability and protein-degradation pathways and modulating transcription factors, signaling transduction networks, and kinases. It facilitates the survival of cells during stress response and exhibits a pronounced antiapoptotic and stabilization effect. HSP performs various functions in the transportation and refolding of proteins from the cytoplasm into the mitochondrial matrix and has been linked to diabetes, stress response, cancer, and certain types of immunological disorders.

The book *Heat Shock Proteins in Inflammatory Diseases* provides the most comprehensive highlight and insight of the expression, function, and therapeutic activity of sHsp in inflammatory diseases including sepsis, psoriasis, neurodegenerative diseases, cancers, viral infection, and autoimmune rheumatic diseases. Using an integrative approach to the understanding of HSP structure, function, and immunobiology, the contributors provide a synopsis of novel mechanisms by which HSP is involved in the regulation of various human inflammatory diseases. HSP alters the inflammation modules through inhibition of cytokines, pro-inflammatory factors, by which playing the significant functions in the inflammatory diseases. HSP is found to be secreted into extracellular milieu, generates autoantibodies, and also acts as immune modulators. The therapeutic potential of HSP in inflammatory/autoimmune diseases has emerged owing to their ability to induce the regulation of regulatory T cells, which play critical role in induction and dysregulation of immune response.

Key basic and clinical research laboratories from major universities, academic medical hospitals, and biotechnology and pharmaceutical laboratories around the world have contributed chapters that review present research activity and importantly project the field into the future. The book is a must read for graduate students,

medical students, basic science researchers, and postdoctoral scholars in the fields of Translational Medicine, Clinical Research, Human Physiology, Biotechnology, Natural Products, and Cell and Molecular Medicine and pharmaceutical scientists and researchers involved in Drug Discovery.

Toledo, OH, USA

Alexzander A. A. Asea
Punit Kaur

# Contents

# About the Editors

**Prof. Dr. Alexzander A. A. Asea** is a highly innovative and accomplished world-renowned clinical and basic research scientist and visionary executive leader who has exceptional experience spearheading clinical and basic science research, training, education, and commercialization initiatives within top-ranked academic biomedical institutes. Prof. Dr. Asea's initial findings studying the effects of Hsp72 on human monocytes led to the proposal of a novel paradigm that Hsp72, previously known to be intracellular molecular chaperones, can be found in the extracellular milieu where it has regulatory effects on immunocompetent cells – a term now called chaperokine. Prof. Asea has authored over 255 scientific publications including peer-reviewed articles, reviews, books, book chapters, editorials, and news headliners in a wide range of biomedical-related disciplines. Prof. Asea is the Editor-in-Chief of the widely successful book series *Heat Shock Proteins* (Springer Nature Publishing) and is an editorial board member of numerous scientific peer-reviewed journals. Prof. Dr. Asea is at the University of Toledo College of Medicine and Life Sciences in Toledo, USA.

**Dr. Punit Kaur** is an expert in onco-proteogenomics, with extensive training and experience in quantitative mass spectrometry imaging, protein chemistry, and biomarker discovery. Dr. Kaur's main research focus is on the use of heat-induced nanotechnology in combination with radiotherapy and chemotherapy in the cancer stem cell therapy. Dr. Kaur has published more than 80 scientific articles, book chapters, and reviews and currently serves as editorial board member for the *European Journal of Cancer Prevention* and the *Journal of Proteomics and Bioinformatics*. Dr. Kaur is the Associate Editor of the highly successful *Heat Shock Proteins* book series by Springer Nature Publishing. Currently, Dr. Kaur is at the University of Toledo College of Medicine and Life Sciences in Toledo, USA.

# Extracellular Vesicle-Associated Moonlighting Proteins: Heat Shock Proteins and Metalloproteinases


**Takanori Eguchi and Eman Ahmed Taha**

## Abstract

**Introduction** Protein moonlighting has been defined as one protein that plays multiple roles. Intracellular heat shock proteins (HSP) are originally molecular chaperones that assist in the folding of intracellular proteins, whereas extracellular HSP plays a cytokine-like role through binding to receptors expressed on the surface of immune and cancer cells. On the other hand, matrix metalloproteinases (MMP) are originally extracellular proteinases, whereas intracellular MMP alters transcription and gene expression. In here, we therefore propose to define HSP and MMP as moonlight proteins and find these moonlighting proteins in extracellular vesicles (EV), which carry and mediate molecular transfer of the cargo factors from the producer cells to the recipient cells.

**Methods** PubMed and Google databases were searched with keywords including "moonlight proteins, protein moonlighting, HSP, MMP, extracellular vesicles, exosomes, alarmin, danger- or damage-associated molecular patterns (DAMPs).

**Results** Many members of HSP and MMP protein families are overexpressed and extracellularly released in cancers and inflammatory diseases, including rheumatoid

Authors Takanori Eguchi and Eman Ahmed Taha have equally contributed to this chapter.

T. Eguchi (✉)
Department of Dental Pharmacology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
e-mail: eguchi@okayama-u.ac.jp; eguchi.takanori@gmail.com

E. A. Taha
Department of Dental Pharmacology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

Department of Medical Bioengineering, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan

Department of Biochemistry, Faculty of Science, Ain Shams University, Cairo, Egypt

Heat Shock Proteins (2021) 22: 1–18
https://doi.org/10.1007/7515_2020_25

Published Online: 27 September 2020

arthritis (RA). HSP are also known as alarmins that are released upon cell damage as DAMPs in cancers and inflammatory diseases.

**Conclusions** In this chapter, we define HSP and MMP as moonlighting proteins and review their roles in inflammatory diseases such as RA.

**Keywords** Alarmin · DAMP · Extracellular HSP · Extracellular vesicle · Intracellular MMP · Moonlighting protein · Rheumatoid arthritis

## Abbreviations

| | |
|---|---|
| APC | antigen-presenting cell |
| ATP | adenosine triphosphate |
| B | B lymphocyte |
| BBB | blood-brain barrier |
| CCL | C-C motif chemokine ligand |
| CRPC | castration-resistant prostate cancer |
| DAMP | damage-associated molecular pattern |
| DC | dendritic cell |
| ECM | extracellular matrix |
| EV | extracellular vesicle |
| HA | hyaluronan |
| HMGB | high-mobility group protein with a 'Box' domain |
| HSP | heat shock protein |
| IL | interleukin |
| IL-1R | interleukin 1 receptor |
| L-EV | large extracellular vesicle |
| LRP1 | low-density lipoprotein receptor-related protein 1 |
| MAPK | microtubule associated protein kinase |
| MEV | metastatic oral cancer-derived extracellular vesicle |
| MHC | major histocompatibility complex |
| MMP | matrix metalloproteinase |
| Mφ | macrophages |
| NALP3 | NACHT, LRR and PYD domains-containing protein 3 |
| NK | natural killer |
| NLR | nucleotide-binding leucine-rich repeat |
| NLS | nuclear localization signal |
| NOD | nucleotide-binding oligomerization domain |
| OA | osteoarthritis |
| OSCC | oral squamous cell carcinoma |
| P2 | purinergic type 2 receptor |
| PAMP | pathogen-associated molecular pattern |
| PC-3 | prostate cancer |
| PI3K/AKT | phosphatidylinositol 3-kinase and protein kinase B |

| | |
|---|---|
| PRR | pattern recognition receptor |
| PTM | post-translational modification |
| RA | rheumatoid arthritis |
| RAGE | receptor for advanced glycation end-products |
| RASF | rheumatoid arthritis synovial fluid |
| SASP | senescence-associated secretory phenotype |
| s-EV | small extracellular vesicle |
| SR | scavenger receptor |
| SRA1 | steroid receptor RNA activator 1 |
| SREC-1 | scavenger receptor expressed by endothelial cells-I |
| T | T lymphocyte |
| TLR | toll-like receptor |
| Treg | regulatory T cell |

## 1 Introduction

Protein moonlighting has been defined as that one protein processes multiple tasks and play multiple roles [49]. For example, heat shock proteins (HSP) are originally molecular chaperones that assist in folding of intracellular proteins [26], while, additionally, extracellular roles of HSP have been discovered to play a cytokine-like role, so-called chaperokine, through binding to surface receptors such as CD94, LRP1/CD91, toll-like receptors (TLRs), and a scavenger receptor (SR) expressed on endothelial cells 1 (SREC-1) expressed on immune cells and cancer cells [27, 105]. Moreover, many types of HSP are often found in extracellular vesicles (EV) such as exosomes, including HSP90, HSP70, heat shock cognate 70 (HSC70), HSP27, HSP110, and chaperonins [80, 105]. HSP are also known as alarmins or damage-/danger-associated molecular patterns (DAMPs), which are released from cells upon cell damage or cell stress. The molecular patterns of extracellular HSP are recognized by pattern recognition receptors (PRRs) such as TLRs expressed on dendritic cells (DC), macrophages (Mφ) and epithelial cells, SR expressed on Mφ and endothelial cells, LRP1/CD91 expressed on immune cells and cancer cells, and CD94 expressed on natural killer (NK) cells [105] (Table 1). The variety of the recipient cells and types of receptors of HSP are the evidence and mechanism of how HSP plays multiple roles as a moonlighting protein.

On the other hand, matrix metalloproteinases (MMP) are originally extracellular proteinases [57], whereas MMP3 also regulates transcription and gene expression in cellular nuclei [28–30, 78]. Classically, MMP are found to cleave extracellular matrix (ECM) proteins, growth factors, and their receptors and alter their extracellular microenvironment in inflammatory diseases and cancers, as well as during development and organogenesis. Extracellular MMP are also known as one of the senescence-associated secretory phenotype (SASP) that includes IL-1β, IL-1α, IL-6, IL-8, chemokine (C-C motif) ligand 2 (CCL2), MMP3, and MMP2 [1, 2, 38, 42, 62]. Cellular senescence is known as a stress response associated with DNA damage,

**Table 1** Alarmins, their receptors, and main impacts

| Alarmins | Receptors | Main impacts |
|---|---|---|
| **HMGB1** | TLR2/TLR4 [84], TLR5 [20], RAGE [25], and CD24/Siglec-10 [18] | Recruitment and activation of immune cells; inducing pro-inflammatory cytokine secretion [14, 119] |
| **HSP** | TLR2/TLR4 [122], CD14 [3, 56], CD40 [5], CD91/LRP1/A2MR [4], CD94 [36], SRA1 [8], SREC-1 [73, 75], and LOX-1 [22] | 1.1.1.1. DC activation and promote the generation of innate and adaptive immune responses [9] |
| **Hsp70** | | |
| **Hsp90** | | |
| **Gp96/Grp94/Hsp96** | | 2. Promoting the production of proinflammatory cytokines [60, 61] |
| | | 3. Supporting Treg expansion and functions [71] |
| **MMP** | Integrin [11] | 1. Extracellular proteolysis [83, 115]<br>2. Transcriptional regulation [29, 30];<br>3. Intracellular proteolysis [59, 68, 120] |
| **IL-1α** | IL-1R [23] | 1. Mediate APC/DC activation [6, 76] and<br>2. Promoting the inflammatory and immune responses [104, 109] |
| **IL-33** | ST2R [96] | A potent mediator of type 2 and regulatory immune responses [96, 110] |
| **S100 proteins** | TLR4 [32] and RAGE [117] | Trigger immune cell recruitment and activation, and induce the production of pro-inflammatory cytokines [94, 117] |
| **ECM components** | | |
| **(a) Hyaluronan** | TLR2 and TLR4 [51, 107] | Degraded HA products activate APC to stimulate chemokine and cytokine production [51, 107] |
| **(b) Fibronectin** (EDA domain) | TLR2 and TLR4 [88] | Cartilage degradation and pathologic changes in the joint connective tissues, also it provokes IL-1, MMP-1, MMP-3, and MMP-9 in chondrocytes and synoviocytes [88] |
| **Non-protein alarmins** | | |
| **(a) ATP** | P2X7 [100], P2Y2 [72], P2Y6 [121], P2Y12 [43], and P2X4 [45] | Promoting the maturation of APCs and orchestrating both innate and adaptive immune responses [70, 72, 121] |
| **(b) Uric acid** | NALP3 [70] | |

mitochondria dysfunction and cancer to maintain cell and tissue homeostasis [13, 37]. However, more recently, intracellular roles of MMP have been discovered to regulate gene expression [28–30] and to cleave intracellular proteins [52]. Moreover, recent studies discovered MMP as a cargo of EV [90]. Proteolytic roles of MMP on the surface of EV can lead the EV to migrate and invade through the

tissues, destruct barriers such as BBB, epithelial barriers, and membranes in living bodies, and lead multiple cargo factors of EV to reach distant organs. Therefore, the classical proteinase has a large potential as a novel moonlighting proteinase (Fig. 1). It has been shown that very small changes such as post-translational modifications (PTM) in a protein's covalent structure can change its biochemical function [49]. To insight into the moonlighting roles of HSP and MMP, we review their roles in inflammatory diseases, especially RA in this chapter.

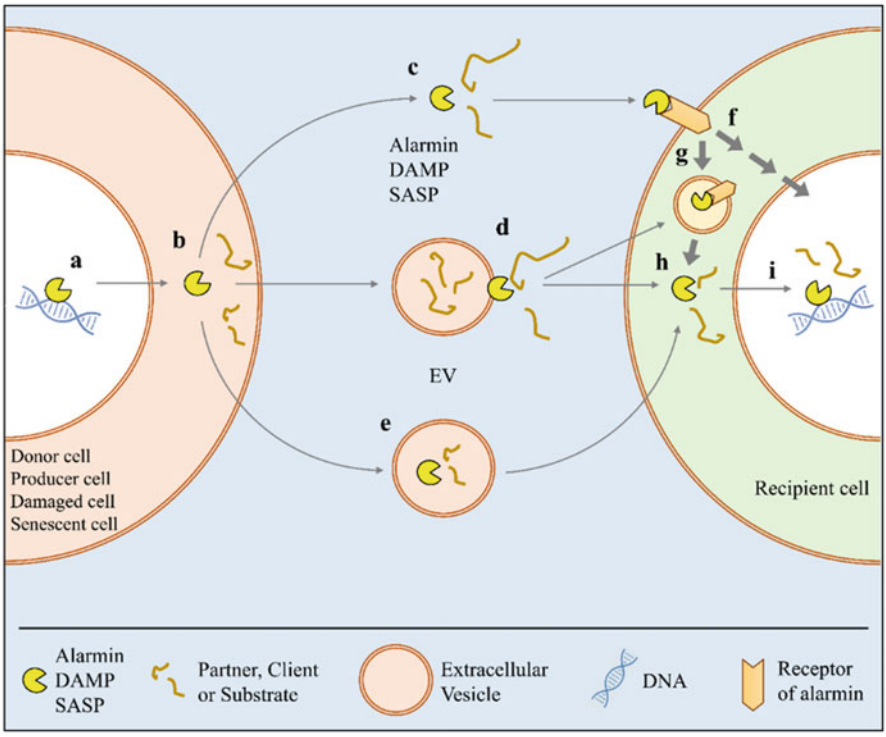



**Fig. 1 Travel of moonlighting proteins.** Alarmins are originally intracellular proteins, including intranuclear proteins in a donor cell, producer cell or damaged cell (**a**, **b**). Upon cell stress or damage, alarmins are released to extracellular space as a free form (**c**), an EV-associated form (**d**) or a cargo of EV (**e**). The free alarmin could bind to and activate a cell-surface receptor that stimulates intracellular signaling pathways (**f**) or endocytosis (**g**). Alarmins can be then released to the cytosol through membrane fusion between EV and cells or the endosomal escape pathway in the recipient cells in the local and distant milieu (**h**). Proteins containing nuclear localization signals (NLSs) such as HMGB1 and MMP3 could then translocate into nuclei of the recipient cells (**i**). HSP can promote the folding of client proteins in the recipient cells. EV-associated MMP can cleave extracellular proteins and destruct barriers such as biological membranes, BBB or epithelial barriers, and thus promote migration and invasion of EV through the barrier tissues in living bodies. MMP3 is also known as one of the SASP

## 1.1 Alarmins, DAMPs and Their Receptors

Alarmins are endogenic molecules rapidly released from damaged or infected tissues or necrotic cells into the extracellular milieu. The release of alarmins often causes inflammation. Alarmins are also known as danger signals and belong to the DAMPs [10, 40, 77, 82]. The representative alarmins include intracellular proteins, such as HSP [64], high-mobility group box 1 (HMGB1) [16], and S100 proteins, cytokines such as IL-1α and IL-33, and materials derived from the extracellular matrix that are generated following tissue injury, such as hyaluronan fragments [29]. Non-protein DAMPs include ATP [60, 65], uric acid [54] heparan sulfate, and DNA [40] (Table 1). The released alarmins bind to the surface receptors of cells that are involved in maintaining tissue repair and host defense. These cell surface receptors include the TLRs, the receptor for advanced glycosylation end products (RAGE), as well as chemokine receptors, integrins, SRs, CD91/LRP1, CD2, and CD44 [54, 93].

By contrast, pathogen-associated molecular patterns (PAMPs) initiate and sustain infectious pathogen-induced inflammatory responses. The cell surface receptors of PAMPs are TLRs, while the nucleotide-binding oligomerization domain (NOD)-like receptors (NOD-like receptors or NLRs) are intracellular sensors of PAMPs that enter the cell via phagocytosis or pores.

The binding of a single danger molecule can simultaneously activate multiple downstream signaling pathways, thus tight regulation of receptor signaling is required to prevent aberrant inflammatory reactions [40, 63, 64, 82]. Notably, in the absence of tissue injury or infection, alarmins play important intracellular roles such as regulating the DNA transcription, cell proliferation, differentiation, and also preventing proteins aggregation and denaturation as well as calcium homeostasis [10]. It has been reported that high extracellular levels of alarmins have been associated with various inflammatory and autoimmune diseases (such as arthritis, sepsis, atherosclerosis, and inflammatory bowel disease) and cancer [16].

## 1.2 The Moonlighting Functions of HSP

HSP is a large family of proteins that are expressed in all living organisms and exhibit a high level of conservation across species [123]. Some members of the HSP are constitutively expressed, including HSC70, while some are stress inducible which make them important biomarkers of diseases [55, 95, 98]. The stress inducible HSP are expressed in response to stress stimuli such as extreme temperature, hypoxia, oxidative stress, and inflammation [26, 105, 118]. Based on their molecular sizes, HSP are classified into six major families including HSP110, HSP90, HSP70, HSP60, HSP40, and small HSP (approximately 15–30 kDa) [19, 66].

HSP function as molecular chaperones due to their physiological and protective roles in cells [91]. For instance, HSP are involved in the maintenance of proper

protein folding, protein trafficking, and assembly/disassembly of protein complex, and degradation [12, 58, 91]. Thus, HSP play a key role in the cellular proteostasis under physiological and stressful conditions [85, 97]. Additionally, they are implicated in the modulation of host immune response, development of autoimmune diseases [24], in antigen processing and presentation [74], stabilization of transcription factors, assembly and disassembly of transcription machinery, DNA repair, and the cell cycle regulation [17]. Thus, HSP are considered as moonlighting proteins (or multifunctional proteins) as they exhibit several physiological functions within the cells [17, 48, 50, 67].

## *1.3 Roles of HSP in Rheumatoid Arthritis*

Rheumatoid Arthritis (RA) is a systemic inflammatory disease that is characterized by irreversible destruction of the cartilage, tendon, and bone that comprise synovial joints. Alarmins that are involved in inflammatory arthritis include the HMGB1 molecule, phagocyte-derived S100-proteins, HSP, and IL-33. It should be noted that the level of these molecules is significantly higher in the synovial fluid, synovium, and in the serum, of arthritis patients, besides they are correlated with the disease severity [33, 54, 60].

The inflamed synovial tissue of RA patients possesses many characteristics of a stressed tissue which resulted in an increase in HSP expression in the synovial tissue [101]. For instance, the inflamed rheumatoid joint is hypoxic, subjected to regular episodes of hypoxic-reperfusion injury (damage caused when blood supply returns to tissue after lack of oxygen), produces many reactive oxygen species, and it is a potent source of inflammatory cytokines [35]. This cellular stress would induce high expression of HSP and their secretion into the extracellular space (e.g. HSP60 and HSP70), therefore, HSP are excellent signals of pathological stress and tissue damage [34]. Notably, there are several potential receptors for HSP such as CD91, TLRs, CD36, CD40, or CD14 [102], which promote cytokine and chemokine release (e. g. TNF-α, IL-1, and IL-12) [34, 53, 86] by cells of the innate immune system or induce maturation of antigen-presenting cells (APC, e.g. DC) and thereby other immune cells including T cells and B cells [34].

In addition, HSP can modulate the adaptive immune response through the major histocompatibility complex (MHC)-dependent presentation of HSP-derived peptides to the TLR, resulting in the stimulation of cytotoxic T cells or proliferation of CD4+/CD25+/FOXP3+ regulatory T cells that exhibit anti-inflammatory properties such as secretion of IL-4, IL-10, and TGF-β [21, 34, 53, 86].

It has been reported that the stress-inducible HSP70 (iHSP70) was highly expressed on the surface of RA synovial fluid (RASF) myeloid DC and this occurred concurrently with the expression CD91 and CD14 HSP receptors [69]. Furthermore, Gp96 (a homolog of HSP90 present in the endoplasmic reticulum) was elevated in RA synovial tissue and it was correlated with lining thickness and inflammation [46]. Gp96 was purified from RA synovial macrophages and it was significantly

induced the transcription of TLR2, TNFα, and IL-8 [46]. Moreover, HSP60 has been involved in RA by activating monocytes of RA patients via TLR2 or TLR4 [9, 47]. Besides, HSP22 was identified in RA synovial tissue where it mediated DC activation through TLR4 [92]. HSP90 also contributed to the pathogenesis of RA via inducing a tumor-like synovial overgrowth by potentiating both actin cytoskeletal rearrangement through stabilizing integrin-linked kinase (ILK) and survival of synovial cells via MAPK and PI3K/AKT signaling pathways [41].

In addition, HSP have potential to attenuate inflammatory responses [113, 116] by activating CD4+/CD25+/FOXP3 + Treg that directly suppress the proinflammatory effector T cells via the release of IL-4, IL-10, and TGF-β [53, 86]. The transfer of these HSP-specific Treg inhibited inflammation in animal models of arthritis [87, 112].

The exact role of HSP in inflammatory arthritis has not been clearly defined, both pro-inflammatory and anti-inflammatory effects induced by several HSP families were described [15]. A possible explanation could be that studies reported the pro-inflammatory effects of HSP were performed with recombinant HSP produced in bacteria. Bacterial contaminants are considered to be responsible for the reported pro-inflammatory effects. Indeed, the purified bacterial HSP failed to induce pro-inflammatory responses in the absence of foreign PAMPs [7, 39, 103, 114].

## *1.4 Roles of MMP in Rheumatoid Arthritis*

MMP are zinc-dependent endopeptidases that are involved in the remodeling of the ECM under physiological (i.e. angiogenesis and wound healing) and pathological processes, i.e. cancers, RA, and osteoarthritis (OA) [29]. Notably, MMP3 is a proteolytic enzyme, a key player in the destruction of bone and cartilage components in RA and OA by breaking down various extracellular components, including collagens (types III, IV, V, IX, and XI), matrix proteins and proteoglycans and activating other pro-MMP such as pro-MMP-7, pro-MMP-8, and pro-MMP-9 [65]. It has been reported that RA patients have a significantly elevated MMP3 level in the serum and synovial fluid, making it an early predictor of progressive joint destruction and a strong prognostic marker of disease activity [33, 111] (Table 1). It was shown that suppression of proteolytically active, but not total MMP-3, was associated with treatment response in phase III clinical study of rheumatoid arthritis [99].

Importantly, intracellular transcriptional roles of MMP3 was originally found in normal cartilage and OA model, while MMP3 can be more abundantly released from synovial fibroblasts in the joint tissues in RA patients. Elevated levels of MMP3 in sera in arthritis patients can be a result of tissue damage and inflammation [111]. However, it has not been clarified whether MMP3 is free forms or EV-associated forms that carry EV cargo factors systemically. The roles of EV-associated MMP may be common between inflammatory diseases and cancers as a manner of alarmin, DAMP, SASP, or the moonlighting protein.

## 1.5 Tumorigenic and Oncogenic Properties of HSP-Enriched EV

Tumor-derived EV have recently emerged as putative biological mediators in cancer [89]. EV are highly specialized molecules in cellular communication, as they carry several oncogenic proteins, nucleic acids, and signaling molecules that can be transferred horizontally to the target cells and modulate the tumor microenvironment for supporting tumor growth, invasion, and metastasis [44, 89, 108].

It should be mentioned that HSP can be released from cells in EV such as exosomes and oncosomes, and they are involved in intercellular communication in cancer, immunity, and various pathological conditions [27, 105]. Proteomics analysis of EV released from oral squamous cell carcinoma (OSCC) revealed their enrichment with HSP90 [80]. The si-RNA mediated double knockdown of HSP90α and HSP90β remarkably reduced the survival of the metastatic OSCC cells, while the single knockdown of HSP90 was ineffective [80]. Likewise, the metastatic oral cancer-derived EV (MEV) were demonstrated to be enriched with HSP90α, HSP90β, and cancer-initiating cell marker CD326/EpCAM [81]. These MEV initiated epithelial cell transformation- EMT, promoted the tumorigenic, migratory, and invasive abilities of oral cancer cells and induced macrophages M2-like polarization. The triple knockdown of these chaperone molecules markedly diminished their levels, reduced their MEV transmission into macrophages, as well as attenuated the EMT events exerted through EV release [81].

Furthermore, proteome analysis of castration-resistant prostate cancer (CRPC)-EV showed that HSP90α, HSP90β, and their potential receptor CD91/LRP1 were enriched at high levels in CRPC cell-derived EV. Moreover, it has demonstrated that the prostate cancer (PC-3) cells release two types of vesicles, small s-EV (30–200 nm) under a non-heated condition, large L-EV (200–500 nm) and membrane-damaged EV [31]. importantly, both membrane-damaged EV and L-EV were co-released upon the heat shock stress, indicating that vesicular membranes were damaged by the stress [31].

## 1.6 Tumorigenic and Oncogenic Properties of MMP3-Enriched EV

Recently we have reported that oncosomes (oncogenic EV) enriched with MMP3 were highly transmissive and protumorigenic in vitro and in vivo [79]. EV derived from LuM1 (a rapidly metastatic murine cancer cell line which derived from a parental cancer cell line Colon26 (aka CT26)) were found to significantly promote the distant tumor growth through body fluids and cause cachexia in mice, followed by a systemic distribution of LuM1-EV into multiple organs, including the liver, lung, and head. Moreover, MMP3-enriched LuM1 EV were transferred into recipient cell nuclei where they altered their features in vitro, through the immediate

induction of an additional protumorigenic factor, CCN2/CTGF (connective tissue growth factor, aka cellular communication network factor 2). CCN2/CTGF is a matricellular protein essential for ECM integrity, tumor-stromal interaction, and possibly for EV integrity. On the other hand, the EV-derived from the MMP3 knockout cells abolished this transactivating effect [79]. Interestingly, the transferred MMP3 was stabilized from 15 min to 9 h post-EV-addition, while the induction of CCN2/CTGF was sustained from 15 min to 1 h, suggesting that CCN2/CTGF might be secreted from the cells upon synthesis or switched off by regulatory mechanisms. Furthermore, the migration and invasion properties of the tumor cells, as well as CCN2/CTGF promoter activity were significantly decreased after the knockout of *Mmp3* using the CRISPR/Cas9 genome editing [79].

Most recently, we demonstrated that the CRISPR/Cas9 mediated knockout of *Mmp3* gene significantly reduced the 3D-tumoroids formation in vitro, reduced the tetraspanins (CD9 and CD63) in EV, and resulted in destabilizing the EV structural integrity [106]. Indeed, the *Mmp3* gene loss was associated with abnormal disorganized shapes of EV such as crescent moon-like and broken EV. We found that MMP3-enriched EV were highly penetrative and deeply transferred to the recipient MMP3-null tumoroids. Moreover, we confirmed the EV-mediated molecular transfer of MMP3 into the MMP3-KO tumoroids under the 3D culture system by treating MMP3-KO cells with LuM1-EV or LuM1-condition medium (CM). Interestingly, MMP3 was restored and detected in the cytoplasmic and nuclear regions of MMP3-KO recipient cells after the addition of LuM1-EV or LuM1-CM [106]. Furthermore, CD9 that was low expressed in MMP3-KO cells became abundantly expressed on the cell surface and well co-localized with endogenous palmT (a membrane lipid-binding palmitoylation (palm) signal fused with fluorescent tandem dimer Tomato proteins) as honeycomb shape after the addition of LuM1-EV or LuM1-CM, suggesting that CD9 contributed to cell-cell adhesion in the recipient tumoroids.

The addition of MMP3-enriched EV fostered the tumorigenicity and increased the proliferation of MMP3-null cells as judged by the highly significant increase in Ki-67 expression index. Thus, we concluded that MMP3-enriched EV were highly transmissive and protumorigenic in vitro [106].

## 2 Conclusion

Protein moonlighting has been defined as that a single protein plays multiple functional roles. Among these proteins, we here focused on the HSP and MMP as moonlighting proteins, as well as alarmins involved in RA. HSP are originally molecular chaperones that essential for proper folding of intracellular proteins, whereas extracellular HSP plays cytokine-like roles through binding to receptors expressed on the surface of immune and cancer cells. On the other hand, MMP are primary extracellular proteinases that cleave ECM proteins, growth factors, and membrane receptors. However, intracellular MMP alter gene expression by regulating transcription. Many members of HSP and MMP protein families are often found

in EV and are overexpressed and extracellularly released in cancers and inflammatory diseases, including RA. Furthermore, HSP are also known as alarmins or DAMPs, which are endogenous molecules released into the extracellular microenvironment upon cellular damage. Extracellular MMP are known as one of the SASP associated with DNA damage, mitochondria dysfunction, and cancer. The multifunctionality of HSP and MMP provide evidence as moonlighting proteins.

**Acknowledgements** The authors thank Chiharu Sogawa, Yuka Okusha, Yanyin Lu, Manh Tien Tran, Keisuke Nakano, and Hotaka Kawai for illuminating discussion and experimentation. The authors appreciate Masaharu Takigawa, Stuart K. Calderwood, Kuniaki Okamoto, Ken-ichi Kozaki, and Ayano Satoh for mentorship and support. T.E. was supported by JSPS KAKENHI, grant numbers, JP17K11642-TE), JP17K11669-KOh, JP18K09789-KN, 19H04051-HO, 19H03817-MT, and by Suzuki Kenzo Memorial Foundation. E.A.T. was supported by the Egypt-Japan Education Partnership (EJEP) grant.

**Disclosure of Interests** The authors declare no conflict of interest with the content of this study.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Abubucker S, Segata N, Goll J, Schubert AM, Izard J, Cantarel BL et al (2015) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 7(2):1–9. https://doi.org/10.1016/j.molcel.2012.08.033
2. Aoshiba K, Tsuji T, Kameyama S, Itoh M, Semba S, Yamaguchi K, Nakamura H (2013) Senescence-associated secretory phenotype in a mouse model of bleomycin-induced lung injury. Exp Toxicol Pathol 65(7–8):1053–1062. https://doi.org/10.1016/j.etp.2013.04.001
3. Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW et al (2000) HSP70 stimulates cytokine production through a CD 14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6(4):435–442. https://doi.org/10.1038/74697
4. Basu S, Binder RJ, Ramalingam T, Srivastava PK (2001) CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity 14(3):303–313. https://doi.org/10.1016/S1074-7613(01)00111-X
5. Becker T, Hartl F-U, Wieland F (2002) CD40, an extracellular receptor for binding and uptake of Hsp70-peptide complexes. J Cell Biol 158(7):1277–1285. https://doi.org/10.1083/jcb.200208083
6. Ben-Sasson SZ, Hu-Li J, Quiel J, Cauchetaux S, Ratner M, Shapira I et al (2009) IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. Proc Natl Acad Sci U S A 106(17):7119–7124. https://doi.org/10.1073/pnas.0902745106
7. Bendz H, Marincek BC, Momburg F, Ellwart JW, Issels RD, Nelson PJ, Noessner E (2008) Calcium signaling in dendritic cells by human or mycobacterial Hsp70 is caused by contamination and is not required for Hsp70-mediated enhancement of cross-presentation. J Biol Chem 283(39):26477–26483. https://doi.org/10.1074/jbc.M803310200

8. Berwin B, Hart JP, Rice S, Gass C, Pizzo SV, Post SR, Nicchitta CV (2003) Scavenger receptor-A mediates gp96/GRP94 and calreticulin internalization by antigen-presenting cells. EMBO J 22(22):6127–6136. https://doi.org/10.1093/emboj/cdg572
9. Bethke K, Staib F, Distler M, Schmitt U, Jonuleit H, Enk AH et al (2002) Different efficiency of heat shock proteins (HSP) to activate human monocytes and dendritic cells: superiority of HSP60. J Immunol 169(11):6141–6148. https://doi.org/10.4049/jimmunol.169.11.6141
10. Bianchi ME (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 81(1):1–5. https://doi.org/10.1189/jlb.0306164
11. Brooks PC, Silletti S, von Schalscha TL, Friedlander M, Cheresh DA (1998) Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. Cell 92(3):391–400. https://doi.org/10.1016/s0092-8674(00)80931-9
12. Bukau B, Weissman J, Horwich A (2006) Molecular chaperones and protein quality control. Cell 125(3):443–451. https://doi.org/10.1016/j.cell.2006.04.014
13. Campisi J (2013) Aging, cellular senescence, and cancer. Annu Rev Physiol 75(1):685–705. https://doi.org/10.1146/annurev-physiol-030212-183653
14. Castiglioni A, Canti V, Rovere-Querini P, Manfredi AA (2011) High-mobility group box 1 (HMGB1) as a master regulator of innate immunity. Cell Tissue Res 343(1):189–199. https://doi.org/10.1007/s00441-010-1033-1
15. Celis L, Vandevyver C, Geusens P, Dequeker J, Raus J, Zhang J (1997) Clonal expansion of mycobacterial heat-shock protein-reactive T lymphocytes in the synovial fluid and blood of rheumatoid arthritis patients. Arthritis Rheum 40(3):510–519. https://doi.org/10.1002/art.1780400317
16. Chan JK, Roth J, Oppenheim JJ, Tracey KJ, Vogl T, Feldmann M et al (2012) Alarmins: awaiting a clinical response. J Clin Invest 122(8):2711–2719. https://doi.org/10.1172/JCI62423
17. Chen C, Jeffery C (2019) Moonlighting functions of heat shock protein 90. In: Kaur P, Asea AAA (eds) Heat shock protein 90 in human diseases and disorders, 19th edn. Springer, Cham. https://doi.org/10.1007/978-3-030-23158-3_13
18. Chen GY, Tang J, Zheng P, Liu Y (2009) CD24 and siglec-10 selectively repress tissue damage – induced immune responses. Science 323(5922):1722–1725. https://doi.org/10.1126/science.1168988
19. Ciocca DR, Calderwood SK (2005, June) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 10:86–103. https://doi.org/10.1379/CSC-99r.1
20. Das N, Dewan V, Grace PM, Gunn RJ, Tamura R, Tzarum N et al (2016) HMGB1 activates proinflammatory signaling via TLR5 leading to allodynia. Cell Rep 17(4):1128–1140. https://doi.org/10.1016/j.celrep.2016.09.076
21. de Kleer IM, Wedderburn LR, Taams LS, Patel A, Varsani H, Klein M et al (2004) CD4 + CD25 bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. J Immunol 172(10):6435–6443. https://doi.org/10.4049/jimmunol.172.10.6435
22. Delneste Y (2004) Scavenger receptors and heat-shock protein-mediated antigen cross-presentation. Biochem Soc Trans 32(4):633–635. https://doi.org/10.1042/BST0320633
23. Dinarello CA (1994) The biological properties of interleukin-1. Eur Cytokine Netw 5:517–531
24. Dreiza CM, Komalavilas P, Furnish EJ, Flynn CR, Sheller MR, Smoke CC et al (2010) The small heat shock protein, HSPB6, in muscle function and disease. Cell Stress Chaperones 15:1–11. https://doi.org/10.1007/s12192-009-0127-8
25. Dumitriu IE, Baruah P, Bianchi ME, Manfredi AA, Rovere-Querini P (2005) Requirement of HMGB1 and RAGE for the maturation of human plasmacytoid dendritic cells. Eur J Immunol 35(7):2184–2190. https://doi.org/10.1002/eji.200526066
26. Eguchi T, Lang BJ, Murshid A, Prince T, Gong J, Calderwood SK (2018) Regulatory roles for Hsp70 in cancer incidence and tumor progression. In: Role of molecular chaperones in structural folding, biological functions, and drug interactions of client proteins: frontiers in

structural biology, vol 1. Bentham Science Publishers Ltd, Sharjah, pp 1–22. https://doi.org/10.2174/9781681086156118010003
27. Eguchi T, Ono K, Kawata K, Okamoto K, Calderwood SK (2019) Regulatory roles of HSP90-rich extracellular vesicles. In: Kaur P, Asea AAA (eds) Heat shock protein 90 in human diseases and disorders, 19th edn. https://doi.org/10.1007/978-3-030-23158-3_1
28. Eguchi T, Calderwood SK, Takigawa M, Kubota S, Kozaki KI (2017) Intracellular MMP3 promotes HSP gene expression in collaboration with chromobox proteins. J Cell Biochem 118 (1):43–51. https://doi.org/10.1002/jcb.25607
29. Eguchi T, Kubota S, Kawata K, Mukudai Y, Uehara J, Ohgawara T et al (2008) Novel transcription-factor-like function of human matrix metalloproteinase 3 regulating the CTGF/CCN2 gene. Mol Cell Biol 28(7):255–264. https://doi.org/10.1128/MCB.01288-07
30. Eguchi T, Kubota S, Kawata K, Mukudai Y, Uehara J, Ohgawara T et al (2010) Novel transcriptional regulation of CCN2/CTGF by nuclear translocation of MMP3. In: CCN proteins in health and disease, pp 255–264. https://doi.org/10.1007/978-90-481-3779-4_19
31. Eguchi T, Sogawa C, Ono K, Matsumoto M, Tran MT, Okusha Y et al (2020) Cell stress induced Stressome release including damaged membrane vesicles and extracellular HSP90 by prostate cancer cells. Cells 9(3):755. https://doi.org/10.3390/cells9030755
32. Ehrchen JM, Sunderkötter C, Foell D, Vogl T, Roth J (2009) The endogenous toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J Leukoc Biol 86(3):557–566. https://doi.org/10.1189/jlb.1008647
33. Fadda S, Abolkheir E, Afifi R, Gamal M (2016) Serum matrix metalloproteinase-3 in rheumatoid arthritis patients: correlation with disease activity and joint destruction. Egypt Rheumatol 38(3):153–159. https://doi.org/10.1016/j.ejr.2016.01.001
34. Foell D, Wittkowski H, Roth J (2007, July) Mechanisms of disease: a "DAMP" view of inflammatory arthritis. Nat Clin Pract Rheumatol 3:382–390. https://doi.org/10.1038/ncprheum0531
35. Panayi GS, Corrigal VM (2003) Heat shock proteins and rheumatoid arthritis. In: van W Eden, D. of Immunology, D. of I. D. and Immunology, F. of V. Medicine, U. of Utrecht, Y. . . . T. Netherlands (ed) Heat shock proteins and inflammation. Birkhäuser, Basel, pp 109–137. https://doi.org/10.1007/978-3-0348-8028-2
36. Gastpar R, Gross C, Rossbacher L, Ellwart J, Riegger J, Multhoff G (2004) The cell surface-localized heat shock protein 70 epitope TKD induces migration and Cytolytic activity selectively in human NK cells. J Immunol 172(2):972–980. https://doi.org/10.4049/jimmunol.172.2.972
37. Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C et al (2019, October 31) Cellular senescence: defining a path forward. Cell 179:813–827. https://doi.org/10.1016/j.cell.2019.10.005
38. Gu L, Kitamura M (2012) Sensitive detection and monitoring of senescence-associated secretory phenotype by SASP-RAP assay. PLoS One 7(12). https://doi.org/10.1371/journal.pone.0052305
39. Bausinger H, Lipsker D, Ziylan U, Manie S, Briand J-P, Cazenave J-P et al (2002) Endotoxin-free heat-shock protein 70 fails to induce APC activation. Eur J Immunol 32(12):3708–3713. https://doi.org/10.1002/1521-4141(200212)32:12<3708::AID-IMMU3708>3.0.CO;2-C
40. Harris HE, Raucci A (2006) Alarmin(g) news about danger: workshop on innate danger signals and HMGB1. EMBO Rep 7(8):774–778. https://doi.org/10.1038/sj.embor.7400759
41. Hashiramoto A, Murata M, Kawazoe T, Yoshida K, Akiyama C, Shiozawa K, Shiozawa S (2011) Heat shock protein 90 maintains the tumour-like character of rheumatoid synovial cells by stabilizing integrin-linked kinase, extracellular signal-regulated kinase and protein kinase B. Rheumatology (Oxford) 50(5):852–861. https://doi.org/10.1093/rheumatology/keq385
42. Hassona Y, Cirillo N, Heesom K, Parkinson EK, Prime SS (2014) Senescent cancer-associated fibroblasts secrete active MMP-2 that promotes keratinocyte dis-cohesion and invasion. Br J Cancer 111(6):1230–1237. https://doi.org/10.1038/bjc.2014.438

43. Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D (2006) The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat Neurosci 9 (12):1512–1519. https://doi.org/10.1038/nn1805
44. Higginbotham JN, Demory Beckler M, Gephart JD, Franklin JL, Bogatcheva G, Kremers GJ et al (2011) Amphiregulin exosomes increase cancer cell invasion. Curr Biol 21(9):779–786. https://doi.org/10.1016/j.cub.2011.03.043
45. Horvath RJ, DeLeo JA (2009) Morphine enhances microglial migration through modulation of P2X 4 receptor signaling. J Neurosci 29(4):998–1005. https://doi.org/10.1523/JNEUROSCI.4595-08.2009
46. Huang Q-Q, Sobkoviak R, Jockheck-Clark AR, Shi B, Mandelin AM, Tak PP et al (2009) Heat shock protein 96 is elevated in rheumatoid arthritis and activates macrophages primarily via TLR2 Signaling. J Immunol 182(8):4965–4973. https://doi.org/10.4049/jimmunol.0801563
47. Iwahashi M, Yamamura M, Aita T, Okamoto A, Ueno A, Ogawa N et al (2004) Expression of toll-like receptor 2 on CD16+ blood monocytes and synovial tissue macrophages in rheumatoid arthritis. Arthritis Rheum 50(5):1457–1467. https://doi.org/10.1002/art.20219
48. Jeffery CJ (1999) Moonlighting proteins. Trends Biochem Sci 24:8–11. https://doi.org/10.1016/S0968-0004(98)01335-8
49. Jeffery CJ (2016) Protein species and moonlighting proteins: very small changes in a protein's covalent structure can change its biochemical function. J Proteome 134:19–24. https://doi.org/10.1016/j.jprot.2015.10.003
50. Jeffery CJ (2018) Protein moonlighting: what is it, and why is it important? Philos Trans R Soc B Biol Sci 373:20160523. https://doi.org/10.1098/rstb.2016.0523
51. Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y et al (2005) Regulation of lung injury and repair by toll-like receptors and hyaluronan. Nat Med 11(11):1173–1179. https://doi.org/10.1038/nm1315
52. Jobin PG, Butler GS, Overall CM (2017) New intracellular activities of matrix metalloproteinases shine in the moonlight. Biochim Biophys Acta, Mol Cell Res 1864:2043–2055. https://doi.org/10.1016/j.bbamcr.2017.05.013
53. Kamphuis S, Kuis W, De Jager W, Teklenburg G, Massa M, Gordon G et al (2005) Tolerogenic immune responses to novel T-cell epitopes from heat-shock protein 60 in juvenile idiopathic arthritis. Lancet 366(9479):50–56. https://doi.org/10.1016/S0140-6736(05)66827-4
54. Kerkhoff C, Radon Y, Flaßkamp H (2014) Alarmins. In: Encyclopedia of inflammatory diseases, pp 1–12. https://doi.org/10.1007/978-3-0348-0620-6_78-1
55. Khalil AA, Kabapy NF, Deraz SF, Smith C (2011) Heat shock proteins in oncology: diagnostic biomarkers or therapeutic targets? Biochim Biophys Acta Rev Cancer 1816:89–104. https://doi.org/10.1016/j.bbcan.2011.05.001
56. Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA (2000) Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. J Immunol 164(1):13–17. https://doi.org/10.4049/jimmunol.164.1.13
57. Kondo S (2002) Connective tissue growth factor increased by hypoxia may initiate angiogenesis in collaboration with matrix metalloproteinases. Carcinogenesis 23(5):769–776. https://doi.org/10.1093/carcin/23.5.769
58. Kriegenburg F, Ellgaard L, Hartmann-Petersen R (2012) Molecular chaperones in targeting misfolded proteins for ubiquitin-dependent degradation. FEBS J 279:532–542. https://doi.org/10.1111/j.1742-4658.2011.08456.x
59. Kwan JA, Schulze CJ, Wang W, Leon H, Sariahmetoglu M, Sung M et al (2004) Matrix metalloproteinase-2 (MMP-2) is present in the nucleus of cardiac myocytes and is capable of cleaving poly (ADP-ribose) polymerase (PARP) in vitro. FASEB J 18(6):690–692. https://doi.org/10.1096/fj.02-1202fje

60. Lavric M, Miranda-García MA, Holzinger D, Foell D, Wittkowski H (2017) Alarmins firing arthritis: helpful diagnostic tools and promising therapeutic targets. Joint Bone Spine 84 (4):401–410. https://doi.org/10.1016/j.jbspin.2016.06.010
61. Lewthwaite JC, Coates ARM, Tormay P, Singh M, Mascagni P, Poole S et al (2001) Mycobacterium tuberculosis chaperonin 60.1 is a more potent cytokine stimulator than chaperonin 60.2 (Hsp 65) and contains a CD14-binding domain. Infect Immun 69 (12):7349–7355. https://doi.org/10.1128/IAI.69.12.7349-7355.2001
62. Lim H, Park H, Kim HP (2015) Effects of flavonoids on senescence-associated secretory phenotype formation from bleomycin-induced senescence in BJ fibroblasts. Biochem Pharmacol 96(4):337–348. https://doi.org/10.1016/j.bcp.2015.06.013
63. Liu-Bryan R, Terkeltaub R (2015) Emerging regulators of the inflammatory process in osteoarthritis. Nat Rev Rheumatol 11(1):35–44. https://doi.org/10.1038/nrrheum.2014.162
64. Loeser RF, Goldring SR, Scanzello CR, Goldring MB (2012) Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum 64(6):1697–1707. https://doi.org/10.1002/art.34453
65. Ma J-D, Zhou J-J, Zheng D-H, Chen L-F, Mo Y-Q, Wei X et al (2014) Serum matrix metalloproteinase-3 as a noninvasive biomarker of histological synovitis for diagnosis of rheumatoid arthritis. Mediat Inflamm 2014:179284. https://doi.org/10.1155/2014/179284
66. Macario AJL, De Macario EC (2007) Molecular chaperones: multiple functions, pathologies, and potential applications. Front Biosci *12*(7):2588–2600. https://doi.org/10.2741/2257
67. Mani M, Chen C, Amblee V, Liu H, Mathur T, Zwicke G et al (2014) MoonProt: a database for proteins that are known to moonlight. Nucleic Acids Res 43:277–282. https://doi.org/10.1093/nar/gku954
68. Marchant DJ, Bellac CL, Moraes TJ, Wadsworth SJ, Dufour A, Butler GS et al (2014) A new transcriptional role for matrix metalloproteinase-12 in antiviral immunity. Nat Med 20 (5):493–502. https://doi.org/10.1038/nm.3508
69. Martin CA, Carsons SE, Kowalewski R, Bernstein D, Valentino M, Santiago-Schwarz F (2003) Aberrant extracellular and dendritic cell (DC) surface expression of heat shock protein (hsp)70 in the rheumatoid joint: possible mechanisms of hsp/DC-mediated cross-priming. J Immunol 171(11):5736–5742. https://doi.org/10.4049/jimmunol.171.11.5736
70. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440(7081):237–241. https://doi.org/10.1038/nature04516
71. Massa M, Passalia M, Manzoni SM, Campanelli R, Ciardelli L, Yung GP et al (2007) Differential recognition of heat-shock protein dnaJ-derived epitopes by effector and Treg cells leads to modulation of inflammation in juvenile idiopathic arthritis. Arthritis Rheum 56 (5):1648–1657. https://doi.org/10.1002/art.22567
72. Müller T, Robaye B, Vieira RP, Ferrari D, Grimm M, Jakob T et al (2010) The purinergic receptor P2Y2 receptor mediates chemotaxis of dendritic cells and eosinophils in allergic lung inflammation. Allergy Eur J Allergy Clin Immunol 65(12):1545–1553. https://doi.org/10.1111/j.1398-9995.2010.02426.x
73. Murshid A, Gong J, Calderwood SK (2010) Heat shock protein 90 mediates efficient antigen cross presentation through the scavenger receptor expressed by endothelial cells-I. J Immunol 185(5):2903–2917. https://doi.org/10.4049/jimmunol.0903635
74. Murshid A, Gong J, Calderwood SK (2012) The role of heat shock proteins in antigen cross presentation. Front Immunol 3:63. https://doi.org/10.3389/fimmu.2012.00063
75. Murshid A, Gong J, Calderwood SK (2014) Hsp90-peptide complexes stimulate antigen presentation through the class II pathway after binding scavenger receptor SREC-I. Immunobiology 219(12):924–931. https://doi.org/10.1016/j.imbio.2014.08.001
76. Nakae S, Naruse-Nakajima C, Sudo K, Horai R, Asano M, Iwakura Y (2001) IL-1α, but not IL-1β, is required for contact-allergen-specific T cell activation during the sensitization phase in contact hypersensitivity. Int Immunol 13(12):1471–1478. https://doi.org/10.1093/intimm/13.12.1471

77. Nefla M, Holzinger D, Berenbaum F, Jacques C (2016) The danger from within: Alarmins in arthritis. Nat Rev Rheumatol 12(11):669–683. https://doi.org/10.1038/nrrheum.2016.162
78. Okusha Y, Eguchi T, Sogawa C, Okui T, Nakano K, Okamoto K et al (2018) The intranuclear PEX domain of MMP involves proliferation, migration, and metastasis of aggressive adenocarcinoma cells. J Cell Biochem 119:(April), 1–(April),14. https://doi.org/10.1002/jcb.27040
79. Okusha Y, Eguchi T, Tran MT, Sogawa C, Yoshida K, Itagaki M et al (2020) Extracellular vesicles enriched with moonlighting metalloproteinase are highly transmissive, pro-tumorigenic, and trans-activates cellular communication network factor (Ccn2/ctgf): CRISPR against cancer. Cancers 12(4):1–27. https://doi.org/10.3390/cancers12040881
80. Ono K, Eguchi T, Sogawa C, Calderwood SK, Futagawa J, Kasai T et al (2018) HSP-enriched properties of extracellular vesicles involve survival of metastatic oral cancer cells. J Cell Biochem 119(9):7350–7362. https://doi.org/10.1002/jcb.27039
81. Ono K, Sogawa C, Kawai H, Tran MT, Taha EA, Lu Y et al (2020) Triple knockdown of CDC37, HSP90-alpha, and HSP90-beta diminishes extracellular vesicles-driven malignancy events and macrophage M2 polarization in oral cancer. J Extracell Vesicles 9:1769373
82. Oppenheim JJ, Yang D (2005) Alarmins: chemotactic activators of immune responses. Curr Opin Immunol 17(4):359–365. https://doi.org/10.1016/j.coi.2005.06.002
83. Page-McCaw A, Ewald AJ, Werb Z (2007, March) Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol 8:221–233. https://doi.org/10.1038/nrm2125
84. Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, Abraham E (2004) Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem 279(9):7370–7377. https://doi.org/10.1074/jbc.M306793200
85. Pearl LH (2016) Review: the HSP90 molecular chaperone – an enigmatic ATPase. Biopolymers 105:594–607. https://doi.org/10.1002/bip.22835
86. Prakken BJ, Roord S, Van Kooten PJS, Wagenaar JPA, Van Eden W, Albani S, Wauben MHM (2002) Inhibition of adjuvant-induced arthritis by interleukin-10-driven regulatory cells induced via nasal administration of a peptide analog of an arthritis-related heat-shock protein 60 T cell epitope. Arthritis Rheum 46(7):1937–1946. https://doi.org/10.1002/art.10366
87. Prakken BJ, Samodal R, Le TD, Giannoni F, Yung GP, Scavulli J et al (2004) Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. Proc Natl Acad Sci U S A 101(12):4228–4233. https://doi.org/10.1073/pnas.0400061101
88. Przybysz M, Borysewicz K, Katnik-Prastowska I (2013) Fibronectin molecular status determination useful to differentiate between rheumatoid arthritis and systemic lupus erythematosus patients. Rheumatol Int *33*(1):37–43. https://doi.org/10.1007/s00296-011-2269-0
89. Rak J, Guha A (2012) Extracellular vesicles – vehicles that spread cancer genes. BioEssays 34 (6):489–497. https://doi.org/10.1002/bies.201100169
90. Ramteke A, Ting H, Agarwal C, Mateen S, Somasagara R, Hussain A et al (2015) Exosomes secreted under hypoxia enhance invasiveness and stemness of prostate cancer cells by targeting adherens junction molecules. Mol Carcinog 54(7):554–565. https://doi.org/10.1002/mc.22124
91. Rappa F, Farina F, Zummo G, David S, Campanella C, Carini F et al (2012) HSP-Molecular chaperones in cancer biogenesis and tumor therapy: an overview. Anticancer Res 32 (12):5139–5150
92. Roelofs MF, Boelens WC, Joosten LAB, Abdollahi-Roodsaz S, Geurts J, Wunderink LU et al (2006) Identification of small heat shock protein B8 (HSP22) as a novel TLR4 ligand and potential involvement in the pathogenesis of rheumatoid arthritis. J Immunol 176 (11):7021–7027. https://doi.org/10.4049/jimmunol.176.11.7021
93. Rosin DL, Okusa MD (2011) Dangers within: DAMP responses to damage and cell death in kidney disease. J Am Soc Nephrol 22(3):416–425. https://doi.org/10.1681/ASN.2010040430

94. Ryckman C, Vandal K, Rouleau P, Talbot M, Tessier PA (2003) Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. J Immunol 170(6):3233–3242. https://doi.org/10.4049/jimmunol.170.6.3233
95. Santos-Junior V d A, Lollo PCB, Cantero MA, Moura CS, Amaya-Farfan J, Morato PN (2018) Heat shock proteins: protection and potential biomarkers for ischemic injury of cardiomyocytes after surgery. Braz J Cardiovasc Surg 33:291–302. https://doi.org/10.21470/1678-9741-2017-0169
96. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK et al (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 23(5):479–490. https://doi.org/10.1016/j.immuni.2005.09.015
97. Schopf FH, Biebl MM, Buchner J (2017, June 1) The HSP90 chaperone machinery. Nat Rev Mol Cell Biol 18:345–360. https://doi.org/10.1038/nrm.2017.20
98. Shekhawat SD, Purohit HJ, Taori GM, Daginawala HF, Kashyap RS (2016) Evaluation of host Hsp(s) as potential biomarkers for the diagnosis of tuberculous meningitis. Clin Neurol Neurosurg 140:47–51. https://doi.org/10.1016/j.clineuro.2015.11.008
99. Siebuhr AS, Kjelgaard-Petersen CF, Sun S, Byrjalsen I, Christiansen C, Karsdal MA, Bay-Jensen AC (2018) Suppression of active, but not total MMP-3, is associated with treatment response in a phase III clinical study of rheumatoid arthritis. Clin Exp Rheumatol 36(1):94–101
100. Sluyter R, Shemon AN, Wiley JS (2004) Glu496 to Ala polymorphism in the P2X7 receptor impairs ATP-induced IL-1 beta release from human monocytes. J Immunol 172 (6):3399–3405. https://doi.org/10.4049/jimmunol.172.6.3399
101. Spierings J, Van Eden W (2017) Heat shock proteins and their immunomodulatory role in inflammatory arthritis. Rheumatology 56(2):198–208. https://doi.org/10.1093/rheumatology/kew266
102. Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev Immunol 2:185–194. https://doi.org/10.1038/nri749
103. Stocki P, Wang XN, Dickinson AM (2012) Inducible heat shock protein 70 reduces T cell responses and stimulatory capacity of monocyte-derived dendritic cells. J Biol Chem 287 (15):12387–12394. https://doi.org/10.1074/jbc.M111.307579
104. Suwara MI, Green NJ, Borthwick LA, Mann J, Mayer-Barber KD, Barron L et al (2014) IL-1a released from damaged epithelial cells is sufficient and essential to trigger inflammatory responses in human lung fibroblasts. Mucosal Immunol 7(3):684–693. https://doi.org/10.1038/mi.2013.87
105. Taha EA, Ono K, Eguchi T (2019) Roles of extracellular HSP as biomarkers in immune surveillance and immune evasion. Int J Mol Sci 20(18). https://doi.org/10.3390/ijms20184588
106. Taha EA, Sogawa C, Okusha Y, Kawai H, Oo MW, Elseoudi A et al (2020) Knockout of MMP3 weakens solid tumor organoids and cancer extracellular vesicles. Cancer 12(5):1260, 1–29. https://doi.org/10.3390/cancers12051260
107. Tesar BM, Jiang D, Liang J, Palmer SM, Noble PW, Goldstein DR (2006) The role of hyaluronan degradation products as innate alloimmune agonists. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg 6(11):2622–2635. https://doi.org/10.1111/j.1600-6143.2006.01537.x
108. Tovar-Camargo OA, Toden S, Goel A (2016) Exosomal microRNA biomarkers: emerging Frontiers in colorectal and other human cancers. Expert Rev Mol Diagn 16:553–567. https://doi.org/10.1586/14737159.2016.1156535
109. Tracy EC, Bowman MJ, Henderson BW, Baumann H (2012) Interleukin-1α is the major alarmin of lung epithelial cells released during photodynamic therapy to induce inflammatory mediators in fibroblasts. Br J Cancer 107(9):1534–1546. https://doi.org/10.1038/bjc.2012.429
110. Turnquist HR, Zhao Z, Rosborough BR, Liu Q, Castellaneta A, Isse K et al (2011) IL-33 expands suppressive CD11b+ Gr-1(int) and regulatory T cells, including ST2L+ Foxp3+ cells,

and mediates regulatory T cell-dependent promotion of cardiac allograft survival. J Immunol 187(9):4598–4610. https://doi.org/10.4049/jimmunol.1100519
111. Uemura Y, Hayashi H, Takahashi T, Saitho T, Umeda R, Ichise Y et al (2015) [MMP-3 as a biomarker of disease activity of rheumatoid arthritis]. Rinsho Byori. Jpn J Clin Pathol 63 (12):1357–1364. http://www.ncbi.nlm.nih.gov/pubmed/27089651
112. Van Eden W, Van Der Zee R, Prakken B (2005) Heat-shock proteins induce T-cell regulation of chronic inflammation. Nat Rev Immunol 5:318–330. https://doi.org/10.1038/nri1593
113. Van Eden W, Van Herwijnen M, Wagenaar J, Van Kooten P, Broere F, Van Der Zee R (2013) Stress proteins are used by the immune system for cognate interactions with anti-inflammatory regulatory T cells. FEBS Lett 587:1951–1958. https://doi.org/10.1016/j.febslet.2013.05.024
114. Wallin RPA, Lundqvist A, Moré SH, Von Bonin A, Kiessling R, Ljunggren HG (2002) Heat-shock proteins as activators of the innate immune system. Trends Immunol 23:130–135. https://doi.org/10.1016/S1471-4906(01)02168-8
115. Wang XB, Bozdagi O, Nikitczuk JS, Zu WZ, Zhou Q, Huntley GW (2008) Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. Proc Natl Acad Sci U S A 105(49):19520–19525. https://doi.org/10.1073/pnas.0807248105
116. Wieten L, Broere F, van der Zee R, Koerkamp EK, Wagenaar J, van Eden W (2007) Cell stress induced HSP are targets of regulatory T cells: a role for HSP inducing compounds as anti-inflammatory immuno-modulators? FEBS Lett 581:3716–3722. https://doi.org/10.1016/j.febslet.2007.04.082
117. Wolf R, Howard OMZ, Dong H-F, Voscopoulos C, Boeshans K, Winston J et al (2008) Chemotactic activity of S100A7 (Psoriasin) is mediated by the receptor for advanced glycation end products and potentiates inflammation with highly homologous but functionally distinct S100A15. J Immunol 181(2):1499–1506. https://doi.org/10.4049/jimmunol.181.2.1499
118. Wu J, Liu T, Rios Z, Mei Q, Lin X, Cao S (2017) Heat shock proteins and cancer. Trends Pharmacol Sci 38(3):226–256. https://doi.org/10.1016/j.tips.2016.11.009
119. Yang D, Chen Q, Yang H, Tracey KJ, Bustin M, Oppenheim JJ (2007) High mobility group box-1 protein induces the migration and activation of human dendritic cells and acts as an alarmin. J Leukoc Biol 81(1):59–66. https://doi.org/10.1189/jlb.0306180
120. Yang Y, Candelario-Jalil E, Thompson JF, Cuadrado E, Estrada EY, Rosell A et al (2010) Increased intranuclear matrix metalloproteinase activity in neurons interferes with oxidative DNA repair in focal cerebral ischemia. J Neurochem 112(1):134–149. https://doi.org/10.1111/j.1471-4159.2009.06433.x
121. Yebdri FB, Kukulski F, Tremblay A, Sévigny J (2009) Concomitant activation of P2Y2 and P2Y6 receptors on monocytes is required for TLR1/2-induced neutrophil migration by regulating IL-8 secretion. Eur J Immunol 39(10):2885–2894. https://doi.org/10.1002/eji.200939347
122. Yokota S i, Minota S, Fujii N (2006) Anti-HSP auto-antibodies enhance HSP-induced pro-inflammatory cytokine production in human monocytic cells via toll-like receptors. Int Immunol 18(4):573–580. https://doi.org/10.1093/intimm/dxh399
123. Zininga T, Ramatsui L, Shonhai A (2018) Heat shock proteins as immunomodulants. Molecules 23(11):2846. https://doi.org/10.3390/molecules23112846

# Heat Shock Proteins and Periodontitis – Cross-Reaction Between Bacterial and Human HSP in Periodontal Infection Linking with Cardiovascular Diseases

**Tadashi Yamamoto and Takanori Eguchi**

## Abstract

**Introduction** Periodontitis is a globally prevalent chronic inflammatory disease caused by oral dysbiotic biofilm. The host immune and inflammatory responses against the biofilm play a crucial role in the initiation and progression of periodontitis. Many members of heat shock proteins (HSP) are upregulated by varieties of stresses in periodontal pathogenic bacteria, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. HSP are immunodominant antigens in periodontitis, however, it has been unclear whether/how anti-HSP reactions involve the pathogenesis of periodontitis as well as cardiovascular diseases. In here, we aim to clarify whether/how anti-HSP reactions involve the pathogenesis of periodontitis and cardiovascular diseases.

**Methods** We review the current knowledge of oral bacterial and mammalian HSP and discuss immunoreaction to HSP in periodontitis.

**Results** Among bacterial HSP, GroEL is well investigated in auto-immune reactions because of the sequence similarity with human Hsp60. The antibody titers to

Authors Tadashi Yamamoto and Takanori Eguchi have equally contributed to this chapter.

T. Yamamoto (✉)
Department of Pathophysiology – Periodontal Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
e-mail: tadashii@md.okayama-u.ac.jp

T. Eguchi (✉)
Department of Dental Pharmacology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

Advanced Research Center for Oral and Craniofacial Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
e-mail: eguchi@okayama-u.ac.jp; eguchi.takanori@gmail.com

Heat Shock Proteins (2021) 22: 19–32
https://doi.org/10.1007/7515_2020_24

Published Online: 13 September 2020

Hsp60 and bacterial GroEL were significantly higher in the periodontitis patients and these antibodies were also detected in the gingival tissue and atherosclerotic plaques.

**Conclusions** The anti-HSP reactions may be involved in the pathogenesis of periodontitis as well as cardiovascular diseases. The precise mechanism of the cross-reaction between bacterial and mammalian HSP remains unclear. However, the elevated levels of the antibodies play important roles in periodontal immune and inflammatory response and may correlate the pathogenesis and the severity of periodontal and cardiovascular diseases. Further understanding of the roles of HSP might lead to a new theranostic strategy of precision medicine for systemic health linked periodontitis.

**Keywords** *Aggregatibacter actinomycetemcomitans* · Autoimmunity · Bacterial HSP · Cardiovascular disease · Periodontitis · *Porphyromonas gingivalis*

## Abbreviations

| | |
|---|---|
| *A. actinomycetemcomitans* | *Aggregatibacter actinomycetemcomitans* |
| CPN | chaperonin |
| EPF | early-pregnancy factor |
| HSP | heat shock protein |
| LDL | low-density lipoprotein |
| MAA-LDL | malondialdehyde-acetaldehyde-modified LDL |
| NEF | nucleotide exchange factor |
| OxLDL | oxidized LDL particles |
| *P. gingivalis* | *Porphyromonas gingivalis* |
| TLR | Toll-like receptor |

## 1 Introduction

Periodontitis is a chronic inflammatory disease caused by oral polymicrobial infection. More than 700 species of oral bacteria that develop into a biofilm, called dental plaque, have been identified. The inter- and intra-individual variation in microbiota depends on the environment, genetics, age, and lifestyle of the host. These risk factors can spatiotemporally influence the microbiota composition and colonization, resulting in dysbiosis; disruption in the normal balance between the oral commensal flora and the innate immune status of periodontal tissue. The subgingival dysbiotic biofilm mediates the destruction of supporting connective tissue and alveolar bone around the teeth and triggers the periodontal inflammation, consequently leading to tooth loss. Periodontitis is one of the most prevalent infectious diseases and can affect up to 90% of the worldwide population. Although moderate periodontal inflammation and bone loss are common in older persons, severe periodontitis should not be regarded as a natural consequence of aging. High susceptibility to periodontitis, termed as aggressive periodontitis, has been reported in children and young adult patients that are characterized by rapid destruction of the periodontal

tissues in otherwise healthy individuals. [10, 18, 39, 42]. The considerable burden of massive tooth loss includes structural disorder in occlusion and temporomandibular joint, physiological dysfunction in mastication and nutrient intake, and even troubles of pronunciation, esthetics, emotion, and oral health-related quality of life. Importantly, periodontitis does not only cause tooth loss, but also adversely affects systemic health.

Over the past two decades, a new paradigm shift has occurred in periodontology with the concept "Periodontal medicine". Periodontal medicine is a collective term used to describe how periodontal infection/inflammation may affect extraoral health [5]. Periodontitis has been potentially linked to over 50 systemic diseases and conditions may increase the patients' risk particularly for cardiovascular disease including atherosclerosis, aspiration pneumonia, diabetes mellitus, adverse pregnancy outcomes, rheumatoid arthritis, and Alzheimer's disease (Fig. 1), even after

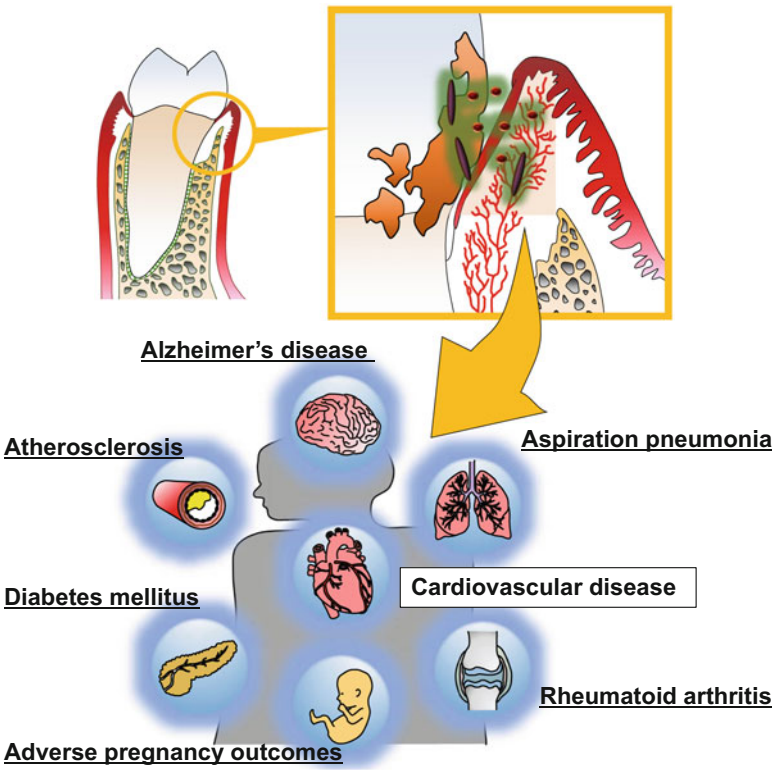



**Fig. 1 Periodontal medicine – a relationship between periodontitis and systemic diseases**. Periodontitis is pathologically correlated with many systemic disease states, such as cardiovascular diseases including atherosclerosis, aspiration pneumonia, diabetes mellitus, adverse pregnancy outcomes, rheumatoid arthritis, and Alzheimer's disease. Periodontal polymicrobial infection and the pro-inflammatory products circulatory spread to the whole body and adversely affect the systemic conditions. Chronic low-grade inflammations contribute to the generation of a systemic inflammatory phenotype. Epidemiological and experimental studies have suggested the most strong link between periodontitis and cardiovascular disease among such systemic diseases

adjusting for other known risk factors such as age, gender, smoking, body mass, *etc*. Although the underlying mechanisms linking these diseases are not fully understood, accumulated data have indicated that periodontitis can elicit a systemic inflammatory response, occurred during transient and recurrent bacteremia caused presumably by periodontal infections. The ulcerated epithelium of the periodontal pocket facilitates the proximity of the subgingival dysbiotic biofilm to the underlying connective tissue and alveolar bone. Periodontal inflammation by bacterial invasion and influx of immune cells leads to leakage of bacterial products, inflammatory mediators, and pathogenic oral bacteria into the blood circulation through which they are transported to distal sites. Eventually, the dissemination of bacterial spread and chronic low-grade inflammatory condition adversely affect systemic diseases (Figs. 1 and 2). More importantly, increasing shreds of evidence have suggested that periodontal treatment can mitigate the risk of systemic diseases. Periodontal treatment consists of mechanical debridement and antimicrobial chemotherapy to remove oral dysbiotic biofilms and may consequently reduce bacteremia and local and systemic inflammatory response [24].

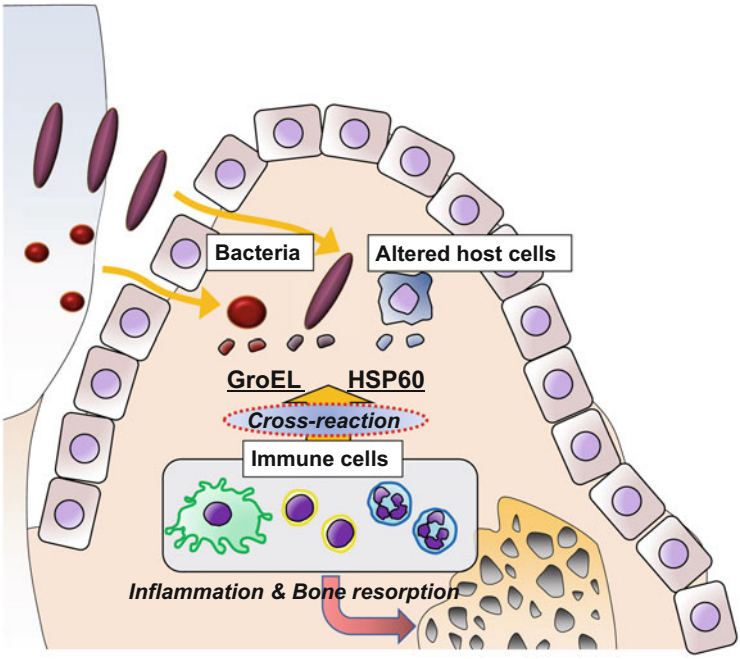



**Fig. 2 Immune inflammatory response in the periodontium.** The junctional epithelium of gingiva closely adheres to the tooth surface through the hemidesmosome and provides a physiological barrier to bacterial exposure. Bacteria and bacterial virulence factors disrupt the integrity of gingival epithelium, leading bacterial invasion and inflammatory infiltration of immune cells in the sub-connective tissue. Periodontal immune responses are highly cross-reactive against bacterial GroEL and HSP60 synthesized in the stressed host cell, resulting in the progression of inflammatory responses and bone resorption around the teeth

Gram-negative anaerobes and spirochetes are closely associated with periodontitis and are referred to as periodontal bacteria [47]. Although the bacterial biofilm plays a crucial role in the initiation and progression of periodontitis, the prolonged inflammation and severity of the disease mainly depend on the host immune and inflammatory responses. The immune-inflammatory response is complex and involves both innate and acquired immunity and the cytokine and inflammatory mediator networks. In the early lesion, both the host and bacteria in the periodontal biofilm release proteolytic enzymes that damage gingival tissue. They also release cytokines and chemokines that recruit neutrophils into sites of infection. If the recruited neutrophils fail to control the dysbiotic microbiota, the chronic lesion is initiated, characterized by the phagocytosis and digestion of bacteria and bacterial products by macrophages and neutrophils that can recognize pathogen *via* toll-like receptors (TLR). Complement proteins are also activated. If the early lesion persists without resolution, the advanced lesion is induced with a transition from innate immune response to the acquired immune response. The antigen-antibody reaction is a major host-protective mechanism that is processed and presented by lymphocytes, macrophages, and dendritic cells. In the inflamed periodontal tissue, macrophages, plasma cells, and T and B lymphocytes are dominant, with IgG1 and IgG3 subclasses of B lymphocytes also present. Tissue destruction is thought to be mediated by many mediators and cytokines, including prostaglandin E2 (PGE2), interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNF-α), which induce the production of matrix metalloproteinases by fibroblasts and receptor activator of nuclear factor kappa-B ligand (RANKL) resulting in collagen and extracellular matrix degradation and bone resorption. All information indicates that the tissue destruction during periodontitis is not primarily due to infectious agents, but rather the result of a persistent but not effective immune and inflammatory response. Excellent reviews of more detailed mechanisms exist elsewhere, e.g. [8, 34].

## *1.1 Bacterial HSP as Potent Antigens in Periodontitis*

There is evidence that heat shock proteins (HSP) are immunodominant antigens derived from many periodontal bacteria [31]. HSP are families of highly conserved proteins and are abundantly found in virtually all living organisms, from bacteria to humans. HSP are commonly grouped into subfamilies based on molecular weight: small HSP, GroES-homologue proteins also known as HSP10 (~ 10 kDa), DnaJ-homologue proteins also called HSP40 (~ 40 kDa), GroEL-homologue proteins also known as HSP60 (~ 60 kDa), DnaK-homologue proteins also called HSP70 (~ 70 kDa), HptG-homologue proteins also called HSP90 (~ 90 kDa), and Clp ATP-dependent proteases also called HSP100. HSP belonging to the same subfamily share the strong amino acid sequence identity [16] (Table 1). These proteins were first described as a response to heat shock and the main role is to allow microorganisms to survive under stress conditions, but they are also expressed during tissue remodeling or wound healing and are associated with several pathological

**Table 1** Bacterial HSP and corresponding human homologs

| Bacterial homolog | Human homolog and alternative names | Functions |
|---|---|---|
| DnaK | Hsp70 | (i) Ubiquitously expressed HSP |
| | | (ii) Forms machinery important for protein folding, and help to protect cells from stress |
| DnaJ | Hsp40 | (i) An enzyme that couples cycles of ATP binding, hydrolysis, and ADP release to cycles of sequestration and release of unfolded proteins |
| | | (ii) The J domain interacts with Hsp70. DnaJ plays a role in regulating the ATPase activity of Hsp70 |
| GroEL | Hsp60, Cpn60, HspD1, mitochondrial matrix protein P1, p60 lymphocyte protein | (i) A mitochondrial chaperonin, which is typically responsible for the transportation and refolding of proteins from the cytoplasm into the mitochondrial matrix |
| | | (ii) A chaperonin to assist in folding linear amino acid chains into their respective three-dimensional structure |
| | | (iii) Involved in diabetes, stress response, cancer, and other immunological disorders |
| GroES | Hsp10, Cpn10, EPF, HspE1 | (i) Required for the proper folding of many proteins |
| | | (ii) Requires the cochaperonin protein complex GroES |
| GrpE | HSP 20-30kD | GrpE dimer serves as the nucleotide exchange factor (NEF) for DnaK |
| HtpG | – | Class III HSP (molecular chaperone) |

*Cpn* chaperonin, *EPF* early-pregnancy factor

functions in inflammatory diseases. HSP act as molecular chaperones that assist in proper protein folding and re-folding as well in the transport of damaged or toxic unfolded proteins to degradation systems [16, 54]. Periodontal bacteria are often exposed to a wide range of stresses (temperature, pH, redox potential, oxidative stress, etc.) that may affect their growth and virulence and induce a stress response. Many HSP are quickly upregulated under stressful conditions. Pre-existing immunity to HSP from various pathogens could cross-react with host natural HSP causing the autoimmune reactions. The high sequence similarity of HSP among species explains the cross-reaction possibilities between host and pathogenic HSP [21].

Periodontal immune responses against HSP also can be highly cross-reactive among bacterial and mammalian species and capable of recognizing both foreign and self-stress proteins [2, 16]. Homologs of specific stress protein families have been demonstrated to be present in many periodontal bacteria. Among them, HSP of *Porphyromonas gingivalis* (*P. gingivalis*) and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) have been extensively studied [16]. *P. gingivalis* shows a strong association with periodontitis, although only

present at a low frequency, and is pathogenic capable of inducing dysbiosis and thereby act as a keystone pathogen [11]. *A. actinomycetemcomitans* is also a major causative bacterium by promoting bacterial invasion into the gingival tissue and elicits an excessive immune response [12]. The JP2 clone of *A actinomycetemcomitans* is considered as a pathogen of aggressive periodontitis [19]. *P. gingivalis* and *A. actinomycetemcomitans* possess several HSP and the syntheses of GroEL, GroES, DnaK, and HtpG are all up-regulated following heat stress [16] (Table 1). The GroEL homologs are known to be key molecules in autoimmune reactions because of the sequence similarity with human Hsp60 and the reactions by *P. gingivalis* GroEL may be one of the causes for the initiation of periodontitis [33]. *P. gingivalis* GroEL and *A. actinomycetemcomitans* GroEL also share a high degree of homology. Human Hsp60 but not *P. gingivalis* or *A. actinomycetemcomitans* GroEL demonstrated a stimulatory activity on the production of tumor necrosis factor-alpha (TNF-α) in THP-1 monocytic cells. Therefore, Hsp60 family proteins from different bacteria species have different proinflammatory activities to induce proinflammatory cytokines [52].

HSP family proteins lack an N-terminal hydrophobic signal sequence, characteristic of most secreted proteins, and therefore, cannot be released from cells by the conventional secretion pathways. However, HSP can be secreted into the extracellular milieu *via* secretory lysosomes or lipid vesicles including ectosomes and exosomes. Formation of the microvesicles consists of complex processes including the internalization of portions of the plasma membrane and subsequent release of microvesicles containing a variety of functional mRNA, microRNA, and intracellular proteins, including HSP [7]. *A. actinomycetemcomitans* GroEL is located on the cell surface, in surface-associated material, and on outer membrane vesicles (OMV) produced by *A. actinomycetemcomitans* meanwhile, *P. gingivalis* GroEL is not encapsulated in the OMV of *P. gingivalis*, possibly suggesting the difference in the virulence of the bacteria [16, 46]. Even so, both GroEL homologs are immunodominant antigens in humans and are presented to macrophages as foreign antigens by lymphocytes and involved in the mediation of bone resorption [22, 28, 44, 45] (Fig. 2). As the molecular chaperone functions, Hsp60 and Hsp70 have been reported to play important roles in antigen presentation and cross-presentation, activation of macrophages, lymphocytes, and dendritic cells to induce proinflammatory mediators. Therefore, it has been suggested that the HSP provides the link between innate and adaptive immune systems. In periodontitis patient's sera, high levels of circulating antibodies to periodontal pathogen and host-derived antigens, and the levels of antibodies to *P. gingivalis* correlated with IgG antibodies to Hsp60 as well as Hsp70 [6]. In addition, these innate immune responses are mediated by TLR2, TLR4, or TLR2-TLR4 heterodimers. The cytokine production activity of hsp60 was inhibited by anti-CD14 and anti-TLR4 antibodies in THP-1 monocytic cells [52]. Nevertheless, there is controversy about whether these effects are due to the contamination of recombinant HSP with pathogen-associated molecules such as lipopolysaccharide [7, 51].

## 1.2 HSP Increased in Periodontitis Patients

Accumulating evidence shows that Hsp60 is highly expressed in inflamed periodontal tissues in periodontitis patients. Hsp60 was highly expressed in inflamed tissue samples from periodontitis patients compared with samples from periodontally healthy individuals [2, 20, 32, 52]. Moreover, it has been shown that gingival tissues extracted from periodontitis patients contained antibodies to the *P. gingivalis* GroEL [48]. Autoimmunity with cross-reaction between human Hsp60 and *P. gingivalis* GroEL has been suggested to be a feature of periodontal disease [21] (Table 1). The antibody titers to Hsp60 and *P. gingivalis* GroEL were significantly higher in the periodontitis patients compared to a control group and those antibodies were detected in all patient-derived samples of gingival tissue extracts of periodontitis [48]. In periodontitis patients, Hsp60 or *P. gingivalis* GroEL-reactive T-cell clones and periodontitis lesion-infiltrating T cells share the same receptors, suggesting that Hsp60-specific and *P. gingivalis* GroEL cross-reactive T-cells accumulate in periodontitis lesion. Analysis of the cytokine profiles demonstrated that Hsp60-reactive T-cell clones from periodontitis patients produced significantly higher levels of gamma interferon compared with cells from healthy control, whereas *P. gingivalis* GroEL did not induce. These data suggest that periodontal infection may activate self Hsp60-reactive T cells with Th1 cytokine profile. Subsequent activation of macrophage and Th2 cytokine secretion may induce antibody production specific to bacteria as well as endogenous Hsp60 [56]. *A. actinomycetemcomitans* GroEL is also an immunodominant antigen in humans. Immunological cross-reactivity between fibronectin and *A. actinomycetemcomitans* GroEL that may lead to an autoimmune response and contribute to tissue destruction during the progression of periodontitis [57]. *A. actinomycetemcomitans* GroEL can modulate acquired and adaptive immune responses [35] although the detailed mechanism remains elusive. Sera collected from patients with periodontitis reacted strongly with *A. actinomycetemcomitans* GroEL [23], however, antisera *A. actinomycetemcomitans* GroEL highly cross-react with *E. coli* GroEL and weakly cross-react with Hsp60, suggesting that the part of serum antibody to *A. actinomycetemcomitans* GroEL could be derived from the cross-reactivity with enterobacteria *E. coli* GroEL and self-Hsp60 [49] (Table 1).

## 1.3 HSP in Cardiovascular Disease

Cardiovascular disease, including atherosclerosis, myocardial infarction, and stroke, is the leading cause of death worldwide, and atherosclerosis is the major underlying etiology. Atherosclerosis is generally initiated by mononuclear cell infiltration into the intima at arterial branches and curves. Infiltration precedes the accumulation of cholesterol and recruitment of macrophages and vascular smooth muscle cells and occurs when endothelial cells are stressed by traditional risk factors such as cigarette smoking, hypertension, elevated lipid levels, and diabetes mellitus. A sizable percentage of patients may not have any of the risk factors, therefore, chronic infection

and inflammation have been focused on the initiation and progression of cardiovascular events. Epidemiologic data have suggested a potential link between periodontitis and cardiovascular disease [4, 24]. Low-grade chronic systemic inflammation and increased circulating cytokines such as C-reactive protein (CRP), IL-1, IL-6, TNF-α, and PGE2, are linked to adverse cardiovascular outcomes and anti-inflammatory agents can prevent cardiovascular disease [41]. Periodontitis is associated with elevated levels of CRP and other inflammatory biomarkers and periodontal therapy can lower the serum levels of the inflammatory biomarkers and reduce the risk factors by improving endothelial function [36, 50]. Periodontal bacteria and their products are also considered to have a role either directly through the cytotoxicity or indirectly by inducing inflammation in the progression of cardiovascular disease, as atherosclerotic plaque contains viable and invasive periodontal bacteria, such as *P. gingivalis* and *A. actinomycetemcomitans* [25]. These bacteria are proposed to exert atherogenic effects by accumulating at sites of plaque development and provoke the local vascular inflammatory response [14].

Indirect interactions of periodontal pathogens in cardiovascular disease include both innate and acquired immune responses. High combined IgG response to *A. actinomycetemcomitans* and *P. gingivalis* was associated with prevalent cardiovascular disease although it is not entirely clear how the antibodies participate in the pathogenesis [38]. Another immunologic mechanism to explain the association is the antigen mimicry between GroEL and human HSP. GroEL cross-reacts with endogenous Hsp60 expressed on endothelial cells, leading to endothelial dysfunction and atherogenesis [37, 53]. Circulating levels of Hsp60 have been correlated with susceptibility to cardiovascular disease [55] whereas antibodies to Hsp60 accelerate and perpetuate the atherosclerotic process by promoting infiltration of mononuclear cells into the intima and significant correlation was observed between anti-Hsp60 antibody and high morbidity and mortality of atherosclerosis [54]. Hsp60 was selectively found in arterial endothelial cells and lymphocytes in atherosclerotic lesions rather than non-atherosclerotic areas, while GroEL and periodontal bacteria were detected within intimal cells in carotid endarterectomy specimens, suggesting that Hsp60 and GroEL closely associate with inflammatory infiltrate of activated T cells and macrophages [13]. Epidemiologic data have reported significant positive correlations between anti-*P. gingivalis* GroEL levels and anti-Hsp60 in cardiovascular patient's sera, as *P. gingivalis* GroEL proteins are highly homologous to Hsp60 [27]. Previous studies have identified T-cell immune responses specific to *P. gingivalis* GroEL in patients suffering from atherosclerosis and periodontitis [9]. Cross-reactive antibodies and T-cells between Hsp60 and *P. gingivalis* GroEL have been detected in the peripheral blood of patients with atherosclerosis as well as in the atherosclerotic plaques themselves [15] (Table 1). The autoimmune concept reinforced the concept that the development of tolerance against Hsp60 and its peptides could be useful for the prevention of atherosclerosis. Several studies demonstrated the protective effect by using recombinant Hsp60 or Hsp peptides vaccination. Sublingual vaccination with recombinant GroEL is also effective for the prevention of *P. gingivalis*-associated atherosclerosis in an experimental mice model. However, clinical studies by using antibiotic therapy have been investigated gave equivocal results for effects on atherosclerosis [17, 54].

Lipid-modification is a principal therapy for the prevention of atherosclerosis. There are several available and emerging therapies for lowering low-density lipoprotein (LDL) cholesterol [40]. In the atherosclerotic intima of the artery wall, LDL particles go through oxidative modification which creates oxidized LDL particles (OxLDL) and subsequently malondialdehyde-acetaldehyde-modified LDL (MAA-LDL) particles are created. The exact role of antibodies to OxLDL in atherosclerosis is not fully understood. Since IgM antibodies to OxLDL inhibit the cholesterol uptake of macrophages through scavenger receptors, IgM antibodies to OxLDL are considered to have protective properties, whereas IgG antibodies to OxLDL are more heterogeneous but mainly considered pro-atherogenic [26]. The MAA adducts have potent immunogenicity and dose-dependent direct cellular toxicity [3]. Increased levels of MAA adducts were detected in cardiovascular disease and periodontitis lesion and salivary IgA antibodies to MAA-LDL cross-react with *P. gingivalis*, Rgp44 (gingipain A hemagglutinin domain of *P. gingivalis*) and *A. actinomycetemcomitans* in patients with cardiovascular disease [1]. Moreover, *A. actinomycetemcomitans* GroEL cross-reacts with Hsp60 and MAA-LDL, which could be a potential mechanism in the progression of cardiovascular disease [26] (Fig. 3). Therefore, controlling immunoreaction to LDL and HSP in periodontitis is beneficial for the prevention and management of cardiovascular diseases.

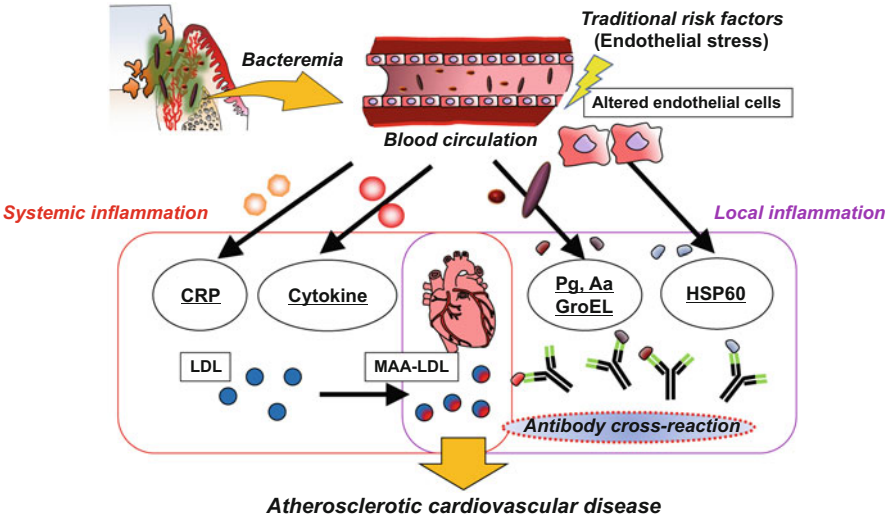



**Fig. 3 Autoimmune concept in periodontitis-associated atherosclerosis.** Periodontal bacteremia can spread into coronary lesions through blood circulation. Endothelial cells in the lesion are stressed by traditional risk factors such as cigarette smoking, hypertension, elevated lipid levels, diabetes mellitus, and consequently synthesized HSP60. Low-grade chronic systemic inflammation and increased circulating cytokines such as CRP, are linked to the modification of LDL to generate malondialdehyde-acetaldehyde-modified LDL (MAA-LDL). GroEL, HSP60, and MAA-LDL released by *P. gingivalis* and *A. actinomycetemcomitans* are highly immunogenic and cross-reactive each other. Eventually, the dissemination of bacterial spread and chronic inflammatory conditions adversely enhances atherosclerotic cardiovascular disease

As mentioned so far, many molecular and immunologic mechanisms have been proposed, the exact mechanism linking between periodontitis and cardiovascular disease is yet to be fully known. However, it has become increasingly evident that periodontal bacterial HSP generate antibodies that can cross-react with human HSP and these antibodies activate inflammatory cytokine production, as well as monocyte and endothelial cell activation in the atherogenic process [43]. Although much evidence of epidemiological linkage between two diseases has been reported, the significance of the correlation depends on the types of study design. According to an American Heart Association statement, the association is independent of known confounders [30]. The most recent Cochrane review described that there is no reliable evidence for primary and secondary prevention of cardiovascular disease by periodontal treatment. Further trials are needed to reach conclusions about whether periodontal treatment can help prevent the occurrence or recurrence of cardiovascular disease [29].

## 2 Conclusions

The precise mechanism of the cross-reaction between bacterial and mammalian HSP remains unclear. However, the elevated levels of the antibodies play important roles in periodontal immune and inflammatory response and may correlate the pathogenesis and the severity of periodontal and cardiovascular diseases. Longitudinal intervention and pathogenic mechanism studies are required. Further understanding of the roles of HSP and LDL might lead to a new theranostic strategy of precision medicine for systemic health linked periodontitis. Importantly, reducing the systemic risk associated with periodontitis requires new biomarkers and diagnostic tools that can link the pathological condition of the diseases and improve diagnostic capability, and establishing clinical guidelines for the treatment.

**Acknowledgments** The authors thank Drs. Hiroshi Maeda, Shogo Takashiba, and Stuart K. Calderwood for illuminating discussion, mentorship, and support. We also thank Dr. Shinsuke Kochi for schematic drawings. T.E. was supported by JSPS KAKENHI, grant numbers, JP17K11642-TE, JP17K11669-KOh, JP18K09789-KN, 19H04051-HO, 19H03817-MT, and by Suzuki Kenzo Memorial Foundation.

**Disclosure of Interests** The authors declare no conflict of interest with the content of this study.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References


1. Akhi R, Wang C, Nissinen AE, Kankaanpää J, Bloigu R, Paju S, Mäntylä P, Buhlin K, Sinisalo J, Pussinen PJ et al (2019) Salivary IgA to MAA-LDL and oral pathogens are linked to coronary disease. J Dent Res 98:296–303
2. Ando T, Ogiuchi H, Kato T, Ishihara K, Okuda K (1995) Heat shock proteins in the human periodontal disease process. Microbiol Immunol 39:321–327
3. Antoniak DT, Duryee MJ, Mikuls TR, Thiele GM, Anderson DR (2015) Aldehyde-modified proteins as mediators of early inflammation in atherosclerotic disease. Free Radic Biol Med 89:409–418
4. Beck JD, Offenbacher S (2005) Systemic effects of periodontitis: epidemiology of periodontal disease and cardiovascular disease. J Periodontol 76:2089–2100
5. Beck JD, Papapanou PN, Philips KH, Offenbacher S (2019) Periodontal medicine: 100 years of progress. J Dent Res 98:1053–1062
6. Buhlin K, Holmer J, Gustafsson A, Hörkkö S, Pockley AG, Johansson A, Paju S, Klinge B, Pussinen PJ (2015) Association of periodontitis with persistent, pro-atherogenic antibody responses. J Clin Periodontol 42:1006–1014
7. Calderwood SK, Gong J, Murshid A (2016) Extracellular HSPs: the complicated roles of extracellular HSPs in immunity. Front Immunol 7:159
8. Cekici A, Kantarci A, Hasturk H, Van Dyke TE (2014) Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000(64):57–80
9. Choi JI, Chung SW, Kang HS, Rhim BY, Kim SJ, K.S. (2002) Establishment of Porphyromonas gingivalis Heat-shock-protein- specific T-cell lines from atherosclerosis patients. J Dent Res 81:344–348
10. Darveau RP (2010) Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbiol 8:481–490
11. Darveau RP, Hajishengallis G, Curtis MA (2012) Porphyromonas gingivalis as a potential community activist for disease. J Dent Res 91:816–820
12. Fine DH, Kaplan JB, Kachlany SC, S.H. (2006) How we got attached to Actinobacillus actinomycetemcomitans: a model for infectious diseases. Periodontol 2000(42):114–157
13. Ford PJ, Gemmell E, Chan A, Carter CL, Walker PJ, Bird PS, West MJ, Cullinan MP, Seymour GJ (2006) Inflammation, heat shock proteins and periodontal pathogens in atherosclerosis: an immunohistologic study. Oral Microbiol Immunol 21:206–211
14. Genco RJ, Van Dyke TE (2010) Prevention: reducing the risk of CVD in patients with periodontitis. Nat Rev Cardiol 7:479–480
15. Goldstein FW (2007) Combating resistance in a challenging, changing environment. Clin Microbiol Infect 13:2–6
16. Goulhen F, Grenier D, Mayrand D (2003) Oral microbial heat-shock proteins and their potential contributions to infections. Crit Rev Oral Biol Med 14:399–412
17. Hagiwara M, Kurita-Ochiai T, Kobayashi R, Hashizume-Takizawa T, Yamazaki K, Yamamoto M (2014) Sublingual vaccine with groel attenuates atherosclerosis. J Dent Res 93:382–387
18. Hajishengallis G (2015) Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol 15:30–44
19. Haubek D, Ennibi OK, Poulsen K, Væth M, Poulsen S, Kilian M (2008) Risk of aggressive periodontitis in adolescent carriers of the JP2 clone of Aggregatibacter (Actinobacillus) actinomycetemcomitans in Morocco: a prospective longitudinal cohort study. Lancet 371:237–242
20. Honda T, Domon H, Okui T, Kajita K, Amanuma R, Yamazaki K (2006) Balance of inflammatory response in stable gingivitis and progressive periodontitis lesions. Clin Exp Immunol 144:35–40
21. Kaur G, Mohindra K, Singla S (2017) Autoimmunity—basics and link with periodontal disease. Autoimmun Rev 16:64–71

22. Kirby AC, Meghji S, Nair SP, White P, Reddi K, Nishihara T, Nakashima K, Willis AC, Sim R, Wilson M, H.B. (1995) The potent bone-resorbing mediator of Actinobacillus actinomycetemcomitans is homologous to the molecular chaperone GroEL. J Clin Invest 96:1185–1194
23. Koga T, Kusuzaki T, Asakawa H, Senpuku S, Nishihara T, Noguchi T (1993) The 64-kilodalton GroEL-like protein of Actinobacillus Actinomycetemcomitans. J Periodontal Res 28:475–477
24. Konkel JE, O'Boyle C, Krishnan S (2019) Distal consequences of oral inflammation. Front Immunol 10:1–16
25. Kozarov EV, Dorn BR, Shelburne CE, Dunn WA Jr, P.-F.A. (2005) Human atherosclerotic plaque contains viable invasive Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. Arterioscler Thromb Vasc Biol 25:e17–e18
26. Kyrklund M, Bildo M, Akhi R, Nissinen AE, Pussinen P, Hörkkö S, Wang C (2020) Humoral immune response to heat shock protein 60 of Aggregatibacter actinomycetemcomitans and cross-reactivity with malondialdehyde acetaldehyde-modified LDL. PLoS One 15:1–16
27. Leishman SJ, Ford PJ, Do HL, Palmer JE, Heng NCK, West MJ, Seymour GJ, Cullinan MP (2012) Periodontal pathogen load and increased antibody response to heat shock protein 60 in patients with cardiovascular disease. J Clin Periodontol 39:923–930
28. Lin FY, Hsiao FP, Huang CY, Shih CM, Tsao NW, Tsai CS, Yang SF, Chang NC, Hung SL, Lin YW (2014) Porphyromonas gingivalis GroEL induces osteoclastogenesis of periodontal ligament cells and enhances alveolar bone resorption in rats. PLoS One 9:e102450
29. Liu W, Cao Y, Dong L, Zhu Y, Wu Y, Lv Z, Iheozor-Ejiofor Z, L.C. (2019) Periodontal therapy for primary or secondary prevention of cardiovascular disease in people with periodontitis. Cochrane Database Syst Rev 12:1–44
30. Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, Taubert KA, Newburger JW, Gornik HL, Gewitz MH et al (2012) Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association?: A scientific statement from the American heart association. Circulation 125:2520–2544
31. Lopatin DE, Shelburne CE, Van Poperin N, Kowalski CJ, Bagramian RA (1999) Humoral immunity to stress proteins and periodontal disease. J Periodontol 70:1185–1193
32. Lundqvist C, Baranov V, Teglund S, Hammarström S, Hammarström ML (1994) Cytokine profile and ultrastructure of intraepithelial gamma delta T cells in chronically inflamed human gingiva suggest a cytotoxic effector function. J Immunol 153:2302–2312
33. Maeda H, Miyamoto M, Kokeguchi S, Kono T, Nishimura F, Takashiba S, Murayama Y (2000) Epitope mapping of heat shock protein 60 (GroEL) from Porphyromonas gingivalis. FEMS Immunol Med Microbiol 28:219–224
34. Moutsopoulos NM, Madianos PN (2006) Low-grade inflammation in chronic infectious diseases: paradigm of periodontal infections. Ann N Y Acad Sci 1088:251–264
35. Nalbant A, Saygılı T (2016) IL12, IL10, IFNγ and TNFα expression in human primary monocytes stimulated with bacterial heat shock GroEL (Hsp64) protein. PLoS One 11:e0154085
36. Offenbacher S, Beck JD, Moss K, Mendoza L, Paquette DW, Barrow DA, Couper DJ, Stewart DD, Falkner KL, Graham SP et al (2009) Results from the Periodontitis and Vascular Events (PAVE) study: a pilot Multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. J Periodontol 80:190–201
37. Perschinka H, Mayr M, Millonig G, Mayerl C, Van Der Zee R, Morrison SG, Morrison RP, Xu Q, Wick G (2003) Cross-reactive B-cell epitopes of microbial and human heat shock protein 60/65 in atherosclerosis. Arterioscler Thromb Vasc Biol 23:1060–1065
38. Pietiäinen M, Liljestrand JM, Kopra E, Pussinen PJ (2018) Mediators between oral dysbiosis and cardiovascular diseases. Eur J Oral Sci 126:26–36
39. Pihlstrom BL, Michalowicz BS, Johnson NW (2005) Periodontal diseases. Lancet 366:1809–1820
40. Ray KK, Corral P, Morales E, Nicholls SJ (2019) Pharmacological lipid-modification therapies for prevention of ischaemic heart disease: current and future options. Lancet 394:697–708

41. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH (1997) Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 336:973–979
42. Rosier BT, Marsh PD, Mira A (2018) Resilience of the oral microbiota in health: mechanisms that prevent dysbiosis. J Dent Res 97:371–380
43. Sanz M, Marco del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, Chapple I, Dietrich T, Gotsman I, Graziani F et al (2020) Periodontitis and cardiovascular diseases: consensus report. J Clin Periodontol 47:268–288
44. Saygili T, Akincilar SC, Akgül B, Nalbant A (2012) Aggregatibacter actinomycetemcomitans GroEL protein promotes conversion of human CD4+ T cells into IFNγ IL10 producing Tbet+ Th1 cells. PLoS One 7:1–8
45. Segain JP, De la Blétière DR, Sauzeau V, Bourreille A, Hilaret G, Cario-Toumaniantz C, Pacaud P, Galmiche JP, Loirand G (2003) Rho kinase blockade prevents inflammation via nuclear factor κB inhibition: evidence in Crohn's disease and experimental colitis. Gastroenterology 124:1180–1187
46. Seyama M, Yoshida K, Yoshida K, Fujiwara N, Ono K, Eguchi T, Kawai H, Guo J, Weng Y, Haoze Y et al (2020) Outer membrane vesicles of Porphyromonas gingivalis attenuate insulin sensitivity by delivering gingipains to the liver. Biochim Biophys Acta – Mol Basis Dis 1866:165731
47. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL (1998) Microbial complexes in subgingival plaque. J Clin Periodontol 25:134–144
48. Tabeta K, Yamazaki K, Hotokezaka H, Yoshie H, Hara K (2000) Elevated humoral immune response to heat shock protein 60 (hsp60) family in periodontitis patients. Clin Exp Immunol 120:285–293
49. Tabeta K, Yoshie H, Yamazaki K (2001) Characterization of serum antibody to Actinobacillus actinomycetemcomitans GroEL-like protein in periodontitis patients and healthy subjects. Oral Microbiol Immunol 16:290–295
50. Tonetti MS, Tonetti MS, Nibali L, Nibali L, Donald A, Donald A, Storry C, Storry C, Parkar M, Parkar M et al (2007) Treatment of periodontitis and endothelial function. N Engl J Med 356:911–920
51. Tsan M-F, Gao B (2009) Heat shock proteins and immune system. J Leukoc Biol 85:905–910
52. Ueki K, Tabeta K, Yoshie H, Yamazaki K (2002) Self-heat shock protein 60 induces tumour necrosis factor-α in monocyte-derived macrophage: possible role in chronic inflammatory periodontal disease. Clin Exp Immunol 127:72–77
53. Wick G, Perschinka H, Xu Q (1999) Autoimmunity and atherosclerosis. Am Heart J 138:S444–S449
54. Wick G, Jakic B, Buszko M, Wick MC, Grundtman C (2014) The role of heat shock proteins in atherosclerosis. Nat Rev Cardiol 11:516–529
55. Xu Q, Schett G, Perschinka H, Mayr M, Egger G, Oberhollenzer F, Willeit J, Kiechl S, Wick G (2000) Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. Circulation 102:14–20
56. Yamazaki K, Ohsawa Y, Tabeta K, Ito H, Ueki K, Oda T, Yoshie H, Seymour GJ (2002) Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. Infect Immun 70:2492–2501
57. Yoshioka M, Grenier D, Hinode D, Fukui M, Mayrand D (2004) Antigenic cross-reactivity and sequence homology between Actinobacillus actinomycetemcomitans GroEL protein and human fibronectin. Oral Microbiol Immunol 19:124–128

# Protective Role of Heat Shock Proteins During Neurodegeneration in Parkinson's Disease


**Amr Ghit**

## Abstract

**Introduction** Parkinson's disease (PD) is one of the most common diseases after Alzheimer's disease (AD) causing neurodegeneration. It affects approximately 1% of individuals above age 60. PD is characterized by intracellular aggregation of misfolded α-Synuclein (α-Syn), loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and subsequent reduction of dopamine levels in the striatum. Aggregation of misfolded α-Syn proteins plays major roles in the onset, progression and severity of PD. This chapter reviews the current understanding of the protective roles of HSPs to prevent the incidence and progression of PD.

**Methods** A review of the previously published articles related to the topic in addition to NCBI and UniProt databases.

**Results** α-Syn monomers can interact causing the formation of toxic α-Syn species, such as oligomers and amyloid-β sheet fibrils that aggregate into Lewy Bodies (LBs). LBs are spherical inclusions promote synaptic dysfunction and impair neuronal connections, ultimately causing synaptopathy and subsequent death of DA neurons. Degradation and clearance of these toxic proteins come through different degradation systems include molecular chaperone activity, autophagy-lysosomal pathways (ALP), and the ubiquitin-proteasome system (UPS). Heat shock proteins (HSPs) play a key role in these mechanisms suppressing protein misfolding and inhibiting the apoptotic activity of DA neurons.

**Conclusions** HSPs represent a class of proteins potentially involved in PD pathogenesis. Dysregulation of HSPs is likely the first step driving the toxic accumulation

A. Ghit (✉)
Department of Biotechnology, Institute of Graduate Studies and Research (IGSR), Alexandria University, Alexandria, Egypt

Department of Biology and Biotechnology, University of Pavia, Pavia, Italy
e-mail: amr.saadshehatariz01@universitadipavia.it; amr.ghit@hotmail.com

Heat Shock Proteins (2021) 22: 33–51
https://doi.org/10.1007/7515_2020_23

Published Online: 7 November 2020

of α-Syn in dopaminergic neurons impairing their functions and connections. HSPs, therefore, protect dopaminergic neurons from the degeneration that causes PD.

**Keywords** Autophagy-lysosomal pathways · Heat shock proteins · Lewy bodies · Molecular chaperones · Neurodegenerative diseases · Parkinson's disease · Ubiquitin proteasome system · α-Synuclein

## Abbreviations

| | |
|---|---|
| Ach | Acetylcholine |
| AD | Alzheimer's disease |
| ALP | Autophagy-lysosomal pathways |
| ALS | Amyotrophic lateral sclerosis |
| AZ | Active zone |
| BAG1 | BAG family molecular chaperone regulator 1 |
| CHIP | Carboxyl terminus of HSC70-interacting protein |
| CMA | Chaperone-mediated autophagy |
| DA | Dopamine/dopaminergic |
| DAT | Dopamine transporter |
| DLB | Dementia with Lewy Bodies |
| EOPD | Early-onset Parkinson's disease |
| ER | Endoplasmic reticulum |
| FTD | Frontotemporal dementia |
| GPe | External globus pallidus |
| GPi | Internal globus pallidus |
| HD | Huntington's disease |
| HIP | HSC70-interacting protein |
| HOP | HSP40 and HSP70–HSP90 organizing protein |
| HSC70 | Heat shock cognate 71 kda protein |
| HSEs | HSP Sequence-binding Elements |
| HSF | Heat shock factors |
| HSP | Heat shock protein |
| LAMP2A | Lysosome-associated membrane protein type 2A |
| LBs | Lewy bodies |
| LC3 | Light chain 3/ALP marker |
| MPTP | 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine |
| MW | Molecular weight |
| NAC | Non-amyloid-β component |
| NT | Neurotransmitter |
| PD | Parkinson's disease |
| PSI | Peptidyl aldehyde selective inhibitor |
| PTM | Post-translational modification |
| RAC | Ribosome-associated complex |
| ROS | Reactive oxygen species |

| | |
|---|---|
| sHSP | Small Heat shock protein |
| SNpc | Substantia nigra pars compacta |
| SNpr | Substantia nigra pars reticulata |
| STN | Subthalamic nucleus |
| SV | Synaptic vesicle |
| Ub | Ubiquitin |
| UCH-L1 | Ubiquitin carboxy-terminal hydrolase L1 |
| UPS | Ubiquitin-proteasome system |
| VMAT2 | Vesicular monoamine transporter 2 |
| α-Syn | α-Synuclein |

## 1 Introduction

Proteins are macromolecules involved in a vast array of biological functions of the cell. In the nucleus, DNA is transcribed into mRNA which is exported to the cytoplasm for translation. MRNA is translated by ribosomes into a sequence of amino acids bound together by peptide bonds forming a polypeptide chain, which is called the primary structure of the protein. The secondary structure defines the folding of a polypeptide chain through hydrogen bonding to produce α-helix and β-pleated sheet structures. The tertiary structure refers to the three-dimensional folding of a polypeptide chain, where the interior of the most of proteins consists of non-polar, hydrophobic amino acid residues and the outside consists of polar hydrophilic residues. The quaternary structure is a group of two or more polypeptide chains held together by non-covalent interactions forming a protein complex [60].

As the primary structure of the protein is being synthesized by the ribosome, it begins to fold into its tertiary structure, the native state of the protein, which is biologically functional. Some proteins fail to fold into its native structure producing misfolded proteins which are biologically inactive. Many proteins, once they leave the ribosome, undergo post-translational modification (PTM) where the amino acid side chains are modified by some chemical modifications [26].

Molecular chaperones or heat shock proteins (HSPs) are a class of proteins that assist in the proper folding of other proteins. They interact with the primary structure of the target proteins, even when they are being synthesized by the ribosome, and fold them into their native three-dimensional structure. HSPs also have critical roles in refolding of partially denatured or misfolded proteins [26]. Mutations or lack of the normal folding process lead to the production of misfolded proteins. Aggregation of misfolded proteins in the cell leads to the formation of amyloid-like structures that pose a threat to the various biological functions in the cell, which ultimately leads to cell degeneration and death [12].

Degradation and clearance of these misfolded protein aggregates are achieved through two principal pathways; ubiquitin-proteasome system (UPS), and autophagy-lysosomal pathways (ALP). HSPs are involved in both the UPS pathway

and an autophagy mechanism, known as chaperone-mediated autophagy (CMA). Therefore, HSPs are considered as intracellular lifeguards of the proteome as well as the cell and the organism itself [56]. Several experimental data revealed that misfolded aggregates are thought to be the major cause of synaptic dysfunction and neuronal death in many neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Frontotemporal Dementia (FTD). Importantly, HSPs which are constitutively expressed in the nervous system, play a key role in reducing the risk of formation of these aggregates [13, 28]. Therefore, activation of HSP to tackle these misfolded protein aggregates could be a promising strategy in drug development against different neurodegenerative diseases. This book chapter was written to declare the role of HSPs to prevent the incidence of PD through maintaining dopaminergic neurons from degeneration and death.

## *1.1 Heat Shock Proteins*

Heat shock proteins (HSPs) are a large family of proteins function as molecular chaperones for other proteins. They are named according to the molecular weight (MW). In 1962, HSPs were first reported by Italian geneticist Ferruccio Ritossa in Drosophila cells under heat stress. Several studies have shown that HSPs have a vital role in cellular protein homeostasis that includes protein maturation, refolding, and degradation. This role not only against heat shock but also against other environmental stresses, such as cold stress and ultraviolet irradiation and also during wound healing [10, 33, 38, 49].

HSPs superfamily is categorized based on their MW into HSP40, HSP60, HSP70, HSP90, HSP100-110 (Table 1), and small heat shock proteins (sHSPs) such as HSP10, HSP20, HSP25/26 and HSP27 (Table 2). Most HSPs require the aid of co-chaperones to function (Table 3; [60]). They can be also categorized into two families: (1) ATP-dependent HSPs or HSPs with high MW; (2) ATP-independent or HSPs with low MW. HSPs can be localized in the cytosol, nucleus and cellular organelles such as mitochondria and endoplasmic reticulum (ER) [4, 16].

### 1.1.1 HSP40

HSP40 also known as DnaJ, is a molecular chaperone protein play a pivotal role in protein synthesis, folding, refolding, translocation and degradation. HSP40s are divided into three subtypes, 1, 2 and 3, in which subtype 1 and 2 are considered as co-chaperones, whereas subtype 3 defines as a chaperone. HSP40s act as co-chaperones for HSP70 and HSP90. They can interact with HSPA/HSP70 via the J-domain and regulates ATPase activity for the HSP70s. They can also bind with and deliver many client proteins to HSP70. Interestingly, HSP70s and certain types of HSP40s assist in protein translation by forming a stable complex known as

**Table 1** HSP subtypes, their cellular localization, and function

| Protein | Gene | MW | Alternative name(s) | Cellular localization | Function |
|---|---|---|---|---|---|
| HSP10 | HSPE1 | 10 | CPN10 Early-pregnancy factor (EPF) | Mitochondrion | Co-chaperonin help Hsp60 for protein folding |
| HSP40 | DNAJB1 | 40 | DnaJB1 hDj-1 | Nucleus Cytoplasm | Co-chaperonin help HSP70 for protein folding |
| HSP60 | HSPD1 | 60 | CPN60 HuCHA60 | Mitochondria | Implicated in mitochondrial protein import and macromolecular assembly. |
| HSP70 | HSPA1A HSPA1B HSPA1L | 70 | HSP70-1 HSP70-2 HSP70-Hom | Cytosol, ER, nucleus, mitochondria | Plays a pivotal role in protein homeostasis in the cell. |
| HSC70 | HSPA8 | 70 | LPS-associated protein 1 | Cytosol, nucleus, cell membrane | Chaperone mediated autophagy, protein degradation |
| HSP90A | HSP90AA1 HSP90AA2 | 90 | HSP86 | Cytoplasm | Help in protein folding, refolding and degradation, signal transduction and prevent cancer growth |
| HSP90B | HSP90AB1 | | HSP84 | ER | |
| HSP110 | HSPH1 HSP110 HSP105 | 110 | Heat shock protein 110 kDa Heat shock protein 105 kDa | Cytoplasm, nucleus | Promote the release of ADP from HSPA1A/B inhibits HSPA8/HSC70 ATPase and chaperone activities |

Data compiled from UniProtKB

ribosome-associated complex (RAC). Importantly, HSP40s increase the stability of protein interactions and prevent their aggregation, and are also involved in proteasomal degradation pathway (Table 1; [35]).

### 1.1.2 HSP60

HSP60 is a mitochondrial chaperone responsible for refolding and translocation of the newly synthesized proteins from the cytoplasm into the mitochondrial matrix. It is involved in folding of amino acid chains into their native three-dimensional structure and also in intracellular protein trafficking. HSP60 cooperate with HSP10, HSP70 and other chaperones for protein folding. Besides, it can recognize misfolded or aggregated proteins and refold them to their native structure. Sometimes HSP60 interacts with anti-apoptotic proteins in the cytosol to prevent cell death (Table 1; [35]).

**Table 2** sHSP subtypes, their cellular localization, and function

| Protein | Gene | MW | Alternative name(s) | Cellular localization | Function |
|---|---|---|---|---|---|
| HSPB1 | HSPB1 | 22.3 | HSP27 HSP28 | Cytoplasm, nucleus | Through its molecular chaperone activity may regulate phosphorylation and the axonal transport of neurofilament proteins |
| HSPB2 | HSPB2 | 20.2 | MKBP | Cytoplasm, nucleus | May regulate the kinase DMPK |
| HSPB3 | HSPB3 | 17 | HSP17 | Cytoplasm, nucleus | Inhibitor of actin polymerization. |
| HSPB4 | CRYAA | 19.9 | Alpha-crystallin A chain | Cytoplasm, nucleus | Has chaperone-like activity, and contributes to the transparency and refractive index of the lens |
| HSPB5 | CRYAB | 20.2 | α/β-crystalline | Cytoplasm, nucleus | Has chaperone-like activity, and contributes to the transparency and refractive index of the lens |
| HSPB6 | HSPB6 | 17.1 | Heat shock 20 kDa-like protein p20 | Cytoplasm, nucleus | Has chaperone-like activity, and plays a role in regulating muscle function |
| HSPB7 | HSPB7 CVHSP | 18.6 | cvHsp | Cytoplasm, nucleus | Has chaperone-like activity, and plays a role in heart development and contraction |
| HSPB8 | HSPB8 HSP22 | 21.6 | HSP22 | Cytoplasm, nucleus | Displays temperature-dependent chaperone activity |
| HSPB9 | HSPB9 | 17.5 | Cancer/testis antigen 51 (CT51) | Cytoplasm, nucleus | Unknown |
| HSPB10 | HSPB10 ODF1 | 28.4 | Outer dense fiber protein 1 (ODF1) | Cytoplasm, nucleus | Component of the outer dense fibers (ODF) of spermatozoa |
| HSPB11 | HSPB11 IFT25 | 16.3 | Placental protein 25 (PP25) | Cytoplasm cilium | Has a role in cell differentiation and spermatogenesis |

Data compiled from UniProtKB

**Table 3** Major HSPs and their co-chaperones in human

| HSPs | Co-chaperone |
|---|---|
| HSP90 | P23, AHA1, HOP, CDC37, CYP40, FKBP51, FKBP52 |
| HSP70 | HSPBP1, HOP, HIP, BAG1–6, HSP40, CHIP |
| HSC70 | BAG1–6, CHIP, HOP, HSP40 |
| HSP60 | HSP10 |

Data compiled from [60] and UniProtKB

### 1.1.3 HSP70

HSP70 is the most conserved chaperones in all living organisms. It is mainly localized in the cytosol, ER, nucleus, and mitochondria. It can also interact with

other chaperones such as HSP40 and HSP90 for its chaperone activities. HSP70 show neuroprotective effects in neurodegenerative diseases such as PD and AD where it can bind with unfolded or partially misfolded proteins, and refold them into their native conformation. Interestingly, in cellular stress condition, HSP70 can inhibit caspases, thus prevents apoptosis (Table 1; [35]).

### 1.1.4 HSC70

HSC70 is a member of the HSP70 family. It has 86% identity to inducible HSP70 and performs similar chaperoning activities. HSC70s cooperates in protein folding and degradation machinery in the cell. In a process called chaperone-mediated autophagy (CMA), HSC70 recognize misfolded or denatured proteins and along with a set of co-chaperones unfold, translocate and target these proteins to the lysosome for degradation (Table 1; [60]).

### 1.1.5 HSP90

HSP90s are among the most abundant proteins in eukaryotes. They can be classified according to their subcellular localization. Heat shock protein HSP90-α (HSP90A), the inducible form of HSP90, is localized in the cytosol whereas Heat shock protein HSP90-β (HSP90B), the constitutive form, is localized in the ER. It requires ATP for its chaperone activities. Importantly, HSP90A can bind with HSF-1, and induce expression of the other HSPs such as HSP70, HSP60 and HSP40 (Table 1; [35]).

### 1.1.6 Small HSP (sHSP)

They are a set of proteins with small molecular weight (15–30 kDa), localized in the cytosol, ER and nucleus. Although in mammals, sHSP family includes at least eleven different subtypes (Table 2), HSP27 (HSPB1) is the most abundant and well understood. They are activated through different environmental, physiological or pathological stresses, and their levels are increased in different neurodegenerative diseases. Small HSPs are involved in many essential functions in the cell, for example, they assist in ubiquitin-proteasome degradation of misfolded or denatured proteins. They also act as molecular chaperones and co-chaperones with HSP70 for inhibition of misfolded or unfolded protein aggregation. Besides, they help in cell development and differentiation and have a role in the inhibition of apoptotic cell death. They can also interact with actin and intermediate filaments to protect the cell from cytoarchitectural damage [35].

## 1.2 Functions of HSP

HSPs have several functions related to the protection of cells against stress conditions such as (i) They work as intra-cellular chaperones for other proteins. They play an important role in the proper folding of nascent proteins and refolding of misfolded or unfolded proteins to their native conformation; (ii) They assist in the degradation of undesirable protein and inhibit its accumulation in the cell through UPS and CMA machineries (Fig. 2); (iii) They modulate protein-protein interactions and prevent their aggregation; (iv) They have a role in cytoskeletal organization and transportation of proteins across membranes within the cell [14].

UPS is a major mechanism in eukaryotes by which cells can degrade misfolded, unfolded or damaged proteins and also regulate particular protein concentration [37]. It consists of the proteasome itself and some other factors that include ubiquitin (Ub), ubiquitin ligases (E3), ubiquitin hydrolases, and ubiquitin-like molecules. We can summarize fundamental pathways as the following (Fig. 1). In reaction depends heavily on ATP, E1 enzymes, which is also called ubiquitin-activating enzymes,

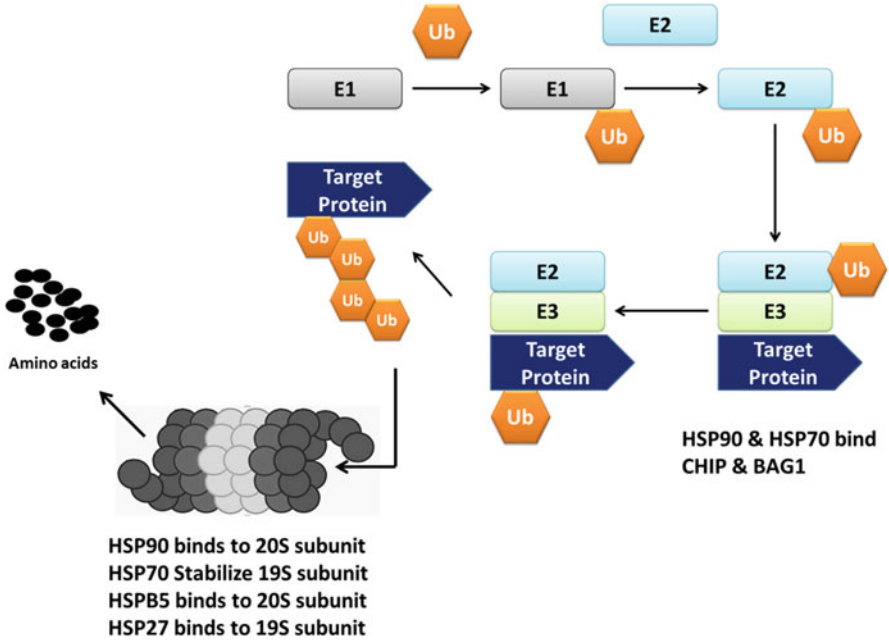



**Fig. 1 Role of HSPs in the ubiquitin-proteasome system.** Protein degradation via Ubiquitin-proteasome system (UPS) requires coordinated reactions by three enzymes E1, E2 and E3. E1 hydrolyzes and activates the ubiquitin (Ub) which is transferred to E2. Finally, E3 catalyzes the transfer of ubiquitin from E2 to the target protein. Additional ubiquitin molecules are conjugated onto the first Ub. Poly-ubiquitinated proteins are recognized by the proteasome 26S subunit for degradation. HSPs can bind to different components of the proteasome subunits, the 20S, 11S, and 19S

form a thioester bond with ubiquitin (Ub). Then, Ub is transferred to bind with ubiquitin conjugases (E2) with a new thioester bond. E3 recognizes specific motifs in the targeted protein and catalyzes the transfer of ubiquitin from E2 to the target protein. Ubiquitin molecules are conjugated onto the first forming a poly-ubiquitinated protein, which ultimately is recognized by the 26S proteasome for degradation [24].

HSPs have a fundamental role in both the ubiquitination as well as the degradation machinery done by the UPS. Direct interactions between HSPs and the proteasome itself were reported in several studies. CHIP (carboxyl terminus of HSC70-interacting protein) in association with HSP90, HSP70 and E3 can form a complex with target protein facilitating its ubiquitination. BAG1 (BAG family molecular chaperone regulator 1) is a co-chaperon binds with HSP70 promoting the ubiquitination process. It can also interact and provide a bridge between the 26S subunit of the proteasome and HSP70/HSC70. During the ubiquitination process, HSP27 bind with ubiquitin, and also with the target protein, however, the purpose of this action is unknown. Moreover, HSP90 and a sHSP known as αβ-crystallin or HSPB5 bind with 20S subunit, in the same time, HSP27 and HSP70 bind with 19S subunit accelerating the degradation of ubiquitinated proteins (Fig. 1; [60]).

It has been reported that a lack of clearance of misfolded proteins or mutation in genes associated with UPS has a prominent role in cellular toxicity and neurodegeneration. Several neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease (HD) share dysfunction of the UPS and aggregation of misfolded or toxic proteins as pathological hallmarks [24].

Some forms of misfolded proteins are too large to pass through proteasome cylinder, and therefore cannot be degraded through UPS. Autophagy lysosomal pathway (ALP) is an alternative machinery present in every cell for degrading large toxic aggregates. ALP includes macroautophagy for the degradation of large aggregates, microautophagy for the degradation of small cytosolic proteins, and chaperone-mediated autophagy (CMA).

CMA is more specific, performing its activity through the selective degradation of protein having KFERQ-like motif which is delivered to the LAMP2A (lysosome-associated membrane protein 2a) receptor on lysosome via HSC70 and co-chaperones, such as CHIP, HOP (HSP40 and HSP70–HSP90 organizing protein) and HSP40. After passing inside lysosome, the target protein is degraded by lysosomal enzymes into amino acids residues (Fig. 2; [5, 30]).

## *1.3 Regulation of HSP Expression*

Heat shock factors (HSFs) are transcription factors that activate the expression of HSP genes [53]. During cellular stress, expression of HSF increase, and translocate to the nucleus where they bind specifically to HSF Sequence-binding Elements (HSEs) throughout the genome, and help to express different types of HSP

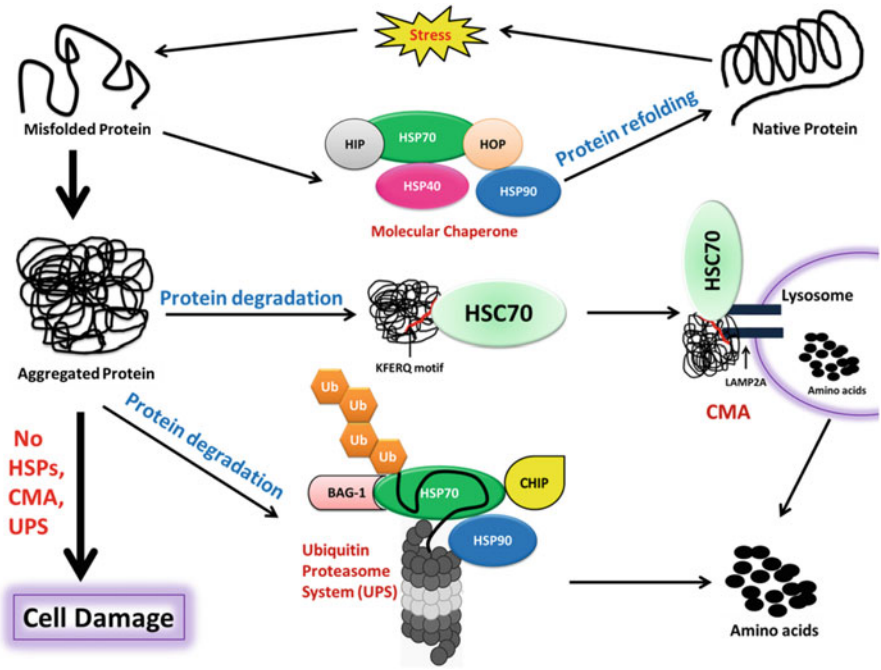



**Fig. 2 Role of HSPs in cellular protein homeostasis**. HSPs assist in protein maturation, re-folding, and degradation through molecular chaperone activity, the ubiquitin-proteasome system (UPS) and chaperone-mediated autophagy (CMA). Dysregulation of HSPs is associated with the accumulation of misfolded proteins leading to degeneration and subsequent death of the cell

depending on the cellular requirements [25]. Enough amount of cytosolic HSP refold the misfolded proteins, then they interfere with further translocation of HSF to the nucleus and stop HSP gene expression by feedback mechanism [61]. However, not all chaperones are target genes of HSF. For example, the major constitutively expressed HSC70 is transcriptionally independent of HSF [60].

## *1.4 Parkinson's Disease (PD)*

Parkinson's disease (PD) is the second most common idiopathic neurodegenerative disease after Alzheimer's disease (AD), first identified by James Parkinson two hundred years ago. It affects approximately 1% of individuals above age 60. Younger people may also be vulnerable to early-onset PD (EOPD) [2, 47]. PD characterized by two main pathological features: (1) loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) leading to a severe reduction of dopamine (DA) levels in the striatum, (2) abnormal deposition of α-synuclein (α-Syn) protein in the cytoplasm of certain neurons in several different brain regions [37]. PD

characterized by motor symptoms (tremor at rest, bradykinesia, muscle rigidity, and postural instability), and non-motor symptoms (anxiety, fatigue, depression, sleep disturbance, cognitive impairment, and gastrointestinal dysfunction) [46].

The exact mechanism of dopaminergic neuron loss in PD is not yet entirely clear. However, the most accepted theory is the accumulation of amyloid proteins forming Lewy bodies (LBs), which trigger synaptic dysfunction that results in neurodegeneration. In addition to that, mitochondrial damage, energy failure, oxidative stress, and excitotoxicity, may be involved in the onset and progression of PD [42].

### 1.4.1 Historical Review of Discovering Degeneration of Dopaminergic Neurons in PD

In 1817, James Parkinson published a valuable work "*An Essay on the Shaking Palsy"* in which he described six individuals with symptoms of a disease he called paralysis agitans. Jean-Martin Charcot and his colleagues at La Salpêtrière hospital in Paris contributed significantly to the detection of major symptoms and pathology of PD. They also argued that the term paralysis agitans that Parkinson used in his essay was improper and suggested to change the name of the disease to be "Parkinson's disease" or "PD" [45].

In 1893, Paul Blocq and Georges Marinesco noted an encapsulated tumour localized in the SNpc of a tuberculosis patient suffering from tremor. They published their work concluding the SNpc is the main pathological site in PD patients [45].

In 1912, Fritz Heinrich Lewy discovered abnormal aggregations of proteins inside neurons of PD patients. Later, these aggregates became the so-called "Lewy bodies" or "LBs" which are considered as a characteristic indicator of PD and dementia with Lewy bodies (DLB) [50]. In 1938, Rolf Hassler studied autopsies of PD patients and he noted neurodegeneration and accumulation of LBs in the SNpc [45].

In 1959, Arvid Carlsson (Nobel Prize in Physiology or Medicine, 2000) presented his work at the international pharmacology meeting and showed that dopamine was somehow responsible for PD. In 1960, Oleh Hornykiewicz published a landmark paper showing for the first time a marked depletion of dopamine in the caudate and putamen brain regions of PD patients [18]. In 1961, Hornykiewicz and Birkmayer published a work declare the therapeutic effect of intravenous administration of amino acid precursor of dopamine (Levodopa or L-DOPA) in PD patients [6].

### 1.4.2 Mechanisms of α-Syn Protein Induced Dopaminergic Neuron Death in PD

α-Syn is a cytoplasmic protein consisting of 140 amino acids encoded by the SNCA gene. Its protein domains include an amphipathic region (N-terminal), non-amyloid-β component (NAC) domain and an acidic tail (C-terminal) (Fig. 3a). α-Syn is

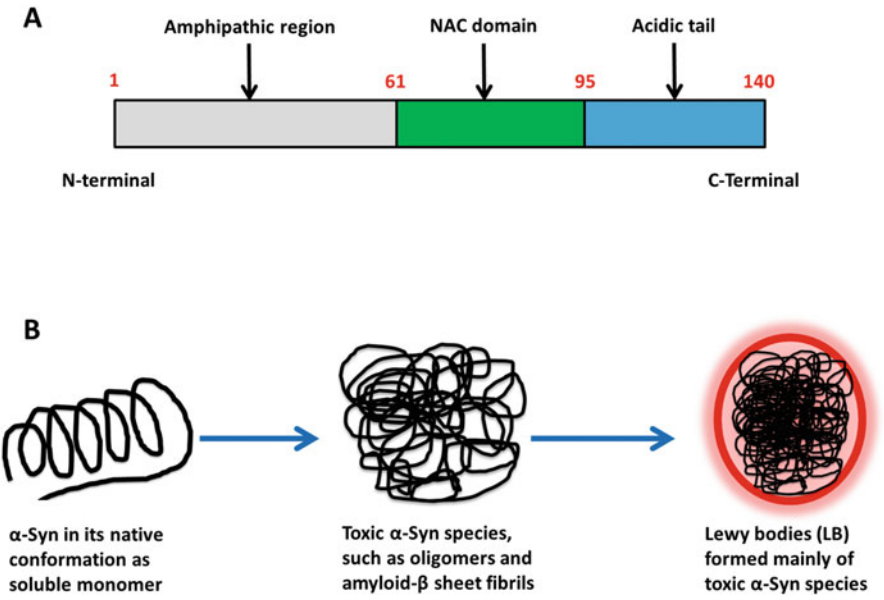



**Fig. 3 α-synuclein protein structure.** (**a**) The protein is composed of 140 amino acids includes three distinct domains, an amphipathic region (N-terminal), non-amyloid-β component (NAC) domain and an acidic tail (C-terminal). (**b**) Under normal physiological conditions, α-Syn in their native conformation as soluble monomers. In diseased conditions α-Syn monomers can interact causing the formation of toxic α-Syn species, such as oligomers and amyloid-β sheet fibrils that aggregate forming Lewy bodies (LBs)

expressed throughout the brain and enriched at presynaptic terminals, while few amounts are distributed in the heart, muscles and other tissues. Synuclein protein family includes 3 subtypes, α-Syn, β-Syn and γ-Syn. However, α-Syn is the only one in the family to be found in LB inclusions, the pathological hallmark in PD. α-Syn is found throughout the brain but dopaminergic (DA) neurons are most susceptible to the accumulation of its toxic species.

So far, the function of α-Syn has not been fully discovered. However, under normal physiological conditions, α-Syn monomers play a role in synaptic vesicle trafficking, recycling and neurotransmitter release. In PD patient, α-Syn monomers can interact causing the formation of toxic α-Syn species, such as oligomers and amyloid-β sheet fibrils that aggregate into LBs (Fig. 3b) affecting processes required for dopamine release and neuronal communication (Fig. 4; [7, 55]). Additionally, it has been demonstrated that deregulated dopamine triggers the formation of toxic α-Syn [43]. Therefore, there is a vicious cycle of α-Syn accumulation and deregulated dopamine promotes synaptic dysfunction which impairs neuronal connections and ultimately causes synaptopathy and subsequent death of DA neurons in PD.

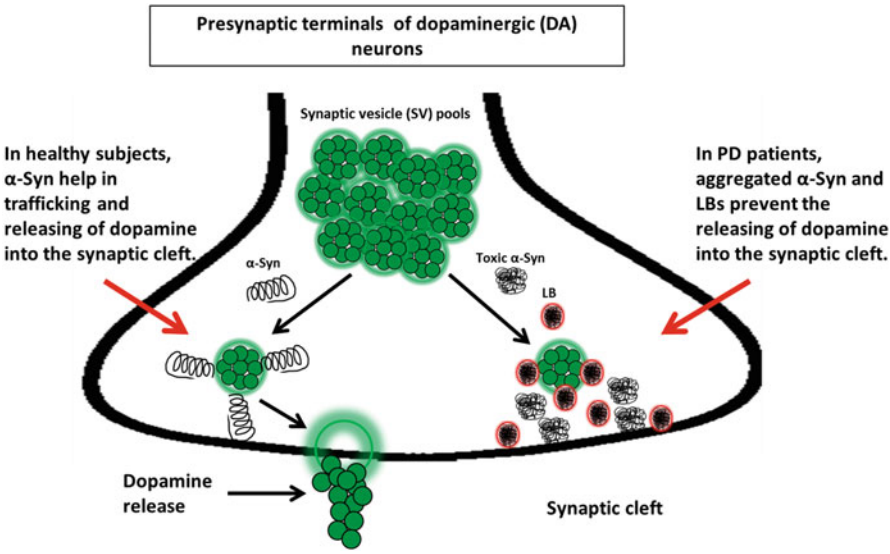



**Fig. 4 The behaviour of α-Syn at the presynaptic terminals of dopaminergic (DA) neurons in healthy subjects and PD patients.** α-Syn in their native conformation as soluble monomers play a role in synaptic vesicle trafficking, recycling and dopamine release. In PD patient, α-Syn monomers can interact causing the formation of toxic α-Syn species that aggregate to form LBs, which prevent dopamine release and neuronal communication

### 1.4.3 Loss of DA Neurons Promotes Dysfunction of Basal Ganglia Motor Circuit in PD

The basal ganglia include the neostriatum (caudate nucleus and putamen), the external and internal segments of globus pallidus (GPe, GPi), the subthalamic nucleus (STN), and the substantia nigra with its pars compacta (SNpc) and pars reticulata (SNpr) [22]. The release of dopamine in striatum initiates and facilitates movement. It stimulates the GABA output via D1 receptors and inhibits the GABA output via D2 receptors. On the other hand, acetylcholine (Ach) in the striatum is thought to do the opposite of DA [27]. In the striatum of healthy individuals, the balance between dopamine and acetylcholine is maintained. Loss of this balance due to the death of the dopaminergic neurons in the SNpc leads to a hypodopaminergic-hypercholinergic condition which describes the abnormal motor features in PD patients [34].

## *1.5 Role of HSPs in PD*

PD is characterized by intracellular accumulation of misfolded α-Syn protein and death of dopaminergic neurons in the brain. HSPs are found to be down-regulated in

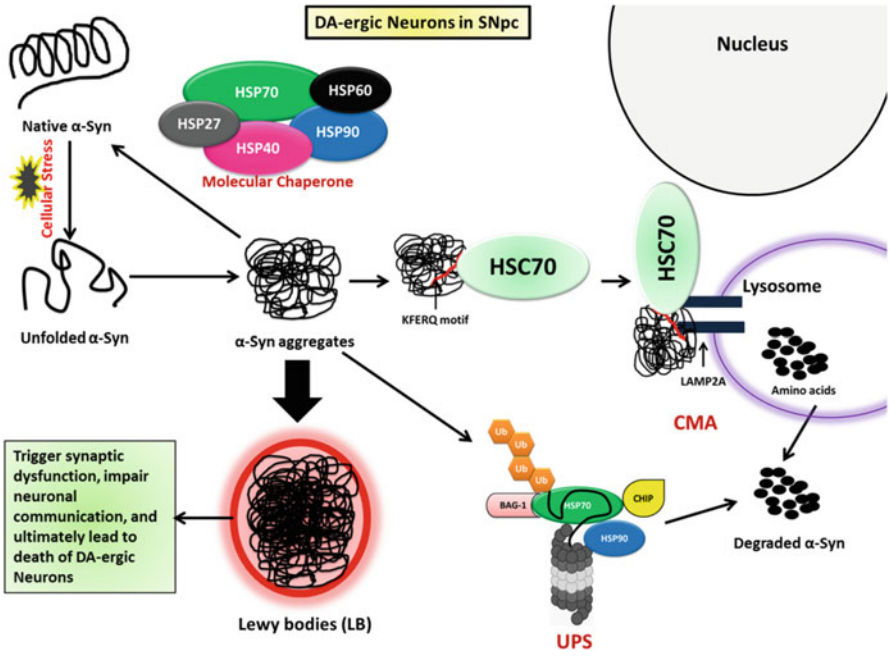



**Fig. 5 Role of HSPs to prevent dopaminergic neuronal loss.** HSPs are involved in protein folding and refolding of misfolded $\alpha$-Syn protein. They also, assist in clearance pathways of toxic $\alpha$-Syn aggregates, ubiquitin-proteasomal system (UPS), and chaperone-mediated autophagy (CMA). HSPs, therefore, protect dopaminergic neurons from degeneration and subsequent death which in turn prevent the incidence of PD

neurons of PD patients and also other neurodegenerative diseases [29, 36]. HSPs are the most efficient, highly conserved cellular defence mechanisms which maintain α-Syn protein homeostasis in neurons through proper folding, refolding of partially misfolded, and degradation of toxic aggregates (Fig. 5; [37, 62]). HSPs also play pivotal roles in the clearance of aggregated $\alpha$-Syn protein through UPS and CMA machineries (Fig. 5; [37]). HSPs involved in the regulation function of UPS and CMA are HSP27, HSP40, HSP60, HSP70, HSP90 and HSP100 [8]. Furthermore, sHSP such as α/β-crystalline (HSPB5), HSP20, HSP27, HSPB8 and HSPB2B3 also interact with α-Syn proteins and inhibit their aggregation [9]. Interestingly, certain co-chaperones such as Bag1, CHIP, HOP and HSC70-interacting protein (HIP) also inhibit α-Syn aggregation (Table 3; Fig. 5; [39]). Therefore, HSPs are regarded as anti-apoptotic factors that prevent dopaminergic neuron degeneration in PD [37].

### 1.5.1 Impairment of Molecular Chaperone Activity Leads to PD

Several studies have reported dysregulation of HSPs in PD. For example, HSP70 co-expression in Drosophila and yeast models of PD decreases aggregation of toxic

α-Syn protein and prevents dopaminergic neuron death [3], whereas mutations at ATPase domain of HSP70 increase aggregation of toxic α-Syn [20]. Similarly, in vitro and in vivo models of PD induced by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), over-expression of HSP70 decreases PD symptoms and neurotoxicity [19, 58]. On the other hand, co-expression of HSP27, HSP40, HSP70 or HSP104 in vitro model of PD observed a reduction of total misfolded α-Syn aggregates [31].

### 1.5.2 Impairment of Degradation Pathways Lead to PD

Ubiquitin Proteasome System (UPS)

Impairment or failure of the UPS has a key role in PD pathogenesis. In fact, aggregation of toxic α-Syn proteins increases neurodegeneration in the SNpc [51]. Inactivation of ubiquitin hydrolases with ubiquitin aldehyde showed toxicity in neuronal cell cultures [40]. A study on ventral mesencephalic cells of fetal rat used lactacystin (an organic compound synthesized by bacteria) to bind and inhibit specific subunits of the proteasome. This led to the loss of dopaminergic neurons and the accumulation of LBs. Similarly, intrastriatal administration of lactacystin led to a degeneration of dopaminergic neurons in rodent brains [21, 48]. A previous study on neuroblastoma cells using UPS inhibitors for several weeks showed an increase in toxic protein aggregates and reactive oxygen species (ROS) and mitochondrial dysfunction [54]. Also, subcutaneous injections of epoxomicin, a natural proteasome inhibitor or PSI (peptidyl aldehyde selective inhibitor), a synthetic proteasome inhibitor in adult rats led to severe motor dysfunction and death of dopaminergic neurons [41].

There are some other UPS-related proteins involved in PD pathogenesis, such as parkin and UCH-L1 (Ubiquitin carboxy-terminal hydrolase L1). A parkin-mutated mouse is a genetic model of PD successfully developed by researchers to prove the link between UPS dysfunction and PD [23]. Parkin mutations in Drosophila lead to loss of dopaminergic neurons and locomotion dysfunction [11]. Further, inactivation of UCHL-1 in mice results in motor ataxia but without degeneration in DA neurons [52].

Chaperone-Mediated Autophagy (CMA)

α-Syn protein is selectively degraded by CMA and therefore the failure of CMA machinery increase levels of α-Syn aggregates, which lead to degeneration of dopaminergic neurons. The downregulation of HSC70 or LAMP2A prevents autophagosome formation or inhibits its binding with lysosomes [15, 37]. In a previous study on brain mice, poly-ubiquitinated proteins were aggregated due to down-regulation of Atg5 or Atg7, autophagy-related genes [32]. Significant evidence from human post-mortem studies showed the dysfunction of autophagy in the

brain of PD patients. For example, levels of ALP marker (LC3 or Light chain 3) and autophagy vacuoles in the SNpc of PD patients and temporal cortex of DLB patients increased sharply compared to age-matched healthy individuals, suggesting ALP dysfunction is linked with PD progression [1, 57, 59]. Also, lower levels of HSC70, LAMP1 and LAMP2A have been observed in the SNpc of PD patients, indicating defective CMA [44]. Further, postmortem studies have shown that some autophagy downstream signaling pathways, such as mTOR and PI3K/AKT signaling, were severely inhibited in brain tissues of PD patients [17].

## 2 Conclusions

In the nervous system, protein homeostasis maintains a persistent environment for neurons to be healthy and alive. This can be accomplished through various mechanisms include regulation of protein translation, protein folding and protein degradation. Aggregations of misfolded α-Syn proteins forming LBs in SNpc are the hallmark pathologies noted in PD. HSPs are molecular chaperones that can refold α-Syn aggregates to their native structure or degrade them through different protein clearance pathways. Consequently, defective HSPs prevent proper folding, refolding and degradation machinery that lead to the aggregation of misfolded α-Syn in dopaminergic neurons. α-Syn aggregates, such as oligomers and amyloid-β sheet fibrils contribute to the formation of LBs, which lead to synaptic dysfunction, impaired neuronal communication, and ultimately dopaminergic neuron death. HSPs, therefore, protect dopaminergic neurons from degeneration and death which in turn prevent the incidence of PD.

**Acknowledgements** This work was supported by the PhD program of biotechnology at the Institute of Graduate Studies and Research (IGSR), Alexandria University, Alexandria, Egypt.

**Compliance with Ethical Standards**

**Funding:** This article did not receive any specific grant from funding agencies.

**Disclosure of Interests:** There are no conflicts of interest regarding this work.

**Ethical Approval for Studies involving Humans:** This article does not contain any studies with human participants performed by the author.

**Ethical Approval for Studies involving Animals:** This article does not contain any studies with animals performed by the author.

## References


1. Anglade P, Vyas S, Javoy-Agid F, Herrero MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC, Agid Y (1997) Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol 12(1):25–31
2. Arshad AR, Sulaiman SA, Saperi AA, Jamal R, Mohamed Ibrahim N, Abdul Murad NA (2017) MicroRNAs and target genes as biomarkers for the diagnosis of early onset of Parkinson disease. Front Mol Neurosci 10:352
3. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM (2002) Chaperone suppression of alpha-synuclein toxicity in a drosophila model for Parkinson's disease. Science 295 (5556):865–868
4. Bakthisaran R, Tangirala R, Rao CM (2015) Small heat shock proteins: role in cellular functions and pathology. Biochim Biophys Acta, Proteins Proteomics 1854(4):291–319
5. Bandyopadhyay U, Cuervo AM (2007) Chaperone-mediated autophagy in aging and neurodegeneration: lessons from alpha-synuclein. Exp Gerontol 42(1–2):120–128
6. Birkmayer W, Hornykiewicz O (1961) The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia. Wien Klin Wochenschr 73:787–788
7. Bridi JC, Hirth F (2018) Mechanisms of α-Synuclein induced Synaptopathy in Parkinson's disease. Front Neurosci 12:80
8. Broer L, Koudstaal PJ, Amin N, Rivadeneira F, Uitterlinden AG, Hofman A, Oostra BA, Breteler MMB, Ikram A, van Duijn CM (2011) Association of heat shock proteins with Parkinson's disease. Eur J Epidemiol 26:933–935
9. Bruinsma IB, Bruggink KA, Kinast K, Versleijen AA, Segers-Nolten IM, Subramaniam V, Kuiperij HB, Boelens W, de Waal RM, Verbeek MM (2011) Inhibition of alpha-synuclein aggregation by small heat shock proteins. Proteins 79:2956–2967
10. Cao Y, Ohwatari N, Matsumoto T, Kosaka M, Ohtsuru A, Yamashita S (1999) TGF-beta1 mediates 70-kDa heat shock protein induction due to ultraviolet irradiation in human skin fibroblasts. Pflugers Arch 438(3):239–244
11. Cha GH, Kim S, Park J, Lee E, Kim M, Lee SB, Kim JM, Chung J, Cho KS (2005) Parkin negatively regulates JNK pathway in the dopaminergic neurons of drosophila. Proc Natl Acad Sci U S A 102(29):10345–10350
12. Chaudhuri TK, Paul S (2006) Protein-misfolding diseases and chaperone-based therapeutic approaches. FEBS J 273(7):1331–1349
13. Ciechanover A, Kwon YT (2015) Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. Exp Mol Med 47:e147
14. Ciechanover A, Kwon YT (2017) Protein quality control by molecular chaperones in neurodegeneration. Front Neurosci 11:185
15. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D (2004) Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science 305(5688):1292–1295
16. Dattilo S, Mancuso C, Koverech G, Di Mauro P, Ontario ML, Petralia CC, Petralia A, Maiolino L, Serra A, Calabrese EJ, Calabrese V (2015) Heat shock proteins and hormesis in the diagnosis and treatment of neurodegenerative diseases. Immun Ageing 12:20
17. Dijkstra AA, Ingrassia A, de Menezes RX, van Kesteren RE, Rozemuller AJ, Heutink P, van de Berg WD (2015) Evidence for immune response, axonal dysfunction and reduced endocytosis in the substantia Nigra in early stage Parkinson's disease. PLoS One 10(6):e0128651
18. Fahn S (2008) The history of dopamine and levodopa in the treatment of Parkinson's disease. Mov Disord 23(Suppl 3):S497–S508
19. Fan GH, Zhou HY, Yang H, Chen SD (2006) Heat shock proteins reduce alpha-synuclein aggregation induced by MPP+ in SK-N-SH cells. FEBS Lett 580(13):3091–3098
20. Flower TR, Chesnokova LS, Froelich CA, Dixon C, Witt SN (2005) Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. J Mol Biol 351 (5):1081–1100

21. Fornai F, Lenzi P, Gesi M, Ferrucci M, Lazzeri G, Busceti CL, Ruffoli R, Soldani P, Ruggieri S, Alessandri MG et al (2003) Fine structure and biochemical mechanisms underlying nigrostriatal inclusions and cell death after proteasome inhibition. J Neurosci 23(26):8955–8966
22. Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250(4986):1429–1432
23. Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, Meloni EG, Wu N, Ackerson LC, Klapstein GJ et al (2003) Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J Biol Chem 278(44):43628–43635
24. Gong B, Radulovic M, Figueiredo-Pereira ME, Cardozo C (2016) The ubiquitin-proteasome system: potential therapeutic targets for Alzheimer's disease and spinal cord injury. Front Mol Neurosci 9:4
25. Guertin MJ, Lis JT (2010) Chromatin landscape dictates HSF binding to target DNA elements. PLoS Genet 6(9):e1001114
26. Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475(7356):324–332
27. Hoebel BG, Avena NM, Rada P (2007) Accumbens dopamine-acetylcholine balance in approach and avoidance. Curr Opin Pharmacol 7(6):617–627
28. Jones DR, Moussaud S, McLean P (2014) Targeting heat shock proteins to modulate alphasynuclein toxicity. Ther Adv Neurol Disord 7:33–51
29. Karunanithi S, Brown IR (2015) Heat shock response and homeostatic plasticity. Front Cell Neurosci 9:68
30. Kaushik S, Cuervo AM (2018) The coming of age of chaperone-mediated autophagy. Nat Rev Mol Cell Biol 19(6):365–381
31. Klucken J, Shin Y, Hyman BT, McLean PJ (2004) A single amino acid substitution differentiates Hsp70-dependent effects on alpha-synuclein degradation and toxicity. Biochem Biophys Res Commun 325(1):367–373
32. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E et al (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441(7095):880–884
33. Laplante AF, Moulin V, Auger FA, Landry J, Li H, Morrow G, Tanguay RM, Germain L (1998) Expression of heat shock proteins in mouse skin during wound healing. J Histochem Cytochem 46(11):1291–1301
34. Lester DB, Rogers TD, Blaha CD (2010) Acetylcholine-dopamine interactions in the pathophysiology and treatment of CNS disorders. CNS Neurosci Ther 16(3):137–162
35. Maiti P, Manna J (2019) Dysregulation of heat shock proteins in neurodegenerative diseases: restorative roles of small molecules and natural compounds. In: Asea A, Kaur P (eds) Heat shock proteins in neuroscience. Heat shock proteins, vol 20. Springer, Cham
36. Maiti P, Manna J, Veleri S, Frautschy S (2014) Molecular chaperone dysfunction in neurodegenerative diseases and effects of curcumin. Biomed Res Int 2014:495091
37. Maiti P, Manna J, Dunbar GL (2017) Current understanding of the molecular mechanisms in Parkinson's disease: targets for potential treatments. Transl Neurodegener 6:28
38. Matz JM, Blake MJ, Tatelman HM, Lavoi KP, Holbrook NJ (1995) Characterization and regulation of cold-induced heat shock protein expression in mouse brown adipose tissue. Am J Phys 269(1 Pt 2):R38–R47
39. Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670–684
40. McNaught KS, Mytilineou C, Jnobaptiste R, Yabut J, Shashidharan P, Jennert P, Olanow CW (2002) Impairment of the ubiquitin-proteasome system causes dopaminergic cell death and inclusion body formation in ventral mesencephalic cultures. J Neurochem 81(2):301–306
41. McNaught KS, Perl DP, Brownell AL, Olanow CW (2004) Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. Ann Neurol 56(1):149–162

42. Michel PP, Hirsch EC, Hunot S (2016) Understanding dopaminergic cell death pathways in Parkinson disease. Neuron 90(4):675–691
43. Mor DE, Tsika E, Mazzulli JR, Gould NS, Kim H, Daniels MJ, Doshi S, Gupta P, Grossman JL, Tan VX, Kalb RG, Caldwell KA, Caldwell GA, Wolfe JH, Ischiropoulos H (2017) Dopamine induces soluble α-synuclein oligomers and nigrostriatal degeneration. Nat Neurosci 20 (11):1560–1568
44. Murphy KE, Gysbers AM, Abbott SK, Spiro AS, Furuta A, Cooper A, Garner B, Kabuta T, Halliday GM (2015) Lysosomal-associated membrane protein 2 isoforms are differentially affected in early Parkinson's disease. Mov Disord 30(12):1639–1647
45. Parent M, Parent A (2010) Substantia Nigra and Parkinson's disease: a brief history of their long and intimate relationship. Can J Neurol Sci 37(3):313–319
46. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag AE, Lang AE (2017) Parkinson disease. Nat Rev Dis Primers 3:17013
47. Reeve A, Simcox E, Turnbull D (2014) Ageing and Parkinson's disease: why is advancing age the biggest risk factor? Ageing Res Rev 14:19–30
48. Rideout HJ, Lang-Rollin IC, Savalle M, Stefanis L (2005) Dopaminergic neurons in rat ventral midbrain cultures undergo selective apoptosis and form inclusions, but do not up-regulate iHSP70, following proteasomal inhibition. J Neurochem 93(5):1304–1313
49. Ritossa F (1996) Discovery of the heat shock response. Cell Stress Chaperones 1(2):97–98
50. Rodrigues e SAM, Geldsetzer F, Holdorff B, Kielhorn FW, Balzer-Geldsetzer M, Oertel WH, Hurtig H, Dodel R (2010) Who was the man who discovered the "Lewy bodies"? Mov Disord 25(12):1765–1773
51. Rubinsztein DC (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. Nature 443(7113):780–786
52. Saigoh K, Wang YL, Suh JG, Yamanishi T, Sakai Y, Kiyosawa H, Harada T, Ichihara N, Wakana S, Kikuchi T et al (1999) Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in gad mice. Nat Genet 23(1):47–51
53. Sorger PK (1991) Heat shock factor and the heat shock response. Cell 65(3):363–366
54. Sullivan PG, Dragicevic NB, Deng JH, Bai Y, Dimayuga E, Ding Q, Chen Q, Bruce-Keller AJ, Keller JN (2004) Proteasome inhibition alters neural mitochondrial homeostasis and mitochondria turnover. J Biol Chem 279(20):20699–20707
55. Sun J, Wang L, Bao H, Premi S, Das U, Chapman ER, Roy S (2019) Functional cooperation of α-synuclein and VAMP2 in synaptic vesicle recycling. Proc Natl Acad Sci U S A 116 (23):11113–11115
56. Sweeney P, Park H, Baumann M, Dunlop J, Frydman J, Kopito R, McCampbell A, Leblanc G, Venkateswaran A, Nurmi A, Hodgson R (2017) Protein misfolding in neurodegenerative diseases: implications and strategies. Transl Neurodegener 6:6
57. Tanji K, Mori F, Kakita A, Takahashi H, Wakabayashi K (2011) Alteration of autophagosomal proteins (LC3, GABARAP and GATE-16) in Lewy body disease. Neurobiol Dis 43 (3):690–697
58. Tantucci M, Mariucci G, Taha E, Spaccatini C, Tozzi A, Luchetti E, Calabresi P, Ambrosini MV (2009) Induction of heat shock protein 70 reduces the alteration of striatal electrical activity caused by mitochondrial impairment. Neuroscience 163(3):735–740
59. Toulorge D, Schapira AH, Hajj R (2016) Molecular changes in the postmortem parkinsonian brain. J Neurochem 139(Suppl 1):27–58
60. Vahid S, Thaper D, Zoubeidi A (2017) Chaperoning the Cancer: the Proteostatic functions of the heat shock proteins in Cancer. Recent Pat Anticancer Drug Discov 12(1):35–47
61. Westerheide SD, Morimoto RI (2005) Heat shock response modulators as therapeutic tools for diseases of protein conformation. J Biol Chem 280:33097–33100
62. Wyttenbach A (2004) Role of heat shock proteins during polyglutamine neurodegeneration: mechanisms and hypothesis. J Mol Neurosci 23(1–2):69–96

# HSP Stimulation on Macrophages and Dendritic Cells Activates Innate Immune System


**Yanyin Lu and Takanori Eguchi**

## Abstract

**Introduction** Heat shock proteins (HSP) are found in both prokaryotes and eukaryotes. Microbial HSP can activate innate immune system by closely regulating the antigen presentation function of dendritic cells and macrophages that produce proinflammatory cytokines. However, it is not organized how microbial and host HSP activate macrophages and dendritic cells in human and mouse. Besides, knowing the functions of HSP in innate immunology and tumor biology is also necessary for a deep understanding of inflammatory diseases and cancers. Here, we review; (i) how bacterial and mammalian HSP activate the innate immune system through activation of macrophages, (ii) how HSP interrelate with professional APC (dendritic cells) and participate in antigen presentation, and (iii) how HSP play key roles in innate immune system and tumor immunology.

**Methods** Studies containing keywords "dendritic cells, heat shock protein; innate immune system; macrophage; mitogen-activated protein kinase; proinflammatory cytokines; toll-like receptor" on Pubmed and Google data base were searched and summarized.

**Results** Microbial HSP60 is capable of activating macrophages by regulating CD14 and toll/IL-1 receptor (TIR) signaling pathway. HSP produced in mammalian hosts

Yanyin Lu and Takanori Eguchi contributed equally with all other contributors.

Y. Lu
Department of Dental Pharmacology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

Department of Dental Anesthesiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

T. Eguchi (✉)
Department of Dental Pharmacology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
e-mail: eguchi@okayama-u.ac.jp

Heat Shock Proteins (2021) 22: 53–67
https://doi.org/10.1007/7515_2020_26

Published Online: 23 November 2020

stimulate the activation of macrophages through various promoters, receptors, and pathways such as enhancing their antioxidant defense, activating the toll-like receptors (TLRs) and inducing the production of IL-12 and TNF-α. HSP can be complexed with peptides and form HSP-peptides complexes, while HSP-peptides complexes can be taken up and re-presented by APCs such as macrophages and dendritic cells (DCs) by MHC class I molecules and induce specific CD8+ cytolytic T lymphocytes (CTLs) responses. HSP are also involved in promoting the production of proinflammatory cytokines and modulating client proteins, which is closely related to inflammatory diseases and tumor survival, proliferation, and progression.

**Conclusions** HSP are involved in the modulation of innate immunity. Both microbial and host HSP are capable of activating macrophages and DCs by provoking the production of proinflammatory cytokines and various receptors for different pathways. HSP engage in the specific immunological function in the progression of protein folding, cytoprotection, antigen presentation, and even tumor proliferation and progression by various pathways or mechanisms. Further studies on the functions of HSP are necessary to develop new therapeutics against tumors and infectious diseases.

**Keywords** Dendritic cells · Heat shock protein · Innate immune system · Macrophage · Mitogen-activated protein kinase · Proinflammatory cytokines · Toll-like receptor

## Abbreviations

| | |
|---|---|
| 17-AAG | 17-allylamino-17-demethoxygeldanamycin |
| APC | antigen-presenting cell |
| CRPC | castration-resistant prostate cancer |
| CTL | cytotoxic T lymphocyte |
| DAMP | danger-associated molecular pattern |
| DC | dendritic cell |
| ELAM-1 | endothelial cell leukocyte adhesion molecule-1 (also known as E-selectin) |
| ER | endoplasmic reticulum |
| ERK | extracellular signal-regulated kinase |
| EV | extracellular vesicle |
| HSP | heat shock proteins |
| ICAM | intercellular adhesion molecule |
| IL | interleukin |
| iNOS | inducible NO synthase |
| JNK/SAPK | c-Jun N-terminal kinase |
| LOX | low-density lipoprotein receptor |
| LPS | lipopolysaccharide (also known as endotoxin) |
| MAPK | mitogen-activated protein kinase |
| MHC | major histocompatibility complex |

| | |
|---|---|
| MIF | Macrophage migration inhibitory factor |
| MMP | matrix metalloproteinase |
| MyD88 | myeloid differentiation primary response 88 |
| NBD | nucleotide-binding domain |
| NF-κB | nuclear factor-kappaB |
| NO | nitrogen oxide |
| OSCC | oral squamous cell carcinoma |
| SAPK | stress-activated protein kinase |
| TAP | transporter-associated antigen processing |
| TIR | toll/IL-1 receptor |
| TLR | toll-like receptor |
| TNF | tumor necrosis factor |
| TRAF | TNF receptor-associated factor |
| VCAM | vascular cell adhesion molecule |

## 1 Introduction

HSP is a sort of ancient and stable protein which exists in almost all creatures. Microbial HSP are significant activator of the innate immune system in human bodies. Therefore, understanding the mechanism of how microbial HSP activate macrophages becomes vital. Besides, HSP produced by host cells can also promote the activation of macrophages.

In addition to macrophages, HSP are also relevant to the professional antigen-presenting cells (APCs) called dendritic cells (DCs). DCs are able to take up, process, and present exogenous antigens with major histocompatibility complex (MHC) class I molecules to CTLs, which is called antigen cross-presentation and is interrelated with HSP. By making a molecular complex with specific peptides, HSP can be taken up by DCs and participate in the MHC class I molecules, which allows them to make a firm connection between innate and adaptive immune systems.

Besides, HSP are involved in tumor survival, proliferation, and progression. Targeting a particular property of HSP may achieve an anti-tumor effect.

In this chapter, we review (i) how bacterial and mammalian HSP activate the innate immune system through activation of macrophages and (ii) how HSP interrelates with DCs and participate in antigen presentation, (iii) how HSP play key roles in the innate immune system and tumor immunology.

## 2 Microbial HSP60 Activate Macrophages in Human and Mouse

Microbial HSP have been identified to be a potent activator of the innate immune system in hosts [54] and are able to induce the production of proinflammatory cytokines by the monocyte-macrophage system [50]. Of note, members of bacterial

HSP60 have been implicated to activate macrophages and induce the production of matrix metalloproteinases (MMPs) and tumor necrosis factor-alpha (TNF-α) [11, 18]. However, a later study has reported that recombinant human HSP60 did not induce the release of TNF-α from murine macrophages [16]. According to Amir Kol's research group, chlamydial HSP60 was positively correlated with increased expression in interleukin-6 (IL-6), nuclear factor-kappaB (NF-κB), and adhesion molecules such as endothelial-leukocyte adhesion molecule-1 (ELAM-1 also known as E-selectin), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) in human [19]. Their group also found that bacterial HSP60 is capable of activating human monocyte-derived macrophages through CD14 and p38 mitogen-activated protein kinase (MAPK) [20]. Bacterial HSP60 was able to activate macrophages by the Toll/IL-1 receptor (TIR) signaling pathway stimulating MyD88 and TRAF6, which can also activate NF-κB, JNK-1/2, p38 MAPK, and extracellular signal-regulated kinase (ERK)1/2 [52].

Bacterial HSP60 can trigger tissue regeneration and wound healing by regulating inflammation and cell proliferation by promoting the differentiation of intermediate monocytes to M2 macrophages [34]. A recent study found that a chemical constitution of the C-terminus of HSP60 can activate mouse peritoneal macrophages by stimulating lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) on the cell surface [24].

Thus, microbial HSP60 is able to promote the production of proinflammatory cytokines and stimulate diverse pathways to induce the differentiation and activation of macrophages to arouse the innate immune system in hosts.

## 3 HSP Expressed in Host Cells Activate Macrophages

Apart from the microbial HSP60, mammalian HSP70 is one of the most prominent and best characterized HSP, which is expressed at a higher level in macrophages than other cell types.

By generating HSP70, macrophages can enhance their antioxidant defenses and prevent themselves from undergoing apoptosis under oxidative stress [5]. Increased expression of HSP60 and HSP70 in bacteria-induced apoptotic neutrophils stimulate macrophages activation by potentiating the effect of lipopolysaccharide (LPS) on macrophages activation [56]. HSP, in this case, can elicit activation of human macrophages by potentiating LPS-stimulated production of TNF-α [56]. It has also been shown that HSP70 induced activation of IL-12 and ELAM-1 promoters in macrophages and this induction was regulated by MyD88 and TRAF6 signaling pathways through activation of toll-like receptor (TLR)-2/4 [53].

Distinctively from their extracellular roles, intracellular HSP have been noted for the cytoprotective function. For instance, it has been shown that HSP70 assist correct protein folding as well as transport, which is called chaperoning [39] and its function of inhibiting the apoptotic pathway is also of great significance for cytoprotection

[37]. These abilities of HSP to act as not only a chaperone but also a cytokine can be termed chaperokine [3].

Gp96 (also known as Grp94 (glucose-regulated protein 94)/endoplasmin/HSP90B1/HSP96) expressed in host cells is also an important HSP associated with macrophages. Gp96 is an endoplasmic reticulum (ER) chaperone essential for cell-surface TLRs. TLR1, TLR2, and TLR4 were found functionally related to ER chaperone gp96 [35]. Studies on gp96-deficient macrophages indicated that gp96 binds directly to not only TLR4 but also TLR9 [55]. Besides, HSP70, HSP90, and gp96 all stimulated the secretion of IL-1β, TNF-α, and IL-12 from $CD11b^+$ cells, which also proved that HSP cytokines could induce the macrophages to secret cytokines [6].

Hence, HSP of different subfamilies released from not only microbial but also host cells are able to stimulate the activation of macrophages through various promoters, receptors, and pathways (Table 1 and Fig. 1).

**Table 1** Heat shock proteins relevant to macrophages and dendritic cells

| Species | HSP family member | Functions | Author, Year |
|---|---|---|---|
| Microbial | HSP60 | Produce IL-12. | Skeen et al. [41] |
| | | Promote CD14 signaling and p38 MAPK production. | Kol et al. [20] |
| | | 1) activate the TIR signaling pathway. | Vabulas et al. [52] |
| | | 2) induce the secretion of IL-6 and NF-κB. | |
| | | 3) increase the expression of adhesion molecules such as E-selectin, ICAM-1 and VCAM-1. | |
| | | 1) trigger tissue regeneration and wound healing. | Pei et al. [34] |
| | | 2) promote the differentiation of monocytes to M2 macrophages. | |
| Mammalian | | Activate macrophages and induce the production of MMPs and TNF-α. | Kol et al. [18] and Chen et al. [11] |
| | HSP70 | 1) activate NF-κB pathway. | Basu et al. [6], Kuppner et al. [21] and Singh-Jasuja et al. [40] |
| | HSP90 | 2) stimulate the secretion of cytokines such as IL-1β, TNF-α, and IL-12 in macrophages. | |
| | gp96 | 3) induce expression of antigen-presenting and co-stimulatory molecules on the DCs and promote maturation. | |

(continued)

**Table 1** (continued)

| Species | HSP family member | Functions | Author, Year |
|---|---|---|---|
| | HSP-peptides complex | 1) induce specific CTLs and CD4+ cells. | Blachere et al. [10], Srivastava et al. [43], Tamura et al. [48] and Basu et al. [7] |
| | | 2) participate in MHC class I and class II molecules. | |
| | | 3) induce the secretion of proinflammatory cytokines. | |
| | | 4) mediate migration and maturation of DCs | |
| | | 5) take part in the antigen cross-presentation. | |
| | HSP70 | 1) enhance antioxidant defenses in macrophages. | Bachelet et al. [5] |
| | | 2) prevent macrophages form undergoing apoptosis. | |
| | HSP70 | 1) assist correct protein folding as well as transport. | Schmitt et al. [37] and Shi and Thomas [39] |
| | | 2) inhibit the apoptotic pathway. | |
| | HSP70 | Mediate migration and maturation of DCs | Basu et al. [6], Singh-Jasuja et al. [40] and Asea [1] |
| | HSP70 | Induce activation of IL-12 and ELAM-1 promoters (regulated by MyD88 and TRAF6 pathways and TLR 2/4).. | Vabulas et al. [53] |
| | HSP70 | Produce TNF-α, IL-1β, IL-6 and IL-12. | Asea et al. [2–4] and Svenssona et al. [44] |
| | HSP70 | Increase the release GM-CSF, chemokines such as MIP-1, MCP-1 and RANTES. | Lehner et al. [23], Panjwani et al. [33] and Srivastava [42] |
| | HSP70 | Present potent endogenous signals through the TLR2 receptor. | Zhou et al. [57] |
| | Gp96 | Act as ER chaperone essential for TLR1, TLR2, TLR4, TLR9. | Randow and Seed [35] and Yang et al. [55] |
| | Gp96 and HSP70 | Induce the production of iNOS and produce NO. | Panjwani et al. [33] |
| | HSP60 | Potentiate LPS-stimulated production of TNF-α. | Zheng et al. [56] |
| | HSP70 | | |

Microbial and host HSP are able to stimulate the activation of macrophages and DCs through various promoters, receptors, and pathways. This table shows some of the mechanisms and function of them by classifying the species, the HSP family and publishing time

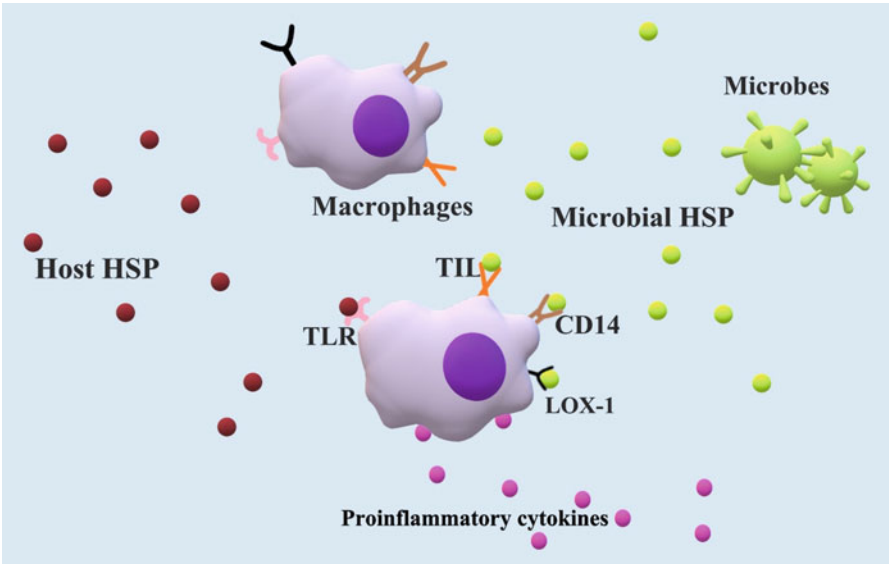



**Fig. 1** HSP activate macrophages by stimulating surface receptors
Microbial HSP (shown as green spheres) activate macrophages by stimulating cell-surface receptors such as Toll/IL-1 receptor (TIR), lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) and CD14, which can promote the production of proinflammatory cytokines such as TNF-α and activate the innate immune system
Host HSP (shown as red spheres) can interreact with other cell-surface receptors such as toll-like receptor (TLR)-2/4, regulating the MyD88 and TRAF6 signaling pathways and induce the production of proinflammatory cytokines like IL-12
Thus, HSP are capable of activating macrophages and increasing the production of proinflammatory cytokines (shown as pink spheres)

## 4 HSP Interrelate with DCs and Participate in Antigen Presentation

Apart from the ability to activate macrophages, HSP have been considered as a bridge between innate and adaptive immunity because of their capability of correlating with DCs and eliciting specific immunity in antigen presentation.

A number of studies showed that necrotic cell death, as well as cellular damage, leads to the release of HSP as damage- /danger-associated molecular patterns (DAMPs). The released extracellular HSP can be complexed with peptides. HSP-peptides derived from HSP90, HSP70, and gp96 can activate the highly conserved NF-κB pathway, stimulate macrophages to secrete cytokines, and induce the maturation and expression of antigen-presenting and co-stimulatory molecules in DCs [21, 40]. Consequently, these HSP species can interact with not only macrophages but also other APCs such as DCs [6].

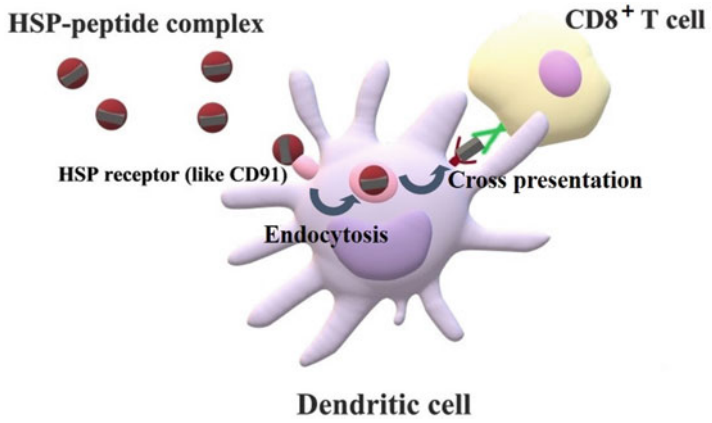



**Fig. 2** HSP-peptide complexes induce antigen cross-presentation of dendritic cells to $CD8^+$ cytotoxic T cells

The ability of DCs to take up, process, and present exogenous antigens with major histocompatibility complex (MHC) class I molecules to $CD8^+$ cytolytic T lymphocytes (CTLs) is called antigen cross-presentation. Studies found specific HSP-peptides complexes can induce specific CTLs and T-helper ($CD4^+$) cells mediated responses by participating in MHC class I molecules [10, 43, 48] (Fig. 2).

HSP-peptides of gp96, HSP70, and HSP90 were found to be taken up and re-presented by macrophages and DCs [7, 10]. These processes were considered to be related to receptor-mediated endocytosis, and studies furtherly discovered that CD91 (also known as low-density lipoprotein receptor-related protein 1 (LRP1) or α2-macroglobulin) was a common HSP receptor on APCs for several HSP members such as HSP70, HSP90, gp96 and calreticulin [7, 9].

By mediating the expression of CD14, CD40, CD83, CD86, and MHC class II molecules, HSP70 can also induce the maturation and migration of DCs [1, 6, 40]. HSP70 was able to chaperone a tyrosinase peptide and transfer it to immature DCs via a receptor-dependent uptake [28]. HSP70 could also enhance the cross-presentation of exogenous antigens by supporting complex formation and uptake of antigen [8]. Even in the absence of innate signals like TNF-α, the enhancement of HSP70-mediated peptides in cross-presentation still existed and the T-cell stimulation didn't require additional exogenous maturation signals, which implied HSP may be involved in various functions in immune modulation [8, 27].

In tumor environment, the peptide complex of HSP70 can be internalized and re-presented by the bone marrow-derived DCs. DCs pulsed with tumor-derived Hsp70 was also able to induce tumor-specific CTLs responses, which was dependent on transporter-associated antigen processing (TAP) [51].

In the occasion of HSP90-peptide complexes, evidence showed the cross-presentation of extracellular Hsp90–ovalbumin (OVA) protein complexes to specific $CD8^+$ T cells involved both classical TAP-dependent and TAP-independent endosomal pathways [32]. In the TAP-independent pathway, HSP90-peptide

complexes were efficiently cross-presented through the recycling endosome pathway by targeting static early endosomes within human monocyte-derived DCs [22]. Static early endosome was also associated with HSP90-chaperoned self-DNA and CpG-oligodeoxynucleotide and necessary for signaling through TLR9 for alpha-type interferon (IFN-α) production [29].

Briefly, HSP from various subfamilies such as HSP70, HSP90, and gp96 released from different host cells are able to induce the activation, maturation, and migration of DCs. HSP-peptides complexes can activate the CTLs and participate in antigen presentation through efficient pathways.

## 5 Innate Immunological Functions of HSP

Except for APCs activation and antigen presentation, HSP still have a variety of functions in the innate immune system. Macrophages and DCs stimulated with the gp96 and HSP70 can lead to the induction of inducible NO synthase (iNOS) and produce a synergistic production of NO [33]. The iNOS induction appeared to be in response to stimuli like TNF-α, IFN-γ, and ligation of CD40 [26], while NO was a major tumoricidal mechanism of macrophages and an immunomodulator of helper T cell function [33]. HSP interrelated with NO secreted by HSP-activated macrophages and DCs also implied the vital roles of HSP in the innate response to the functions of APCs.

HSP70 was able to cause various immunoregulatory effects. By the pathways depending on CD14 and intracellular calcium, the releases of IL-1β, IL-6, IL-12, and TNF-α can be increased after 2 to 4 h post-exposure of APC to exogenous HSP70 in human monocytes. However, by means of the CD14-independent and calcium-dependent pathways, only TNF-α showed an increasing release. These suggested that CD14 may be a potent co-receptor in HSP70-activated pathways [2, 3]. Besides, GM-CSF, chemokines such as MIP-1, MCP-1, and RANTES showed a significant release under the stimulation of extracellular HSP70 ([23, 33, 42]). These functions of HSP70 were derived by binding with high affinity to the plasma membrane and elicit a rapid intracellular calcium flux. HSP70-mediated pro-inflammatory cytokine release was modulated by the MyD88/NF-κB pathway and could exploit both TLR2 and TLR4 to accomplish the proinflammatory signal transduction, which was also CD14-dependent [4].

Recently, a study in the plant showed that the HopBF1 family of bacterial effectors can phosphorylate and inactivate eukaryotic HPS90 but also mimic HSP90 client by adopting a protein kinase folding to achieve specificity and hence cause many disease symptoms, which also indicates HSP90 to be an essential component of immunity [25].

Thus, HSP can take part in specific immunological functions like inducing the secretion of proinflammatory cytokines such as NO, IL-1β, IL-6, IL-12, TNF-α, GM-CSF, and chemokines. These indicate that HSP play an important role in modulating innate immune system and eliciting inflammatory diseases.

## 6 HSP Are Involved in Tumor Proliferation and Progression

HSP90 is a critical molecular chaperone that could be targeted by an anti-cancer agent because its client proteins such as HER2 (human epithelial growth factor receptor 2), EGFR (epithelial growth factor receptor), AKT and RAF1 assisted the survival, proliferation, and progression of tumors [47].

HSP90 complexed with a human tumor antigen peptide derived from survivin-2B can induce specific CTLs [49], which could be expected to be a natural activator for cancer vaccines.

Macrophage migration inhibitory factor (MIF) was also one of the client proteins of HSP90. MIF was a kind of cancer-promoting proinflammatory cytokine and performed a central role in the innate immune response in various diseases [38]. HSP90 could prevent MIF from degradation, and studies in the mouse model of breast cancer had proved MIF to be a stable client of HSP90 in vivo. In 1997, Tamura reported a method of tumor immunotherapy with autologous tumor-derived gp96 and HSP70 [48].

In tumor progression, the secretion of extracellular vesicles (EVs) are often closely related to HSP. A recent study examined the EV proteome of oral squamous cell carcinoma (OSCC) cells and found metastatic OSCC cells profoundly secrete HSP90-rich EVs. Double knockdown of HSP90α and HSP90β can decline the survival of metastatic OSCC cells. These findings implied that HSP90 level in EVs may be potential prognostic biomarkers and double targeting of HSP90α and HSP90β can become a new therapeutic in metastatic OSCC [30]. Moreover, CDC37, a kinases-specific co-chaperone of HSP90, is also a significant in the release of EV proteins such as CD9. Therefore, the effect of triple targeting of CDC37, HSP90α and HSP90β is more anticipating. Tumorigenicity of castration-resistant prostate cancer (CRPC) was markedly weakened after triple knockdown of CDC37/ HSP90α/ HSP90β [15]. In the case of metastatic oral cancer cells, metastatic oral cancer-derived EVs transmission into macrophages was also effectively reduced after triple knockdown of CDC37/ HSP90α/ HSP90β [31]. Besides, three-dimensional (3D) nano-environment was more close to in-vivo tumor status and found to promote secretion of HSP90 and EVs [14]. These studies indicate triple depletion of CDC37/ HSP90α/HSP90β could be a novel therapeutic strategy for EV-mediated malignancy events.

Apart from HSP90, stress-inducible HSP70 (also called HSP72) is significant in refolding disrupted proteins, which can furtherly prevent cellular injury, and restore cellular function [17]. A research found that HSP72 can be applied to predict the survival of patients in small cell lung cancer (SCLC) by monitoring the different expression levels of HSP72 in the cells [45]. The cellular expression of HSP72 is increased by kinds of stimuli like heat, ischemia, cytokines, etc. [36], while low expression levels of HSP72 in cells of SCLC patients carrying specific HSP72 genotype may suffer a higher risk of carcinogenesis and more rapid progression than a high expression group [45]. Besides, gene expression of HSP72 were found

regulated by intracellular MMP3 because of its ability to control transcription [12, 13].

Thus, targeting the HSP system using the HSP inhibitors could be a considerable method for anti-tumor treatment. For instance, targeting HSP90 with HSP90 inhibitors such as 17-allylamino-17-demethoxygeldanamycin (17-AAG), ganetespib, AUY922, and retaspimycin provided a pleiotropic and tumor-specific method for cancer treatment. As for HSP70, various inhibitors targeting different homologous members can be efficient, such as VER155008, MKT-077, and YK5 targeting the N-terminal nucleotide-binding domain (NBD) which contains ATPase activity [46].

Hence, the HSP family, including the HSP70 and HSP90 subfamilies, with specific tumor-related client proteins should be noticed, since they are related to a variety of functions on tumor proliferation and progression. By using specific inhibitors, targeting the HSP can become a notable and anticipated method in anti-tumor treatment.

## 7 Conclusions

HSP are involved in the modulation of innate immune system. Both microbial and host HSP are capable of stimulating the activation of macrophages and DCs through various promoters, receptors, and pathways such as enhancing their antioxidant defense, activating the toll-like receptors (TLRs) and inducing the production of proinflammatory cytokines.

HSP can be complexed with peptides and form HSP-peptides complexes, while HSP-peptides complexes can be taken up and re-presented by APCs such as macrophages and DCs by MHC class I molecules and induce specific CTL responses. HSP also engage in the specific immunological function of progress of protein folding, cytoprotection, antigen presentation, and even tumor proliferation and progression by different pathways or mechanisms. Further studies on the functions of HSP are necessary to develop new therapeutics against tumors and infectious diseases.

HSP-peptides complexes (shown as red spheres with grey cuboid) can be taken into dendritic cells (DCs) by receptor (shown as a pink sphere)-mediated endocytosis and re-presented by MHC class I molecules. CD91 is known as a common HSP receptor on APCs for endocytosis on several HSP members such as HSP70, HSP90, gp96 and calreticulin. Antigens (shown as grey cuboids) can be cross-presented by DCs and promote the activation of cytotoxic T lymphocytes especially CD8+ T cells

**Acknowledgments** The authors thank Takuya Miyawaki, Chiharu Sogawa, Kuniaki Okamoto, Yuka Okusha, Eman A. Taha for their cooperation, mentorship, or support. T.E. was supported by JSPS KAKENHI, grant numbers, JP17K11642-TE, JP17K11669-KOh, JP18K09789-KN, 19H04051-HO, 19H03817-MT, and by Suzuki Kenzo Memorial Foundation. Y.L. was supported by Rotary Yoneyama Memorial Foundation.

**Disclosure of Interests** The authors declare no conflict of interest with the content of this study.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References


1. Asea A (2007) Mechanisms of HSP72 release. J Biosci 32(3):579–584. https://doi.org/10.1007/s12038-007-0057-5
2. Asea A, Kabingu E, Stevenson MA, Calderwood SK (2000a) HSP70 peptide-bearing and peptide-negative preparations act as chaperokines. Cell Stress Chaperones 5(5):425–431. https://doi.org/10.1379/1466-1268(2000)005<0425:HPBAPN>2.0.CO;2
3. Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW et al (2000b) HSP70 stimulates cytokine production through a CD 14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6(4):435–442. https://doi.org/10.1038/74697
4. Asea A, Rehli M, Kabingu E, Boch JA, Baré O, Auron PE et al (2002) Novel signal transduction pathway utilized by extracellular HSP70. Role of toll-like receptor (TLR) 2 and TLR4. J Biol Chem 277(17):15028–15034. https://doi.org/10.1074/jbc.M200497200
5. Bachelet M, Adrie C, Polla BS (1998) Macrophages and heat shock proteins. Res Immunol 149 (7–8):727–732. https://doi.org/10.1016/S0923-2494(99)80047-9
6. Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK (2000) Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-κB pathway. Int Immunol 12(11):1539–1546. https://doi.org/10.1093/intimm/12.11.1539
7. Basu S, Binder RJ, Ramalingam T, Srivastava PK (2001) CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity 14(3):303–313. https://doi.org/10.1016/S1074-7613(01)00111-X
8. Bendz H, Ruhland SC, Pandya MJ, Hainzl O, Riegelsberger S, Bräuchle C et al (2007) Human heat shock protein 70 enhances tumor antigen presentation through complex formation and intracellular antigen delivery without innate immune signaling. J Biol Chem 282 (43):31688–31702. https://doi.org/10.1074/jbc.M704129200
9. Binder RJ, Han DK, Srivastava PK (2000) CD91: a receptor for heat shock protein gp96. Nat Immunol 1(2):151–155. https://doi.org/10.1038/77835
10. Blachere NE, Li Z, Chandawarkar RY, Suto R, Jaikaria NS, Basu S et al (1997) Heat shock protein-peptide complexes, reconstituted in vitro, elicit peptide-specific cytotoxic T lymphocyte response and tumor immunity. J Exp Med 186(8):1315–1322. https://doi.org/10.1084/jem.186.8.1315
11. Chen W, Syldath U, Bellmann K, Burkart V, Kolb H (1999) Human 60-kDa heat-shock protein: a danger signal to the innate immune system. J Immunol 162(6):3212–3219. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10092772
12. Eguchi T, Taha E (2020) Extracellular vesicle-associated moonlighting proteins: heat shock proteins and Metalloproteinases. In: Heat shock proteins in human diseases; heat shock proteins, vol 22. Springer Nature
13. Eguchi T, Calderwood SK, Takigawa M, Kubota S, Kozaki KI (2017) Intracellular MMP3 promotes HSP gene expression in collaboration with Chromobox proteins. J Cell Biochem 118 (1):43–51. https://doi.org/10.1002/jcb.25607

14. Eguchi T, Sogawa C, Okusha Y, Uchibe K, Iinuma R, Ono K et al (2018) Organoids with cancer stem cell-like properties secrete exosomes and HSP90 in a 3D nanoenvironment. PLoS One 13(e0191109). https://doi.org/10.1371/journal.pone.0191109
15. Eguchi T, Sogawa C, Ono K, Matsumoto M, Tran MT (2020) Cell stress induced Stressome release including. Cell 9(755)
16. Gao B, Tsan MF (2003) Recombinant human heat shock protein 60 does not induce the release of tumor necrosis factor α from murine macrophages. J Biol Chem 278(25):22523–22529. https://doi.org/10.1074/jbc.M303161200
17. Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381(6583):571–580. https://doi.org/10.1038/381571a0
18. Kol A, Sukhova GK, Lichtman AH, Libby P (1998) Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor-α and matrix metalloproteinase expression. Circulation 98(4):300–307. https://doi.org/10.1161/01.CIR.98.4.300
19. Kol A, Bourcier T, Lichtman AH, Libby P (1999) Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. J Clin Investig 103(4):571–577. https://doi.org/10.1172/JCI5310
20. Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA (2000) Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. J Immunol 164(1):13–17. https://doi.org/10.4049/jimmunol.164.1.13
21. Kuppner MC, Gastpar R, Gelwer S, Nössner E, Ochmann O, Scharner A, Issels RD (2001) The role of heat shock protein (hsp70) in dendritic cell maturation: Hsp70 induces the maturation of immature dendritic cells but reduces DC differentiation from monocyte precursors. Eur J Immunol 31(5):1602–1609. https://doi.org/10.1002/1521-4141(200105)31:5<1602::AID-IMMU1602>3.0.CO;2-W
22. Kurotaki T, Tamura Y, Ueda G, Oura J, Kutomi G, Hirohashi Y et al (2007) Efficient cross-presentation by heat shock protein 90-peptide complex-loaded dendritic cells via an endosomal pathway. J Immunol 179(3):1803–1813. https://doi.org/10.4049/jimmunol.179.3.1803
23. Lehner T, Bergmeier LA, Wang Y, Tao L, Sing M, Spallek R, Van Der Zee R (2000) Heat shock proteins generate β-chemokines which function as innate adjuvants enhancing adaptive immunity. Eur J Immunol 30(2):594–603. https://doi.org/10.1002/1521-4141(200002)30:2<594::AID-IMMU594>3.0.CO;2-1
24. Liu B, Li S, Xiu B (2019) C-terminus of heat shock protein 60 can activate macrophages by lectin-like oxidized low-density lipoprotein receptor 1. Biochem Biophys Res Commun 508(4):1113–1119. https://doi.org/10.1016/J.BBRC.2018.12.008
25. Lopez VA, Park BC, Nowak D, Sreelatha A, Zembek P, Fernandez J et al (2019) A bacterial effector mimics a host HSP90 client to undermine immunity. Cell 179(1):205–218.e21. https://doi.org/10.1016/j.cell.2019.08.020
26. MacMicking J, Xie Q, Nathan C (1997) Nitric oxide and macrophage function. Annu Rev Immunol 15(1):323–350. https://doi.org/10.1146/annurev.immunol.15.1.323
27. Milani V, Noessner E, Ghose S, Kuppner M, Ahrens B, Scharner A et al (2002) Heat shock protein 70 role in antigen presentation and immune stimulation. Int J Hyperth 6736. https://doi.org/10.1080/0265673021016614
28. Noessner E, Gastpar R, Milani V, Brandl A, Hutzler PJS, Kuppner MC et al (2002) Tumor-derived heat shock protein 70 peptide complexes are cross-presented by human dendritic cells. J Immunol 169(10):5424–5432. https://doi.org/10.4049/jimmunol.169.10.5424
29. Okuya K, Tamura Y, Saito K, Kutomi G, Torigoe T, Hirata K, Sato N (2010) Spatiotemporal regulation of heat shock protein 90-chaperoned self-DNA and CpG-Oligodeoxynucleotide for type I IFN induction via targeting to static early endosome. J Immunol 184(12):7092–7099. https://doi.org/10.4049/jimmunol.1000490
30. Ono K, Eguchi T, Sogawa C (2018) HSP-enriched properties of extracellular vesicles involve survival of metastatic oral cancer cells. J Cell Biochem 119(9):7350–7362. https://doi.org/10.1002/jcb.27039

31. Ono K, Sogawa C, Kawai H, Tran MT, Taha EA, Lu Y et al (2020) Triple knockdown of CDC37, HSP90-alpha, and HSP90-beta diminishes extracellular vesicles-driven malignancy events and macrophage M2 polarization in oral cancer. J Extracell Vesicles. https://doi.org/10.1080/20013078.2020.1769373
32. Oura J, Tamura Y, Kamiguchi K, Kutomi G, Sahara H, Torigoe T et al (2011) Extracellular heat shock protein 90 plays a role in translocating chaperoned antigen from endosome to proteasome for generating antigenic peptide to be cross-presented by dendritic cells. Int Immunol 23 (4):223–237. https://doi.org/10.1093/intimm/dxq475
33. Panjwani NN, Popova L, Srivastava PK (2002) Heat shock proteins gp96 and hsp70 activate the release of nitric oxide by APCs. J Immunol 168(6):2997–3003. https://doi.org/10.4049/jimmunol.168.6.2997
34. Pei W, Tanaka K, Huang SC, Xu L, Liu B, Sinclair J et al (2016) Extracellular HSP60 triggers tissue regeneration and wound healing by regulating inflammation and cell proliferation. Regen Med 1(1):1–11. https://doi.org/10.1038/npjregenmed.2016.13
35. Randow F, Seed B (2001) Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. Nat Cell Biol 3(10):891–896. https://doi.org/10.1038/ncb1001-891
36. Schlesinger MJ (1990) Heat shock proteins. In: Maloy S, Hughes K (eds) Brenner's encyclopedia of genetics, vol 3, 2nd edn. Elsevier Inc., pp 402–405. https://doi.org/10.1016/B978-0-12-374984-0.00685-9
37. Schmitt E, Gehrmann M, Brunet M, Multhoff G, Garrido C (2007) Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J Leukoc Biol 81 (1):15–27. https://doi.org/10.1189/jlb.0306167
38. Schulz R, Moll UM (2014) Targeting the heat shock protein 90: a rational way to inhibit macrophage migration inhibitory factor function in cancer. Curr Opin Oncol 26(1):108–113. https://doi.org/10.1097/CCO.0000000000000036
39. Shi Y, Thomas JO (1992) The transport of proteins into the nucleus requires the 70-kilodalton heat shock protein or its cytosolic cognate. Mol Cell Biol 12(5):2186–2192. https://doi.org/10.1128/mcb.12.5.2186
40. Singh-Jasuja H, Scherer HU, Hilf N, Arnold-Schild D, Rammensee HG, Toes REM, Schild H (2000) The heat shock protein gp96 induces maturation of dendritic cells and down-regulation of its receptor. Eur J Immunol 30(8):2211–2215. https://doi.org/10.1002/1521-4141(200030:8<2211::AID-IMMU2211>3.0.CO;2-0
41. Skeen MJ, Miller MA, Shinnick TM, Ziegler HK (1996) Regulation of murine macrophage IL-12 production. Activation of macrophages in vivo, restimulation in vitro, and modulation by other cytokines. J Immunol 156(3):1196–1206. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8557998
42. Srivastava P (2002) Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Annu Rev Immunol 20(1):395–425. https://doi.org/10.1146/annurev.immunol.20.100301.064801
43. Srivastava PK, Udono H, Blachere NE, Li Z (1994) Heat shock proteins transfer peptides during antigen processing and CTL priming, pp 93–98
44. Svenssona P-A, Asea A, Englund MCO, Bausero MA et al (2007) Major role of HSP70 as a paracrine inducer of cytokine production in human oxidized LDL treated macrophages. Atherosclerosis 185(1):32–38
45. Szondy K, Rusai K, Szabó AJ, Nagy A, Gal K, Fekete A et al (2012) Tumor cell expression of heat shock protein (HSP) 72 is influenced by HSP72 [HSPA1B a(1267)G] polymorphism and predicts survival in small cell lung Cancer (SCLC) patients. Cancer Investig 30(4):317–322. https://doi.org/10.3109/07357907.2012.657815
46. Taha EA, Ono K, Eguchi T (2019) Roles of extracellular HSPs as biomarkers in immune surveillance and immune evasion. Int J Mol Sci 20(18). https://doi.org/10.3390/ijms20184588
47. Takeuchi S, Fukuda K, Arai S, Nanjo S, Kita K, Yamada T et al (2016) Organ-specific efficacy of HSP90 inhibitor in multiple-organ metastasis model of chemorefractory small cell lung cancer. Int J Cancer 138(5):1281–1289. https://doi.org/10.1002/ijc.29858

48. Tamura Y, Peng P, Liu K, Daou M, Srivastava PK (1997) Immunotherapy of Tumors with autologous tumor-derived heat shock protein preparations. Science 278(5335):117 LP–117120. https://doi.org/10.1126/science.278.5335.117
49. Tanaka T, Okuya K, Kutomi G, Takaya A, Kajiwara T, Kanaseki T et al (2015) Heat shock protein 90 targets a chaperoned peptide to the static early endosome for efficient cross-presentation by human dendritic cells. Cancer Sci 106(1):18–24. https://doi.org/10.1111/cas.12570
50. Tsan MF, Gao B (2004) Heat shock protein and innate immunity. Cell Mol Immunol 1 (4):274–279. https://doi.org/10.0000/014198799329495
51. Ueda G, Tamura Y, Hirai I, Kamiguchi K, Ichimiya S, Torigoe T et al (2004) Tumor-derived heat shock protein 70-pulsed dendritic cells elicit-tumor-specific cytotoxic T lymphocytes (CTLs) and tumor immunity. Cancer Sci 95(3):248–253. https://doi.org/10.1111/j.1349-7006.2004.tb02211.x
52. Vabulas RM, Ahmad-Nejad P, Da Costa C, Miethke T, Kirschning CJ, Häcker H, Wagner H (2001) Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/Interleukin-1 receptor Signaling pathway in innate immune cells. J Biol Chem 276 (33):31332–31339. https://doi.org/10.1074/jbc.M103217200
53. Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, Wagner H (2002) HSP70 as endogenous stimulus of the toll/interleukin-1 receptor signal pathway. J Biol Chem 277 (17):15107–15112. https://doi.org/10.1074/jbc.M111204200
54. Wallin RPA, Lundqvist A, Moré SH, Von Bonin A, Kiessling R, Ljunggren HG (2002) Heat-shock proteins as activators of the innate immune system. Trends Immunol 23(3):130–135. https://doi.org/10.1016/S1471-4906(01)02168-8
55. Yang Y, Liu B, Dai J, Srivastava PK, Zammit DJ, Lefrançois L, Li Z (2007) Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages. Immunity 26(2):215–226. https://doi.org/10.1016/j.immuni.2006.12.005
56. Zheng L, He M, Long M, Blomgran R, Stendahl O (2004) Pathogen-induced apoptotic neutrophils express heat shock proteins and elicit activation of human macrophages. J Immunol 173(10):6319–6326. https://doi.org/10.4049/jimmunol.173.10.6319
57. Zhou F, Xing D, Chen WR (2009) Regulation of HSP70 on activating macrophages using PDT-induced apoptotic cells. Int J Cancer 125(6):1380–1389. https://doi.org/10.1002/ijc.24520

# Heat Shock Factor 1 and Its Small Molecule Modulators with Therapeutic Potential


**Naibedya Dutta, Koustav Pal, and Mahadeb Pal**

## Abstract

**Introduction** Heat shock response (HSR), a crucial component of cellular protein quality control mechanism has been shown to be deregulated in various protein conformation diseases such as Parkinson's disease and cancer. Therefore, heat shock factor 1 (HSF1), being a master regulator of HSR has been in consideration for targeting for potential therapeutic intervention of those diseases. Our objective is to review the literature in the area and sum up the progress in this book chapter.

**Methods** Pubmed was searched using key words such as heat shock response (HSR), heat shock factor 1 (HSF1), and small molecule modulators of HSR/HSF1 to select relevant set of publications to assess research progress in the area.

**Results** Literature showed identification of growing number of small molecule modulators such as activators and inhibitors of HSF1. Increasing number of studies has shaded lights on the molecular basis of their actions as well.

**Conclusions** In this book chapter a brief discussion on our understanding on cellular HSF1 regulation was made followed by highlighting various small molecule modulators of HSF1 and their underlying basis of the activities as became available.

**Keywords** Heat shock element · Heat shock factor 1 · Heat shock protein · Heat shock response · Natural product · Small molecule

N. Dutta · M. Pal (✉)
Division of Molecular Medicine, Bose Institute, Kolkata, India
e-mail: palmahadeb@gmail.com; mahadeb@jcbose.ac.in

K. Pal
Pursuing MBBS, Jawaharlal Institute of Post Graduate Medical Education and Research, Pondicherry, India

Heat Shock Proteins (2021) 22: 69–88
https://doi.org/10.1007/7515_2020_15

Published Online: 27 August 2020

## Abbreviations

| | |
|---|---|
| DBD | DNA binding domain |
| HSE | heat shock element |
| HSF1 | heat shock factor 1 |
| HSP | heat shock protein |
| HSR | heat shock response |
| RD | regulatory domain |
| TAD | trans activation domain |

## 1 Introduction

Study about the heat shock response has begun in 1962 with an accidental discovery by a young Italian geneticist Ferruccio Ritossa. He noticed a different type of puffing pattern in the polytene chromosome of Drosophila larvae, and a rapid synthesis of new RNA under exposure to an elevated temperature, or dinitrophenol [1, 2]. Later this phenomenon which known as heat shock response (HSR) has been observed to be active in all living organism starting from bacteria to human. This conserved cellular process is orchestrated by a transcription activator, heat shock factor (HSF), which acts through binding to a sequence motif on its target gene regulatory regions called heat shock elements (HSE) to control the expression of different molecular chaperones including those of inducible heat shock proteins (HSP), co-chaperones and ubiquitin in response to exposure to an increased temperature or any other condition that will compromise normal structure and function of protein [3]. Notably, while the HSE unit occurring in different organisms are highly conserved, the HSF proteins are significantly different in their sizes and structures [4]. In addition, the number of variants of HSF protein varies from organism to organism with single HSF in baker's yeast and fruit fly to six in human.

### *1.1 Structure and Regulation of HSF1 Activity*

HSF1 is a 529 amino acid long protein with ~57kD molecular weight. This is the most well characterized protein among six member family of heat shock factors in human. Human genome encodes HSF1, HSF2, HSF4, HSF5, HSFX and HSFY with some of these expressed only in certain cell type with very specialized function [5]. In non-stress condition HSP70, HSP90 and TRiC/CCT proteins associate to stabilize HSF1 in an inactive monomeric state in the cytoplasm [6, 7]. Upon exposure to an activation signal which includes an elevated temperature or hypoxia or any other proteotoxic stressor, inactive monomeric HSF1 rapidly converted to an active

homotrimeric form which then translocates to the nucleus to bind to an alternately inverted sequence unit (5′-nGAAn-3′) in the regulatory region of target genes [8, 9].

HSF1 has four major structural/functional domains implicated in the regulation of its activity. The best characterized DNA binding domain (DBD), which forms a looped helix-turn-helix structure spans from aa15 to aa110 in the amino terminal end. The carboxy terminus of DBD wraps around the DNA. The DNA-HSF1 complex is stabilized by interaction of amphipathic helical region with the hydrophobic DNA pockets as well as specific interaction of Arg and Lys residues with the DNA backbone [5, 46]. The α-helix rich trimerization or oligomerization domain is located next to the DBD spanning region from aa130 to aa203. Oligomerization domain named LZ1-3 is divided into two sub-domains, HR-A and HR-B. Upon activation, the intermolecular hydrophobic interactions between the oligomerization regions of three HSF1 molecule form an unusual coiled-coil configuration [11]. An additional hydrophobic region HR-C spanning from aa384 to aa409 as called LZ4, located between the transactivation domain and regulatory domain and inhibits the spontaneous oligomerization of HSF1 monomers [12]. The Trans activation domain (TAD) is localized in the extreme C-terminus of HSF1 (from aa409 to aa529). Like oligomerization domain TAD is also sub-divided into two regions, TAD1 (spanning from aa402 to aa420) and TAD2 (spanning from aa431 to aa529) [13]. Acidic residues present at TAD1 region are responsible for the transcriptional initiation whereas the hydrophobic residues present at the TAD2 region help in transcriptional elongation process by recruiting specific transcription factors and chromatin remodelers [14–16]. The regulatory domain (RD) of HSF1 spanning from aa203 to aa384 is present in between HR-A/B and HR-C regions. RD can sense the heat stress and subjected to the various forms of post translational modifications for the complete activation of HSF1. In non-stress condition RD suppress the function of TAD [17].

For the completion of HSF1 activation cycle, several post translational modifications including phosphorylation, acetylation, and sumoylation are essential. Activation of HSF1 is highly dependent on hyper-phosphorylation of specific serine residues which are present at the regulatory region of HSF1. Different events of activatory and inhibitory phosphorylations of HSF1 have been identified to date. Stimulatory phosphorylation events take place at Ser230, Ser419, Thr142, Ser320, Ser326 positions where as inhibitory phosphorylations occurs at Ser303, Ser307, Ser363 and Ser121 [18]. Hyper-phosphorylation and the consequence of those phosphorylations can alter in response to different physiological conditions and environmental stressors [19]. It was also found that the phosphorylation dependent sumoylation at Lys298 residue leads to the inhibition of HSF1 transcriptional activity [20]. Acetylation at Lys80 residue of DBD by p300 negatively regulates the DNA binding activity of HSF1. Sirtuin 1 or SIRT1, a $NAD^+$ dependent protein deacetylase helps to maintain the DNA binding competent state of HSF1 by deacetylation of Lys80 residue [19, 21].

The activation cycle of HSF1 is limited by feedback regulation established by interaction with chaperons like Hsp70 and Hsp90 to restore the activity to its basal level. Though it is considered that the sequence of HSF1 activation events are

universal across different cell lines, some studies indicate the cell line specific differences of HSF1 activation and the expression of its target gene products.

## *1.2 Association of HSF1 with Neurodegenerative Diseases*

Pathogenesis of most of the neurodegenerative diseases such as Huntington's and Parkinson's disease which affect majorly the older population are linked with inefficient functioning of protein quality control mechanism associated with HSF1 function. In fact, functions of protein quality control machineries including HSF1 are progressively reduced with aging. The stability of HSF1 protein was found to be compromised in these diseases as demonstrated in a number of studies [5]. Phosphoryltion of distinct serine residues in the regulatory domain of HSF1 through different kinases such as CK2 was linked with Huntington's disease [5, 22]. Expressing constitutively active HSF1 could prevent the α-synuclein aggregation mediated loss of dopaminergic neurones in Drosophila [23]. Neuronal precursor cell expressed developmentally down-regulated protein 4 or NEDD4, an E3 ligase mediated degradation of HSF1 which aggravates α-synucleinopathy, was shown to be counteracted by pharmacological activation of HSF1 through SIRT1 mediated deacetylation [24]. Activation of HSF1 through SIRT1 mediated deacetylation was shown to ameliorate Parkinson's disease developed in mouse model through induced expression of mutant α-synuclein [25]. Accumulation of mutant superoxide dismutase 1 (SOD1) aggregation and hyper-phosphorylated TAR DNA binding protein 43 (TARDBP or TDP43) associate with amyotrophic lateral sclerosis (ALS). It was observed that *HSF1* knockout mice carry increased insoluble TDP43 aggregates leading to the development of ALS-associated phenotypes [26]. Aggregation of SOD1 cause oxidative stress and severely affect motor neurons which can be protected by HSF1 dependent activation of molecular chaperones and taurine transporter (TauT) [27]. Increased expression of Hsp70 and Hsp25 through pharmacological activation of HSF1 could remarkably reduce the neuronal loss caused by the accumulation of toxic β-amyloid peptide' enhancing spatial memory in hippocampus and cerebellum region of the brain [28–30]. Activation of protein chaperons by inducing HSF1 activity facilitated protein refolding and enhancement of the solubilisation of toxic polyQ proteins [31]. In contrast to a mouse with normal HSF1 function, an increased level of mutant Huntingtin's protein correlating with shorter lifespan was observed in *HSF1* deficient mouse model of Huntington's disease [32]. Similarly $HSF1^{+/-}$ mouse appear with higher pathogenic polyQ repeat expansion in androgen receptor which conduct higher neurodegeneration compared to its genetically unmodified counterpart in spinal and bulbar muscular atrophy (SBMA) [33]. It is widely documented that upregulation of protein quality control mechanism by inducing HSF1 activity helped to ameliorate protein misfolding and associated toxicity in neurodegenerative disease models in cell, fly and animals [5, 31].

## 1.3 Association of HSF1 with Cancer

Molecular chaperones encoded by HSF1 target genes help to stabilize many proteins needed by cancer cells for their proliferation. Protein chaperones such as HSP90, stabilizes many oncogenes such as AKT, RAF1, and BCR-ABL [34]. Cancer cells depend for their survival on a constitutive upregulation of HSF1 activity [6, 39]. About a decade ago a surprising role of HSF1 in cancer progression has become apparent revealing that HSF1 mediates expression of genes, independent of heat shock, that encode many functions such as helping cancer cells in metabolism, proliferation, cell signalling, and resistance to apoptotic signals in distinct ways [35]. Independent studies demonstrated a higher level of HSF1 mRNA and protein expression with slower turnover in different cancer types [36]. MEK which is activated in cancer cells activates HSF1 though phosphorylation at serine 326. MEK activation results in many cancer cells due to loss of tumor suppressor neurofibromin (NF1) [37]. MEK mediated phosphorylation of HSF1 at Ser326 combined with downregulation of tumour suppressor filamin A-interacting protein 1-like (FILIP1 L), which helps in HSF1 stabilization in tumor cells [38–40]. F-box and tryptophan/aspartic acid (WD) repeat domain-containing 7 (FBXW7), another tumour suppressor in the class of E3 ligase promotes degradation of HSF1 is inhibited in various cancer types [22]. HSF1 antagonizes HER2/NEU mediated cellular senescence to promote malignant transformation [41]. It was also found that HSF1 is directly associated with activation of oncogenic RAS signalling and inactivation of tumour suppressor p53 pathway [42, 43]. In $HSF1^{-/-}$ cell line an impaired p53 ubiquitination pathway was found [43]. Apart from its transcriptional activation property, HSF1 upon phosphorylation at ser216 by PLK1 promoting aneuploidy [43]. In cancer cells HSF1 through inhibition of JNK induces mTORC1 activity to facilitate protein translation and proliferation [44]. Given strong association with many aspects of cancer cell metabolism, growth and survival, HSF1 has been considered as a promising next generation anticancer therapeutics.

## 1.4 Small Molecule Modulators of HSF1

### 1.4.1 Small Molecule Activators of HSF1

Accumulated evidence since last two decades suggest that forceful activation of heat shock response helps to ameliorate misfolded protein aggregation in various neurodegenerative disease conditions [45]. There are several naturally occurring as well as chemically synthesized small molecule HSF1 activators have been identified which represent a diverse set of structurally unrelated compounds (Fig. 1).

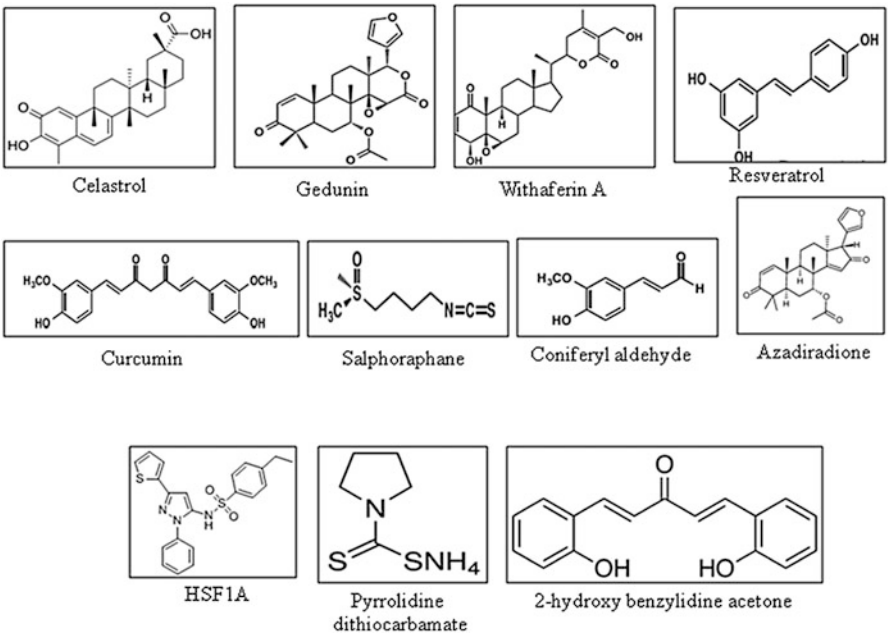



**Fig. 1 Structures of natural and synthetic HSF1 activators.** (**a**) Celastrol, Gedunin, Withaferin A, Resveratrol, Curcumin, Salphoraphane, Coniferyl aldehyde and Azadiradione were isolated from different plants of Indian or Chinese origin (**b**) HSF1A, Pyrrolidine dithiocarbamate, and 2-hydroxy benzylidine acetone are among the most studied synthetic HSF1 activators

### 1.4.2 Phytochemicals

Celastrol

Celastrol, a triterpene is present majorly in the celastraceae family of plants. It was first isolated from the root extracts of a very common Chinese medicinal plant *Tripterygium wilfordii*. At the beginning of the twentieth century, celastrol was identified as an antioxidant and anti-inflammatory drug which further established as a potent neuroprotective agent [47]. Richard Morimoto and his group showed for the first time that celastrol stimulates HSF1 by disrupting the association between HSP90 and its co-chaperone CDC37 leading to activation of heat shock response in different types of human cell lines to maintain cellular protein homeostasis [48, 49]. Celastrol was shown to induce HSP70 and facilitate the amelioration of the MPTP induced loss of dopamine concentration in the mice model of Parkinson's disease. Celastrol significantly reduced the volume of striatal lesion induced by 3-nitropropionic acid by activating HSF1 in a rat model of Huntington's disease [50].

### Gedunin

Gedunin, a pentacyclic triterpenoid which has been used in numerous homeopathic medicine for a long time, is one of the major compound present in Indian medicinal plant Neem (*Azadirachta indica*) [51]. It is well known for its anticancer, antifungal, and antiparasitic activity [52, 53]. Recently it was found that gedunin induces the HSF1 activity through inhibiting HSP90, a negative regulator of HSF1 activity [53, 54].

### Withaferin A

Withaferin A is a naturally occurring steroidal lactone found mainly in the Indian plant *Withania somnifera.* It is a well-known molecule for its anticancer and neuroprotective activity [55]. By a cell based reporter assay withaferin A was identified as a HSF1 inducer which leads to the activation of HSR pathway through inhibiting the function of HSP90 [56, 57].

### Curcumin

Curcumin, a biologically active small molecule, has been traditionally used as an antibacterial and anti-inflammatory agent against wound and inflammation. It was isolated from Indian medicinal plant *Curcuma longa*, commonly known as turmeric [58]. Curcumin induces HSF1 activity by inhibiting HSP90 ATPse activity [59]. Currently curcumin is under investigation in different clinical trials for its potential activity against Alzheimer's disease [60].

### Salforaphane

Salphoraphane [1-isothiocyanato-4-(methyl-sulfinyl)butane] is a well-established anticancer agent majorly found in cruciferous vegetables such as broccoli, cabbages, and cauliflower [61]. Sulphoraphane was also found as a promising neuroprotective compound which can significantly reduce the load of β-amyloid peptide deposition in the mice model of Alzheimer's disease [62]. It stimulates proteasome activity by inducing the expression of heat shock proteins, mainly HSP27 to clear the misfolded protein aggregates formed under proteotoxic stress [63].

### Resveratrol

Resveratrol [3,5,4′-trihydroxy-trans-stilbene] is a strong antioxidant with potent anticarcinogenic, neuroprotective, cardio-protective and anti-aging activities [64]. Resveratrol is found majorly in different fruits which include grapes,

blueberries, and peanuts. It is found as a potent compound to clear β-amyloid aggregations by stimulating a $NAD^+$ dependent protein deacetylase SIRT1 activity on HSF1 function [65, 66].

Coniferyl Aldehyde

Coniferyl aldehyde present in *Eucommia ulmoides*, a plant used in traditional medicine in Korea, Japan and China. Treatment with this glucoside stimulates activating phosphorylation of HSF1 at S326 involving MAP Kinase ERK1/2. An effect, coniferyl Aldehyde includes enhanced stability of HSF1 protein [67].

Azadiradione

Azadiradione found in the neem (*Azadirachta indica*) seed extract was shown for the first time to stimulate HSF1 activity through enhancing the affinity of HSF1 to its recognition site (HSE) of its target gene promoters. They have identified the heat shock response inducing activity of Azadiradione by a cell-based reporter assay. Their study revealed that Azadiradione can significantly ameliorates protein aggregates both in cell line and Droshophila model of PolyQ disease [68]. HSF1 inducing activity of Azadiradione was shown to be independent of cellular HSP90 and proteasome functions. Azadiradione was also found to ameliorate Huntington's disease in genetic model of mice with the activity correlating with upregulation HSF1, Ube3a and inducible protein chaperones [69].

### 1.4.3 Synthetic Compounds

HSF1A

HSF1A is a benzyl pyrazole derivative which can activate HSF1 without inhibiting HSP90 and proteasome activity. Neef et al. have screened over 10,000 compounds from a chemical library by a humanized yeast-based high throughput system and identified HSF1A for the first time as a HSF1 activator to clear the misfolded protein aggregation load in both animal cell line and Droshoplia model of PolyQ disease. HSF1A binds and inhibits the activity of TRiC/CCT, one of the negative regulator of HSF1and disrupt the interaction between TRiC/CCT and HSF1 [7, 70].

Sulfoxy Thiocarbamate Alkyne

Sulfoxy thiocarbamate alkyne is an another class of synthetic HSF1 activator which derived from sulforaphane, a naturally occurring HSF1 inducer [71]. It inhibits HSP90 by modifying specific cysteine residues which inhibits interaction with its clients [72].

Pyrrolidine Dithiocarbamate

It was identified as a HSF1 inducer by Kim et al. Their results suggested that PDTC, a known NFkB inhibitor, could induce HSF1 activity due to its pro-oxidant and thiol group modulating activity [73].

Bis (2-Hydroxybenzylidine) Acetone

Bis-(2-hydroxybenzylidine) acetone, a synthetic derivative of bis-(benzylidine) acetone causes a robust induction of HSF1 activity through inhibition of HSP90 action via interacting with sulfohydryl group. It is more efficient than compound A1, a closely related analogue of bis-(benzylidine) acetone [74].

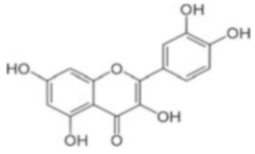
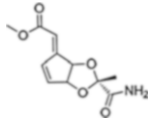
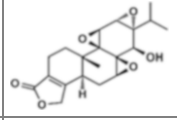
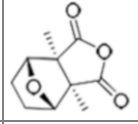
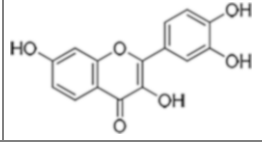
### 1.4.4 Small Molecule Inhibitors of HSF1

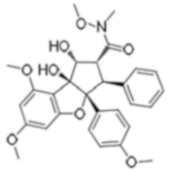
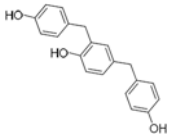
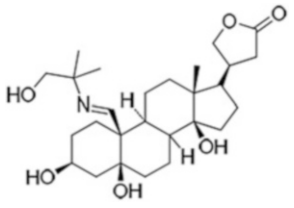
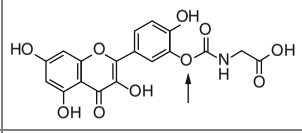
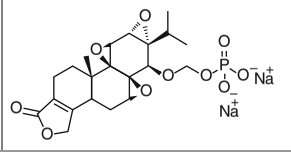
Many research groups have independently come up with different small molecule inhibitors of HSF1, several of which showed promising therapeutic potential against cancer. Understanding molecular mechanisms of action of many of these molecules are currently ongoing as noted in Table 1.

## 2 Conclusions

HSF1 is an indispensable molecule to maintain optimal proteostatic condition in cell. HSF1 not only coordinates the transcriptional activation of heat shock proteins but also found as an important determinant of other cellular processes. As discussed, beneficial effects of pharmacological activation of HSF1 has been observed in different disease models such as Hungtington's disease and Parkinson's disease. Because of pro-proliferative/antiapoptotic properties, HSF1 activation as a therapeutic purpose has to be considered with a caution especially for the population with genetic susceptibility to various cancers. On the other hand, identification/development of a small molecule with cancer cell specific HSF1 inhibitory activity which can extinguish oncogenic potential of HSF1 without hampering cellular HSR can be a plausible approach to fight against cancer.

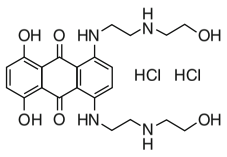
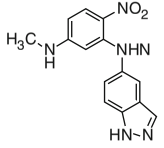
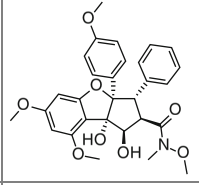
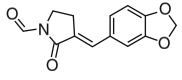
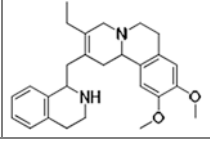
**Table 1** Compounds of natural and synthetic origin with inhibitory activity towards HSF1

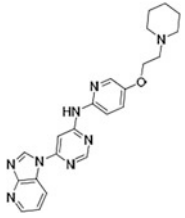
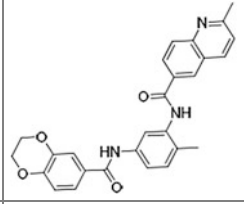
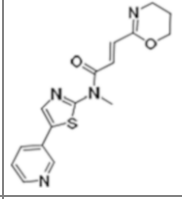
| Si | Name | Chemical Structure | Source | Function | References |
|---|---|---|---|---|---|
| **Natural Inactivators of HSF1** | | | | | |
| **1** | Quercetin |  | Various plants;<br>Apples, Berries, Brassica, Onions, Tea, Capers<br>Medicinal plants;<br>Ginkgo biloba, Sambucus candansis | Kinase inhibition of JNK, p38 MAPK<br>Hsp70 inhibition<br>Reduces phosphorylation of HSF1 | [74, 75] |
| **2** | Stresgenin B |  | Culture broth of Streptomyces sp. AS-9strain | Similar to Quercetin<br>Inhibits HSP gene expression.<br>4.9 times more potent than quercetin | [76] |
| **3** | Triptolide |  | Triptergium wilfordii (Thundergod vine) | NFKB suppression,<br>Inhibits HSF1 dependent transcription | [77] |
| **4** | Cantharidin |  | Male Blister Beetle; Oral Secretions<br>Family Meloidae | Inhibits HSF1 binding to its target gene HSP70 promoter | [78] |
| **5** | Fisetin |  | Found in many fruits, vegetables, eg. Strawberry, Apples, Grapes | Blocks HSF1 from binding to the HSP70 promoter | [79] |

| | | | | | |
|---|---|---|---|---|---|
| **6** | Rocaglamide A |  | Secondary metabolite of Aglaia plants. | Inhibits translation initiation factors like eIF4A. Reduced binding of HSF1 to targets induced by heat shock as well as those inducing by non-heat shock | [80] |
| **7** | 2,4-Bis (4-hydroxy benzyl) phenol |  | Gastroida elata | Induces dephosphorylation at Serine 326 of HSF1 leading to decreased stability, and degradation. | [81] |
| **8** | CL-43 |  | Acokanthera ouabaio | Inhibits HSF1 function with mechanism unclear<br>Some similarity with action as of triplotide | [82] |
| **9** | QC12 (Quercitin) |  | Synthetic, Prodrug | Inhibition of JNK, p38 MAPK<br>Hsp70 inhibition<br>Reduces the phosphorylation of HSF1 | [83] |
| **10** | Minnelide (Triplotide) |  | Synthetic, Prodrug | NFkB suppression,<br>Inhibits HSF1, HSP90 dependent chaperoning of kinases | [84] |

(continued)

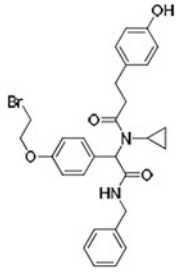
**Table 1** (continued)

| Si | Name | Chemical Structure | Source | Function | References |
|---|---|---|---|---|---|
| | **Synthetic inhibitors** | | | | |
| **1** | PW3405 |  | Synthetic | Inhibits phosphorylation at serine 326 of HSF1 | [85] |
| **2** | KRIBB11 |  | Synthetic | Inhibits positive transcription elongation factor b | [86] |
| **3** | Rohinitib |  | Synthetic | Inhibits binding to HSE binding to HSF1 DNA binding domain | [87] |
| **4** | KNK437 |  | Synthetic | Blocks activation of HSF1, Inhibits transcription of Hsp70 | [88, 89] |
| **5** | NZ-28 |  | Synthetic | Inhibits transcription function of HSF1, Sp1 and NFkB | [90] |

| | | | | | |
|---|---|---|---|---|---|
| **6** | 2,4-Bis (4-hydroxybenzyl) phenol |  | Synthetic | Promotes degradation of HSF1 through stimulating serine 326 phosphorylation | [81] |
| **7** | CCT251236 |  | Synthetic | Inhibits HSF1 activation pathway | [91] |
| **8** | $I_{HSF}$-115 |  | Synthetic | Inhibits transcription activity of HSF1 without inhibiting DNA binding and trimerization ability. | [92] |
| **9** | Acyl amino Carboxamides | | Synthetic | Destabilizes HSF1<br>Impaired translation of HSF1 mRNA | [93] |

(continued)

**Table 1** (continued)

| Si | Name | Chemical Structure | Source | Function | References |
|---|---|---|---|---|---|
| | |  | | | |

**Acknowledgements** The authors thank all the members of Prof. Pal's laboratory for their support and valuable suggestions to prepare the manuscript. We are grateful to DBT, SERB, and Bose Institute for financial support. ND is an ICMR SRF.

**Disclosure of Interests** None of this funding relates to the scientific content of this chapter.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia. https://doi.org/10.1007/BF02172188
2. Ritossa F (1996) Discovery of the heat shock response. Cell Stress Chaperones 1:97–98. https://doi.org/10.1379/1466-1268(1996)001<0097:dothsr>2.3.co;2
3. Anckar J, Sistonen L (2011) Regulation of HSF1 function in the heat stress response: implications in aging and disease. Annu Rev Biochem 80:1089–1115. https://doi.org/10.1146/annurev-biochem-060809-095203
4. Zimarino V, Wu C (1987) Induction of sequence-specific binding of Drosophila heat shock activator protein without protein synthesis. Nature 327:727–730. https://doi.org/10.1038/327727a0
5. Gomez-Pastor R, Burchfiel ET, Thiele DJ (2018) Regulation of heat shock transcription factors and their roles in physiology and disease. Nat Rev Mol Cell Biol 19:4–19. https://doi.org/10.1038/nrm.2017.73
6. Shi Y, Mosser DD, Morimoto RI (1998) Molecular chaperones as HSF1-specific transcriptional repressors. Genes Dev 12:654–666. https://doi.org/10.1101/gad.12.5.654
7. Neef DW et al (2014) A direct regulatory interaction between chaperonin TRiC and stress-responsive transcription factor HSF1. Cell Rep 9:955–966. https://doi.org/10.1016/j.celrep.2014.09.056
8. Dayalan Naidu S, Dinkova-Kostova AT (2017) Regulation of the mammalian heat shock factor 1. FEBS J 284:1606–1627. https://doi.org/10.1111/febs.13999
9. Vihervaara A, Sistonen L (2014) HSF1 at a glance. J Cell Sci 127:261–266. https://doi.org/10.1242/jcs.132605
10. Littlefield O, Nelson HC (1999) A new use for the 'wing' of the 'winged' helix-turn-helix motif in the HSF-DNA cocrystal. Nat Struct Biol 6:464–470. https://doi.org/10.1038/8269
11. Peteranderl R et al (1999) Biochemical and biophysical characterization of the trimerization domain from the heat shock transcription factor. Biochemistry 38:3559–3569. https://doi.org/10.1021/bi981774j
12. Rabindran SK, Haroun RI, Clos J, Wisniewski J, Wu C (1993) Regulation of heat shock factor trimer formation: role of a conserved leucine zipper. Science 259:230–234. https://doi.org/10.1126/science.8421783
13. Newton EM, Knauf U, Green M, Kingston RE (1996) The regulatory domain of human heat shock factor 1 is sufficient to sense heat stress. Mol Cell Biol 16:839–846. https://doi.org/10.1128/mcb.16.3.839
14. Corey LL, Weirich CS, Benjamin IJ, Kingston RE (2003) Localized recruitment of a chromatin-remodeling activity by an activator in vivo drives transcriptional elongation. Genes Dev 17:1392–1401. https://doi.org/10.1101/gad.1071803

15. Sullivan EK, Weirich CS, Guyon JR, Sif S, Kingston RE (2001) Transcriptional activation domains of human heat shock factor 1 recruit human SWI/SNF. Mol Cell Biol 21:5826–5837. https://doi.org/10.1128/mcb.21.17.5826-5837.2001
16. Brown SA, Weirich CS, Newton EM, Kingston RE (1998) Transcriptional activation domains stimulate initiation and elongation at different times and via different residues. EMBO J 17:3146–3154. https://doi.org/10.1093/emboj/17.11.3146
17. Green M, Schuetz TJ, Sullivan EK, Kingston RE (1995) A heat shock-responsive domain of human HSF1 that regulates transcription activation domain function. Mol Cell Biol 15:3354–3362. https://doi.org/10.1128/mcb.15.6.3354
18. Guettouche T, Boellmann F, Lane WS, Voellmy R (2005) Analysis of phosphorylation of human heat shock factor 1 in cells experiencing a stress. BMC Biochem 6:4–4. https://doi.org/10.1186/1471-2091-6-4
19. Dai C (2018) The heat-shock, or HSF1-mediated proteotoxic stress, response in cancer: from proteomic stability to oncogenesis. Philos Trans R Soc Lond Ser B Biol Sci 373:20160525. https://doi.org/10.1098/rstb.2016.0525
20. Hietakangas V et al (2006) PDSM, a motif for phosphorylation-dependent SUMO modification. Proc Natl Acad Sci U S A 103:45–50. https://doi.org/10.1073/pnas.0503698102
21. Westerheide SD, Anckar J, Stevens SM Jr, Sistonen L, Morimoto RI (2009) Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. Science 323:1063–1066. https://doi.org/10.1126/science.1165946
22. Kourtis N et al (2015) FBXW7 modulates cellular stress response and metastatic potential through HSF1 post-translational modification. Nat Cell Biol 17:322–332. https://doi.org/10.1038/ncb3121
23. Auluck PK, Chan HYE, Trojanowski JQ, Lee VMY, Bonini NM (2002) Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. Science 295:865–868. https://doi.org/10.1126/science.1067389
24. Kim E et al (2016) NEDD4-mediated HSF1 degradation underlies α-synucleinopathy. Hum Mol Genet 25:211–222. https://doi.org/10.1093/hmg/ddv445
25. Donmez G et al (2012) SIRT1 protects against α-Synuclein aggregation by activating molecular chaperones. J Neurosci 32:124–132. https://doi.org/10.1523/jneurosci.3442-11.2012
26. Chen H-J et al (2016) The heat shock response plays an important role in TDP-43 clearance: evidence for dysfunction in amyotrophic lateral sclerosis. Brain 139:1417–1432. https://doi.org/10.1093/brain/aww028
27. Kalmar B, Greensmith L (2017) Cellular chaperones as therapeutic targets in ALS to restore protein homeostasis and improve cellular function. Front Mol Neurosci 10:251–251. https://doi.org/10.3389/fnmol.2017.00251
28. Mavroudis IA et al (2010) Morphological changes of the human purkinje cells and deposition of neuritic plaques and neurofibrillary tangles on the cerebellar cortex of Alzheimer's disease. Am J Alzheimers Dis Other Dement 25:585–591. https://doi.org/10.1177/1533317510382892
29. Chen Y et al (2014) Hsp90 chaperone inhibitor 17-AAG attenuates Aβ-induced synaptic toxicity and memory impairment. J Neurosci 34:2464–2470. https://doi.org/10.1523/JNEUROSCI.0151-13.2014
30. Bobkova NV et al (2014) Therapeutic effect of exogenous hsp70 in mouse models of Alzheimer's disease. J Alzheimers Dis 38:425–435. https://doi.org/10.3233/JAD-130779
31. Neef DW, Jaeger AM, Thiele DJ (2011) Heat shock transcription factor 1 as a therapeutic target in neurodegenerative diseases. Nat Rev Drug Discov 10:930–944. https://doi.org/10.1038/nrd3453
32. Fujimoto M et al (2005) Active HSF1 significantly suppresses polyglutamine aggregate formation in cellular and mouse models. J Biol Chem 280:34908–34916. https://doi.org/10.1074/jbc.M506288200
33. Kondo N et al (2013) Heat shock factor-1 influences pathological lesion distribution of polyglutamine-induced neurodegeneration. Nat Commun 4:1405–1405. https://doi.org/10.1038/ncomms2417

34. Dai C, Sampson SB (2016) HSF1: Guardian of Proteostasis in Cancer. Trends Cell Biol 26:17–28. https://doi.org/10.1016/j.tcb.2015.10.011
35. Mendillo ML et al (2012) HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. Cell 150:549–562. https://doi.org/10.1016/j.cell.2012.06.031
36. Santagata S et al (2011) High levels of nuclear heat-shock factor 1 (HSF1) are associated with poor prognosis in breast cancer. Proc Natl Acad Sci U S A 108:18378–18383. https://doi.org/10.1073/pnas.1115031108
37. Dai C et al (2012) Loss of tumor suppressor NF1 activates HSF1 to promote carcinogenesis. J Clin Invest 122:3742–3754. https://doi.org/10.1172/jci62727
38. Tang Z et al (2015) MEK guards proteome stability and inhibits tumor-suppressive amyloidogenesis via HSF1. Cell 160:729–744. https://doi.org/10.1016/j.cell.2015.01.028
39. Hu Y, Mivechi NF (2011) Promotion of heat shock factor Hsf1 degradation via adaptor protein filamin A-interacting protein 1-like (FILIP-1L). J Biol Chem 286:31397–31408. https://doi.org/10.1074/jbc.M111.255851
40. Kwon M, Libutti SK (2014) Filamin a interacting protein 1-like as a therapeutic target in cancer. Expert Opin Ther Targets 18:1435–1447. https://doi.org/10.1517/14728222.2014.957181
41. Meng L, Gabai VL, Sherman MY (2010) Heat-shock transcription factor HSF1 has a critical role in human epidermal growth factor receptor-2-induced cellular transformation and tumorigenesis. Oncogene 29:5204–5213. https://doi.org/10.1038/onc.2010.277
42. Dai C et al (2012) Loss of tumor suppressor NF1 activates HSF1 to promote carcinogenesis. J Clin Invest 122:3742–3754. https://doi.org/10.1172/JCI62727
43. Jin X, Moskophidis D, Hu Y, Phillips A, Mivechi NF (2009) Heat shock factor 1 deficiency via its downstream target gene alphaB-crystallin (Hspb5) impairs p53 degradation. J Cell Biochem 107:504–515. https://doi.org/10.1002/jcb.22151
44. Su K-H et al (2016) HSF1 critically attunes proteotoxic stress sensing by mTORC1 to combat stress and promote growth. Nat Cell Biol 18:527–539. https://doi.org/10.1038/ncb3335
45. Westerheide SD, Morimoto RI (2005) Heat shock response modulators as therapeutic tools for diseases of protein conformation. J Biol Chem 280:33097–33100. https://doi.org/10.1074/jbc.R500010200
46. Allison AC, Cacabelos R, Lombardi VR, Alvarez XA, Vigo C (2001) Celastrol, a potent antioxidant and anti-inflammatory drug, as a possible treatment for Alzheimer's disease. Prog Neuro-Psychopharmacol Biol Psychiatry 25:1341–1357. https://doi.org/10.1016/s0278-5846(01)00192-0
47. Westerheide SD et al (2004) Celastrols as inducers of the heat shock response and cytoprotection. J Biol Chem 279:56053–56060. https://doi.org/10.1074/jbc.M409267200
48. Zhang T et al (2008) A novel Hsp90 inhibitor to disrupt Hsp90/Cdc37 complex against pancreatic cancer cells. Mol Cancer Ther 7:162–170. https://doi.org/10.1158/1535-7163.MCT-07-0484
49. Cleren C, Calingasan NY, Chen J, Beal MF (2005) Celastrol protects against MPTP- and 3-nitropropionic acid-induced neurotoxicity. J Neurochem 94:995–1004. https://doi.org/10.1111/j.1471-4159.2005.03253.x
50. Subapriya R, Nagini S (2005) Medicinal properties of neem leaves: a review. Curr Med Chem Anticancer Agents 5:149–146
51. Misra S et al (2011) Gedunin and photogedunin of Xylocarpus granatum possess antifilarial activity against human lymphatic filarial parasite Brugia malayi in experimental rodent host. Parasitol Res 109:1351–1360. https://doi.org/10.1007/s00436-011-2380-x
52. Brandt GE, Schmidt MD, Prisinzano TE, Blagg BS (2008) Gedunin, a novel hsp90 inhibitor: semisynthesis of derivatives and preliminary structure-activity relationships. J Med Chem 51:6495–6502. https://doi.org/10.1021/jm8007486
53. Patwardhan CA et al (2013) Gedunin inactivates the co-chaperone p23 protein causing cancer cell death by apoptosis. J Biol Chem 288:7313–7325. https://doi.org/10.1074/jbc.M112.427328

54. Zhang X, Timmermann B, Samadi AK, Cohen MS (2012) Withaferin a induces proteasome-dependent degradation of breast cancer susceptibility gene 1 and heat shock factor 1 proteins in breast cancer cells. ISRN Biochem 2012:707586. https://doi.org/10.5402/2012/707586
55. Santagata S et al (2012) Using the heat-shock response to discover anticancer compounds that target protein homeostasis. ACS Chem Biol 7:340–349. https://doi.org/10.1021/cb200353m
56. Yu Y et al (2010) Withaferin a targets heat shock protein 90 in pancreatic cancer cells. Biochem Pharmacol 79:542–551. https://doi.org/10.1016/j.bcp.2009.09.017
57. Marathe SA, Dasgupta I, Gnanadhas DP, Chakravortty D (2011) Multifaceted roles of curcumin: two sides of a coin! Expert Opin Biol Ther 11:1485–1499. https://doi.org/10.1517/14712598.2011.623124
58. Teiten MH, Reuter S, Schmucker S, Dicato M, Diederich M (2009) Induction of heat shock response by curcumin in human leukemia cells. Cancer Lett 279:145–154. https://doi.org/10.1016/j.canlet.2009.01.031
59. Hamaguchi T, Ono K, Yamada M (2010) REVIEW: Curcumin and Alzheimer's disease. CNS Neurosci Ther 16:285–297. https://doi.org/10.1111/j.1755-5949.2010.00147.x
60. Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P (1994) Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci U S A 91:3147–3150. https://doi.org/10.1073/pnas.91.8.3147
61. Zhang J et al (2017) Beneficial effects of Sulforaphane treatment in Alzheimer's disease may be mediated through reduced HDAC1/3 and increased P75NTR expression. Front Aging Neurosci 9:121–121. https://doi.org/10.3389/fnagi.2017.00121
62. Gan N et al (2010) Sulforaphane activates heat shock response and enhances proteasome activity through up-regulation of Hsp27. J Biol Chem 285:35528–35536. https://doi.org/10.1074/jbc.M110.152686
63. Pangeni R, Sahni JK, Ali J, Sharma S, Baboota S (2014) Resveratrol: review on therapeutic potential and recent advances in drug delivery. Expert Opin Drug Deliv 11:1285–1298. https://doi.org/10.1517/17425247.2014.919253
64. Bastianetto S, Menard C, Quirion R (2015) Neuroprotective action of resveratrol. Biochim Biophys Acta 1852:1195–1201. https://doi.org/10.1016/j.bbadis.2014.09.011
65. Putics A, Végh EM, Csermely P, Soti C (2008) Resveratrol induces the heat-shock response and protects human cells from severe heat stress. Antioxid Redox Signal 10:65–75. https://doi.org/10.1089/ars.2007.1866
66. Kim S-Y, Lee H-J, Nam J-W, Seo E-K, Lee Y-S (2015) Coniferyl aldehyde reduces radiation damage through increased protein stability of heat shock transcriptional factor 1 by phosphorylation. Int J Radiat Oncol Biol Phys 91:807–816. https://doi.org/10.1016/j.ijrobp.2014.11.031
67. Nelson VK et al (2016) Azadiradione ameliorates polyglutamine expansion disease in Drosophila by potentiating DNA binding activity of heat shock factor 1. Oncotarget 7 (48):78281–78296
68. Singh BK et al (2018) Azadiradione restores protein quality control and ameliorates the disease pathogenesis in a mouse model of Huntington's disease. Mol Neurobiol 55:6337–6346. https://doi.org/10.1007/s12035-017-0853-3
69. Neef DW, Turski ML, Thiele DJ (2010) Modulation of heat shock transcription factor 1 as a therapeutic target for small molecule intervention in neurodegenerative disease. PLoS Biol 8: e1000291–e1000291. https://doi.org/10.1371/journal.pbio.1000291
70. Ahn Y-H et al (2010) Electrophilic tuning of the chemoprotective natural product sulforaphane. Proc Natl Acad Sci 107:9590–9595. https://doi.org/10.1073/pnas.1004104107
71. Zhang Y et al (2014) Sulphoxythiocarbamates modify cysteine residues in HSP90 causing degradation of client proteins and inhibition of cancer cell proliferation. Br J Cancer 110:71–82. https://doi.org/10.1038/bjc.2013.710
72. Kim SH et al (2001) Activation of heat shock factor 1 by pyrrolidine dithiocarbamate is mediated by its activities as pro-oxidant and thiol modulator. Biochem Biophys Res Commun 281:367–372. https://doi.org/10.1006/bbrc.2001.4376

73. Zhang Y et al (2011) HSF1-dependent upregulation of Hsp70 by sulfhydryl-reactive inducers of the KEAP1/NRF2/ARE pathway. Chem Biol 18:1355–1361. https://doi.org/10.1016/j.chembiol.2011.09.008
74. Nagai N, Nakai A, Nagata K (1995) Quercetin suppresses heat shock response by down regulation of HSF1. Biochem Biophys Res Commun 208:1099–1105. https://doi.org/10.1006/bbrc.1995.1447
75. Li C et al (2016) Quercetin attenuates cardiomyocyte apoptosis via inhibition of JNK and p38 mitogen-activated protein kinase signaling pathways. Gene 577:275–280. https://doi.org/10.1016/j.gene.2015.12.012
76. Akagawa H et al (1999) Stresgenin B, an inhibitor of heat-induced heat shock protein gene expression, produced by Streptomyces sp. AS-9. J Antibiot (Tokyo) 52:960–970. https://doi.org/10.7164/antibiotics.52.960
77. Westerheide SD, Kawahara TLA, Orton K, Morimoto RI (2006) Triptolide, an inhibitor of the human heat shock response that enhances stress-induced cell death. J Biol Chem 281:9616–9622. https://doi.org/10.1074/jbc.M512044200
78. Kim JA, Kim Y, Kwon B-M, Han DC (2013) The natural compound cantharidin induces cancer cell death through inhibition of heat shock protein 70 (HSP70) and Bcl-2-associated athanogene domain 3 (BAG3) expression by blocking heat shock factor 1 (HSF1) binding to promoters. J Biol Chem 288:28713–28726. https://doi.org/10.1074/jbc.M113.488346
79. Kim JA et al (2015) Fisetin, a dietary flavonoid, induces apoptosis of cancer cells by inhibiting HSF1 activity through blocking its binding to the hsp70 promoter. Carcinogenesis 36:696–706. https://doi.org/10.1093/carcin/bgv045
80. Santagata S et al (2013) Tight coordination of protein translation and HSF1 activation supports the anabolic malignant state. Science 341:1238303–1238303. https://doi.org/10.1126/science.1238303
81. Yoon T, Kang G-Y, Han A-R, Seo E-K, Lee Y-S (2014) 2,4-Bis(4-hydroxybenzyl)phenol inhibits heat shock transcription factor 1 and sensitizes lung cancer cells to conventional anticancer modalities. J Nat Prod 77:1123–1129. https://doi.org/10.1021/np4009333
82. Nikotina AD et al (2018) Discovery and optimization of cardenolides inhibiting HSF1 activation in human colon HCT-116 cancer cells. Oncotarget 9:27268–27279. https://doi.org/10.18632/oncotarget.25545
83. Mulholland PJ et al (2001) Pre-clinical and clinical study of QC12, a water-soluble, pro-drug of quercetin. Ann Oncol 12:245–248. https://doi.org/10.1023/a:1008372017097
84. Chugh R et al (2012) A preclinical evaluation of Minnelide as a therapeutic agent against pancreatic cancer. Sci Transl Med 4:156ra139–156ra139. https://doi.org/10.1126/scitranslmed.3004334
85. Zhang D, Zhang B (2016) Selective killing of cancer cells by small molecules targeting heat shock stress response. Biochem Biophys Res Commun 478:1509–1514. https://doi.org/10.1016/j.bbrc.2016.08.108
86. Yoon YJ et al (2011) KRIBB11 inhibits HSP70 synthesis through inhibition of heat shock factor 1 function by impairing the recruitment of positive transcription elongation factor b to the hsp70 promoter. J Biol Chem 286:1737–1747. https://doi.org/10.1074/jbc.M110.179440
87. Moore CL et al (2016) Transportable, chemical genetic methodology for the small molecule-mediated inhibition of heat shock factor 1. ACS Chem Biol 11:200–210. https://doi.org/10.1021/acschembio.5b00740
88. Ohnishi K, Takahashi A, Yokota S, Ohnishi T (2004) Effects of a heat shock protein inhibitor KNK437 on heat sensitivity and heat tolerance in human squamous cell carcinoma cell lines differing in p53 status. Int J Radiat Biol 80:607–614. https://doi.org/10.1080/09553000412331283470
89. Yokota S, Kitahara M, Nagata K (2000) Benzylidene lactam compound, KNK437, a novel inhibitor of acquisition of thermotolerance and heat shock protein induction in human colon carcinoma cells. Cancer Res 60:2942–2948

90. Zaarur N, Gabai VL, Porco JA Jr, Calderwood S, Sherman MY (2006) Targeting heat shock response to sensitize cancer cells to proteasome and Hsp90 inhibitors. Cancer Res 66:1783–1791. https://doi.org/10.1158/0008-5472.CAN-05-3692
91. Menezes K et al (2017) The novel protein HSF1 stress pathway inhibitor Bisamide CCT361814 demonstrates pre-clinical anti-tumor activity in myeloma. Blood 130:3072–3072. https://doi.org/10.1182/blood.V130.Suppl_1.3072.3072
92. Vilaboa N et al (2017) New inhibitor targeting human transcription factor HSF1: effects on the heat shock response and tumor cell survival. Nucleic Acids Res 45:5797–5817. https://doi.org/10.1093/nar/gkx194
93. Bach M et al (2017) Ugi reaction-derived α-acyl Aminocarboxamides bind to phosphatidylinositol 3-kinase-related kinases, inhibit HSF1-dependent heat shock response, and induce apoptosis in multiple myeloma cells. J Med Chem 60:4147–4160. https://doi.org/10.1021/acs.jmedchem.6b01613

# Heat Shock Proteins, a Key Modulator of Neuroinflammation in Alzheimer's Disease


**Komal Panchal, Vidhi Bhatt, Mahima Raval, and Anand Krishna Tiwari**

## Abstract

**Introduction** Heat shock proteins (Hsp) are a key player to maintain protein homeostasis and folding in neurodegenerative diseases (NDDs) such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), etc. Hsp are associated with NDs via induction of proper folding of toxic misfolded protein. AD is the second most common neurodegenerative disease worldwide and is characterized by accumulation of $A\beta_{42}$ plaques and hyperphosphorylated tau that results in cognitive decline, neuronal death and affects brain structure. From the last past decade, several researchers proved that AD is not restricted to the brain, but it also manipulates the immune response and activation of inflammatory cells. AD is the amalgam of neurobiology and Immunology. One of the core pathologies of AD is neuroinflammation which activates the innate immune response followed by activation of microglia (macrophages), a resident immune cell of CNS and astroglia cells. Amyloid plaques and neurofibrillary tangles activate neuroinflammatory components such as microglia which further induce the production of a variety of proinflammatory cytokines, ROS, nitric oxide, eicosanoids, etc. Previous studies have shown that apart from Hsp molecular chaperone function, it also plays a role in neuroinflammation and disease-related signaling mechanisms. In here, we aim to summarize the details of Hsp as a key modulator of neuroinflammation in Alzheimer's Disease.

**Methods** The authors reviewed most of the relevant papers of Hsp and their role in neuroinflammation in AD.

**Results** Available data suggest that Hsp plays a protective role in neuroinflammation by acting as an immunomodulator in the central nervous system

K. Panchal · V. Bhatt · M. Raval · A. K. Tiwari (✉)
Genetics & Developmental Biology Laboratory, Department of Biological Sciences & Biotechnology, Institute of Advanced Research/IIAR, Koba Institutional Area, Gandhinagar, Gujarat, India
e-mail: aktiwari@iiar.res.in; anandk.tiwari@iar.ac.in; aktbhu@gmail.com

Heat Shock Proteins (2021) 22: 89–145
https://doi.org/10.1007/7515_2020_12

Published Online: 30 August 2020

and is also associated with astrocytes in $A\beta_{42}$ plaques in the brain of AD patients. It has been demonstrated that several signaling pathways are activated by cytokines such as TNF-α, INF-γ, IL-1β, etc. in the brain which exacerbates the AD-related pathologies and overexpression of Hsp decreases the inflammatory cytokines in the brain and decrease the progression and severity of the disease.

**Conclusions** Hsp are significantly involved in the modulation of neuroinflammation via interacting with inflammation-causing molecules and helps in the prevention of neuroinflammation in AD. It is used as a potential therapeutic target for the prevention of AD-related pathologies. The supplementation of compounds, known as inducers/co-inducers of Hsp in AD might be one of the potential therapeutic targets to treat/prolong the AD related pathologies in future. Moreover, membrane lipid rearrangement and nanoparticle-based therapies are also involved in decreasing the neuroinflammation via increasing the Hsp level at the site of neuroinflammation. Thus, apart from the supplementation of drugs to modulate the Hsp level, the interaction of Hsp with inflammatory cells and their affinity to reduce/inactivate them should be a more focused area in the case of AD and need to be extensively studied to get better therapeutic approach to treat the AD.

**Keywords** Alzheimer's disease · Astrocytes · $A\beta_{42}$ plaques · Heat Shock Proteins (Hsp) · Microglia · Neuroinflammation · Nuclear factor-kappa B · Pro-inflammatory cytokines · Tau

## Abbreviations

| | |
|---|---|
| 17-AAG | 17-allylamino-demethoxygeldanamycin |
| AA | arachidonic acid |
| AAVs | adeno-associated viruses |
| ACD | α-crystallin domain |
| AD | Alzheimer's disease |
| AP1 | activator protein 1 |
| ApoE | apolipoprotein E |
| APP | amyloid precursor protein |
| Appl | amyloid precursor protein like |
| ASK1 | apoptosis signal-regulating kinase 1 |
| ATP | adenosine triphosphate |
| Aβ | amyloid-β |
| BACE-1 | beta-site amyloid precursor protein cleaving enzyme – 1 |
| BAX | Bcl-2-associated X protein |
| BBB | blood brain barrier |
| Bcl2 | B-cell lymphoma 2 |
| CD | cluster of differentiation |
| Clp | Casein lytic proteinase |
| CNS | central nervous system |

| | |
|---|---|
| COX-2 | cyclooxygenase-2 |
| CPX | cyclooxygenase – 2 |
| CSF | cerebrospinal fluid |
| CTF | C-terminal fragment |
| CTL | cytotoxic T lymphocytes |
| CTR | c-terminal region |
| CvHsp | cardiovascular heat shock protein |
| DAXX | death domain associated protein |
| DHMN2C | distal hereditary motor neuropathy 2C |
| DMPK | myotonic dystrophy protein kinase |
| DNA | deoxyribonucleic acid |
| DPPC | dipalmitoyl phosphatidyl choline |
| EEVD | Glu-Glu-Val-Asp (Glu- glutamic acid, Val- Valine, Asp-Aspartic acid) |
| eIF4E | eukaryotic translation initiation factor |
| EPF | extracellular protein factor |
| ER | endoplasmic reticulum |
| ERGIC | ER-Golgi intermediate compartment |
| ERK | extracellular-signal regulated kinases |
| FLT3 | FMS-like tyrosine kinase-3 |
| GGA | Geranylgeranyl acetone |
| GRP78 | glucose-regulated protein 78kD |
| HD | Huntington's disease |
| HSE | heat shock elements |
| HSF | heat shock factor |
| Hsp | heat shock proteins |
| HTPG | high-temperature protein G |
| IL | interleukins |
| INF | interferone |
| iNOS | inducible nitric oxide synthase |
| JAK2 | Janus kinase |
| JNK | c-Jun N-terminal kinase |
| kDa | kilo Dalton |
| LPS | lipopolysaccharides |
| MAPK | mitogen-activated protein kinase |
| MCP-1 | macrophage chemo-attractant protein-1 |
| M-CSF | macrophage colony-stimulating factor |
| MHC | major histocompatibility complex |
| MIP-1α | macrophage inflammatory peptide |
| MKBP | myotonic dystrophy kinase binding protein |
| MN | motor neuron |
| MS | multiple sclerosis |
| MtUPR | mitochondrial related unfolding protein response |
| Myd88 | myeloid differentiation factor 88 |

| | |
|---|---|
| NDDs | neurodegenerative diseases |
| NF-kB | nuclear factor-kappa B |
| NFT | neurofibrillary tangles |
| NO | nitric oxide |
| NOD | leucine rich repeat and pyrin containing protein 3 (NLRP3) |
| NTR | N-terminal region |
| ODF1 | outer dense fiber protein 1 |
| PD | Parkinson's disease |
| PG | prostaglandins |
| PI3K | phosphatidylinositol-3-kinase |
| PP1 | protein phosphatase 1 |
| PS | presenilin |
| RAF | rapidly accelerated fibrosarcoma |
| RIP | receptor-interacting kinase |
| ROS | reactive oxygen species |
| SAPK | stress-activated kinases |
| sAPP | soluble amyloid precursor protein |
| sHsp | small heat shock protein |
| STAT-1 | signal transducers and activator of transcription-1 |
| TBI | traumatic brain injury |
| TCTEL1 | T-complex-associated-testis-expressed 1-like 1 |
| TGF | transforming growth factor |
| TLR | toll like receptor |
| TNF | tumor necrosis factor |
| TRAP | tryptophan regulated attenuation protein |
| WD/EPF domain | WD (Tryptophan-aspartic acid (W-D) dipeptide), epf-domain |

## 1 Introduction

Heat Shock Proteins (Hsp), molecular chaperones play a vital role in maintaining protein homeostasis via proper protein folding, degradation and protein trafficking in cellular stress conditions such as neuroinflammation, autoimmune diseases, environmental stress, brain injury, trauma, ischemia, neurodegenerative disorder, metal ions, ethanol, anoxia, UV exposer, etc. [1–4]. Hsp also plays a key role in multiprotein complexes assembly, sorting/transporting proteins into their respective subcellular compartments, inhibition of cell death and oxidative stress, etc. [5–8]. They are highly conserved proteins that confer their thermotolerance in all organisms [9–11]. Hsp were first discovered in the early 1960s by FM Ritossa. He demonstrated that chromosomal puffs in the salivary gland of the fruit flies were generated in the response of heat shock in *Drosophila busckii* [12].

The Hsp has been categorized as large and small Hsp dependent on their molecular weight [10, 13]. Large ATP has range of 40–110 kDa ATP dependent

Hsp such as Hsp100, 90, 70, 60, 47, 40 and small, ATP independent Hsp (sHsp) categorized as Hsp27, HspB5, Hsp20, Hsp22, etc. [10, 13–15]. Among all, Hsp70 is one of the most studied, conserved and ubiquitously expressed Hsp, present in archaebacteria to human [16, 17]. Hsp are very well known for its role in immunity by presenting antigens to the MHC Class I and class II molecule [7, 18, 19]. They also have anti-cancer properties (mediated by the adaptive and innate immune system) [19, 20].

The regulation of Hsp gene expression in stress condition is mediated by the interaction of heat shock factor (HSF) with heat shock elements (HSEs) in the heat shock protein gene promoter regions in DNA (Fig. 1) [21, 22]. In unstressed condition, HSF is unable to bind DNA and remains in monomeric molecules and are bound to Hsp70 and Hsp90 (repressors of HSF) in the cytoplasm [23–25]. When cellular insults induce cellular stress, Hsp70 and Hsp90 interact with misfolded protein and subsequently, HSF monomers get dissociated form Hsp90 and Hsp70 (Fig. 1) [26, 27]. HSF gets hyperphosphorylated at several serine residues 230, 326, and 419 by the mitogen-activated protein kinase (MAPK) subfamilies such as JNK/SAPK, ERK1, p38 protein kinase, etc. in a ras-dependent manner which promotes its transcriptional activity [26, 28–31]. After phosphorylation HSF forms trimer structure and translocate from cytoplasm to the nucleus and bind at the promoter region of Hsp gene on DNA results in robust increase in various Hsp gene expression such as Hsp70, Hsp60 as well as sHsp27 (Fig. 1) [15, 28, 32, 33].

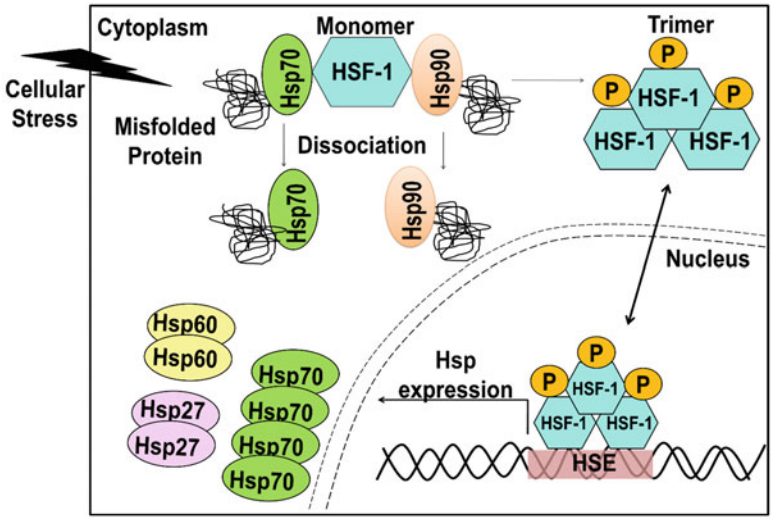



**Fig. 1 Heat shock transcription factor 1 (HSF1) activates Hsp expression**. The above schematic diagram shows that in normal conditions**,** HSF-1 is in monomeric inactive form in the cytoplasm and Hsp70 and Hsp90 remain bounded with HSF-1 and blocking its transcriptional activity. Under stress conditions, misfolded proteins are formed which binds to Hsp70 and Hsp90 and triggers the detachment of it from the HSF-1. HSF-1 forms a phosphorylated trimer structure in the cytoplasm and translates to the nucleus and binds to specific heat shock elements (HSE) sequences on DNA and activates Hsp such as Hsp70, Hsp60, Hsp27 etc. genes transcription

## *1.1 Large Heat Shock Proteins (40-110 kDa) [ATP Dependent]*

As mentioned above, ATP dependent large Hsp are high molecular weight Hsp ranging from 40 to 110 kDa includes Hsp100, 90, 70, 60, 47, 40 kDa (Table 1).

### 1.1.1 Hsp40

Hsp40 (DnaJ) has 40 kDa molecular weight and mostly present in the cytosol and endoplasmic reticulum (ER) [41, 42]. It has three types such as type I, type II and type III [141]. All three types of 40 contain J-domain made up of 70 amino acid residues located on N-terminal in Hsp40 types I and II and at any position in the case of type III [37, 40]. The C-terminal domain of type I and type II Hsp40 has a peptide-binding domain [141, 142]. They are bind to the non-native protein and induce protein folding by acting as cochaperones of Hsp70 [141, 143, 144]. Hsp40 and Hsp70 are the members of the DnaJ-like family which make transient complexes with non-native polypeptides results in protein folding, protein assembly, transport and degradation [56, 160, 333]. Hsp40 type I also known as DnaJ (E.coli), Ydj1 (Yeast) and Hdj2 (Human), all these contain middle two Zinc-finger motifs but it is absent in Hsp40 type II such as Sis1 (yeast) and Hdj1 (human) (Table 1) ([34, 35].

### 1.1.2 Hsp47

Hsp47 (Serpin H1) is 47 kDa molecular weight glycoprotein located in ER as well as cytoplasm [47, 52, 145]. This is a collagen specific chaperon mostly expressed in type I procollagen expressing cells and play a vital role in collagen synthesis [46, 48, 49]. Hsp47 belongs to the serpin family and possesses a serpin loop without serine protease inhibitory activity (Table 1) [145, 146]. It is induced by heat shock and shows the interaction with collagens I, II, III, IV and V [45, 47, 147]. It dissociates from the cis/ER-Golgi intermediate compartment region (ERGIC) after binding with procollagen in the ER. It is bind with procollagen with the help of collagenous repeats (Gly-Xaa-Arg) present on triple-helical procollagen results in inhibition of premature aggregation of procollagen [1, 49, 145, 148]. The previous study demonstrated that knockdown of Hsp47 causes collagen related genetic diseases like osteogenesis imperfect. It is expressed in the case of atherosclerosis involve in the proper folding of procollagen [50, 51, 147].

### 1.1.3 Hsp60

Hsp60 is a 60 kDa molecular weight containing prototypic molecular chaperone abundantly present in bacterial and eukaryotic cells [16]. It is located in

**Table 1** HSP family classification according to their molecular weight and physiological role

| Class | Molecular weight (kDa) | Other name/ Organisms | Location | Structure/Characteristics | Biological Function(s) | Interaction with other Hsp | References |
|---|---|---|---|---|---|---|---|
| **Large Heat Shock Proteins (40-100 kDa) [ATP dependent]** | | | | | | | |
| Hsp40 | 40 | **Type I:** DnaJ (*E. coli*), Ydj1 (Yeast), Hdj2 (Human) **Type II:** Sis1 (Yeast), Hdj1 (Human) | Cytoplasm, Endoplasmic Reticulum (ER) | A J-domain made up of 70 amino acids residues on N-terminal in types I and II and at any position in the case of type III | Protein folding, protein assembly, transport and degradation | Hsp70 | [34–43] |
| Hsp47 | 47 | Serpin H1 | Cytoplasm, ER, Mitochondria | A serpin loop without serine protease inhibitory activity | Inhibits the aggregation of premature procollagen | – | [44–52] |
| Hsp60 | 60 | GroEL (*E. coli*) | Nucleus, Cytoplasm, Mitochondria | A ring-shaped heptameric quaternary structure with two stacked heptameric rings | Mitochondrial protein folding and non-folding function such as cell signaling, anti-inflammatory activity | Hsp10 | [36, 53–65] |
| Hsp70 | 70 | Grp78, Hsc70, Ssa1–4, Kar2 (*S. cerevisiae*), DnaK (*E. coli*) | Ubiquitous [Nucleus Cytoplasm, Mitochondria, ER] | 640 amino acid residues with N-terminal ATPase domain (44 kDa) and C-terminal domain:18 kDa peptide-binding domainand10kDa part contains the Glu-Glu-Val-Asp (EEVD) regulatory motif | Protein folding, protein assembly and refolding | Hsp40, Hsp100, Hsp60, Hsp90, small heat shock proteins | [2, 36, 66–77] |
| Hsp90 | 90 | Hsp90A, Hsp90B, TRAP, HtpG (*E. coli*), | Nucleus, Cytoplasm, Mitochondria, ER | A homodimer structure consists of three flexibly linked regions such as an N-terminal ATP-binding domain, a | Protein folding and signal transduction | Tetratricopeptide repeat (TPR) co-chaperone: | [78–87] |

(continued)

**Table 1** (continued)

| Class | Molecular weight (kDa) | Other name/ Organisms | Location | Structure/Characteristics | Biological Function(s) | Interaction with other Hsp | References |
|---|---|---|---|---|---|---|---|
| | | Hsp82 (*S. cerevisiae*) | | central domain, and a C-terminal dimerization domain | | Hop, Fkbp51 & 2, CHIP, etc. Non-TPR co-chaperones: Aha1, p23, Cdc37, etc. | |
| Hsp100 | 100 | Casein lytic proteinase (Clp) A ClpB (*E. coli*), ClpX (*Helicobacter pylori*), ClpC (Plant), Hsp78 (*S. cerevisiae*) | Nucleus, Cytoplasm | A large hexameric structures contain a N-terminal domain, two different conserved AAA1 and AAA2 AAA+ ATPase domains and a coiled-coil middle regulatory domain | Resolubilizing aggregated protein | Hsp40, Hsp70, Hsp90 | [88–94] |
| **Small Heat Shock Proteins (10–40 kDa) [ATP independent]** | | | | | | | |
| **Class I** | | | | | | | |
| Hsp27 | 22.8 | HspB1, Hsp26, Hsp25 (rodents) | Nucleus, Cytosol | A conserved α-crystallin domain near the C-terminus and N-terminus consists of WD/EPF domain | Protein folding, antioxidant activity, anti-apoptotic activity suppress kinases, actin polymerization | Hsp70 | [95–101] |
| HspB5 | ~20 | αB-crystallin | Nucleus, Cytosol | A monomeric structure containing three regions such as α-crystallin domain (ACD) (a conserved central domain), the flanking N-terminal | Protein refolding, protein remodeling of cytoskeletal, anti-apoptotic activity | Hsp70 | [43, 102–104] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | region (NTR) and the C-terminal region (CTR) | | | |
| Hsp20 | ~17 | α-crystallin, HspB6 | Nucleus, Cytosol | A homodimer made up of combined groove at the interface via extending a beta sheet and the shared groove contains two symmetry-related C-terminal extensions with peptide in polyproline II conformation | Protein refolding and inhibit protein aggregation, protection from endotoxin-induced myocardial dysfunction, myocardial ischemia/reperfusion (I/R) injury, platelet aggregation inhibition | HspB8 | [38, 105–109] |
| Hsp22 | ~20 | α-crystallin C, HspB8 | Nuclease, Cytosol, Mitochondria | A conservative α-crystallin domain at C-terminaland hydrophobic N-terminal domain | Mitochondrial related Unfolding Protein Response (mtUPR), anti-aging activity, anti-oxidant activity, cell proliferation, carcinogenesis | Hsp60, Hsp70 | [100, 110–116] |
| **Class II** | | | | | | | |
| HspB2 | ~20.3 | Myotonic Dystrophy Kinase Binding Protein (MKBP) | Nucleus, Cytoplasm, Mitochondria | A conservative α-crystallin domain at the C-terminal part of the molecule | Maintains muscle structure and function, prevent aggregation of $A\beta_{42}$ plaques | HspB8 | [117–124] |
| HspB3 | 17 | sHsp 27-like protein (HspL27) | Nucleus, Cytoplasm | A monomeric protein lacks a flexible extension of the C-terminal structure | MNs survival, protein refolding and inhibits toxic protein aggregation | HspB2 | [119, 123, 125, 126] |
| HspB4 | ~20 | αA-crystallin CRYAA | Nucleus, Cytoplasm | A90 amino acid long α-crystallin domain" (ACD) flanked by a variable hydrophilic C-terminal domain and hydrophobic N-terminal domain | Proper substrate protein folding, anti-apoptotic activity | HspB5 | [127–130] |

(continued)

**Table 1** (continued)

| Class | Molecular weight (kDa) | Other name/ Organisms | Location | Structure/Characteristics | Biological Function(s) | Interaction with other Hsp | References |
|---|---|---|---|---|---|---|---|
| HspB7 | 18.6 | Cardiovascular HSP (cvHsp) | Nucleus, Cytoplasm | A conserved α-crystallin bordered by variable N- and C-terminal extensions | Tumor suppression in p53 pathway, cardiac morphogenesis, thin filament structural regulation, inhibits polyQ aggregation | HspB1 | [131–136] |
| HspB9 | 17.5 | Cancer/testis antigen 51 (CT51) | Nucleus, Cytoplasm | A conserved α-crystallin flanked by variable N- and C-terminal extensions. | Protein folding, maintains integrity of sperm flagella | – | [102, 137, 138, 140] |
| HB10 | ~28 | Outer dense fiber protein 1 (ODF1) | Nucleus, Cytoplasm | A conserved α-crystallin flanked by variable N- and C-terminal extensions. C terminal tail like keratins with high content of cystein | Spermatogenesis, structural role in sperm tail | – | [101, 137, 138–140] |

mitochondria [36, 64]. It forms a football like crystal structure with an oligomer composed of monomer arranged in two stacked heptameric rings [65, 149]. GroEL is one of the most studied family among all chaperons, *E. coli*. Hsp60 [150, 151]. Hsp60 has a barrel-like structure and can entrap 50 kDa of proteins (Table 1) [62, 152]. Its capacity increases when the co-chaperone GroEL comes into picture which works for closing the structure [62]. This group of proteins shares similarities with other families in terms of the ATP-mediated protein folding mechanism [153, 154]. It is also known as a mitochondrial chaperonin protein because it induces proper folding of nuclear-encoded protein which is imported into the mitochondria with the help of co-chaperonin Hsp10 [53, 56, 63]. Thus, it is important for maintaining mitochondria protein homeostasis [64, 155, 156]. Apart from mitochondria, it is also present in cytosol, nucleus, extracellular space, intracellular vesicles, etc. [54, 55, 57–59]. Hsp60 act as moon lightning protein as it performs multiple functions apart from protein folding, the non-folding functions such as cell-signaling molecule, in the immune system, act as a receptor for several ligands, etc. [60, 61].

### 1.1.4 Hsp70

This family of heat shock proteins has 70 kDa molecular weight and they are most studied chaperons among all which are involved in the protein folding, protein assembly, protein refolding and interacts with other proteins to achieve proper folding in case of stressed conditions [70, 71, 75]. It has ATP-mediated chaperone activity as it relies on ATP for conformational changes and subsequently proper protein folding [75]. There are 13 members of Hsp70 present in humans i.e. HspA1A, HspA1B, HspA1L, HspA2, HspA3, HspA4, HspA5, HspA5BP1, HspA6, HspA7, HspA8, HspA9B and HspA10 [2, 73, 157, 158]. These Hsp are universally conserved shows high structural similarity [159, 160]. It is made up of 640 amino acid residues with two main domains such as the N-terminal ATPase domain and the C-terminal domain which is divided in two parts such as peptide-binding domain and a Glu-Glu-Val-Asp (EEVD) regulatory motif [66, 68, 76]. The N-terminal ATPase domain binds to ATP and hydrolyzes it while the C-terminal domain binds with the client protein and refold the non-native polypeptides [68, 76]. They are ubiquitously present in the cell such as cytoplasm, nucleus, mitochondria, ER, etc. (Table 1) [67, 69, 73, 77]. Partner proteins such as Hsp40, Hsp100 and nucleotide exchange factors involved in the Hsp70 mechanism [158, 161]. These co-chaperones have J domain made up of long helical hairpin formed by ~70 residues along with a flexible loop and a conserved His-Pro-Asp motif and is required for the ATP hydrolysis by Hsp70 [76, 162]. Hsp70 also exhibits its protein folding and unfolding function by interacting with other chaperones such as Hsp40, Hsp60 chaperonins, Hsp90, small heat shock proteins and Hsp100 AAA+ [36, 72, 74, 77].

### 1.1.5 Hsp90

Hsp90 belongs to the family of molecular chaperones with 90 kD molecular weight of proteins [86]. Hsp90 family members are encoded by 17 known genes in humans [86, 156]. The Hsp90 family exhibits 4 different classes: Hsp90A (cytosolic), Hsp90B (ER) and TRAP (Tryptophan Regulated Attenuation Protein) (mitochondria) and isoforms are known to localize to the mitochondria, chloroplasts, cytosol, nucleoplasm and ER (Table 1) [163–165]. The structure of Hsp90 contains homodimer and three flexibly linked regions such as a N-terminal ATP-binding domain, a central domain, and a C-terminal dimerization domain [84, 166]. The different members of the family identified by its functions and its subcellular localization. Hsp90A and Hsp90B present in all eukaryotes whereas High-temperature protein G (HTPG), TRAP and Hsp90C occur only in Animalia, Bacteria and Plantae respectively [163]. Hsp90A is duplicated into Hsp90AA and Hsp90AB in vertebrates and Hsp90C duplicated into Hsp90C1 and Hsp90C2 in higher plants [167]. It is an ATP-dependent chaperone that changes its conformation with ATP triggering and binds to unfolded and folded proteins and induce proper protein folding [10, 168]. It has a conserved function in protein folding and signal transduction [79, 169].

### 1.1.6 Hsp100

Hsp100 protein has a molecular weight of 100 kDa also known as Casein lytic proteinase (Clp) and is located in the cytoplasm and present in bacteria, yeast, plants, humans and animals [91, 170]. The most studied Hsp100 family chaperons are bacterial ClpB and yeast Hsp104 (Table 1) [91, 171]. This family plays a crucial role in resolubilizing aggregated protein with the help of co-chaperones Hsp40, Hsp70 and Hsp90 [91, 172]. Hsp100 chaperones are members of AAA+ ATPases, a super large family of energy-driven conformational “machines” [173]. They form large hexameric structures in the presence of ATP and possess unfoldase activity [92, 174]. The structure of Hsp100 contains a highly mobile N-terminal domain that helps in substrate recruitment, two different conserved AAA1, and 2AAA+ ATPase domains and a coiled-coil middle regulatory domain which forms a belt around the AAA1 tier [94, 175]. In yeast, disaggregates the protein plaques were first studied with Hsp104 chaperons by Lindquist and coworkers [91, 176, 177]. Moreover, the bacterial ClpB is homologous of yeast Hsp104 which reactivates the aggregate proteins with the help of the DnaK/DnaJ/GrpE system [91, 178]. So, these unique properties of Hsp100 make it unique from conventional molecular chaperones that cannot reactivate the protein so efficiently like Hsp100 [91]. Moreover, Hsp110 is located in the cytosol and nucleus and help in the proper folding of proteins folding in stress condition with co-chaperone Hsp70 or Glucose-regulated protein 78 (GRP78) [10, 179].

## *1.2 Small Heat Shock Proteins (sHsp) [ATP Independent]*

Small heat shock proteins (sHsp) are present ubiquitously and are ATP-independent molecular chaperones [124, 180]. They have a molecular weight between 12 and 30 kDa, with a core conserved α-crystallin domain (ACD) flanked by variable N- and C-terminal domains [124, 181, 182]. sHsp have a more confined mode of action than other Hsp such as they have a large binding capacity and can efficiently bind to the non-native proteins from peptides to large-size proteins to prevent their aggregation irreversibly [124]. sHsp perform multiple cellular functions including protein refolding and degradation [183].

Apart from protein folding, sHsp also act as anti-apoptotic, anti-inflammatory, neuroprotective agents [184, 185]. sHsp distributed in the different tissues and specific cell types results in cell survival under stress conditions [186, 187]. There are two classes of sHsp such as class I and class II. Class I sHsp includes Hsp27 (HspB1), αB-crystallin (HspB5), Hsp20 and Hsp22 (α-crystallin C) and class II sHsp includes HspB2, HspB3, αA-crystallin (HspB4), HspB7, HspB9 and HspB10 (Table 1) [186, 188, 189]. Class I sHsp express ubiquitously whereas the Class II sHsp are primarily expressed in myogenic and testicular lineages [190].

### 1.2.1 Class I sHsp

Class I sHsp includes Hsp27 (HspB1), HspB5 (αB-crystallin), Hsp20 (α-crystallin) and Hsp22 (α-crystallin C) (Table 1).

### 1.2.2 Hsp27 (HspB1)

Hsp27 has a molecular weight of 22.8 kDa and express ubiquitously but mainly expressed in cardiac, skeletal, smooth muscles (Table 1) [98, 191]. It is a redox-sensitive molecular chaperone and has a homologous highly conserved α-crystallin domain near the C-terminus and N-terminus consists WD/EPF domain ([99, 186, 192]. Activation of Hsp27 activated by phosphorylation in unphosphorylated oligomer which contains Ser-15, Ser-78 and Ser-82 sites for phosphorylation [193, 194]. It induces proper protein folding and inhibits the aggregation of toxic protein in the stress condition [195, 196]. The previous studies have shown that phosphorylation of Hsp27 causes conformation changes and suppress kinases and inhibits the growth of hepatocellular carcinoma [100]. Further, unphosphorylated Hsp27 (HspB1) regulates translation initiation via a direct association with eIF4E in osteoblast [98]. Hsp27 acts as an anti-oxidant agent in oxidative stress conditions by lowering the intracellular ROS level [197–199]. The previous study by Charette et al. [200] has shown that Hsp27 also acts as an anti-apoptotic agent and prevents Fas-FasL mediated apoptosis via binding to Death domain associated protein (DAXX) and prevent the binding of Apoptosis signal regulating kinase 1 (ASK1)

to DAXX ([199, 201]. It also inhibits the mitochondrial-dependent apoptosis by binding to the Bax and cytochrome c [202, 203]. Additionally, it is an actin capping protein and helps in the regulation of actin cytoskeletal by promoting actin polymerization [96, 204, 205].

### 1.2.3 HspB5 (αB-crystallin)

HspB5 is also known as αB-crystallin and has ~20 kDa molecular weight. It has a monomeric structure containing three regions such as α-crystallin domain (ACD) (a conserved central domain), the flanking N-terminal region (NTR) and the C-terminal region (CTR) (Table 1) [43, 206, 207]. It is mostly expressed in the eye lens but is also present in the brain, skeletal and cardiac muscles [206, 208]. It has chaperonin activity that promotes protein refolding and degradation and also participates in cytoskeletal remodeling in stress conditions as well as during development [43, 101]. It assists ATP dependent Hsp70 chaperone in protein folding and degradation via the ubiquitin-proteasome and autophagic lysosomal pathways [103]. In stress conditions such as oxidative stress, heat shock and ischemia, it regulates the apoptosis in the cells [97, 104]. HspB5 acts as an anti-apoptotic agent, binds to procaspase-3, p53 and Bax which inhibits their translocation to the mitochondria and results in a decrease in apoptosis [97]. Moreover, it also blocks the RAF/MEK/ERK signaling pathway, BAX, Bcl-2 mitochondrial translocation and inhibits caspase-3 maturation in the cells [209, 210].

### 1.2.4 Hsp20 (α-crystallin)

The Hsp20 also known as α-crystallin, has molecular mass ~ 20 kDa with conserved 100 residues C terminal domain (Table 1) [107, 109, 211]. It is also known as HspB6, a small heat shock protein family that includes 10 members such as HspB1-B10 with 15–30 kDa molecular mass and protects the cell in stress conditions [33, 212]. Hsp20 has a homodimer structure formed by a combined groove at the interface via extending a beta-sheet and the shared groove contains two symmetry-related C-terminal extensions with peptide in polyproline II conformation [107, 213]. It is evolutionarily related to alpha-crystallin, an abundant constituent of eye lenses of vertebrate species and plays a key role in the correction of the refractive index of the lens [3, 129]. Hsp20 is abundantly present in the cytoplasm, mammalian heart, skeletal and various muscle cells [35, 38, 185, 207]. It has a role in protection of heart from endotoxin-induced myocardial dysfunction, myocardial ischemia/reperfusion (I/R) injury via inhibition of Akt, Bax, α-actin, NF-kβ, ASK1, etc. [212, 214, 215]. It also plays a key role in platelet aggregation inhibition and acts as a negative regulator of type 1 protein phosphatase (PP1) (a negative regulator of cardiac function) activity in the heart [38, 106].

### 1.2.5 Hsp22 (α-crystallin C)

Hsp22 has a molecular weight of ~20 kDa which is involved in the aging process [46, 216]. It contains a conservative alpha-crystallin domain at C-terminal and present in the cytoplasm, nucleus and mitochondria (Table 1) [32, 112, 217–219]. It is also present in the heart, skeletal and smooth muscle and brain along with prostate, lung, and kidney in some extent [110, 220–223]. Apart from aging, it plays a role in cardiac hypertrophy, cell proliferation, apoptosis, and carcinogenesis [146, 224, 225]. In aging, particularly the expression of Hsp22 was increased [216, 226]. In *Drosophila*, it is expressed during the metamorphosis of larvae to pupa development [216, 227]. It is also involved in increasing the lifespan of fly probably via histone deacetylation by Hsp22 [82, 111]. Together, the upregulation of Hsp22 shows a helpful effect in aging and also used as a biomarker for aging which indicates stress and improper homeostasis [216, 228]. The previous study by Tower et al. [229] has shown that Hsp22 decreases the adverse effect of aging via reducing mitochondrial metabolism. In *Drosophila*, it has been reported that Hsp22 alter some gene expressions, protein translation and shows a beneficial effect in maintaining mitochondrial structure and integrity during aging and oxidative stress condition [216, 230, 231]. Hsp22 is also involved in the mitochondrial related unfolding protein response (mtUPR) (a response against protein misfolding in mitochondria) with the help of Hsp60 and mitochondrial Hsp70 [231, 232]. During protein misfolding in mitochondria, Hsp22 decreases the ROS production, increases the lifespan, and enhance mtUPR signaling along with mitochondrial and nuclear signaling [114, 223, 233].

### 1.2.6 Class II sHsp

Class II sHsp includes HspB2, HspB3, HspB4 (αA-crystallin), HspB7, HspB9 & HspB10 (Table 1).

### 1.2.7 HspB2

HspB2 is a new member of the sHsp family with ~20.3 molecular weight and also known as Myotonic Dystrophy Kinase Binding Protein (MKBP), expressed mostly in the heart and skeletal muscles [118, 121]. It is present in the nucleus, cytoplasm and mitochondria [118, 122] and contains a conservative alpha-crystallin domain at the C-terminal part of the molecule (Table 1) [123, 124]. It is associated with Myotonic Dystrophy Protein Kinase (DMPK), a serine/threonine-protein kinase and maintains muscle structure and function [120, 234]. The previous study by Prabhu et al. [120] has shown that HspB2 exhibits molecular chaperone activity by inhibiting the aggregation of $A\beta_{42}$.

### 1.2.8 HspB3

HspB3 (Heat Shock Protein Family B (small) Member 3) is sHsp27 like the smallest sHsp protein with 17 kDa monomeric mass and shows high sequence homology with HspB1 [126, 207]. It lacks a flexible extension of the C-terminal structure (Table 1) [123, 125]. It is localized in the spinal cord, brain cortex and nerves of chicken, mouse and human [119, 235]. HspB3 is linked to neurological and muscular diseases in humans and helps in MNs survival [235, 236]. It shows chaperone-like activity with the help of yeast alcohol dehydrogenase [237]. The mutation of HspB3 associated with distal hereditary motor neuropathy 2C (dHMN2C) [43, 217, 238]. It is associated with HspB2 in the heart. The previous study by [123] has shown that the crystal structure of a tetrameric heterocomplex of HspB2/HspB3 found in muscle cells.

### 1.2.9 HspB4 (αA-crystallin)

HspB4 has ~20 kDa molecular weight and mainly expressed in eye ocular lens along with spleen and thymus in some amount [130, 239]. It produces from the duplication process of an ancestral α-crystallin gene and exhibits 57% amino acid sequence homology with HspB5 αB crystallin [207, 240]. It has 90 amino acid long α-crystallin domain (ACD) flanked by a variable hydrophilic C-terminal domain and hydrophobic N-terminal domain [129]. It acts as a molecular chaperone, possess anti-apoptotic activity and helps in proper substrate protein folding (Table 1) [128, 130].

### 1.2.10 HspB7

HspB7 is a member of the sHspB family which heterodimerize with other similar Hsp (Table 1) [97, 134]. It is made up of 170 amino acids with 18.6 kDa molecular mass and mostly expresses in the heart [131, 135] and also known as cardiovascular heat shock protein (cvHsp). It facilitates sarcomeric proteostasis with the help of Filamin C and Titin [134, 136]. It is mainly present in the nucleus, cajal body, cytoplasm, developing and adult heart [131, 132, 136]. Mutation of this gene results in heart failure, renal carcinoma, induction of autophagic pathways etc. [241–243]. The function of this gene includes tumor suppression in the p53 pathway, cardiac morphogenesis along with left-right asymmetry and thin filament structural regulation [133, 135]. The previous study by Wu et al. [244] has shown that HspB7 decreases the polyQ aggregation by its unique N-terminal domain by binding with the HspB1 alpha-crystallin domain.

### 1.2.11 HspB9 & HspB10

HspB9 & HspB10 are the members of sHsp family B also known as Cancer/testis antigen 51 (CT51) [140, 245]. HspB9 has 17.5 kDa molecular mass and a continuous open reading frame encoding a protein of 159 residues [102, 139]. HspB10 is also known as outer dense fiber protein 1 (ODF1) [138, 139] and has 27 kDa molecular mass with C terminal tail like keratins with high content of cysteine (Table 1) [139, 194]. These two Hsp are the testis-specific expressed sHsp [101, 139]. HspB10 mostly found in sperm tail and also localized in the nucleus, cytoplasm and tumor cells [243, 246]. Both Hsp response to environmental heat stress conditions [139]. HspB10 plays a key structural role in the sperm tail (Table 1) [243, 247]. The previous study has shown that HspB10 interacts with the T-complex-associated-testis-expressed 1-like 1 (TCTEL1) gene in spermatogenesis [139]. HspB9 and B10 expression gradually increases with the age and remain constant after sexual maturity [139]. HspB9 expressed in spermatogonia, spermatocytes, round spermatids and interact with the TCTEL1 gene in spermatogenesis. HspB10 expressed in elongated spermatids [102, 139].

## *1.3 Neuroinflammation in Alzheimer's Disease*

Inflammation is a biological response of body tissue that can be triggered by various factors such as injury, injured cells, pathogen attack, exposure to toxic compounds, etc. [248–250]. Neuroinflammation is the inflammation of central nervous system (CNS) and is characterized by activation of glial cells, release of cytokines chemokines and infiltration of blood cells to the brain parenchyma [251–255]. The neuroinflammation response is induced by microglia, astrocytes, neutrophils, mast cells, macrophages, lymphocytes, etc. (Fig. 2) [256–258]. During neuroinflammation condition, activated microglia, astrocytes, macrophage and lymphocytes releases the inflammatory mediators such as pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, and Tumor necrosis factor-α (TNF-α), ROS, macrophage chemo-attractant protein-1 (MCP-1), neurotransmitters, pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), etc. (Fig. 2) [252, 255].

Further in the CNS, microglia and astrocytes are the two main types of cells involved in the inflammatory response [252, 250]. Microglial cells are a type of macrophage and predominantly found in CNS. Approximately 10% of these cells play a vital role in regeneration, neuronal plasticity, neurogenesis and mounting the immune response in case of injury to the brain [252, 253]. The astrocytes are the abundant glial cells that are crucial for homeostasis of the brain, and help for synaptic plasticity/synapse formation, regulate neurotransmitters and ion balance extracellularly [260, 261].

Neuroinflammation plays an important role in neurodegenerative diseases (NDDs) such as Alzheimer's disease (AD), Parkinson's disease (PD), Multiple

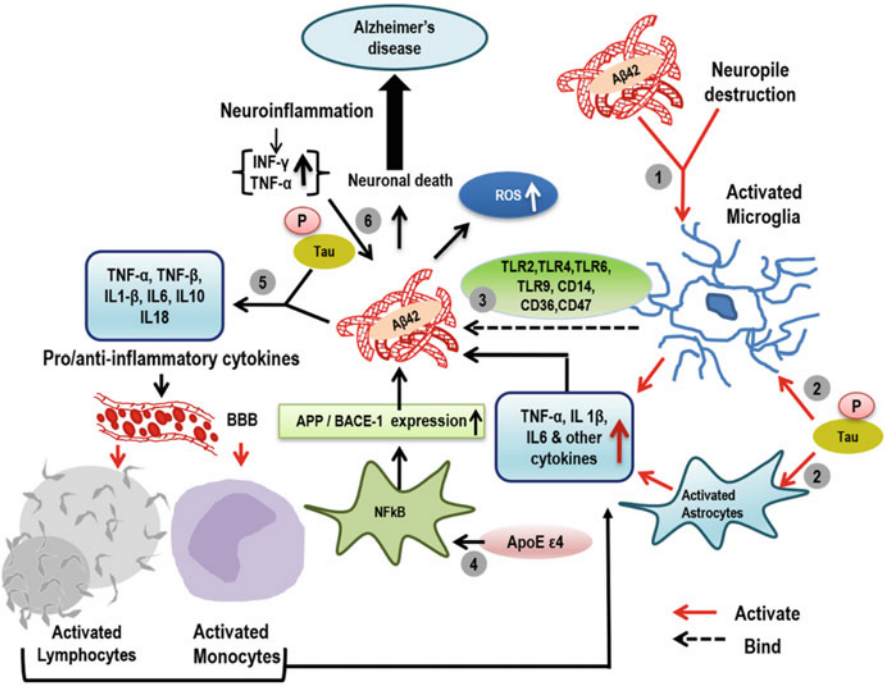



**Fig. 2 Neuroinflammation in Alzheimer's disease**. Schematic diagram showing various neuroinflammatory mechanisms involved in AD, such as cytokines, TLR receptors and other proteins on amyloid beta (Aβ) production leading to Alzheimer's disease (AD). (1) Neuropil destruction and Aβ aggregates activate the microglial cells which further releases cytokines (IL-1β, IL-6, TNF-α) that promotes an increase in Aβ plaques aggregation. (2) Hyperphosphorylated Tau also activates microglial cells, astrocyte cells and release various cytokines that increase the Aβ plaque formation. (3) Activated microglial also interacts with Aβ peptides using receptors such as- TLR-2, TLR-4, TLR-6, TLR-9, CD-14, CD-36 and CD-47, thus increasing ROS levels. (4) NFκB a transcription factor when activated in the presence of ApoE-ε4, upregulates APP and BACE-1 protein production. This further increases Aβ production in AD. (5) Hyperphosphorylated Tau and Aβ plaques stimulate the release of pro/anti-inflammatory factors such as- TNF-α, TNF-β, IL-1β, IL-6, IL-10, and IL-18. These inflammatory factors cross the blood brain barrier and activate the lymphocytes and monocytes leading to the upregulation of Cytokines (IL-1β, Il-6, TNF-α) and promote Aβ depositions. (6) In the case of neuroinflammation, TNF-α, IFN- γ levels are increased and this leads to Aβ production and eventually AD and neuronal death

sclerosis (MS) and Huntington's disease (HD) [252, 259]. These NDDs are correlated to high levels of pro-inflammatory cytokines [252, 259, 262]. AD is a neurodegenerative disease and the most common cause of mental deterioration in elderly people. It is classified as a type of dementia that was first discovered by Alois Alzheimer in 1907 [263, 264]. Two main neuropathological hallmarks of AD are extracellular peptide aggregates of senile plaques; mainly composed of amyloid-β (Aβ) peptides and formation of intracellular neurofibrillary tangles (NFT) associated with tau protein (microtubule-binding protein) [85, 252, 265–267]. In non-disease condition, amyloid precursor protein (APP) is a transmembrane protein cleaved by

non-amyloidogenic pathway in which it is cleaved by α- and γ- secretases and generates large, soluble, secreted fragments (sAPPα and sAPPγ) along with membrane-associated C-terminal fragments (CTFs) [268, 269]. APP has two isoforms amyloid precursor-like protein 1 (APLP1) and amyloid precursor protein 2 (APLP2) in humans and in flies (*Drosophila melanogaster*) it is present as Appl [266]. In AD, APP is cleaved by the amyloidogenic pathway via β and γ-secretase at the extracellular domain produces two insoluble $A\beta_{42}$ fragments [268, 269]. Amyloid plaques are predominantly found in the basal forebrain and spread to the cortex; which are associated with sensory or motor areas of the brain [270, 271]. Thus, this concludes that $A\beta_{42}$ plaques and NFTs along with microglial activation plays an important role in neuroinflammation in neurodegeneration [252, 272].

Disclosing evidence recommends that inflammatory response contributes to the progression of AD, accelerating the course of the disease [273]. During inflammation process, glial cells like astrocytes and microglia release cytokines such as IL-1β and IL-12 and these are related to the progression of AD pathology (Fig. 2) [262, 274, 275] Aβ plaques and neurofibrillary tangles present in the brain are known to activate inflammatory cells such as astrocytes, microglia and tissue levels of pro and anti-inflammatory mediators like cytokines and chemokines [276]. These inflammatory molecules and mediators are associated with $A\beta_{42}$ plaque aggregation in the brain [273, 276]. This release of mediators activate monocytes and lymphocytes through the blood-brain barrier (BBB, the barrier between blood and brain which are composed of endothelial cells, astrocytic end-feet and pericytes) along with activation of microglia, improve their proliferation and releases more inflammatory factors (Fig. 2) [252, 273, 277]. Activated microglial cells and reactive oxygen species (ROS) contribute to the loss of neurons, apolipoprotein E (ApoE) and nuclear factor-kβ (NF-kβ) as they all are involved in the inflammatory process related to AD (Fig. 2) [278].

Microglia and astrocytes contribute to a pivotal role in the inflammatory response in the AD brain [279]. Microglia interacts with Aβ peptide to produce ROS and other inflammatory mediators such as cytokines and chemokines; these are known to cause damage to the neuronal cells [278, 279]. NFk-B is a well-studied transcription factor and located in the cytoplasm, is responsible for the regulation of cytokine-producing genes [280]. The production of different inflammatory mediators is enhanced when activated NF-kB enters the nucleus. Several molecules can activate NFk-B such as TNFα, Aβ, and secreted *APP* [278, 281]. *APP* and *BACE-1* levels are increased when NF-kB is activated which increases the production of Aβ [224, 278]. Recent studies have demonstrated that in the presence of apolipoprotein E4 (APOE e4) NF-kB levels were increased; also, Aβ peptides increases APOE production via NFk-B dependent pathway [224, 278]. Cytokine production is associated with TNF and by T-lymphocytes and activated microglia [282, 283]. Transforming Growth Factor-β (TGF-β) is an inflammation regulator [274] and is known to have pro-apoptotic and anti-apoptotic effects [283, 284]. In the AD brain, TNF-α has also been shown to have a neuroprotective role. Further, cytokines have a vital role in AD [283, 284]. An interaction of chemokines and cytokines with Aβ was shown recently in an in-vitro study. It showed that Aβ

production and APP processing can be regulated by TNF-α [283, 284]. In the cerebrospinal fluid (CSF) of AD patient's brains; increased TGF-β was correlated with amyloid plaques aggregates. So, in a way cytokine plays a dual role in AD. Another known inflammatory regulator in the case of neuroinflammation is the IL12 and IL-23. Pathway of IL-12/IL-23 attenuates the pathologies of AD [274, 285]. It has been shown that in AD patients IL-12p40 subunit and its receptor activity was decreased due to cognitive deficits, this study also found that in CSF of AD patients IL-12p40 concentration was increased. In a deletion study of AD-related deficits, behavioral deficits in APP/PS1 mice and altered synaptic integrity was shown to be triggered by anti-inflammatory cytokine IL-10 [274, 286]. Chakrabarty et al. [287] have shown that in APP transgenic mouse model, there was an increased amyloid aggregation, synaptic alterations, behavioral deficits and impaired microglial phagocytosis of Aβ when IL-10 levels were up-regulated by using adeno-associated viruses (AAVs) [274, 287].

An important source of cytokines is microglia and astrocytes in AD. Thus, cytokines are the most important aspects of neuroinflammation [253]. The anti-inflammatory and pro-inflammatory processes are started as a result of the response of microglia to the aggregation of $A\beta_{42}$ plaques, chemoattraction and neuronal injury [253, 288]. Immunohistochemistry studies proved that AD trigger neuroinflammatory components in the presence of activated microglia that further express major histocompatibility complex (MHC) and releases the pro-inflammatory mediators such as inflammatory cytokines which are associated with amyloid plaques in AD patient's brain [289, 290]. Microglial cells have an important functions in the brain that tend to protect and support the neurons and neuronal survival [251, 273, 289].

The neuropil destruction process in AD patient's triggers microglial activity as per clinical studies [291, 292]. In an in-vitro study, Aβ peptide generated an inflammatory type response concerning fibrillar Aβ, and they can bind to complement factor-C and activate the complementary pathway in an antibody independent fashion [276]. Genetics and epidemiological studies showed positive signs that inflammatory mechanisms are involved in the AD [293, 294]. Also, these studies point out the linkage of polymorphisms of plaques with pro-inflammatory cytokines i.e., acute-phase proteins [α1-antichymotrypsin], IL-1, IL-6, and TNF-α are risk factors in AD [276, 295, 296]. AD pathogenesis was closely tied to IL-1 in the case of neuroinflammation [297]. Increased expression of IL-1 by microglia was seen in amyloidal plaques' surroundings. Thus IL-1 was associated with AD pathogenesis [277, 297].

In AD patients before neuropil destruction, microglial activation was found to take place in neuropathological and neuroradiological studies [276, 292]. Microglia and astrocytes were related to Aβ aggregation as per mammalian associated studies. Moreover, β-secretase activity was found to be increased due to cytokines in inflammatory conditions, this result was found in correlation with increased Aβ aggregation too [252, 298]. The inflammatory reactions in AD take place with the help of receptors, such as class A scavenger receptor A1, a6β1 integrin, toll-like receptors i.*e* toll-like receptors 2 (TLR2), TLR4, TLR6 and TLR9 and CD14, CD47,

CD36 [210, 299, 300]. Using this mechanism, microglial cells bind to Aβ fibrils and soluble amyloid β (Aβ) oligomers [253, 301].

Inefficient removal of Aβ is identified with AD sporadic cases. Downregulation of Aβ phagocytosis receptors leads to increased levels of cytokine which causes the relative loss of microglial phagocytic capacity [258, 302]. Early response in AD is represented by astroglial atrophy that has further effects on synaptic connections. In synaptic transmission astrocytes predominantly contribute to cognitive defects as per animal model studies [303–305]. Also, cytokines, cytotoxic molecules, nitric oxide and interleukins are released by microglia and astrocytes cells when exposed to Aβ aggregates, thus, increasing the neuroinflammation in the brain [258]. Astrocytes can increase the microglial activity by lipidation and require ApoE60 for the removal of Aβ [258, 306]. Thus, astrocytes play an important role in the degradation of Aβ. While adult astrocytes increase the production of Aβ-degrading proteases like insulin-degrading enzyme, neprilysin, angiotensin-converting enzyme-1 (ACE-1) and endothelin-converting enzyme-2 [258].

In a wider aspect multiple factors such as anti and pro-inflammation, neuronal injury, microglia cells aggregation are associated to cytokine production in presence of Aβ peptides, for example, one such study pointed out the increase in production of pro-inflammatory cytokines (i.e., pro-IL-1α, IL-6, TNF-α), macrophage inflammatory peptide (MIP-1α) and macrophage colony-stimulating factor (M-CSF) due to the exposure of microglia to pre-aggregated $A\beta_{42}$ [258, 307].

## *1.4 Hsp and Its Biological Role in Neuroinflammation*

As mentioned above, Hsp are evolutionarily conserved proteins that expressed in various stress conditions and helps in protein homeostasis by promoting proper protein folding, protein assembly and degradation [134, 308]. Apart from chaperone activity, they play a vital role in neuroinflammation [184, 186, 309, 310]. Heat shock responses (HSR) are triggered due to various cellular stress such as thermal shock, heavy metals, oxidative stress (ROS), etc. [184, 311, 312]. The neuroprotective role of Hsp in the nervous system was first described by [313]. They have shown that in thermal stress conditions, extracellular Hsp migrates from glial cells to neurons in the squid model [190]. Additionally, [314] have also demonstrated Hsp translocation as human glioblastoma cells secrets HspA1 in heat stress condition that is taken up by the human neuroblastoma cells [315]. HspA1 exerts its anti-apoptotic activity in neuroblastoma cells and decreases cell death in neurons [314, 315].

Several studies have demonstrated that apart from chaperone activity, Hsp plays an important role in the prevention of neuroinflammation in various neurodegenerative disease conditions [116, 184]. Hsp are involved in the regulation of neuroinflammation by modulating the expression of pro-inflammatory genes but the detailed study on the role of Hsp in neuroinflammation is still elusive. Hsp has a key role in antigen cross-presentation with its chaperone activity via processing in proteasome and transfer antigenic peptides to MHC class I or class II, followed by

the activation of CD8+ CTL (cytotoxic T lymphocytes) to kill the antigen/virus-infected cells [310, 316, 317]

Furthermore, NF-κB is frequently constitutively activated in neuroinflammatory conditions and activation of NF-kB receptor leads to the production of pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-6 by macrophages which results in neuronal death [242, 318]. The previous study by [319] has shown that Hsp plays a key role in the inhibition of neuroinflammation via inhibiting NF-kB receptor activation [320]. Overexpression of Hsp70 inhibits the NF-kB receptor activation as well as the production of pro-inflammatory molecules such as TNF-α, IFN-γ, IL-6 and prevents neuroinflammation. Hsp70 inhibits NF-kB receptor activation by binding with IKKα and IKKβ, which are required for the NF-κB activation (Fig. 3) [321]. Hsp70 inhibits the IκB kinase (IKK) activation via stabilizing the IκB-α and inhibits the NF-κB activation [322, 323]. Hsp70 inhibits the activity of IκB kinase as well as NF-kB activation and results in decreases TNF-α production and neuroinflammation (Fig. 3) [184, 324–326]. Furthermore, Hsp70 also decreases the NO production stimulated by LPS-activated macrophages [323, 327]. Hsp70 helps in the modulation of neuroinflammation via inhibiting the JNK and NF-κB signaling pathways (Fig. 3) [328, 329]. It suppresses the phosphorylation and activation of JNK as well as IκBα. The inhibition of JNK and IκBα activation by Hsp70 leads to suppress their binding to DNA and also inhibits the production of their transcription factors such as NF-kB, signal transducers and activator of transcription-1 (STAT-1) and activator protein-1 (AP-1) (Fig. 3). The decrease in the expression of pro-inflammatory genes NF-kB, STAT-1 and AP-1 results in decrease neuroinflammation [330–333]. The inflammasomes also plays a key role in neuroinflammation and composed of a receptor and an adaptor through which they activate pro-inflammatory cytokines such as IL-1β or IL-18 [334, 335]. NOD-leucine rich repeat and pyrin containing protein 3 (NLRP3) are most studied inflammasome that activates caspase-1 and produce IL-1β and IL-18 [335–337]. The previous study by Martine et al. [338] have shown that knockdown of Hsp70 increases NLRP3 inflammasome along with the production of pro-inflammatory cytokines such as IL-1β and activates caspase-1 which results in neuroinflammation in mice.

Hsc70 also plays a key role in the prevention of neuroinflammation via inhibiting the activation of NF-kB, and decreases the release of pro-inflammatory molecules, reduces phosphorylation of downstream signaling molecules such as c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases 1/2 ERK, phosphatidylinositol-3-kinase (PI3K/Akt) (Fig. 3) [116, 323, 339]. JNK, ERK and PI3K play a vital role in the neuroinflammation as JNK and ERK level is increased by the activated astrocytes and microglia [340, 341]. They are involved in the central sensitization especially in chronic pain, induced via glial cell stimulated neuroinflammation so the inactivation of JNK by Hsc70 results in the reduction of neuroinflammation related pathologies (Fig. 3) [323, 341]. Hsc70 also shows the anti-inflammatory effect via decreasing the production of iNOS and COX-2 gene expression and ultimately suppress the neuroinflammation [323].

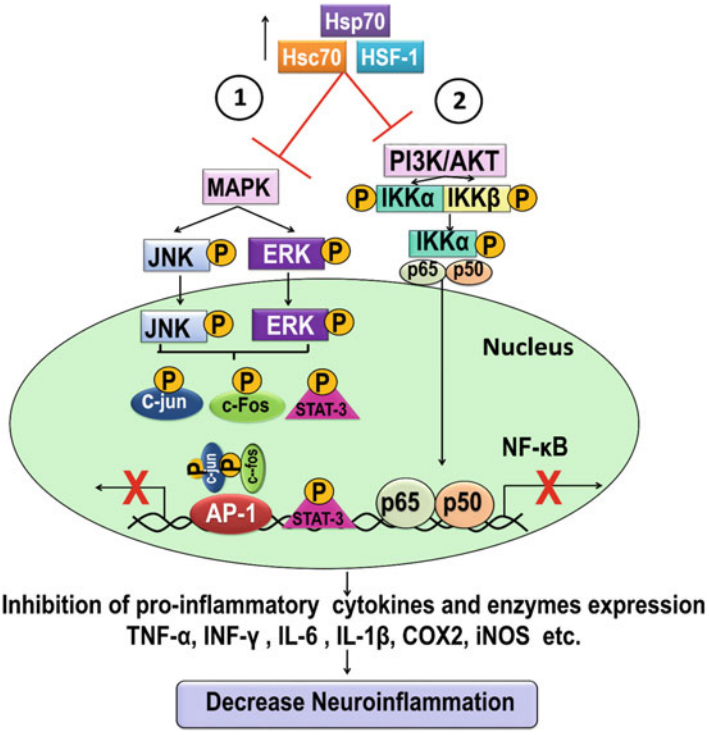



**Fig. 3 Hsp role in neuroinflammation**. The above schematic diagram shows that Hsp (Hsp70, Hsc70) and HSF-1 inhibit the neuroinflammation via two mechanisms. (1) JNK and ERK pathways are activated in neuroinflammation via MAPK which causes the phosphorylation of JNK and ERK. Activated JNK and ERK translocate into the nucleus and activates the various transcription factors such as c-jun, c-fos and STAT3 via phosphorylation. After phosphorylation, activated transcription factors bind to the pro-inflammatory gene promoter region of DNA and induces the expression of different pro-inflammatory cytokines and enzymes such as TNF-α, INF-γ, IL-6, IL-1β, COX2, iNOS etc. Moreover, (2) PI3K/AKT phosphorylates and activates IKKα and IKKβ. Activated IKKα cleaved by the proteasome and formed p56 and p50 subunits. These subunits are bind to DNA at NF-kB promoter region and increase the expression of NF-kβ which leads to the production of pro-inflammatory cytokines and enzymes such as TNF-α, INF-γ, IL-6, IL-1β, COX2, iNOS etc. and increases the neuroinflammation. Hsp plays a protective role in neuroinflammation via inactivating the JNK, ERK and PI3K/AKT signaling pathways and inhibits the production of TNF-α, INF-γ, IL-6, IL-1β, COX2, iNOS, NF-kB, etc.

The previous study by [342] have demonstrated the anti-inflammatory role of HspA1 in ischemic conditions. They have shown that overexpression of HspA1 leads to a decrease in the activity of NF-κB in the brain via inhibiting the IκB phosphorylation [343]. Overexpression of HspA1 also prevents apoptosis in the case of stroke patients [328]. Intravenous administration of HspB1 significantly decreases the neuroinflammation in the case of ischemic injury and autoimmune demyelination in CNS [344, 345].

It has been demonstrated that the Hsp60 level is significantly increased during stress conditions in the cytosol [346, 347]. Several studies demonstrated that intracellular as well as extracellular Hsp60 is associated with the TLR4 receptor mediates apoptosis in microglia and plays a central role in the generation of neuroimmune responses in the case of NDDs [255, 348]. Thus, Hsp60 can be used as a warning signal in case of neuronal damage caused due to the neuroinflammation, as seen in one such study where the TLR4-MyD88 (myeloid differentiation factor 88) signaling pathway was associated with neuronal damage in microglial cells [255, 325].

Additionally, HSF1 is also involved in the directly regulating the neuroinflammation via inhibiting the production of TNF-α and IL-1β in the response of lipopolysaccharide (LPS)-induced shock in a mouse model [349–351]. The previous study by [116] has shown that overexpression of Hsp70 inhibits the activation of astrocytes, stimulated by α-synuclein in PD. Subsequently, it also decreases the release of pro-inflammatory molecules such as TNF-α and IL-1β by astrocytes. Overexpression of Hsp70 also decreases the pro-inflammatory enzymes such as COX-2 and iNOS level and ultimately suppresses the neuroinflammation [116, 323]. A study by Schettet et al. [352] has demonstrated that the downregulation of HSF1 increases the apoptotic cell death and TNF-α mediated inflammatory signaling pathway.

## *1.5 Hsp Role in the Modulation of Neuroinflammation in AD*

As discussed above, neuroinflammation is one of the prominent features of the different brain-related diseases such as traumatic brain injury, ischemic stroke as well as NDDs i.e. AD, PD, HD [184, 353]. It is characterized by activation of glia and astrocytes cells with the increased level of pro-inflammatory molecules such as cytokines and chemokines, etc. [354, 355]. As mentioned above, the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles initiate the neuroinflammation and lead to neuronal death [356, 357]. Different Hsp are known as a key modulator of neuroinflammation via preventing the neuroinflammatory response and release of pro-inflammatory molecules such as IL1, IL1β, TNFα, and IL6 [184, 358]. Through this Hsp help in the prevention of neuroinflammation and decreasing the progression of disease condition [359, 360]. The detailed mechanisms, how Hsp modulates the neuroinflammation in AD are still not well understood.

As mentioned above, neuroinflammation in AD is mainly caused by astrocytes and microglial cells [361, 362]. Extracellular HspA1 or HspC1 stimulates microglial cells and enhances their phagocytic activity against Aβ peptides [184, 363]. Nuclear factor-kappaB (NF-kB) level increases in neuroinflammation in the brains of AD patients and plays an important role in inflammation, apoptosis and oxidative stress [364, 365]. The higher level of NF-κB increases the level of BACE1 and the APP gene which results in the accumulation of $A\beta_{42}$ plaques [364, 366]. The previous study by [342] has shown that HspA1 reduced NF-κB activity and exert anti-

inflammatory effect which might play an important role in AD. NF-κB also increases the level of neurodegenerative disease-related pro-inflammatory enzymes iNOS and COX-2 in AD [275, 365]. Hsp70 tends to inhibit the production of iNOS and COX-2 by macrophages and astrocytes in neuroinflammation which might be helpful in decrease the AD-related pathologies [163, 367].

## *1.6 Therapeutic Strategies Targeting Hsp Anti-inflammatory Role in AD*

As mentioned above, Hsp plays an important role in modulation of AD and serve as potential therapeutic targets to treat/improve neuroinflammation in AD [184, 368]. Activation of microglial and astrocyte cells in the brain is one of the prominent pathologies of neuroinflammation in AD [355]. Therefore, targeting the cells along with inhibition of pro-inflammatory cytokines could be a relevant therapy of AD [184, 369]. The immunosuppressive action of Hsp consists of inactivation of antigen-presenting MHC cells, expansion of regulatory T cells and inactivation of NF-kB activity in the diseased condition [370, 371]. In this paper, we have demonstrated the possible therapeutic strategies targeting the modulation of different Hsp levels to decrease the neuroinflammation in AD. Nowadays, increasing the level of endogenous Hsp and delivering extracellular Hsp into the cell is a promising therapeutic strategy to reduce the neuroinflammation related toxicity in AD [372, 373]. Membrane Lipid Therapy is useful to induce the heat shock response and allows the entry of extracellular Hsp into the cell via modulating the membrane fluidity [184, 374].

## *1.7 Membrane Lipid Therapy*

Membrane lipid therapy is one of the promising therapies that target lipid membrane fluidity as well as its structure and influence the lipid organization via principles of structure-function (Structure and function reciprocally dependent on each other like structure of the cell formed according to its function and function of cell is dependent on the structure) results in change in the localization and function transportation of proteins across the lipid bilayer [375, 376]. It is a novel therapeutic approach for drug development that helps in the maintenance of lipid structure and its composition in the membrane [375, 376]. Membrane lipid therapy would also be applicable for Hsp response in neuroinflammation in AD. It might influence the transport of Hsp across the lipid membrane and help in decreasing the neuroinflammation in AD [312, 377]. Through membrane lipid therapy, alteration in the physical properties and microdomain organization of lipid membrane is possible which has a vital role in the activation of heat shock proteins [225, 311]. Hyperfluidization of lipid

membrane helps in the activation of different Hsp which performs various role in the prevention of neuroinflammation in AD by maintaining the normal proteostasis, decreasing pro-inflammatory molecules, activated microglia and astrocytes apoptosis [342, 378, 379]. The previous study by [374] has shown that membrane lipid therapy is also exerting its beneficial effect in normalizing Hsp expression in diseased conditions. The drugs which interact with lipid raft in plasma membranes such as hydroximic acid derivatives, including BGP-15 and BM, play a role as Hsp co-inducers and help in the prolonged activation of HSF1 [374, 380, 381]. These drugs known for their neuroprotective property via increase transcription of Hsp gene and subsequently decreases in neuroinflammation [184, 382, 383]. The previous study by [184] has demonstrated that HSF1 inactivation leads to the uncontrolled inflammatory process and leads to neuroinflammation. Together, BGP-15 and BM might help in the decrease neuroinflammation by increasing HSF1 expression. Further, several studies suggested that different Hsp inducers and co-inducers help in decreasing the neuroinflammation in AD. Few Hsp inducers and co-inducers are described below:

## *1.8 Hsp Inducers and Co-inducers*

### 1.8.1 Celastrol

Celastrol (tripterine) is a pentacyclic triterpenoid compound that belongs to the family of quinone methides isolated from the root extracts of *Tripterygium wilfordii* (Thunder god vine) and *Celastrusregelii* [384, 385]. Previous studies have shown that celastrol has antioxidant and anti-inflammatory effects. These effects are helpful in the prevention of AD [384, 386]. The anti-inflammatory effect of celastrol is due to suppression of the production of the pro-inflammatory cytokines' TNF-α and IL-1β produced by human monocytes and macrophages which might help in the prevention of AD [384, 387]. Celastrol also acts as a Hsp co inducer as it increases the Hsp32/HO-1 and Hsp70 expression level in AD via activating the HSF1 and HSR (Fig. 4) [388, 389]. Higher expression of Hsp32/HO-1 and Hsp70 shows the anti-inflammatory effect and prevents AD [184, 390–392]. Upregulation of Hsp32/HO-1 and Hsp70 decrease the neuroinflammation by reducing the LPS induced activation of NF-kB signaling cascade and production of TNF-α and INF-γ-induced iNOS expression in rat brain (Fig. 4) [163, 393]. The previous study by [394] has shown that celastrol has the neuroprotective potential through increasing the Hsp level especially Hsp70B in human neurons and showed beneficial effects of celastrol in NDDs including AD. Moreover, celastrol induced expression of Hsp70, Hsp27 and Hsp32 in cerebral cortical cultures of rat and induce Hsp70 expression in the neuronal cell body (Fig. 4) [395, 396]. It shows neuroprotective effect in the case of AD by inhibiting Aβ protein aggregation by in vivo administration of celestrol in a transgenic mouse model of AD [395–398]. The previous

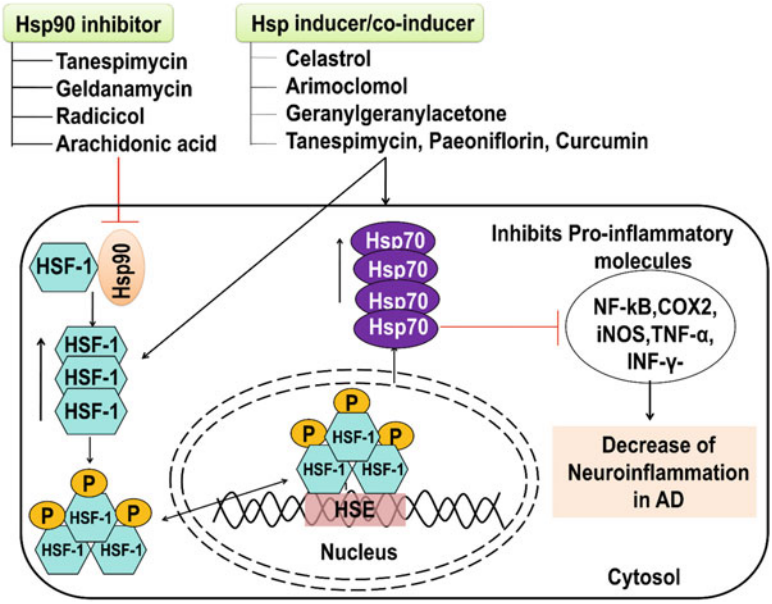



**Fig. 4 Therapeutic effects of Hsp inducers/co-inducers and Hsp90 inhibitor in neuroinflammation.** Hsp90 inhibitors such as tanespimycin, geldanamycin, Radicicol and Arachidonic acid dissociates Hsp90 from the HSF-1 monomer. Free monomers of HSF-1 phosphorylates and forms trimer which can bind to the HSE region on DNA and induces Hsp70 expression. Secondly, the Hsp inducers/co-inducers such as celastrol, arimoclomol, geranylgeranyl acetone, tanespimycin, paeoniflorin and curcumin increases the expression of HSF-1 and Hsp70. In both cases, an increased Hsp70 level reduces the production of pro-inflammatory molecules such as NF-kB, COX2, iNOS, TNF-α, INF-γ, etc. and ultimately decreases the neuroinflammation in AD

study by [399] has demonstrated that celastrol also act as a Hsp90 inhibitor and shows protective effect in AD by suppressing the accumulation of Aβ-induced cell death and also induces Hsp70 expression along with increases the Blood-Brain Barrier (BBB) penetration [400, 401]

### 1.8.2 Arimoclomol

Arimoclomol (BRX-220) is a small new chemical compound synthesized by Biorex pharmaceutical company (Hungary) at the end of the last century [204, 205]. The oral administration of this drug easily penetrates the CNS and shows an anti-inflammatory effect [98, 205]. Previous studies have revealed that arimoclomol acts as a co-inducer of Hsp [402, 403] and increases the expression of HSF1 which binds to heat shock elements (HSEs) in the promoter regions of heat shock genes and increases the Hsp level [394, 404] such as Hsp70, Hsp40, and Hsp27and results in decrease of the neuroinflammation in AD [405, 406]. BRX-220 protects

motor neurons from axotomy-induced cell death and causes upregulation of HspC1 and HspA1 in parallel in glial and neuronal cells [370, 411].

### 1.8.3 Geranylgeranylacetone

Geranylgeranylacetone (GGA) is a non-toxic ulcer drug that induces the expression of Hsp70 [407, 408]. Previous studies have shown that oral administration of GGA significantly decreases the levels of inflammatory cytokines, namely TNF- α, IL-1β and COX-2 in the GGA-treated mice [409, 410] and also improves the cognitive function and other pathological phenotypes in APP/PS1 mice in case of AD. It has been shown that it decreases cerebral ischemic damage in rat brains [410]. The previous study by [411] has shown GGA has cytoprotective and anti-aggregation activities besides the anti-inflammatory effect (Fig. 4). In vivo administration of GGA increases the expression of Hsp70 in neurons and decreases the accumulation of $A\beta_{42}$ plaques in AD. It also exhibits the neuroprotective effect in focal cerebral ischemia by inducing the Hsp expression in the neurons [412]. Oral administration of GGA improved the cognitive defect along with other pathological manifestations in APP23 AD mice [411].

### 1.8.4 Tanespimycin

Tanespimycin [17-allylamino-demethoxygeldanamycin (17-AAG)] is a water-soluble benzoquinone and antibiotic geldanamycin derivatives which are promising new anticancer drugs [413, 414]. 17-AAG up-regulates the expression l of Hsp70 and Hsp27 in neurons (Fig. 4) [415, 416]. It reduces the expression levels of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 and exhibits the neuroprotective effect in a rat model of Traumatic Brain Injury (TBI) [417]. 17-AAG significantly increases the neuronal survival in the cortex region of the brain following trauma [359, 418]. The previous study by [419] has shown that 17-AAG injection decreasing the accumulation of $A\beta_{42}$ plaques and improve behavior activity by increasing the Hsp70 expression level in the rat.

### 1.8.5 Paeoniflorin

Paeoniflorin is a monoterpene glycoside which is one of the major elements of an herbal medicine derived from *Paeonia lactiflora* and it can also be isolated from the freshwater fern *Salvinia molesta* [420–422]. It has the potential to induce Hsp via HSF1 activation (Fig. 4) [423, 424]. Cells treated with paeoniflorin showed enhanced phosphorylation and acquisition of the deoxyribonucleic acid-binding ability of heat shock transcription factor 1 (HSF1), as well as enhanced the formation of characteristic HSF1 granules in the nucleus, resulting in the induction of Hsp by the activation of HSF1 [423]. The previous study by [180] has shown paeoniflorin

function in brain protection from cerebral ischemic injury *via* inhibition of apoptosis. Moreover, the intraperitoneal administration of paeoniflorin induces Hsp70 expression in the mouse stomach (Fig. 4) [425].

### 1.8.6 Curcumin

Curcumin is a biphenolic antioxidant as well as anti-inflammatory molecule present in turmeric, a common seasoning commonly used in Indian food [426, 427]. It plays a tremendous role in cancer, wound repair and inflammatory disorders. Several studies have shown that curcumin as a Hsp co-inducer can induce Hsp such as Hsp27, αB crystallin and Hsp70 expression and help in the prevention of neuroinflammation in AD (Fig. 4) [278, 396, 423, 428, 429]. The previous study by Ma et al. [430] has shown that administration of curcumin in increases Hsp70 expression and decreases the Aβ plaques and tau oligomers accumulation in A-β-infused rats. Moreover, it acts as a homeostatic regulator in inflammatory diseases by association with phagocytic cells like macrophages along with Aβ plaques and induces its clearance in human cells and AD rodent model also [222, 431]. Curcumin decreases the neuroinflammation and hyperphosphorylated Tau toxicity by NF-kB and the Activator Protein 1 (AP1) inhibition [432, 433]. The previous study by Sundaram et al. [434] has demonstrated that curcumin decreases neuroinflammation by inhibiting the p25-induced tau and amyloid-beta pathologies and improve cognitive function in AD p25Tg mice.

The other therapeutic strategy to prevent the neuroinflammation can be treatment by Hsp90 inhibitors is described below:

## *1.9 Hsp90 Inhibition: Potential Therapeutic Strategy to Modulate Neuroinflammation in AD*

Hsp90 interacts with receptor-interacting kinase (RIP) and recruits it to the TNF receptor which activates the NF-kB, a key component of the inflammatory response in AD [435, 436]. The previous studies have shown that overexpression of Hsp such as Hsp27, Hsp70, Hsp60, etc. prevents activation of NF-kB by inducing the degradation of receptor-interacting protein kinase (RIP) and reduce the inflammation [437]. Moreover, Hsp90 directly interacts with HSF1 and inactive it results in a reduction of Hsp levels and neuroinflammation [438]. Hsp90 is also involved in the activation of pro-inflammatory cytokines [439] and increases the neuroinflammation in AD and inhibits the expression of Hsp by binding to HSF1 [440, 441]. Hsp90 inhibitor is one of the prominent therapeutic strategies to cure neuroinflammation in AD [401, 442]. There are several Hsp90 inhibitors available which might be helpful to reduce the neuroinflammation in AD. Some Hsp90 inhibitors are described as below:

### 1.9.1 Tanespimycin

Tanespimycin (17-AAG) is the first potential Hsp90 inhibitor tested in phase II clinical trials in kidney cancer, thyroid and pancreatic patients [443]. It inhibits the Hsp90 by inhibiting its intrinsic ATPase activity via binding to its N-terminal ATP binding domain [444, 445]. The previous study by Zuo et al. [446] has shown that Hsp90 inhibition by 17-AAG reduced the neuroinflammation via decreasing the level of inflammasome NLRP3, caspase-1 and IL-1β level and increase neurogenesis in mice with Subarachnoid Hemorrhage [447, 448]. Moreover, 17-AAG inhibits Hsp90 association with HSF-1 results in expression of Hsp70 and Hsp40 which decreases the neuroinflammation in AD [449–451] (Fig. 4).

### 1.9.2 Geldanamycin

Geldanamycin is a 1,4-benzoquinone ansamycin, acts as Hsp90 inhibitor [452, 453]. This is also popular to have antitumor, antibiotic properties and inhibits the expression of Hsp90 by binding at the N-terminal ATP-binding pocket of Hsp90 [454–456]. It also acts as a Hsp co-inducer because it releases the HSF1 and induces the expression of Hsp70 which is known to suppress the pro-inflammatory signals and activate the anti-inflammatory genes (Fig. 4) [108, 451]. The previous study by [457] has demonstrated that geldanamycin inhibits the aggregation of huntingtin protein in both a mammalian and mouse model by increasing the chaperone expression. So, inhibition of Hsp90 by geldanamycin might be useful in the prevention of neuroinflammation in AD [455, 458].

### 1.9.3 Radicicol

Radicicol is an antifungal macrolactone antibiotic derived from *Diheterospora chlamydosporia* and *Chaetomium chiversii* that inhibits the heat shock protein 90 (Hsp90) and induces the heat shock responses [414, 459, 460]. Radicicol derivatives NXD30001 exhibits higher stability in in-vivo conditions than radicicol and induces the expression of different Hsp such as Hsp70, Hsp60, Hsp40, and Hsp27 via HSF1 activation and helps in decreasing the neuroinflammation in AD [461, 462].

### 1.9.4 Arachidonic Acid

Arachidonic acid (AA) acts as a potential inhibitor of Hsp90 as well as inducer and co-inducer of Hsp72/Hsp70 [389, 463, 464]. It is a polyunsaturated essential fatty acid present in the plasma membrane of human and animal cells [141, 407]. It is found in human fat cells, liver, brain, and glandular organs [465, 466]. It produces

prostaglandins, thromboxanes, and leukotrienes which can act as mediators in several processes such as immune function, leukocyte chemotaxis, inflammatory cytokine production, etc. [467, 468]. The previous study by [469] has shown that the expression of AA induces HSF1 phosphorylation and results in increased Hsp expression. Moreover, AA product prostaglandins (PGs) are a class of naturally occurring cyclic 20-carbon fatty acids. The type A and J prostaglandins (PGA1, PGA2 and PGJ2), could activate HSF1 and induces Hsp72 in the presence of a reactive, unsaturated carbonyl group in the cyclopentane ring (cyclopenteneone) [37, 470–473]. The previous study suggested that the HSF1/Hsp72 pathway exhibits an endogenous anti-inflammatory role through inhibits the prolonged and higher activation of the inflammatory response [473]. Thus, the supplementation of AA in the early stage of AD reduced the symptoms and toxicity of the disease [474].

Further, the intranasal administration of exogenous recombinant human Hsp70 (eHsp70) administration increases the life and cognitive function in a mouse model of AD [93, 475]. Previous studies have shown that Hsp70 could significantly reduce the production of TNF-α, IL-1β, glial fibrillary acidic protein (GFAP), COX-2 as well as Inos [116, 184]. It also modulates astrocytes induced inflammation [343, 476]. This study indicates that eHsp70 could be a potential therapeutic strategy to decrease the neuroinflammation in AD.

The main challenges are in the effective drug delivery to treat the AD because the BBB restricts drug efficacy. Not many drugs can efficiently cross the BBB and give the 100% results in AD so there are some drugs such as polymeric nanoparticles, liposomes, metallic nanoparticles and cyclodextrins form to achieve most promising drug delivery systems [477]. The BBB serves as a physical barrier that can protect the CNS from exogenous substances and on the other side, the BBB helps in the chemical transportation to the CNS [478–480]. But the traditional AD drugs have low penetration capacity and limited transportation through BBB. Therefore, several types of research are done to overcome this limitation by using nanoscale particles to deliver the drug efficiently through BBB via increasing their penetration capacity [421, 481]. One of the strategies to improve the pharmacokinetic profiles of the drug is liposome-mediated nanoparticle therapy.

## *1.10 Liposome-Lipophilic Nanoparticles*

Liposomes are sphere-shaped colloidal particles containing one or more phospholipid bilayers exposing outside [482, 483]. It has a hydrophilic core inside which is useful to encapsulated hydrophilic drugs and lipophilic particles [484, 485]. The liposome is a potential particle to deliver therapeutic molecules across the BBB by incorporating with cell-penetrating peptides and antibodies [486, 487]. One of the anti-inflammatory drugs is Rivastigmine, an FDA approved drug for AD treatment which reduces the level of T-cells and TNF-α and IFN-γ which are implicated in the pathogenesis of AD but it has 1.5 h half-life and its brain penetration is restricted by tight junctions [488, 489]. Previous studies have shown that the administration of the

rivastigmine by using dipalmitoylphosphatidylcholine (DPPC)/cholesterol liposomes increases the penetration of it into the brain as compared to without liposomes [477]. So, it could be a novel and effective therapeutic strategy to prevent neuroinflammation in AD. The above-mentioned therapeutic strategies could help in the modulation of the neuroinflammation and its related toxicity in AD.

## 2 Conclusions

Hsp are evolutionarily conserved proteins that play a vital role in maintaining the protein homeostasis through the induction of protein folding, protein assembly and degradation. It also plays a key role in neurodegenerative diseases such as AD, PD, HD, etc. via decreasing the neuroinflammation and promoting neuronal survival. Neuroinflammation in AD is the result of synchronized prolonged activation of astrocytes, microglia, pro-inflammatory cytokines as well as enzymes along with other CNS cells in the brain that induces chronic inflammation which ultimately promotes tissue injury and disease-related toxicities. Thus, the modulation of neuroinflammation via regulating activation inflammation-related cells is one of the effective strategies to treat the AD. As discussed above, large and small Hsp are significantly involved in the modulation of neuroinflammation via interacting with inflammation-causing molecules and helps in the prevention of neuroinflammation in AD. Hsp also interacted with neuroinflammasome and decreases the neuroinflammation in AD. As mentioned above, Hsp play a neuroprotective role by decreasing the neuroinflammation in AD, thus it is used as a potential therapeutic target for the prevention of AD-related pathologies. The supplementation of compounds known as inducers/co-inducers of Hsp and applications in AD might be one of the potential therapeutic targets to treat/prolong AD related pathologies in the future. Moreover, membrane lipid rearrangement and nanoparticle-based therapies are also involved in decreasing the neuroinflammation via increasing the Hsp level at the site of neuroinflammation. Thus, apart from the supplementation of drugs to modulates the Hsp level, the interaction of Hsp with inflammatory cells and their affinity to reduce/inactivate them should be a more focused area in the case of AD. Overall, this chapter highlights the effect of different Hsp in the modulation of neuroinflammation in AD and how Hsp modulating drugs used for the prevention of neuroinflammation in AD.

**Acknowledgements** The authors are very much thankful to Science and Engineering Research Board (SERB), New Delhi, India (No. EMR/2016/006911/HS), Gujarat Council on Science & Technology (GUJCOST/MRP/2015-16/2680) Gujarat for financial support to AKT and DST-Innovation of Science Pursuit for Inspire Research (INSPIRE), New Delhi for financial support to KP (IF140990).

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References


1. Ikwegbue PC, Masamba P, Oyinloye BE, Kappo AP (2018) Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. Pharmaceuticals 11:2
2. Panchal K, Kumar A, Tiwari AK (2018) Heat shock protein 70 and molecular confession during neurodegeneration. In: HSP70 in human diseases and disorders. Springer, New York pp 3–35
3. Ross CA, Poirier MA (2004) Protein aggregation and neurodegenerative disease. Nat Med 10: S10
4. Verghese J, Abrams J, Wang Y, Morano KA (2012) Biology of the heat shock response and protein chaperones: budding yeast (Saccharomyces cerevisiae) as a model system. Microbiol Mol Biol Rev 76:115–158
5. Arya R, Mallik M, Lakhotia SC (2007) Heat shock genes—integrating cell survival and death. J Biosci 32:595–610
6. Beere HM (2004) The stress of dying': the role of heat shock proteins in the regulation of apoptosis. J Cell Sci 117:2641–2651
7. Li Z, Srivastava P (2003) Heat-shock proteins. Curr Protoc Immunol 58:A. 1T. 1–A. 1T. 6
8. Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, Massie B (2000) The chaperone function of Hsp70 is required for protection against stress-induced apoptosis. Mol Cell Biol 20:7146–7159
9. Candido E (2001) Heat shock proteins
10. Jee H (2016) Size dependent classification of heat shock proteins: a mini-review. J Exerc Rehabil 12:255
11. Lindquist S, Craig E (1988) The heat-shock proteins. Annu Rev Genet 22:631–677
12. Ritossa F (1968) Unstable redundancy of genes for ribosomal RNA. Proc Natl Acad Sci U S A 60:509
13. Miller D, Fort PE (2018) Heat shock proteins regulatory role in neurodevelopment. Front Neurosci 12:821
14. Latchman DS (1998) Heat shock proteins: protective effect and potential therapeutic use. Int J Mol Med 2:375–456
15. Wang X, Chen M, Zhou J, Zhang X (2014) HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy. Int J Oncol 45:18–30
16. Gupta RS, Singh B (1994) Phylogenetic analysis of 70 kD heat shock protein sequences suggests a chimeric origin for the eukaryotic cell nucleus. Curr Biol 4:1104–1114
17. Kim JY, Han Y, Lee JE, Yenari MA (2018) The 70-kDa heat shock protein (Hsp70) as a therapeutic target for stroke. Expert Opin Ther Targets 22:191–199
18. Bolhassani A, Agi E (2019) Heat shock proteins in infection. Clin Chim Acta 498:90–100
19. Multhoff G (2006) Heat shock proteins in immunity. In: Molecular chaperones in health and disease. Springer, Berlin/New York, pp 279–304
20. Tobian AA, Canaday DH, Boom WH, Harding CV (2004) Bacterial heat shock proteins promote CD91-dependent class I MHC cross-presentation of chaperoned peptide to CD8+ T cells by cytosolic mechanisms in dendritic cells versus vacuolar mechanisms in macrophages. J Immunol 172:5277–5286

21. Pockley AG (2001) Heat shock proteins in health and disease: therapeutic targets or therapeutic agents? Expert Rev Mol Med 3:1–21
22. Voellmy R (1994) Transduction of the stress signal and mechanisms of transcriptional regulation of heat shock/stress protein gene expression in higher eukaryotes. Crit Rev Eukaryot Gene Expr 4:357–401
23. Egel R (2013) The molecular biology of Schizosaccharomyces pombe: genetics, genomics and beyond. Springer Science & Business Media, Berlin/Heidelberg
24. Neef DW, Jaeger AM, Thiele DJ (2013) Genetic selection for constitutively trimerized human HSF1 mutants identifies a role for coiled-coil motifs in DNA binding. G3: Genes Genomes Genetics 3:1315–1324
25. Sharp FR, Zhan X, Liu D-Z (2013) Heat shock proteins in the brain: role of Hsp70, Hsp 27, and HO-1 (Hsp32) and their therapeutic potential. Transl Stroke Res 4:685–692
26. Morimoto RI (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev 12:3788–3796
27. Voisine C, Orton K, Morimoto RI (2007) Protein misfolding, chaperone networks, and the heat shock response in the nervous system. Elsevier/Academic Press, United States
28. Åkerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 11:545
29. Guettouche T, Boellmann F, Lane WS, Voellmy R (2005) Analysis of phosphorylation of human heat shock factor 1 in cells experiencing a stress. BMC Biochem 6:4
30. Kline MP, Morimoto RI (1997) Repression of the heat shock factor 1 transcriptional activation domain is modulated by constitutive phosphorylation. Mol Cell Biol 17:2107–2115
31. Naidu SD, Sutherland C, Zhang Y, Risco A, de la Vega L, Caunt CJ, Hastie CJ, Lamont DJ, Torrente L, Chowdhry S (2016) Heat shock factor 1 is a substrate for p38 mitogen-activated protein kinases. Mol Cell Biol 36:2403–2417
32. Mivechi NF, Shi XY, Hahn GM (1995) Stable overexpression of human HSF-1 in murine cells suggests activation rather than expression of HSF-1 to be the key regulatory step in the heat shock gene expression. J Cell Biochem 59:266–280
33. Zhang Y, Chou S-D, Murshid A, Prince TL, Schreiner S, Stevenson MA, Calderwood SK (2011) The role of heat shock factors in stress-induced transcription. In: Molecular chaperones. Springer, New York pp 21–32
34. Caplan AJ, Cyr DM, Douglas MG (1992) YDJ1p facilitates polypeptide translocation across different intracellular membranes by a conserved mechanism. Cell 71:1143–1155
35. Zhong T, Arndt KT (1993) The yeast SIS1 protein, a DnaJ homolog, is required for the initiation of translation. Cell 73:1175–1186
36. Bukau B, Horwich AL (1998) The Hsp70 and Hsp60 chaperone machines. Cell 92:351–366
37. Ohtsuka K, Hata M (2000) Mammalian HSP40/DNAJ homologs: cloning of novel cDNAs and a proposal for their classification and nomenclature. Cell Stress Chaperones 5:98
38. Qian X, Hou W, Zhengang L, Sha B (2001) Direct interactions between molecular chaperones heat-shock protein (Hsp) 70 and Hsp40: yeast Hsp70 Ssa1 binds the extreme C-terminal region of yeast Hsp40 Sis1. Biochem J 361:27–34
39. Hartl FU, Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295:1852–1858
40. Hennessy F, Nicoll WS, Zimmermann R, Cheetham ME, Blatch GL (2005) Not all J domains are created equal: implications for the specificity of Hsp40–Hsp70 interactions. Protein Sci 14:1697–1709
41. Benyair R, Ron E, Lederkremer GZ (2011) Protein quality control, retention, and degradation at the endoplasmic reticulum. Int Rev Cell Mol Biol 292:197–280. Elsevier
42. Sopha P, Ren HY, Grove DE, Cyr DM (2017) Endoplasmic reticulum stress–induced degradation of DNAJB12 stimulates BOK accumulation and primes cancer cells for apoptosis. J Biol Chem 292:11792–11803

43. Delbecq SP, Klevit RE (2019) HSPB5 engages multiple states of a destabilized client to enhance chaperone activity in a stress-dependent manner. J Biol Chem 294:3261–3270
44. Ito H, Kamei K, Iwamoto I, Inaguma Y, Tsuzuki M, Kishikawa M, Shimada A, Hosokawa M, Kato K (2003) Hsp27 suppresses the formation of inclusion bodies induced by expression of R120GαB-crystallin, a cause of desmin-related myopathy. Cellular and Molecular Life Sciences CMLS 60:1217–1223
45. Tasab M, Batten MR, Bulleid NJ (2000) Hsp47: a molecular chaperone that interacts with and stabilizes correctly-folded procollagen. EMBO J 19:2204–2211
46. Brown KE, Broadhurst KA, Mathahs MM, Brunt EM, Schmidt WN (2005) Expression of HSP47, a collagen-specific chaperone, in normal and diseased human liver. Lab Investig 85:789
47. Mala JGS, Rose C (2010) Interactions of heat shock protein 47 with collagen and the stress response: an unconventional chaperone model? Life Sci 87:579–586
48. Ishida Y, Nagata K (2011) Hsp47 as a collagen-specific molecular chaperone. Methods Enzymol 499:167–182. Elsevier
49. Widmer C, Gebauer JM, Brunstein E, Rosenbaum S, Zaucke F, Drögemüller C, Leeb T, Baumann U (2012) Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition. Proc Natl Acad Sci 109:13243–13247
50. Rousseau J, Gioia R, Layrolle P, Lieubeau B, Heymann D, Rossi A, Marini JC, Trichet V, Forlino A (2014) Allele-specific Col1a1 silencing reduces mutant collagen in fibroblasts from Brtl mouse, a model for classical osteogenesis imperfecta. Eur J Hum Genet 22:667
51. Atkinson K (2017) The biology and therapeutic application of mesenchymal cells, 2 volume set. Wiley, Hoboken
52. Song X, Liao Z, Zhou C, Lin R, Lu J, Cai L, Tan X, Zeng W, Lu X, Zheng W (2017) HSP47 is associated with the prognosis of laryngeal squamous cell carcinoma by inhibiting cell viability and invasion and promoting apoptosis. Oncol Rep 38:2444–2452
53. Cheng MY, Hartl F-U, Norwich AL (1990) The mitochondrial chaperonin Hsp60 is required for its own assembly. Nature 348:455
54. Itoh H, Kobayashi R, Wakui H, Komatsuda A, Ohtani H, Miura AB, Otaka M, Masamune O, Andoh H, Koyama K (1995) Mammalian 60-kDa stress protein (chaperonin homolog). Identification, biochemical properties, and localization. J Biol Chem 270:13429–13435
55. Soltys BJ, Gupta RS (1996) Immunoelectron microscopic localization of the 60-kDa heat shock chaperonin protein (Hsp60) in mammalian cells. Exp Cell Res 222:16–27
56. Nielsen KL, McLennan N, Masters M, Cowan NJ (1999) A single-ring mitochondrial chaperonin (Hsp60-Hsp10) can substitute for GroEL-GroES in vivo. J Bacteriol 181:5871–5875
57. Kirchhoff S, Gupta S, Knowlton AA (2002) Cytosolic heat shock protein 60, apoptosis, and myocardial injury. Circulation 105:2899–2904
58. Gupta S, Knowlton AA (2007) HSP60 trafficking in adult cardiac myocytes: role of the exosomal pathway. Am J Phys Heart Circ Phys 292:H3052–H3056
59. Chun JN, Choi B, Lee KW, Lee DJ, Kang DH, Lee JY, Song IS, Kim HI, Lee S-H, Kim HS (2010) Cytosolic Hsp60 is involved in the NF-κB-dependent survival of cancer cells via IKK regulation. PLoS One 5:e9422
60. Zonneveld-Huijssoon E, van Wijk F, Roord S, Delemarre E, Meerding J, de Jager W, Klein M, Raz E, Albani S, Kuis W (2012) TLR9 agonist CpG enhances protective nasal HSP60 peptide vaccine efficacy in experimental autoimmune arthritis. Ann Rheum Dis 71:1706–1715
61. Henderson B, Kaiser F (2013) Do reciprocal interactions between cell stress proteins and cytokines create a new intra-/extra-cellular signalling nexus? Cell Stress Chaperones 18:685–701
62. Finka A, Mattoo RU, Goloubinoff P (2016) Experimental milestones in the discovery of molecular chaperones as polypeptide unfolding enzymes. Annu Rev Biochem 85:715–742

63. Wiechmann K, Müller H, König S, Wielsch N, Svatoš A, Jauch J, Werz O (2017) Mitochondrial chaperonin HSP60 is the apoptosis-related target for myrtucommulone. Cell Chem Biol 24:614–623. e616
64. Meng Q, Li BX, Xiao X (2018) Toward developing chemical modulators of Hsp60 as potential therapeutics. Front Mol Biosci 5:35
65. Vilasi S, Bulone D, Caruso Bavisotto C, Campanella C, Marino Gammazza A, San Biagio PL, Cappello F, Conway de Macario E, Macario AJ (2018) Chaperonin of group I: oligomeric spectrum and biochemical and biological implications. Front Mol Biosci 4:99
66. Freeman BC, Myers M, Schumacher R, Morimoto RI (1995) Identification of a regulatory motif in Hsp70 that affects ATPase activity, substrate binding and interaction with HDJ-1. EMBO J 14:2281–2292
67. Azem A, Oppliger W, Lustig A, Jenö P, Feifel B, Schatz G, Horst M (1997) The Mitochondrial Hsp70 Chaperone System effect of adenine nucleotides, peptide substrate, and mGrpE on the oligomeric state of mHsp70. J Biol Chem 272:20901–20906
68. Sriram M, Osipiuk J, Freeman B, Morimoto R, Joachimiak A (1997) Human Hsp70 molecular chaperone binds two calcium ions within the ATPase domain. Structure 5:403–414
69. Ngosuwan J, Wang NM, Fung KL, Chirico WJ (2003) Roles of cytosolic Hsp70 and Hsp40 molecular chaperones in post-translational translocation of presecretory proteins into the endoplasmic reticulum. J Biol Chem 278:7034–7042
70. Banfi G, Dolci A, Verna R, Corsi MM (2004) Exercise raises serum heat-shock protein 70 (Hsp70) levels. Clin Chem Lab Med 42:1445–1446
71. Ogawa Y, Miura Y, Harazono A, Kanai-Azuma M, Akimoto Y, Kawakami H, Yamaguchi T, Toda T, Endo T, Tsubuki M (2011) Proteomic analysis of two types of exosomes in human whole saliva. Biol Pharm Bull 34:13–23
72. Mogk A, Kummer E, Bukau B (2015) Cooperation of Hsp70 and Hsp100 chaperone machines in protein disaggregation. Front Mol Biosci 2:22
73. Radons J (2016) The human HSP70 family of chaperones: where do we stand? Cell Stress Chaperones 21:379–404
74. Żwirowski S, Kłosowska A, Obuchowski I, Nillegoda NB, Piróg A, Ziętkiewicz S, Bukau B, Mogk A, Liberek K (2017) Hsp70 displaces small heat shock proteins from aggregates to initiate protein refolding. EMBO J 36:783–796
75. Fernández-Fernández MR, Valpuesta JM (2018) Hsp70 chaperone: a master player in protein homeostasis. F1000Research 7:pii: F1000
76. Mayer MP, Gierasch LM (2019) Recent advances in the structural and mechanistic aspects of Hsp70 molecular chaperones. J Biol Chem 294:2085–2097
77. Rosenzweig R, Nillegoda NB, Mayer MP, Bukau B (2019) The Hsp70 chaperone network. Nat Rev Mol Cell Biol 20:665–680
78. Nair SC, Rimerman RA, Toran EJ, Chen S, Prapapanich V, Butts RN, Smith DF (1997) Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor. Mol Cell Biol 17:594–603
79. Pratt WB (1997) The role of TheHsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via map kinase. Annu Rev Pharmacol Toxicol 37:297–326
80. Johnson BD, Schumacher RJ, Ross ED, Toft DO (1998) Hop modulates Hsp70/Hsp90 interactions in protein folding. J Biol Chem 273:3679–3686
81. Mayer MP, Nikolay R, Bukau B (2002) Aha, another regulator for Hsp90 chaperones. Mol Cell 10:1255–1256
82. Zhao Y, Lu J, Sun H, Chen X, Huang W, Tao D, Huang B (2005) Histone acetylation regulates both transcription initiation and elongation of Hsp22 gene in Drosophila. Biochem Biophys Res Commun 326:811–816
83. Li J, Soroka J, Buchner J (2012) The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. Biochim Biophys Acta Mol Cell Res 1823:624–635

84. Buchner J, Li J (2013) Structure, function and regulation of the Hsp90 machinery. Biom J 36:106
85. Biella G, Fusco F, Nardo E, Bernocchi O, Colombo A, Lichtenthaler SF, Forloni G, Albani D (2016) Sirtuin 2 inhibition improves cognitive performance and acts on amyloid-β protein precursor processing in two Alzheimer's disease mouse models. J Alzheimers Dis 53:1193–1207
86. Hoter A, El-Sabban ME, Naim HY (2018) The HSP90 family: structure, regulation, function, and implications in health and disease. Int J Mol Sci 19:2560
87. Kravats AN, Hoskins JR, Reidy M, Johnson JL, Doyle SM, Genest O, Masison DC, Wickner S (2018) Functional and physical interaction between yeast Hsp90 and Hsp70. Proc Natl Acad Sci 115:E2210–E2219
88. Parsell DA, Kowal AS, Singer MA, Lindquist S (1994) Protein disaggregation mediated by heatshock protein Hspl04. Nature 372:475
89. Singh A, Singh U, Mittal D, Grover A (2010) Genome-wide analysis of rice ClpB/HSP100, ClpC and ClpD genes. BMC Genomics 11:95
90. Rath P, K Singh P, K Batra J (2012) Functional and structural characterization of Helicobacter pylori ClpX: a molecular chaperone of Hsp100 family. Protein Pept Lett 19:1263–1271
91. Zolkiewski M, Zhang T, Nagy M (2012) Aggregate reactivation mediated by the Hsp100 chaperones. Arch Biochem Biophys 520:1–6
92. Zeymer C, Werbeck ND, Schlichting I, Reinstein J (2013) The molecular mechanism of Hsp100 chaperone inhibition by the prion curing agent guanidinium chloride. J Biol Chem 288:7065–7076
93. Bobkova NV, Evgen'ev M, Garbuz DG, Kulikov AM, Morozov A, Samokhin A, Velmeshev D, Medvinskaya N, Nesterova I, Pollock A (2015) Exogenous Hsp70 delays senescence and improves cognitive function in aging mice. Proc Natl Acad Sci 112:16006–16011
94. Deville C, Carroni M, Franke KB, Topf M, Bukau B, Mogk A, Saibil HR (2017) Structural pathway of regulated substrate transfer and threading through an Hsp100 disaggregase. Sci Adv 3:e1701726
95. Alderson TR, Roche J, Gastall HY, Dias DM, Pritišanac I, Ying J, Bax A, Benesch JL, Baldwin AJ (2019) Local unfolding of the HSP27 monomer regulates chaperone activity. Nat Commun 10:1068
96. Doshi BM, Hightower LE, Lee J (2009) The role of Hsp27 and actin in the regulation of movement in human cancer cells responding to heat shock. Cell Stress Chaperones 14:445–457
97. Arrigo A-P, Gibert B (2014) HspB1, HspB5 and HspB4 in human cancers: potent oncogenic role of some of their client proteins. Cancers 6:333–365
98. Kuroyanagi G, Tokuda H, Yamamoto N, Matsushima-Nishiwaki R, Kozawa O, Otsuka T (2015) Unphosphorylated HSP27 (HSPB1) regulates the translation initiation process via a direct association with eIF4E in osteoblasts. Int J Mol Med 36:881–889
99. Rajagopal P, Liu Y, Shi L, Clouser AF, Klevit RE (2015) Structure of the α-crystallin domain from the redox-sensitive chaperone, HSPB1. J Biomol NMR 63:223–228
100. Matsushima-Nishiwaki R, Toyoda H, Takamatsu R, Yasuda E, Okuda S, Maeda A, Kaneoka Y, Yoshimi N, Kumada T, Kozawa O (2017) Heat shock protein 22 (HSPB8) reduces the migration of hepatocellular carcinoma cells through the suppression of the phosphoinositide 3-kinase (PI3K)/AKT pathway. Biochim Biophys Acta Mol Basis Dis 1863:1629–1639
101. Mymrikov EV, Daake M, Richter B, Haslbeck M, Buchner J (2017) The chaperone activity and substrate spectrum of human small heat shock proteins. J Biol Chem 292:672–684
102. Kappé G, Verschuure P, Philipsen RL, Staalduinen AA, Van de Boogaart P, Boelens WC, De Jong WW (2001) Characterization of two novel human small heat shock proteins: protein kinase-related HspB8 and testis-specific HspB9. Biochim Biophys Acta Gene Struct Expr 1520:1–6

103. Basha E, O'Neill H, Vierling E (2012) Small heat shock proteins and α-crystallins: dynamic proteins with flexible functions. Trends Biochem Sci 37:106–117
104. Zhang J, Liu J, Wu J, Li W, Chen Z, Yang L (2019) Progression of the role of CRYAB in signaling pathways and cancers. Onco Targets Ther 12:4129
105. Fontaine J-M, Sun X, Benndorf R, Welsh MJ (2005) Interactions of HSP22 (HSPB8) with HSP20, αB-crystallin, and HSPB3. Biochem Biophys Res Commun 337:1006–1011
106. Herzig RP, Scacco S, Scarpulla RC (2000) Sequential serum-dependent activation of CREB and NRF-1 leads to enhanced mitochondrial respiration through the induction of cytochrome c. J Biol Chem 275:13134–13141
107. Bagneris C, Bateman OA, Naylor CE, Cronin N, Boelens W, Keep NH, Slingsby C (2009) Crystal structures of α-crystallin domain dimers of αB-crystallin and Hsp20. J Mol Biol 392:1242–1252
108. Zhang H-M, Dang H, Kamat A, Yeh C-K, Zhang B-X (2012) Geldanamycin derivative ameliorates high fat diet-induced renal failure in diabetes. PLoS One 7:e32746
109. Heirbaut M, Lermyte F, Martin EM, Beelen S, Verschueren T, Sobott F, Strelkov SV, Weeks SD (2016) The preferential heterodimerization of human small heat shock proteins HSPB1 and HSPB6 is dictated by the N-terminal domain. Arch Biochem Biophys 610:41–50
110. Gober MD, Smith CC, Ueda K, Toretsky JA, Aurelian L (2003) Forced expression of the H11 heat shock protein can be regulated by DNA methylation and trigger apoptosis in human cells. J Biol Chem 278:37600–37609
111. Morrow G, Battistini S, Zhang P, Tanguay RM (2004) Decreased lifespan in the absence of expression of the mitochondrial small heat shock protein Hsp22 in Drosophila. J Biol Chem 279:43382–43385
112. Shemetov AA, Seit-Nebi AS, Gusev NB (2008) Structure, properties, and functions of the human small heat-shock protein HSP22 (HspB8, H11, E2IG1): a critical review. J Neurosci Res 86:264–269
113. Guan X, Tu C, Li M, Hu Z (2011) HSP22 and its role in human neurological disease. Curr Neurovasc Res 8:323–333
114. Modem S, Chinnakannu K, Bai U, Reddy GPV, Reddy TR (2011) Hsp22 (HspB8/H11) knockdown induces Sam68 expression and stimulates proliferation of glioblastoma cells. J Cell Physiol 226:2747–2751
115. Rashed E, Lizano P, Dai H, Thomas A, Suzuki CK, Depre C, Qiu H (2015) Heat shock protein 22 (Hsp22) regulates oxidative phosphorylation upon its mitochondrial translocation with the inducible nitric oxide synthase in mammalian heart. PLoS One 10:e0119537
116. Yu W-W, Cao S-N, Zang C-X, Wang L, Yang H-Y, Bao X-Q, Zhang D (2018) Heat shock protein 70 suppresses neuroinflammation induced by α-synuclein in astrocytes. Mol Cell Neurosci 86:58–64
117. Sun X, Fontaine J-M, Rest JS, Shelden EA, Welsh MJ, Benndorf R (2004) Interaction of human HSP22 (HSPB8) with other small heat shock proteins. J Biol Chem 279:2394–2402
118. Nakagawa M, Tsujimoto N, Nakagawa H, Iwaki T, Fukumaki Y, Iwaki A (2001) Association of HSPB2, a member of the small heat shock protein family, with mitochondria. Exp Cell Res 271:161–168
119. Kondaurova EM, Naumenko VS, Sinyakova NA, Kulikov AV (2011) Map 3k1, Il6st, Gzmk, and Hspb3 gene coexpression network in the mechanism of freezing reaction in mice. J Neurosci Res 89:267–273
120. Prabhu S, Raman B, Ramakrishna T, Rao CM (2012) HspB2/myotonic dystrophy protein kinase binding protein (MKBP) as a novel molecular chaperone: structural and functional aspects. PLoS One 7:e29810
121. Dubińska-Magiera M, Jabłońska J, Saczko J, Kulbacka J, Jagla T, Daczewska M (2014) Contribution of small heat shock proteins to muscle development and function. FEBS Lett 588:517–530

122. Morelli FF, Verbeek DS, Bertacchini J, Vinet J, Mediani L, Marmiroli S, Cenacchi G, Nasi M, De Biasi S, Brunsting JF (2017) Aberrant compartment formation by HSPB2 mislocalizes Lamin A and compromises nuclear integrity and function. Cell Rep 20:2100–2115
123. Clark AR, Egberts WV, Kondrat FD, Hilton GR, Ray NJ, Cole AR, Carver JA, Benesch JL, Keep NH, Boelens WC (2018) Terminal regions confer plasticity to the tetrameric assembly of human HspB2 and HspB3. J Mol Biol 430:3297–3310
124. Haslbeck M, Weinkauf S, Buchner J (2019) Small heat shock proteins: simplicity meets complexity. J Biol Chem 294:2121–2132
125. Sugiyama Y, Suzuki A, Kishikawa M, Akutsu R, Hirose T, Waye MM, Tsui SK, Yoshida S, Ohno S (2000) Muscle develops a specific form of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. J Biol Chem 275:1095–1104
126. Nam DE, Nam SH, Lee AJ, Hong YB, Choi BO, Chung KW (2018) Small heat shock protein B3 (HSPB3) mutation in an axonal Charcot-Marie-Tooth disease family. J Peripher Nerv Syst 23:60–66
127. Kolb S, Snyder P, Poi E, Renard E, Bartlett A, Gu S, Sutton S, Arnold W, Freimer M, Lawson V (2010) Mutant small heat shock protein B3 causes motor neuropathy: utility of a candidate gene approach. Neurology 74:502–506
128. Vos MJ, Zijlstra MP, Kanon B, van Waarde-Verhagen MA, Brunt ER, Oosterveld-Hut HM, Carra S, Sibon OC, Kampinga HH (2010) HSPB7 is the most potent polyQ aggregation suppressor within the HSPB family of molecular chaperones. Hum Mol Genet 19:4677–4693
129. Slingsby C, Wistow GJ, Clark AR (2013) Evolution of crystallins for a role in the vertebrate eye lens. Protein Sci 22:367–380
130. Slingsby C, Wistow GJ (2014) Functions of crystallins in and out of lens: roles in elongated and post-mitotic cells. Prog Biophys Mol Biol 115:52–67
131. Krief S, Faivre J-F, Robert P, Le Douarin B, Brument-Larignon N, Lefrere I, Bouzyk MM, Anderson KM, Greller LD, Tobin FL (1999) Identification and characterization of cvHsp A novel human small stress protein selectively expressed in cardiovascular and insulin-sensitive tissues. J Biol Chem 274:36592–36600
132. Elicker KS, Hutson LD (2007) Genome-wide analysis and expression profiling of the small heat shock proteins in zebrafish. Gene 403:60–69
133. Lahvic JL, Ji Y, Marin P, Zuflacht JP, Springel MW, Wosen JE, Davis L, Hutson LD, Amack JD, Marvin MJ (2013) Small heat shock proteins are necessary for heart migration and laterality determination in zebrafish. Dev Biol 384:166–180
134. Juo L-Y, Liao W-C, Shih Y-L, Yang B-Y, Liu A-B, Yan Y-T (2016) HSPB7 interacts with dimerized FLNC and its absence results in progressive myopathy in skeletal muscles. J Cell Sci 129:1661–1670
135. Wu T, Mu Y, Bogomolovas J, Fang X, Veevers J, Nowak RB, Pappas CT, Gregorio CC, Evans SM, Fowler VM (2017) HSPB7 is indispensable for heart development by modulating actin filament assembly. Proc Natl Acad Sci 114:11956–11961
136. Mercer EJ, Lin Y-F, Cohen-Gould L, Evans T (2018) Hspb7 is a cardioprotective chaperone facilitating sarcomeric proteostasis. Dev Biol 435:41–55
137. Yang K, Meinhardt A, Zhang B, Grzmil P, Adham IM, Hoyer-Fender S (2012) The small heat shock protein ODF1/HSPB10 is essential for tight linkage of sperm head to tail and male fertility in mice. Mol Cell Biol 32:216–225
138. Gastmann O, Burfeind P, Güunther E, Hameister H, Szpirer C, Hoyer-Fender S (1993) Sequence, expression, and chromosomal assignment of a human sperm outer dense fiber gene. Mol Reprod Dev 36:407–418
139. Xun W, Shi L, Cao T, Zhao C, Yu P, Wang D, Hou G, Zhou H (2015) Dual functions in response to heat stress and spermatogenesis: Characterization of expression profile of small heat shock proteins 9 and 10 in goat testis. Biomed Res Int 2015:686239
140. MacPhee DJ (2017) The role of heat shock proteins in reproductive system development and function. Springer, Cham

141. Li J, Qian X, Sha B (2009) Heat shock protein 40: structural studies and their functional implications. Protein Pept Lett 16:606–612
142. Cyr DM, Langer T, Douglas MG (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. Trends Biochem Sci 19:176–181
143. Cheetham ME, Caplan AJ (1998) Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. Cell Stress Chaperones 3:28
144. Wittung-Stafshede P, Guidry J, Horne BE, Landry SJ (2003) The J-domain of Hsp40 couples ATP hydrolysis to substrate capture in Hsp70. Biochemistry 42:4937–4944
145. Ito S, Nagata K (2017) Biology of Hsp47 (Serpin H1), a collagen-specific molecular chaperone. Semin Cell Dev Biol 62:142–151. Elsevier
146. Ishikawa Y, Rubin K, Bächinger HP, Kalamajski S (2018) The endoplasmic reticulum–resident collagen chaperone Hsp47 interacts with and promotes the secretion of decorin, fibromodulin, and lumican. J Biol Chem 293:13707–13716
147. Ito S, Nagata K (2019) Roles of the endoplasmic reticulum–resident, collagen-specific molecular chaperone Hsp47 in vertebrate cells and human disease. J Biol Chem 294:2133–2141
148. Koide T, Nishikawa Y, Asada S, Yamazaki CM, Takahara Y, Homma DL, Otaka A, Ohtani K, Wakamiya N, Nagata K (2006) Specific recognition of the collagen triple helix by chaperone HSP47 II. The HSP47-binding structural motif in collagens and related proteins. J Biol Chem 281:11177–11185
149. Nisemblat S, Yaniv O, Parnas A, Frolow F, Azem A (2015) Crystal structure of the human mitochondrial chaperonin symmetrical football complex. Proc Natl Acad Sci 112:6044–6049
150. Hemmingsen SM, Woolford C, van der Vies SM, Tilly K, Dennis DT, Georgopoulos CP, Hendrix RW, Ellis RJ (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 333:330
151. Stevens M, Abdeen S, Salim N, Ray A-M, Washburn A, Chitre S, Sivinski J, Park Y, Hoang QQ, Chapman E (2019) HSP60/10 chaperonin systems are inhibited by a variety of approved drugs, natural products, and known bioactive molecules. Bioorg Med Chem Lett 29:1106–1112
152. Vilasi S, Carrotta R, Mangione MR, Campanella C, Librizzi F, Randazzo L, Martorana V, Gammazza AM, Ortore MG, Vilasi A (2014) Human Hsp60 with its mitochondrial import signal occurs in solution as heptamers and tetradecamers remarkably stable over a wide range of concentrations. PLoS One 9:e97657
153. Horwich AL, Fenton WA, Chapman E, Farr GW (2007) Two families of chaperonin: physiology and mechanism. Annu Rev Cell Dev Biol 23:115–145
154. Okamoto T, Yamamoto H, Kudo I, Matsumoto K, Odaka M, Grave E, Itoh H (2017) HSP60 possesses a GTPase activity and mediates protein folding with HSP10. Sci Rep 7:16931
155. Ostermann J, Horwich AL, Neupert W, Hartl F-U (1989) Protein folding in mitochondria requires complex formation with Hsp60 and ATP hydrolysis. Nature 341:125
156. Reading DS, Hallberg RL, Myers AM (1989) Characterization of the yeast HSP60 gene coding for a mitochondrial assembly factor. Nature 337:655
157. Brocchieri L, De Macario EC, Macario AJ (2008) Hsp70 genes in the human genome: conservation and differentiation patterns predict a wide array of overlapping and specialized functions. BMC Evol Biol 8:19
158. Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower LE (2009) Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 14:105–111
159. Genest O, Wickner S, Doyle SM (2019) Hsp90 and Hsp70 chaperones: collaborators in protein remodeling. J Biol Chem 294:2109–2120
160. Van Eden W, Jansen MA, Ludwig I, van Kooten P, Van Der Zee R, Broere F (2017) The enigma of heat shock proteins in immune tolerance. Front Immunol 8:1599
161. Mayer M, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670

162. Cajo GC, Horne BE, Kelley WL, Schwager F, Georgopoulos C, Genevaux P (2006) The role of the DIF motif of the DnaJ (Hsp40) co-chaperone in the regulation of the DnaK (Hsp70) chaperone cycle. J Biol Chem 281:12436–12444
163. Chen H, Wu Y, Zhang Y, Jin L, Luo L, Xue B, Lu C, Zhang X, Yin Z (2006) Hsp70 inhibits lipopolysaccharide-induced NF-κB activation by interacting with TRAF6 and inhibiting its ubiquitination. FEBS Lett 580:3145–3152
164. Csermely P, Schnaider T, So C, Prohászka Z, Nardai G (1998) The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. Pharmacol Ther 79:129–168
165. Felts SJ, Owen BA, Nguyen P, Trepel J, Donner DB, Toft DO (2000) The Hsp90-related protein TRAP1 is a mitochondrial protein with distinct functional properties. J Biol Chem 275:3305–3312
166. Biebl MM, Buchner J (2019) Structure, function, and regulation of the Hsp90 machinery. Cold Spring Harb Perspect Biol 11:a034017
167. Carretero-Paulet L, Albert VA, Fares MA (2013) Molecular evolutionary mechanisms driving functional diversification of the HSP90A family of heat shock proteins in eukaryotes. Mol Biol Evol 30:2035–2043
168. Yamamoto S, Subedi GP, Hanashima S, Satoh T, Otaka M, Wakui H, Sawada K-i, Yokota S-i, Yamaguchi Y, Kubota H (2014) ATPase activity and ATP-dependent conformational change in the co-chaperone HSP70/HSP90-organizing protein (HOP). J Biol Chem 289:9880–9886
169. Zhao R, Houry WA (2005) Hsp90: a chaperone for protein folding and gene regulation. Biochem Cell Biol 83:703–710
170. Lee U, Rioflorido I, Hong SW, Larkindale J, Waters ER, Vierling E (2007) The Arabidopsis ClpB/Hsp100 family of proteins: chaperones for stress and chloroplast development. Plant J 49:115–127
171. Kummer E, Szlachcic A, Franke KB, Ungelenk S, Bukau B, Mogk A (2016) Bacterial and yeast AAA+ disaggregases ClpB and Hsp104 operate through conserved mechanism involving cooperation with Hsp70. J Mol Biol 428:4378–4391
172. Lackie RE, Maciejewski A, Ostapchenko VG, Marques-Lopes J, Choy W-Y, Duennwald ML, Prado VF, Prado MA (2017) The Hsp70/Hsp90 chaperone machinery in neurodegenerative diseases. Front Neurosci 11:254
173. Grimminger-Marquardt V, Lashuel HA (2010) Structure and function of the molecular chaperone Hsp104 from yeast. Biopolymers 93:252–276
174. Guo F, Maurizi MR, Esser L, Xia D (2002) Crystal structure of ClpA, an Hsp100 chaperone and regulator of ClpAP protease. J Biol Chem 277:46743–46752
175. Carroni M, Kummer E, Oguchi Y, Wendler P, Clare DK, Sinning I, Kopp J, Mogk A, Bukau B, Saibil HR (2014) Head-to-tail interactions of the coiled-coil domains regulate ClpB activity and cooperation with Hsp70 in protein disaggregation. elife 3:e02481
176. Glover JR, Lindquist S (1998) Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell 94:73–82
177. Sanchez Y, Lindquist SL (1990) HSP104 required for induced thermotolerance. Science 248:1112–1115
178. Goloubinoff P, Mogk A, Zvi APB, Tomoyasu T, Bukau B (1999) Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. Proc Natl Acad Sci 96:13732–13737
179. Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381:571
180. Zhang K, Ezemaduka AN, Wang Z, Hu H, Shi X, Liu C, Lu X, Fu X, Chang Z, Yin C-C (2015) A novel mechanism for small heat shock proteins to function as molecular chaperones. Sci Rep 5:8811
181. Treweek TM, Meehan S, Ecroyd H, Carver JA (2015) Small heat-shock proteins: important players in regulating cellular proteostasis. Cell Mol Life Sci 72:429–451
182. Zhu Z, Reiser G (2018) The small heat shock proteins, especially HspB4 and HspB5 are promising protectants in neurodegenerative diseases. Neurochem Int 115:69–79

183. Webster JM, Darling AL, Uversky VN, Blair LJ (2019) Small heat shock proteins, big impact on protein aggregation in neurodegenerative disease. Front Pharmacol 10:1047
184. Dukay B, Csoboz B, Tóth ME (2019) Heat shock proteins in neuroinflammation. Front Pharmacol 10:920
185. Zininga T, Ramatsui L, Shonhai A (2018) Heat shock proteins as immunomodulants. Molecules 23:2846
186. Bakthisaran R, Tangirala R, Rao CM (2015) Small heat shock proteins: role in cellular functions and pathology. Biochim Biophys Acta Proteins Proteomics 1854:291–319
187. Tiwari S, Thakur R, Shankar J (2015) Role of heat-shock proteins in cellular function and in the biology of fungi. Biotechnol Res Int 2015:132635
188. Acunzo J, Katsogiannou M, Rocchi P (2012) Small heat shock proteins HSP27 (HspB1), αB-crystallin (HspB5) and HSP22 (HspB8) as regulators of cell death. Int J Biochem Cell Biol 44:1622–1631
189. McLoughlin F, Basha E, Fowler ME, Kim M, Bordowitz J, Katiyar-Agarwal S, Vierling E (2016) Class I and II small heat shock proteins together with HSP101 protect protein translation factors during heat stress. Plant Physiol 172:1221–1236
190. Taylor RP, Benjamin IJ (2005) Small heat shock proteins: a new classification scheme in mammals. J Mol Cell Cardiol 38:433–444
191. Mymrikov EV, Seit-Nebi AS, Gusev NB (2012) Heterooligomeric complexes of human small heat shock proteins. Cell Stress Chaperones 17:157–169
192. Carver JA, Esposito G, Schwedersky G, Gaestel M (1995) 1H NMR spectroscopy reveals that mouse Hsp25 has a flexible C-terminal extension of 18 amino acids. FEBS Lett 369:305–310
193. Kostenko S, Moens U (2009) Heat shock protein 27 phosphorylation: kinases, phosphatases, functions and pathology. Cell Mol Life Sci 66:3289–3307
194. Zhang D, Wong LL, Koay ES (2007) Phosphorylation of Ser 78 of Hsp27 correlated with HER-2/neu status and lymph node positivity in breast cancer. Mol Cancer 6:52
195. Gobbo J, Gaucher-Di-Stasio C, Weidmann S, Guzzo J, Garrido C (2011) Quantification of HSP27 and HSP70 molecular chaperone activities. In: Molecular chaperones. Springer, New York pp 137–143
196. Rogalla T, Ehrnsperger M, Preville X, Kotlyarov A, Lutsch G, Ducasse C, Paul C, Wieske M, Arrigo A-P, Buchner J (1999) Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor α by phosphorylation. J Biol Chem 274:18947–18956
197. Arrigo A-P, Virot S, Chaufour S, Firdaus W, Kretz-Remy C, Diaz-Latoud C (2005) Hsp 27 consolidates intracellular redox homeostasis by upholding glutathione in its reduced form and by decreasing iron intracellular levels. Antioxid Redox Signal 7:414–422
198. Mehlen P, Hickey E, Weber LA, Arrigo A-P (1997) Large unphosphorylated aggregates as the active form of Hsp27 which controls intracellular reactive oxygen species and glutathione levels and generates a protection against TNFα in NIH-3T3-ras cells. Biochem Biophys Res Commun 241:187–192
199. Vidyasagar A, Wilson NA, Djamali A (2012) Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. Fibrogenesis Tissue Repair 5:7
200. Charette SJ, Lavoie JN, Lambert H, Landry J (2000) Inhibition of Daxx-mediated apoptosis by heat shock protein 27. Mol Cell Biol 20:7602–7612
201. Manero F, Ljubic-Thibal V, Moulin M, Goutagny N, Yvin J-C, Arrigo A-P (2004) Stimulation of Fas agonistic antibody–mediated apoptosis by heparin-like agents suppresses Hsp27 but not Bcl-2 protective activity. Cell Stress Chaperones 9:150
202. Bruey J-M, Paul C, Fromentin A, Hilpert S, Arrigo A-P, Solary E, Garrido C (2000) Differential regulation of HSP27 oligomerization in tumor cells grown in vitro and in vivo. Oncogene 19:4855
203. Tian X, Zhao L, Song X, Yan Y, Liu N, Li T, Yan B, Liu B (2016) HSP27 inhibits homocysteine-induced endothelial apoptosis by modulation of ROS production and mitochondrial caspase-dependent apoptotic pathway. Biomed Res Int 2016:4847874

204. Huot J, Houle F, Spitz DR, Landry J (1996) HSP27 phosphorylation-mediated resistance against actin fragmentation and cell death induced by oxidative stress. Cancer Res 56:273–279
205. Mounier N, Arrigo A-P (2002) Actin cytoskeleton and small heat shock proteins: how do they interact? Cell Stress Chaperones 7:167
206. Dimauro I, Antonioni A, Mercatelli N, Caporossi D (2018) The role of αB-crystallin in skeletal and cardiac muscle tissues. Cell Stress Chaperones 23:491–505
207. Kappé G, Franck E, Verschuure P, Boelens WC, Leunissen JA, de Jong WW (2003) The human genome encodes 10 α-crystallin–related small heat shock proteins: HspB1–10. Cell Stress Chaperones 8:53
208. Neppl RL, Kataoka M, Wang D-Z (2014) Crystallin-αB regulates skeletal muscle homeostasis via modulation of argonaute2 activity. J Biol Chem 289:17240–17248
209. Kamradt MC, Chen F, Cryns VL (2001) The small heat shock protein αB-crystallin negatively regulates cytochrome c-and caspase-8-dependent activation of caspase-3 by inhibiting its autoproteolytic maturation. J Biol Chem 276:16059–16063
210. Liu Y, Walter S, Stagi M, Cherny D, Letiembre M, Schulz-Schaeffer W, Heine H, Penke B, Neumann H, Fassbender K (2005) LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide. Brain 128:1778–1789
211. Maaroufi H, Tanguay RM (2013) Analysis and phylogeny of small heat shock proteins from marine viruses and their cyanobacteria host. PLoS One 8:e81207
212. Fan G-C, Ren X, Qian J, Yuan Q, Nicolaou P, Wang Y, Jones WK, Chu G, Kranias EG (2005) Novel cardioprotective role of a small heat-shock protein, Hsp20, against ischemia/reperfusion injury. Circulation 111:1792–1799
213. Stamler R, Kappé G, Boelens W, Slingsby C (2005) Wrapping the α-crystallin domain fold in a chaperone assembly. J Mol Biol 353:68–79
214. Brophy CM, Lamb S, Graham A (1999) The small heat shock-related protein–20 is an actin-associated protein. J Vasc Surg 29:326–333
215. Tessier DJ, Komalavilas P, Panitch A, Joshi L, Brophy CM (2003) The small heat shock protein (HSP) 20 is dynamically associated with the actin cross-linking protein actinin. J Surg Res 111:152–157
216. Morrow G, Tanguay RM (2015) Drosophila melanogaster Hsp22: a mitochondrial small heat shock protein influencing the aging process. Front Genet 6:103
217. Marunouchi T, Abe Y, Murata M, Inomata S, Sanbe A, Takagi N, Tanonaka K (2013) Changes in small heat shock proteins HSPB1, HSPB5 and HSPB8 in mitochondria of the failing heart following myocardial infarction in rats. Biol Pharm Bull 36:529–539
218. Morrow G, Inaguma Y, Kato K, Tanguay RM (2000) The small heat shock protein Hsp22 of Drosophila melanogaster is a mitochondrial protein displaying oligomeric organization. J Biol Chem 275:31204–31210
219. Nagata K (1996) Hsp47: a collagen-specific molecular chaperone. Trends Biochem Sci 21:23–26
220. Benndorf R, Sun X, Gilmont RR, Biederman KJ, Molloy MP, Goodmurphy CW, Cheng H, Andrews PC, Welsh MJ (2001) HSP22, a new member of the small heat shock protein superfamily, interacts with mimic of phosphorylated HSP27 (3DHSP27). J Biol Chem 276:26753–26761
221. Dreiza CM, Komalavilas P, Furnish EJ, Flynn CR, Sheller MR, Smoke CC, Lopes LB, Brophy CM (2010) The small heat shock protein, HSPB6, in muscle function and disease. Cell Stress Chaperones 15(1):1–11
222. Mishra S, Palanivelu K (2008) The effect of curcumin (turmeric) on Alzheimer's disease: an overview. Ann Indian Acad Neurol 11:13
223. Yu L, Liang Q, Zhang W, Liao M, Wen M, Zhan B, Bao H, Cheng X (2019) HSP22 suppresses diabetes-induced endothelial injury by inhibiting mitochondrial reactive oxygen species formation. Redox Biol 21:101095

224. Ophir G, Amariglio N, Jacob-Hirsch J, Elkon R, Rechavi G, Michaelson DM (2005) Apolipoprotein E4 enhances brain inflammation by modulation of the NF-κB signaling cascade. Neurobiol Dis 20:709–718
225. Horváth I, Glatz A, Nakamoto H, Mishkind ML, Munnik T, Saidi Y, Goloubinoff P, Harwood JL, Vigh L (2012) Heat shock response in photosynthetic organisms: membrane and lipid connections. Prog Lipid Res 51:208–220
226. Landis G, Shen J, Tower J (2012) Gene expression changes in response to aging compared to heat stress, oxidative stress and ionizing radiation in Drosophila melanogaster. Aging (Albany NY) 4:768
227. Michaud S, Morrow G, Marchand J, Tanguay RM (2002) Drosophila small heat shock proteins: cell and organelle-specific chaperones? In: Small stress proteins. Springer, New York pp 79–101
228. Rodgers KJ, Ford JL, Brunk UT (2009) Heat shock proteins: keys to healthy ageing? Redox Rep 14:147–153
229. Tower J (2015) Mitochondrial maintenance failure in aging and role of sexual dimorphism. Arch Biochem Biophys 576:17–31
230. Kim H-J, Morrow G, Westwood JT, Michaud S, Tanguay RM (2010) Gene expression profiling implicates OXPHOS complexes in lifespan extension of flies over-expressing a small mitochondrial chaperone, Hsp22. Exp Gerontol 45:611–620
231. Tower J, Landis G, Gao R, Luan A, Lee J, Sun Y (2013) Variegated expression of Hsp22 transgenic reporters indicates cell-specific patterns of aging in Drosophila oenocytes. J Gerontol Ser Biomed Sci Med Sci 69:253–259
232. Fernandez-Ayala DJ, Chen S, Kemppainen E, O'Dell KM, Jacobs HT (2010) Gene expression in a Drosophila model of mitochondrial disease. PLoS One 5:e8549
233. Dabbaghizadeh A, Morrow G, Amer YO, Chatelain EH, Pichaud N, Tanguay RM (2018) Identification of proteins interacting with the mitochondrial small heat shock protein Hsp22 of Drosophila melanogaster: implication in mitochondrial homeostasis. PLoS One 13:e0193771
234. Pantic B, Trevisan E, Citta A, Rigobello M, Marin O, Bernardi P, Salvatori S, Rasola A (2013) Myotonic dystrophy protein kinase (DMPK) prevents ROS-induced cell death by assembling a hexokinase II-Src complex on the mitochondrial surface. Cell Death Dis 4:e858
235. La Padula V, Staszewski O, Nestel S, Busch H, Boerries M, Roussa E, Prinz M, Krieglstein K (2016) HSPB3 protein is expressed in motoneurons and induces their survival after lesion-induced degeneration. Exp Neurol 286:40–49
236. Adriaenssens E, Geuens T, Baets J, Echaniz-Laguna A, Timmerman V (2017) Novel insights in the disease biology of mutant small heat shock proteins in neuromuscular diseases. Brain 140:2541–2549
237. Asthana A, Raman B, Ramakrishna T, Rao CM (2012) Structural aspects and chaperone activity of human HspB3: role of the "C-terminal extension". Cell Biochem Biophys 64:61–72
238. Boelens WC, Van Boekel MA, De Jong WW (1998) HspB3, the most deviating of the six known human small heat shock proteins. Biochim Biophys Acta Protein Struct Mol Enzymol 1388:513–516
239. Dabbaghizadeh A, Finet S, Morrow G, Moutaoufik MT, Tanguay RM (2017) Oligomeric structure and chaperone-like activity of Drosophila melanogaster mitochondrial small heat shock protein Hsp22 and arginine mutants in the alpha-crystallin domain. Cell Stress Chaperones 22:577–588
240. de Jong WW, Caspers G-J, Leunissen JA (1998) Genealogy of the α-crystallin—small heat-shock protein superfamily. Int J Biol Macromol 22:151–162
241. Liao W-C, Juo L-Y, Shih Y-L, Chen Y-H, Yan Y-T (2017) HSPB7 prevents cardiac conduction system defect through maintaining intercalated disc integrity. PLoS Genet 13:e1006984
242. Liu T, Zhang L, Joo D, Sun S-C (2017) NF-κB signaling in inflammation. Signal Transduct Target Ther 2:17023
243. Yang J, Carra S, Zhu W-G, Kampinga HH (2013) The regulation of the autophagic network and its implications for human disease. Int J Biol Sci 9:1121

244. Wu D, Vonk JJ, Salles F, Vonk D, Haslbeck M, Melki R, Bergink S, Kampinga HH (2019) The N terminus of the small heat shock protein HSPB7 drives its polyQ aggregation–suppressing activity. J Biol Chem 294:9985–9994
245. de Wit NJ, Verschuure P, Kappé G, King SM, de Jong WW, van Muijen GN, Boelens WC (2004) Testis-specific human small heat shock protein HSPB9 is a cancer/testis antigen, and potentially interacts with the dynein subunit TCTEL1. Eur J Cell Biol 83:337–345
246. Fontaine J-M, Rest JS, Welsh MJ, Benndorf R (2003) The sperm outer dense fiber protein is the 10th member of the superfamily of mammalian small stress proteins. Cell Stress Chaperones 8:62
247. Lehti MS, Sironen A (2017) Formation and function of sperm tail structures in association with sperm motility defects. Biol Reprod 97:522–536
248. Ashley NT, Demas GE (2017) Neuroendocrine-immune circuits, phenotypes, and interactions. Horm Behav 87:25–34
249. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L (2018) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9:7204
250. Medzhitov R (2008) Origin and physiological roles of inflammation. Nature 454:428
251. Agostinho P, A Cunha R, Oliveira C (2010) Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. Curr Pharm Des 16:2766–2778
252. Calsolaro V, Edison P (2016) Neuroinflammation in Alzheimer's disease: current evidence and future directions. Alzheimers Dement 12:719–732
253. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol 14:388–405
254. Leyva-López N, Gutierrez-Grijalva EP, Ambriz-Perez DL, Heredia JB (2016) Flavonoids as cytokine modulators: a possible therapy for inflammation-related diseases. Int J Mol Sci 17:921
255. Swaroop S, Sengupta N, Suryawanshi AR, Adlakha YK, Basu A (2016) HSP60 plays a regulatory role in IL-1β-induced microglial inflammation via TLR4-p38 MAPK axis. J Neuroinflammation 13:27
256. Carson MJ, Thrash JC, Walter B (2006) The cellular response in neuroinflammation: The role of leukocytes, microglia and astrocytes in neuronal death and survival. Clin Neurosci Res 6:237–245
257. Cekanaviciute E, Buckwalter MS (2016) Astrocytes: integrative regulators of neuroinflammation in stroke and other neurological diseases. Neurotherapeutics 13:685–701
258. Park B-K, Kim YH, Kim YR, Choi JJ, Yang C, Jang I-S, Lee MY (2019) Antineuroinflammatory and neuroprotective effects of Gyejibokryeong-hwan in lipopolysaccharide-stimulated BV2 microglia. Evid Based Complement Alternat Med 2019:7585896
259. Morales I, Guzmán-Martínez L, Cerda-Troncoso C, Farías GA, Maccioni RB (2014) Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches. Front Cell Neurosci 8:112
260. Colombo E, Farina C (2016) Astrocytes: key regulators of neuroinflammation. Trends Immunol 37:608–620
261. Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638–647
262. Dursun E, Gezen-Ak D, Hanağası H, Bilgiç B, Lohmann E, Ertan S, Atasoy İL, Alaylıoğlu M, Araz ÖS, Önal B (2015) The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. J Neuroimmunol 283:50–57
263. Newcombe EA, Camats-Perna J, Silva ML, Valmas N, Huat TJ, Medeiros R (2018) Inflammation: the link between comorbidities, genetics, and Alzheimer's disease. J Neuroinflammation 15:1–26

264. Ramanan VK, Saykin AJ (2013) Pathways to neurodegeneration: mechanistic insights from GWAS in Alzheimer's disease, Parkinson's disease, and related disorders. Am J Neurodegener Dis 2:145
265. Jeibmann A, Paulus W (2009) Drosophila melanogaster as a model organism of brain diseases. Int J Mol Sci 10:407–440
266. O'Brien RJ, Wong PC (2011) Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci 34:185–204
267. Rajasekhar K, Chakrabarti M, Govindaraju T (2015) Function and toxicity of amyloid beta and recent therapeutic interventions targeting amyloid beta in Alzheimer's disease. Chem Commun 51:13434–13450
268. Cárdenas-Aguayo MdC, Silva-Lucero MdC, Cortes-Ortiz M, Jiménez-Ramos B, Gómez-Virgilio L, Ramírez-Rodríguez G, Vera-Arroyo E, Fiorentino-Pérez R, García U, Luna-Muñoz J (2014) Physiological role of amyloid beta in neural cells: the cellular trophic activity. In: Neurochemistry. IntechOpen, London
269. Wang X, Zhou X, Li G, Zhang Y, Wu Y, Song W (2017b) Modifications and trafficking of APP in the pathogenesis of Alzheimer's disease. Front Mol Neurosci 10:294
270. Albers MW, Gilmore GC, Kaye J, Murphy C, Wingfield A, Bennett DA, Boxer AL, Buchman AS, Cruickshanks KJ, Devanand DP, Duffy CJ, Gall CM, Gates GA, Granholm AC, Hensch T, Holtzer R, Hyman BT, Lin FR, McKee AC, Morris JC, Petersen RC, Silbert LC, Struble RG, Trojanowski JQ, Verghese J, Wilson DA, Xu S, Zhang LI (2015) At the interface of sensory and motor dysfunctions and Alzheimer's disease. Alzheimers Dement 11:70–98
271. Baker-Nigh A, Vahedi S, Davis EG, Weintraub S, Bigio EH, Klein WL, Geula C (2015) Neuronal amyloid-beta accumulation within cholinergic basal forebrain in ageing and Alzheimer's disease. Brain 138:1722–1737
272. Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW (1991) The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. Cereb Cortex 1:103–116
273. Lee Y-J, Han SB, Nam S-Y, Oh K-W, Hong JT (2010) Inflammation and Alzheimer's disease. Arch Pharm Res 33:1539–1556
274. Ardura-Fabregat A, Boddeke E, Boza-Serrano A, Brioschi S, Castro-Gomez S, Ceyzériat K, Dansokho C, Dierkes T, Gelders G, Heneka MT (2017) Targeting neuroinflammation to treat Alzheimer's disease. CNS Drugs 31:1057–1082
275. Sciacca F, Ferri C, Licastro F, Veglia F, Biunno I, Gavazzi A, Calabrese E, Boneschi FM, Sorbi S, Mariani C (2003) Interleukin-1B polymorphism is associated with age at onset of Alzheimer's disease. Neurobiol Aging 24:927–931
276. Eikelenboom P, Bate C, Van Gool W, Hoozemans J, Rozemuller J, Veerhuis R, Williams A (2002) Neuroinflammation in Alzheimer's disease and prion disease. Glia 40:232–239
277. Shaftel SS, Griffin WST, O'Banion MK (2008) The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. J Neuroinflammation 5:7
278. Ray B, Lahiri DK (2009) Neuroinflammation in Alzheimer's disease: different molecular targets and potential therapeutic agents including curcumin. Curr Opin Pharmacol 9:434–444
279. Coraci IS, Husemann J, Berman JW, Hulette C, Dufour JH, Campanella GK, Luster AD, Silverstein SC, El Khoury JB (2002) CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to β-amyloid fibrils. Am J Pathol 160:101–112
280. Barger SW, Mattson MP (1996) Induction of neuroprotective κB-dependent transcription by secreted forms of the Alzheimer's β-amyloid precursor. Mol Brain Res 40:116–126
281. Guo Q, Robinson N, Mattson MP (1998) Secreted β-amyloid precursor protein counteracts the proapoptotic action of mutant presenilin-1 by activation of NF-κB and stabilization of calcium homeostasis. J Biol Chem 273:12341–12351
282. Salminen A, Huuskonen J, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T (2008) Activation of innate immunity system during aging: NF-kβ signaling is the molecular culprit of inflamm-aging. Ageing Res Rev 7:83–105

283. Verri M, Pastoris O, Dossena M, Aquilani R, Guerriero F, Cuzzoni G, Venturini L, Ricevuti G, Bongiorno A (2012) Mitochondrial alterations, oxidative stress and neuroinflammation in Alzheimer's disease. SAGE Publications Sage UK, London
284. Heneka MT, O'Banion MK, Terwel D, Kummer MP (2010) Neuroinflammatory processes in Alzheimer's disease. J Neural Transm 117:919–947
285. Vom Berg J, Prokop S, Miller KR, Obst J, Kälin RE, Lopategui-Cabezas I, Wegner A, Mair F, Schipke CG, Peters O (2012) Inhibition of IL-12/IL-23 signaling reduces Alzheimer's disease–like pathology and cognitive decline. Nat Med 18:1812
286. Guillot-Sestier M-V, Doty KR, Gate D, Rodriguez J Jr, Leung BP, Rezai-Zadeh K, Town T (2015) Il10 deficiency rebalances innate immunity to mitigate Alzheimer-like pathology. Neuron 85:534–548
287. Chakrabarty P, Li A, Ceballos-Diaz C, Eddy JA, Funk CC, Moore B, DiNunno N, Rosario AM, Cruz PE, Verbeeck C (2015) IL-10 alters immunoproteostasis in APP mice, increasing plaque burden and worsening cognitive behavior. Neuron 85:519–533
288. Patel NS, Paris D, Mathura V, Quadros AN, Crawford FC, Mullan MJ (2005) Inflammatory cytokine levels correlate with amyloid load in transgenic mouse models of Alzheimer's disease. J Neuroinflammation 2:9
289. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL (2000) Inflammation and Alzheimer's disease. Neurobiol Aging 21:383–421
290. Rogers J, Luber-Narod J, Styren SD, Civin WH (1988) Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. Neurobiol Aging 9:339–349
291. Jeffrey M, Halliday WG, Bell J, Johnston AR, Macleod NK, Ingham C, Sayers AR, Brown DA, Fraser JR (2000) Synapse loss associated with abnormal PrP precedes neuronal degeneration in the scrapie-infected murine hippocampus. Neuropathol Appl Neurobiol 26:41–54
292. Williams A, Van Dam A-M, Ritchie D, Eikelenboom P, Fraser H (1997) Immunocytochemical appearance of cytokines, prostaglandin E2 and lipocortin-1 in the CNS during the incubation period of murine scrapie correlates with progressive PrP accumulations. Brain Res 754:171–180
293. Robinson M, Lee BY, Hane FT (2017) Recent progress in Alzheimer's disease research, part 2: genetics and epidemiology. J Alzheimers Dis 57:317–330
294. Wyss-Coray T, Rogers J (2012) Inflammation in Alzheimer disease—a brief review of the basic science and clinical literature. Cold Spring Harb Perspect Med 2:a006346
295. McCusker SM, Curran MD, Dynan KB, McCullagh CD, Urquhart DD, MacPhee DJ (2017) The role of heat shock proteins in reproductive system development and function. Springer, New York
296. Papassotiropoulos A, Bagli M, Jessen F, Bayer TA, Maier W, Rao ML, Heun R (1999) A genetic variation of the inflammatory cytokine interleukin-6 delays the initial onset and reduces the risk for sporadic Alzheimer's disease. Ann Neurol 45:666–668
297. Griffin W, Stanley L, Ling C, White L, MacLeod V, Perrot L, White Cr, Araoz C (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in down syndrome and Alzheimer disease. Proc Natl Acad Sci 86:7611–7615
298. Guo K, Kang NX, Li Y, Sun L, Gan L, Cui FJ, Gao MD, Liu KY (2009) Regulation of HSP27 on NF-κB pathway activation may be involved in metastatic hepatocellular carcinoma cells apoptosis. BMC Cancer 9:100
299. Bamberger ME, Harris ME, McDonald DR, Husemann J, Landreth GE (2003) A cell surface receptor complex for fibrillar β-amyloid mediates microglial activation. J Neurosci 23:2665–2674
300. Paresce DM, Ghosh RN, Maxfield FR (1996) Microglial cells internalize aggregates of the Alzheimer's disease amyloid β-protein via a scavenger receptor. Neuron 17:553–565
301. Stewart CR, Stuart LM, Wilkinson K, Van Gils JM, Deng J, Halle A, Rayner KJ, Boyer L, Zhong R, Frazier WA (2010) CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. Nat Immunol 11:155

302. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ (2010) Decreased clearance of CNS β-amyloid in Alzheimer's disease. Science 330:1774–1774
303. Beauquis J, Pavía P, Pomilio C, Vinuesa A, Podlutskaya N, Galvan V, Saravia F (2013) Environmental enrichment prevents astroglial pathological changes in the hippocampus of APP transgenic mice, model of Alzheimer's disease. Exp Neurol 239:28–37
304. Olabarria M, Noristani HN, Verkhratsky A, Rodríguez JJ (2010) Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. Glia 58:831–838
305. Olabarria M, Noristani HN, Verkhratsky A, Rodríguez JJ (2011) Age-dependent decrease in glutamine synthetase expression in the hippocampal astroglia of the triple transgenic Alzheimer's disease mouse model: mechanism for deficient glutamatergic transmission? Mol Neurodegener 6:55
306. Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J, Higgs R, Liu F, Malkani S, Bales KR (2004) Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-β peptides. Nat Med 10:719
307. Lue LF, Rydel R, Brigham EF, Yang LB, Hampel H, Murphy GM Jr, Brachova L, Yan SD, Walker DG, Shen Y (2001) Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. Glia 35:72–79
308. Yu J, Cheng Y, Feng K, Ruan M, Ye Q, Wang R, Li Z, Zhou G, Yao Z, Yang Y (2016) Genome-wide identification and expression profiling of tomato Hsp20 gene family in response to biotic and abiotic stresses. Front Plant Sci 7:1215
309. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE (2009) Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 78:959–991
310. Tóth ME, Gombos I, Sántha M (2015) Heat shock proteins and their role in human diseases. Acta Biologica Szegediensis 59:121–141
311. Balogh G, Péter M, Glatz A, Gombos I, Török Z, Horváth I, Harwood JL, Vígh L (2013) Key role of lipids in heat stress management. FEBS Lett 587:1970–1980
312. Vigh L, Nakamoto H, Landry J, Gomez-Munoz A, Harwood JL, Horvath I (2007) Membrane regulation of the stress response from prokaryotic models to mammalian cells. Ann N Y Acad Sci 1113:40–51
313. Tytell M, Greenberg S, Lasek R (1986) Heat shock-like protein is transferred from glia to axon. Brain Res 363:161–164
314. Guzhova I, Kislyakova K, Moskaliova O, Fridlanskaya I, Tytell M, Cheetham M, Margulis B (2001) In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance. Brain Res 914:66–73
315. Taylor AR, Robinson MB, Gifondorwa DJ, Tytell M, Milligan CE (2007) Regulation of heat shock protein 70 release in astrocytes: role of signaling kinases. Dev Neurobiol 67:1815–1829
316. Li Z, Dai J, Zheng H, Liu B, Caudill M (2002) An integrated view of the roles and mechanisms of heat shock protein gp96-peptide complex in eliciting immune response. Front Biosci 7:731–751
317. Murshid A, Gong J, Calderwood SK (2012) The role of heat shock proteins in antigen cross presentation. Front Immunol 3:63
318. Mussbacher M, Salzmann M, Brostjan C, Hoesel B, Schoergenhofer C, Datler H, Hohensinner P, Basílio J, Petzelbauer P, Assinger A (2019) Cell type-specific roles of NF-κB linking inflammation and thrombosis. Front Immunol 10:85
319. Gupta V, Gupta B, Rastogi A, Agarwal S, Nayak A (2011) Pesticides removal from waste water by activated carbon prepared from waste rubber tire. Water Res 45:4047–4055
320. Kammanadiminti SJ, Chadee K (2006) Suppression of NF-κB activation by Entamoeba histolytica in intestinal epithelial cells is mediated by heat shock protein 27. J Biol Chem 281:26112–26120
321. Buommino E, Schiraldi C, Baroni A, Paoletti I, Lamberti M, De Rosa M, Tufano MA (2005) Ectoine from halophilic microorganisms induces the expression of Hsp70 and Hsp70B′ in

human keratinocytes modulating the proinflammatory response. Cell Stress Chaperones 10:197
322. Aneja R, Odoms K, Dunsmore K, Shanley TP, Wong HR (2006) Extracellular heat shock protein-70 induces endotoxin tolerance in THP-1 cells. J Immunol 177:7184–7192
323. Sulistyowati E, Lee M-Y, Wu L-C, Hsu J-H, Dai Z-K, Wu B-N, Lin M-C, Yeh J-L (2018) Exogenous heat shock cognate protein 70 suppresses LPS-induced inflammation by down-regulating NF-κB through MAPK and MMP-2/-9 Pathways In Macrophages. Molecules 23:2124
324. Ali A, Biswas A, Pal M (2018) HSF1 mediated TNF-α production during proteotoxic stress response pioneers proinflammatory signal in human cells. FASEB J 33:2621–2635
325. Lehnardt S, Schott E, Trimbuch T, Laubisch D, Krueger C, Wulczyn G, Nitsch R, Weber JR (2008) A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. J Neurosci 28:2320–2331
326. Liu L, An D, Xu J, Shao B, Li X, Shi J (2018) Ac2-26 Induces IKKβ Degradation Through Chaperone-Mediated Autophagy Via HSPB1 in NCM-Treated Microglia. Front Mol Neurosci 11:76
327. Rozhkova E, Yurinskaya M, Zatsepina O, Garbuz D, Karpov V, Surkov S, Murashev A, Ostrov V, Margulis B, Evgen'ev M (2010) Exogenous mammalian extracellular HSP70 reduces endotoxin manifestations at the cellular and organism levels. Ann N Y Acad Sci 1197:94–107
328. Kim JY, Kim N, Zheng Z, Lee JE, Yenari MA (2016) 70-kDa heat shock protein downregulates dynamin in experimental stroke: a new therapeutic target? Stroke 47:2103–2111
329. Wang B, Liu Y, Huang L, Chen J, Li JJ, Wang R, Kim E, Chen Y, Justicia C, Sakata K (2017a) A CNS-permeable Hsp90 inhibitor rescues synaptic dysfunction and memory loss in APP-overexpressing Alzheimer's mouse model via an HSF1-mediated mechanism. Mol Psychiatry 22:990
330. Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, Murphy TL, Murphy KM (2002) T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. Nat Immunol 3:549
331. Ghosh S, May MJ, Kopp EB (1998) NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol 16:225–260
332. Kim J, Yenari M, Lee J (2015) Regulation of inflammatory transcription factors by heat shock protein 70 in primary cultured astrocytes exposed to oxygen–glucose deprivation. Neuroscience 286:272–280
333. Shaulian E, Karin M (2001) AP-1 in cell proliferation and survival. Oncogene 20:2390
334. Guo C, Yang L, Wan C-X, Xia Y-Z, Zhang C, Chen M-H, Wang Z-D, Li Z-R, Li X-M, Geng Y-D (2016) Anti-neuroinflammatory effect of Sophoraflavanone G from Sophora alopecuroides in LPS-activated BV2 microglia by MAPK, JAK/STAT and Nrf2/HO-1 signaling pathways. Phytomedicine 23:1629–1637
335. Yang Y, Wang H, Kouadir M, Song H, Shi F (2019) Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis 10:1–11
336. Shao B-Z, Cao Q, Liu C (2018) Targeting NLRP3 inflammasome in the treatment of CNS diseases. Front Mol Neurosci 11:320
337. Tőzsér J, Benkő S (2016) Natural compounds as regulators of NLRP3 inflammasome-mediated IL-1β production. Mediat Inflamm 2016:5460302
338. Martine P, Rébé C (2019) Heat shock proteins and inflammasomes. Int J Mol Sci 20:4508
339. Riedemann NC, Guo R-F, Ward PA (2003) The enigma of sepsis. J Clin Invest 112:460–467
340. Sanna MD, Ghelardini C, Galeotti N (2015) Activation of JNK pathway in spinal astrocytes contributes to acute ultra–low-dose morphine thermal hyperalgesia. Pain 156:1265–1275
341. Zhou K, Chen J, Wu J, Wu Q, Jia C, Xu YXZ, Chen L, Tu W, Yang G, Kong J (2019) Atractylenolide III ameliorates cerebral ischemic injury and neuroinflammation associated

with inhibiting JAK2/STAT3/Drp1-dependent mitochondrial fission in microglia. Phytomedicine 59:152922
342. Van Eden W, Van der Zee R, Prakken B (2005) Heat-shock proteins induce T-cell regulation of chronic inflammation. Nat Rev Immunol 5:318
343. Zheng Z, Kim JY, Ma H, Lee JE, Yenari MA (2008) Anti-inflammatory effects of the 70 kDa heat shock protein in experimental stroke. J Cereb Blood Flow Metab 28:53–63
344. Ousman SS, Tomooka BH, Van Noort JM, Wawrousek EF, O'Conner K, Hafler DA, Sobel RA, Robinson WH, Steinman L (2007) Protective and therapeutic role for αB-crystallin in autoimmune demyelination. Nature 448:474
345. Shimada Y, Shimura H, Tanaka R, Yamashiro K, Koike M, Uchiyama Y, Urabe T, Hattori N (2018) Phosphorylated recombinant HSP27 protects the brain and attenuates blood-brain barrier disruption following stroke in mice receiving intravenous tissue-plasminogen activator. PLoS One 13:e0198039
346. Kalderon B, Kogan G, Bubis E, Pines O (2015) Cytosolic Hsp60 can modulate proteasome activity in yeast. J Biol Chem 290:3542–3551
347. Sarangi U, Singh MK, Abhijnya KVV, Reddy LPA, Prasad BS, Pitke VV, Paithankar K, Sreedhar AS (2013) Hsp60 chaperonin acts as barrier to pharmacologically induced oxidative stress mediated apoptosis in tumor cells with differential stress response. Drug Target Insights 7:DTI. S12513
348. Cheng W, Li Y, Hou X, Zhang N, Ma J, Ding F, Li F, Miao Z, Zhang Y, Qi Q (2014) HSP60 is involved in the neuroprotective effects of naloxone. Mol Med Rep 10:2172–2176
349. Cahill CM, Waterman WR, Xie Y, Auron PE, Calderwood SK (1996) Transcriptional repression of the prointerleukin 1β gene by heat shock factor 1. J Biol Chem 271:24874–24879
350. Singh IS, He J-R, Calderwood S, Hasday JD (2002) A high affinity HSF-1 binding site in the 5′-untranslated region of the murine tumor necrosis factor-α gene is a transcriptional repressor. J Biol Chem 277:4981–4988
351. Xie Y, Chen C, Stevenson MA, Hume DA, Auron PE, Calderwood SK (2002) NF-IL6 and HSF1 have mutually antagonistic effects on transcription in monocytic cells. Biochem Biophys Res Commun 291:1071–1080
352. Schett G, Steiner C, Xu Q, Smolen J, Steiner G (2003) TNF α mediates susceptibility to heatinduced apoptosis by protein phosphatase-mediated inhibition of the HSF1/hsp70 stress response. Cell Death & Differentiation 10:1126–1136
353. Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N (2019) Neuroinflammation as a common feature of neurodegenerative disorders. Front Pharmacol 10:1008
354. Kim JY, Yenari MA (2013) The immune modulating properties of the heat shock proteins after brain injury. Anat Cell Biol 46:1–7
355. Tjalkens RB, Popichak KA, Kirkley KA (2017) Inflammatory activation of microglia and astrocytes in manganese neurotoxicity. In: Neurotoxicity of metals. Springer, New York pp 159–181
356. Roe MS, Wahab B, Török Z, Horváth I, Vigh L, Prodromou C (2018) Dihydropyridines allosterically modulate HSP90 providing a novel mechanism for heat shock protein co-induction and neuroprotection. Front Mol Biosci 5:51
357. Wilhelmus MM, De Waal RM, Verbeek MM (2007) Heat shock proteins and amateur chaperones in amyloid-Beta accumulation and clearance in Alzheimer's disease. Mol Neurobiol 35:203–216
358. Banjara M, Ghosh C (2017) Sterile neuroinflammation and strategies for therapeutic intervention. Int J Inflamm 2017:8385961
359. Jassam YN, Izzy S, Whalen M, McGavern DB, El Khoury J (2017) Neuroimmunology of traumatic brain injury: time for a paradigm shift. Neuron 95:1246–1265
360. Salinaro AT, Pennisi M, Di Paola R, Scuto M, Crupi R, Cambria MT, Ontario ML, Tomasello M, Uva M, Maiolino L (2018) Neuroinflammation and neurohormesis in the pathogenesis of Alzheimer's disease and Alzheimer-linked pathologies: modulation by nutritional mushrooms. Immun Ageing 15:8

361. Fakhoury M (2018) Microglia and astrocytes in Alzheimer's disease: implications for therapy. Curr Neuropharmacol 16:508–518
362. Kelly AM (2018) Exercise-induced modulation of neuroinflammation in models of Alzheimer's disease. Brain Plast 4:81–94
363. Kakimura J-I, Kitamura Y, Takata K, Umeki M, Suzuki S, Shibagaki K, Taniguchi T, Nomura Y, Gebicke-Haerter PJ, Smith MA (2002) Microglial activation and amyloid-β clearance induced by exogenous heat-shock proteins. FASEB J 16:601–603
364. Chen C-H, Zhou W, Liu S, Deng Y, Cai F, Tone M, Tone Y, Tong Y, Song W (2012) Increased NF-κB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease. Int J Neuropsychopharmacol 15:77–90
365. Jones SV, Kounatidis I (2017) Nuclear factor-kappa B and Alzheimer disease, unifying genetic and environmental risk factors from cell to humans. Front Immunol 8:1805
366. Goyzueta MLD, Magalhães AI Jr, Ruan Z, de Carvalho JC, Soccol CR (2019) Industrial production, patent landscape, and market trends of arachidonic acid-rich oil of Mortierella alpina. Biotechnol Res Innov 3(1):103–119
367. Prestes-Carneiro L, Shio M, Fernandes P, Jancar S (2007) Cross-regulation of iNOS and COX-2 by its products in murine macrophages under stress conditions. Cell Physiol Biochem 20:283–292
368. Wilhelmus MM, Boelens WC, Otte-Höller I, Kamps B, de Waal RM, Verbeek MM (2006) Small heat shock proteins inhibit amyloid-β protein aggregation and cerebrovascular amyloid-β protein toxicity. Brain Res 1089:67–78
369. Walker D, Lue L-F (2007) Anti-inflammatory and immune therapy for Alzheimer's disease: current status and future directions. Curr Neuropharmacol 5:232–243
370. Borges TJ, Wieten L, Van Herwijnen MJ, Broere F, Van Der Zee R, Bonorino C, Van Eden W (2012) The anti-inflammatory mechanisms of Hsp70. Front Immunol 3:95
371. Stocki P, Dickinson AM (2012) The immunosuppressive activity of heat shock protein 70. Autoimmune Dis 2012:617213
372. Edkins AL, Price JT, Pockley AG, Blatch GL (2017) Heat shock proteins as modulators and therapeutic targets of chronic disease: an integrated perspective. The Royal Society, London
373. Taha AY, Blanchard HC, Cheon Y, Ramadan E, Chen M, Chang L, Rapoport SI (2017) Dietary linoleic acid lowering reduces lipopolysaccharide-induced increase in brain arachidonic acid metabolism. Mol Neurobiol 54:4303–4315
374. Crul T, Toth N, Piotto S, Literati-Nagy P, Tory K, Haldimann P, Kalmar B, Greensmith L, Torok Z, Balogh G (2013) Hydroximic acid derivatives: pleiotropic HSP co-inducers restoring homeostasis and robustness. Curr Pharm Des 19:309–346
375. Escriba PV, Ferrer-Montiel AV, Ferragut JA, Gonzalez-Ros JM (1990) Role of membrane lipids in the interaction of daunomycin with plasma membranes from tumor cells: implications in drug-resistance phenomena. Biochemistry 29:7275–7282
376. Török Z, Crul T, Maresca B, Schütz GJ, Viana F, Dindia L, Piotto S, Brameshuber M, Balogh G, Peter M (2014) Plasma membranes as heat stress sensors: from lipid-controlled molecular switches to therapeutic applications. Biochim Biophys Acta Biomembranes 1838:1594–1618
377. Ruiz-Gutiérrez V, Muriana FJ, Guerrero A, Cert AM, Villar J (1996) Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. J Hypertens 14:1483–1490
378. Nagy E, Balogi Z, Gombos I, Åkerfelt M, Björkbom A, Balogh G, Török Z, Maslyanko A, Fiszer-Kierzkowska A, Lisowska K (2007) Hyperfluidization-coupled membrane microdomain reorganization is linked to activation of the heat shock response in a murine melanoma cell line. Proc Natl Acad Sci 104:7945–7950
379. Sheppard PW, Sun X, Khammash M, Giffard RG (2014) Overexpression of heat shock protein 72 attenuates NF-κB activation using a combination of regulatory mechanisms in microglia. PLoS Comput Biol 10:e1003471

380. Gombos I, Crul T, Piotto S, Güngör B, Török Z, Balogh G, Péter M, Slotte JP, Campana F, Pilbat A-M (2011) Membrane-lipid therapy in operation: the HSP co-inducer BGP-15 activates stress signal transduction pathways by remodeling plasma membrane rafts. PLoS One 6: e28818
381. Literáti-Nagy Z, Tory K, Literáti-Nagy B, Kolonics A, Török Z, Gombos I, Balogh G, Vígh L, Horváth I, Mandl J (2012) The HSP co-inducer BGP-15 can prevent the metabolic side effects of the atypical antipsychotics. Cell Stress Chaperones 17:517–521
382. Ou J-R, Tan M-S, Xie A-M, Yu J-T, Tan L (2014) Heat shock protein 90 in Alzheimer's disease. Biomed Res Int 2014:796869
383. Penke B, Bogár F, Crul T, Sántha M, Tóth ME, Vígh L (2018) Heat shock proteins and autophagy pathways in neuroprotection: from molecular bases to pharmacological interventions. Int J Mol Sci 19:325
384. Allison AC, Cacabelos R, Lombardi VR, Álvarez XA, Vigo C (2001) Celastrol, a potent antioxidant and anti-inflammatory drug, as a possible treatment for Alzheimer's disease. Prog Neuro-Psychopharmacol Biol Psychiatry 25:1341–1357
385. Cascão R, Fonseca JE, Moita LF (2017) Celastrol: a spectrum of treatment opportunities in chronic diseases. Front Med 4:69
386. Faust K, Gehrke S, Yang Y, Yang L, Beal MF, Lu B (2009) Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a Drosophila model of Parkinson's disease. BMC Neurosci 10:109
387. Ju SM, Youn GS, Cho YS, Choi SY, Park J (2015) Celastrol ameliorates cytokine toxicity and pro-inflammatory immune responses by suppressing NF-κB activation in RINm5F beta cells. BMB Rep 48:172
388. Sharma S, Mishra R, Walker BL, Deshmukh S, Zampino M, Patel J, Anamalai M, Simpson D, Singh IS, Kaushal S (2015) Celastrol, an oral heat shock activator, ameliorates multiple animal disease models of cell death. Cell Stress Chaperones 20:185–201
389. Westerheide SD, Morimoto RI (2005) Heat shock response modulators as therapeutic tools for diseases of protein conformation. J Biol Chem 280:33097–33100
390. Deane CA, Brown IR (2016) Induction of heat shock proteins in differentiated human neuronal cells following co-application of celastrol and arimoclomol. Cell Stress Chaperones 21:837–848
391. Francis S, Kramarenko I, Brandon C, Lee F, Baker T, Cunningham L (2011) Celastrol inhibits aminoglycoside-induced ototoxicity via heat shock protein 32. Cell Death Dis 2:e195
392. Shen H-H, Huang S-Y, Cheng P-Y, Chu Y-J, Chen S-Y, Lam K-K, Lee Y-M (2017) Involvement of HSP70 and HO-1 in the protective effects of raloxifene on multiple organ dysfunction syndrome by endotoxemia in ovariectomized rats. Menopause 24:959–969
393. Ferat-Osorio E, Sánchez-Anaya A, Gutiérrez-Mendoza M, Boscó-Gárate I, Wong-Baeza I, Pastelin-Palacios R, Pedraza-Alva G, Bonifaz LC, Cortés-Reynosa P, Pérez-Salazar E (2014) Heat shock protein 70 down-regulates the production of toll-like receptor-induced pro-inflammatory cytokines by a heat shock factor-1/constitutive heat shock element-binding factor-dependent mechanism. J Inflamm 11:19
394. Chow AM, Brown IR (2007) Induction of heat shock proteins in differentiated human and rodent neurons by celastrol. Cell Stress Chaperones 12:237
395. Chow AM, Tang DW, Hanif A, Brown IR (2014) Localization of heat shock proteins in cerebral cortical cultures following induction by celastrol. Cell Stress Chaperones 19:845–851
396. Lu R-C, Tan M-S, Wang H, Xie A-M, Yu J-T, Tan L (2014) Heat shock protein 70 in Alzheimer's disease. Biomed Res Int 2014:435203
397. Abbott NJ (2002) Astrocyte–endothelial interactions and blood–brain barrier permeability. J Anat 200:629–638
398. Paris D, Ganey NJ, Laporte V, Patel NS, Beaulieu-Abdelahad D, Bachmeier C, March A, Ait-Ghezala G, Mullan MJ (2010) Reduction of β-amyloid pathology by celastrol in a transgenic mouse model of Alzheimer's disease. J Neuroinflammation 7:17

399. Taldone T, Ochiana SO, Patel PD, Chiosis G (2014) Selective targeting of the stress chaperome as a therapeutic strategy. Trends Pharmacol Sci 35:592–603
400. Ansar S, Burlison JA, Hadden MK, Yu XM, Desino KE, Bean J, Neckers L, Audus KL, Michaelis ML, Blagg BS (2007) A non-toxic Hsp90 inhibitor protects neurons from A-β-induced toxicity. Bioorg Med Chem Lett 17:1984–1990
401. Campanella C, Pace A, Caruso Bavisotto C, Marzullo P, Marino Gammazza A, Buscemi S, Palumbo Piccionello A (2018) Heat shock proteins in Alzheimer's disease: role and targeting. Int J Mol Sci 19:2603
402. Kalmar B, Burnstock G, Vrbova G, Urbanics R, Csermely P, Greensmith L (2002) Upregulation of heat shock proteins rescues motoneurones from axotomy-induced cell death in neonatal rats. Exp Neurol 176:87–97
403. Kieran D, Kalmar B, Dick JR, Riddoch-Contreras J, Burnstock G, Greensmith L (2004) Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat Med 10:402
404. Hargitai J, Lewis H, Boros I, Rácz Tm, Fiser A, Kurucz I, Benjamin I, Vígh L, Pénzes Z, Csermely P (2003) Bimoclomol, a heat shock protein co-inducer, acts by the prolonged activation of heat shock factor-1. Biochem Biophys Res Commun 307:689–695
405. Ebrahimi-Fakhari D, Wahlster L, McLean PJ (2011) Molecular chaperones in Parkinson's disease–present and future. J Parkinsons Dis 1:299–320
406. Keppel Hesselink J (2016) Bimoclomol and arimoclomol: HSP-co-inducers for the treatment of protein misfolding disorders, neuropathy and neuropathic pain. J Pain Relief 6:2167–0846.1000279
407. Fujibayashi T, Hashimoto N, Jijiwa M, Hasegawa Y, Kojima T, Ishiguro N (2009) Protective effect of geranylgeranylacetone, an inducer of heat shock protein 70, against drug-induced lung injury/fibrosis in an animal model. BMC Pulm Med 9:45
408. Zhong J-M, Wu S-Y, Bai J, Guo Q, Tao J, Chen H, Zhao N-W, Zhao Z, Fu H (2012) Antidepressant effect of geranylgeranylacetone in a chronic mild stress model of depression and its possible mechanism. Exp Ther Med 4:627–632
409. Ohkawara T, Nishihira J, Takeda H, Miyashita K, Kato K, Kato M, Sugiyama T, Asaka M (2005) Geranylgeranylacetone protects mice from dextran sulfate sodium-induced colitis. Scand J Gastroenterol 40:1049–1057
410. Sun Y, Zhang JR, Chen S (2017) Suppression of Alzheimer's disease-related phenotypes by the heat shock protein 70 inducer, geranylgeranylacetone, in APP/PS1 transgenic mice via the ERK/p38 MAPK signaling pathway. Exp Ther Med 14:5267–5274
411. Hoshino T, Suzuki K, Matsushima T, Yamakawa N, Suzuki T, Mizushima T (2013) Suppression of Alzheimer's disease-related phenotypes by geranylgeranylacetone in mice. PLoS One 8:e76306
412. Yasuda H, Shichinohe H, Kuroda S, Ishikawa T, Iwasaki Y (2005) Neuroprotective effect of a heat shock protein inducer, geranylgeranylacetone in permanent focal cerebral ischemia. Brain Res 1032:176–182
413. Gaspar N, Sharp SY, Pacey S, Jones C, Walton M, Vassal G, Eccles S, Pearson A, Workman P (2009) Acquired resistance to 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) in glioblastoma cells. Cancer Res 69:1966–1975
414. Schulte TW, Neckers LM (1998) The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. Cancer Chemother Pharmacol 42:273–279
415. Nanke Y, Kawamoto M, Yago T, Chiba J, Yamanaka H, Kotake S (2009) Geranylgeranylacetone, a non-toxic inducer of heat shock protein, induces cell death in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Mod Rheumatol 19:379–383
416. Schaefer S, Svenstrup TH, Guerra B (2017) The small-molecule kinase inhibitor D11 counteracts 17-AAG-mediated up-regulation of HSP70 in brain cancer cells. PLoS One 12: e0177706

417. Gu Y, Chen J, Wang T, Zhou C, Liu Z, Ma L (2016) Hsp70 inducer, 17-allylamino-demethoxygeldanamycin, provides neuroprotection via anti-inflammatory effects in a rat model of traumatic brain injury. Exp Ther Med 12:3767–3772
418. Galluzzi L, Bravo-San Pedro JM, Blomgren K, Kroemer G (2016) Autophagy in acute brain injury. Nat Rev Neurosci 17:467
419. Ortega L, Calvillo M, Luna F, Pérez-Severiano F, Rubio-Osornio M, Guevara J, Limón ID (2014) 17-AAG improves cognitive process and increases heat shock protein response in a model lesion with Aβ25–35. Neuropeptides 48:221–232
420. Choudhary MI, Naheed N, Abbaskhan A, Musharraf SG, Siddiqui H (2008) Phenolic and other constituents of fresh water fern Salvinia molesta. Phytochemistry 69:1018–1023
421. He D-Y, Dai S-M (2011) Anti-inflammatory and immunomodulatory effects of Paeonia lactiflora Pall., a traditional Chinese herbal medicine. Front Pharmacol 2:10
422. Zhao X, Chen Z, Zhao J, Zhang P, Pu Y, Jiang S, Hou J, Cui Y, Jia X, Zhang S (2016b) Hsp90 modulates the stability of MLKL and is required for TNF-induced necroptosis. Cell Death Dis 7:e2089
423. Dai Yan KS, Ohmi Y, Fujie N, Ohtsuka K (2004) Paeoniflorin, a novel heat shock protein–inducing compound. Cell Stress Chaperones 9:378
424. Ohtsuka K, Kawashima D, Gu Y, Saito K (2005) Inducers and co-inducers of molecular chaperones. Int J Hyperth 21:703–711
425. Asai M, Kawashima D, Katagiri K, Takeuchi R, Tohnai G, Ohtsuka K (2011) Protective effect of a molecular chaperone inducer, paeoniflorin, on the HCl-and ethanol-triggered gastric mucosal injury. Life Sci 88:350–357
426. Lee W-H, Loo C-Y, Bebawy M, Luk F, Mason RS, Rohanizadeh R (2013) Curcumin and its derivatives: their application in neuropharmacology and neuroscience in the 21st century. Curr Neuropharmacol 11:338–378
427. Prasad S, Aggarwal BB (2011) Turmeric, the golden spice: from traditional medicine to modern medicine. Herbal medicine: Biomolecular and Clinical Aspects, 13
428. Kato K, Ito H, Kamei K, Iwamoto I (1998) Stimulation of the stress-induced expression of stress proteins by curcumin in cultured cells and in rat tissues in vivo. Cell Stress Chaperones 3:152
429. Wieten L, Broere F, van der Zee R, Koerkamp EK, Wagenaar J, van Eden W (2007) Cell stress induced HSP are targets of regulatory T cells: a role for HSP inducing compounds as anti-inflammatory immuno-modulators? FEBS Lett 581:3716–3722
430. Ma Q-L, Zuo X, Yang F, Ubeda OJ, Gant DJ, Alaverdyan M, Teng E, Hu S, Chen P-P, Maiti P (2013) Curcumin suppresses soluble tau dimers and corrects molecular chaperone, synaptic, and behavioral deficits in aged human tau transgenic mice. J Biol Chem 288:4056–4065
431. Zhang L, Fiala M, Cashman J, Sayre J, Espinosa A, Mahanian M, Zaghi J, Badmaev V, Graves MC, Bernard G (2006) Curcuminoids enhance amyloid-β uptake by macrophages of Alzheimer's disease patients. J Alzheimers Dis 10:1–7
432. Singh S, Aggarwal BB (1995) Activation of transcription factor NF-κB is suppressed by curcumin (diferuloylmethane). J Biol Chem 270:24995–25000
433. Xu Y, Pindolia K, Janakiraman N, Chapman R, Gautam S (1997) Curcumin inhibits IL1 alpha and TNF-alpha induction of AP-1 and NF-kβ DNA-binding activity in bone marrow stromal cells. Hematopathol Mol Hematol 11:49–62
434. Sundaram JR, Poore CP, Sulaimee NHB, Pareek T, Cheong WF, Wenk MR, Pant HC, Frautschy SA, Low C-M, Kesavapany S (2017) Curcumin ameliorates neuroinflammation, neurodegeneration, and memory deficits in p25 transgenic mouse model that bears hallmarks of Alzheimer's disease. J Alzheimers Dis 60:1429–1442
435. Lewis J, Devin A, Miller A, Lin Y, Rodriguez Y, Neckers L, Liu Z-g (2000) Disruption of Hsp90 function results in degradation of the death domain kinase, receptor-interacting protein (RIP), and blockage of tumor necrosis factor-induced nuclear factor-κB activation. J Biol Chem 275:10519–10526

436. Zhao D-D, Jiang L-L, Li H-Y, Yan P-F, Zhang Y-L (2016a) Chemical components and pharmacological activities of terpene natural products from the genus Paeonia. Molecules 21:1362
437. Sevin M, Girodon F, Garrido C, de Thonel A (2015) HSP90 and HSP70: implication in inflammation processes and therapeutic approaches for myeloproliferative neoplasms. Mediat Inflamm 2015:970242
438. Ali A, Bharadwaj S, O'Carroll R, Ovsenek N (1998) HSP90 interacts with and regulates the activity of heat shock factor 1 in Xenopus oocytes. Mol Cell Biol 18:4949–4960
439. Ambade A, Catalano D, Lim A, Mandrekar P (2012) Inhibition of Hsp90 attenuates pro-inflammatory cytokines and prevents LPS induced liver injury. Hepatology (Baltimore, Md) 55:1585
440. Sable A, Rai KM, Choudhary A, Yadav VK, Agarwal SK, Sawant SV (2018) Inhibition of Heat Shock proteins HSP90 and HSP70 induce oxidative stress, suppressing cotton fiber development. Sci Rep 8:3620
441. Solárová Z, MOJžiš J, SOLáR P (2015) Hsp90 inhibitor as a sensitizer of cancer cells to different therapies. Int J Oncol 46:907–926
442. Blair LJ, Sabbagh JJ, Dickey CA (2014) Targeting Hsp90 and its co-chaperones to treat Alzheimer's disease. Expert Opin Ther Targets 18:1219–1232
443. Talaei S, Mellatyar H, Asadi A, Akbarzadeh A, Sheervalilou R, Zarghami N (2019) Spotlight on 17-AAG as an Hsp90 inhibitor for molecular targeted cancer treatment. Chem Biol Drug Des 93(5):760–786
444. Pearl LH, Prodromou C, Workman P (2008) The Hsp90 molecular chaperone: an open and shut case for treatment. Biochem J 410:439–453
445. Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 90:65–75
446. Zuo Y, Wang J, Liao F, Yan X, Li J, Huang L, Liu F (2018) Inhibition of heat shock protein 90 by 17-AAG reduces inflammation via P2X7 receptor/NLRP3 Inflammasome pathway and increases neurogenesis after subarachnoid hemorrhage in mice. Front Mol Neurosci:11
447. Bradley E, Zhao X, Wang R, Brann D, Bieberich E, Wang G (2014) Low dose Hsp90 inhibitor 17AAG protects neural progenitor cells from ischemia induced death. J Cell Commun Signal 8:353–362
448. Li J, Csibi A, Yang S, Hoffman GR, Li C, Zhang E, Jane JY, Blenis J (2015) Synthetic lethality of combined glutaminase and Hsp90 inhibition in mTORC1-driven tumor cells. Proc Natl Acad Sci 112:E21–E29
449. Ho SW, Tsui YTC, Wong TT, Cheung SK-K, Goggins WB, Yi LM, Cheng KK, Baum L (2013) Effects of 17-allylamino-17-demethoxygeldanamycin (17-AAG) in transgenic mouse models of frontotemporal lobar degeneration and Alzheimer's disease. Transl Neurodegener 2:24
450. Waza M, Adachi H, Katsuno M, Minamiyama M, Tanaka F, Doyu M, Sobue G (2006) Modulation of Hsp90 function in neurodegenerative disorders: a molecular-targeted therapy against disease-causing protein. J Mol Med 84:635–646
451. Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell 94:471–480
452. Kitson RR, Chang C-H, Xiong R, Williams HE, Davis AL, Lewis W, Dehn DL, Siegel D, Roe SM, Prodromou C (2013) Synthesis of 19-substituted geldanamycins with altered conformations and their binding to heat shock protein Hsp90. Nat Chem 5:307
453. Reigan P, Siegel D, Guo W, Ross D (2011) A mechanistic and structural analysis of the inhibition of the 90-kDa heat shock protein by the benzoquinone and hydroquinone ansamycins. Mol Pharmacol 79:823–832

454. Ochel H-J, Eichhorn K, Gademann G (2001) Geldanamycin: the prototype of a class of antitumor drugs targeting the heat shock protein 90 family of molecular chaperones. Cell Stress Chaperones 6:105
455. Pezzulo AA, Tudas RA, Stewart CG, Buonfiglio LGV, Lindsay BD, Taft PJ, Gansemer ND, Zabner J (2019) HSP90 inhibitor geldanamycin reverts IL-13–And IL-17–induced airway goblet cell metaplasia. J Clin Invest 129:744–758
456. Whitesell L, Robbins N, Huang DS, McLellan CA, Shekhar-Guturja T, LeBlanc EV, Nation CS, Hui R, Hutchinson A, Collins C (2019) Structural basis for species-selective targeting of Hsp90 in a pathogenic fungus. Nat Commun 10:402
457. Sittler A, Lurz R, Lueder G, Priller J, Hayer-Hartl MK, Hartl FU, Lehrach H, Wanker EE (2001) Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. Hum Mol Genet 10:1307–1315
458. Tukaj S, Węgrzyn G (2016) Anti-Hsp90 therapy in autoimmune and inflammatory diseases: a review of preclinical studies. Cell Stress Chaperones 21:213–218
459. Bartsch K, Hombach-Barrigah A, Clos J (2017) Hsp90 inhibitors radicicol and geldanamycin have opposing effects on Leishmania Aha1-dependent proliferation. Cell Stress Chaperones 22:729–742
460. Chang YS, Lee LC, Sun FC, Chao CC, Fu HW, Lai YK (2006) Involvement of calcium in the differential induction of heat shock protein 70 by heat shock protein 90 inhibitors, geldanamycin and radicicol, in human non-small cell lung cancer H460 cells. J Cell Biochem 97:156–165
461. Gomez-Monterrey I, Sala M, Musella S, Campiglia P (2012) Heat shock protein 90 inhibitors as therapeutic agents. Recent Pat Anticancer Drug Discov 7:313–336
462. Louis KS (2014) Investigation of the novel small molecule HSP90 inhibitor, NXD30001, in a mouse model of amyotrophic lateral sclerosis. McGill University Libraries, Montreal
463. Rao JS, Kim H-W, Kellom M, Greenstein D, Chen M, Kraft AD, Harry GJ, Rapoport SI, Basselin M (2012) Increased neuroinflammatory and arachidonic acid cascade markers, and reduced synaptic proteins, in brain of HIV-1 transgenic rats. J Neuroinflammation 9:19
464. Toyomura K, Saito T, Emori S, Matsumoto I, Kato E, Kaneko M, Okuma Y, Nakamura H, Murayama T (2012) Effects of Hsp90 inhibitors, geldanamycin and its analog, on ceramide metabolism and cytotoxicity in PC12 cells. J Toxicol Sci 37:1049–1057
465. Brash AR (2001) Arachidonic acid as a bioactive molecule. J Clin Invest 107:1339–1345
466. Tallima H, El Ridi R (2018) Arachidonic acid: physiological roles and potential health benefits–a review. J Adv Res 11:33–41
467. Calder PC (2010) Omega-3 fatty acids and inflammatory processes. Nutrients 2:355–374
468. Granström E (1984) The arachidonic acid cascade. Inflammation 8:S15–S25
469. Jurivich DA, Sistonen L, Sarge KD, Morimoto RI (1994) Arachidonate is a potent modulator of human heat shock gene transcription. Proc Natl Acad Sci 91:2280–2284
470. Elia G, Polla B, Rossi A, Santoro MG (1999) Induction of ferritin and heat shock proteins by prostaglandin A1 in human monocytes: evidence for transcriptional and post-transcriptional regulation. Eur J Biochem 264:736–745
471. Rossi A, Elia G, Santoro MG (1998) Activation of the heat shock factor 1 by serine protease inhibitors an effect associated with nuclear factor-κB inhibition. J Biol Chem 273:16446–16452
472. West JD, Wang Y, Morano KA (2012) Small molecule activators of the heat shock response: chemical properties, molecular targets, and therapeutic promise. Chem Res Toxicol 25:2036–2053
473. Ianaro A, Ialenti A, Maffia P, Pisano B, Di Rosa M (2001) HSF1/Hsp72 pathway as an endogenous anti-inflammatory system. FEBS Lett 499:239–244
474. Sanchez-Mejia RO, Mucke L (2010) Phospholipase A2 and arachidonic acid in Alzheimer's disease. Biochim Biophys Acta Mol Cell Biol Lipids 1801:784–790

475. Evgen'ev MB, Garbuz DG, Morozov AV, Bobkova NV (2018) Intranasal administration of Hsp70: molecular and therapeutic consequences. In: HSP70 in human diseases and disorders. Springer, New York pp 305–323
476. Hulina A, Rajković MG, Despot DJ, Jelić D, Dojder A, Čepelak I, Rumora L (2018) Extracellular Hsp70 induces inflammation and modulates LPS/LTA-stimulated inflammatory response in THP-1 cells. Cell Stress Chaperones 23:373–384
477. Wong KH, Riaz MK, Xie Y, Zhang X, Liu Q, Chen H, Bian Z, Chen X, Lu A, Yang Z (2019) Review of current strategies for delivering Alzheimer's disease drugs across the blood-brain barrier. Int J Mol Sci 20:381
478. Brzica H, Abdullahi W, Ibbotson K, Ronaldson PT (2017) Role of transporters in central nervous system drug delivery and blood-brain barrier protection: relevance to treatment of stroke. J Cent Nerv Syst Dis 9:1179573517693802
479. Pardridge WM (2012) Drug transport across the blood–brain barrier. J Cereb Blood Flow Metab 32:1959–1972
480. Upadhyay RK (2014) Drug delivery systems, CNS protection, and the blood brain barrier. Biomed Res Int 2014:1–37
481. Singh R, Lillard JW Jr (2009) Nanoparticle-based targeted drug delivery. Exp Mol Pathol 86:215–223
482. Wong KH, Riaz MK, Xie Y, Zhang X, Liu Q, Chen H, Bian Z, Chen X, Lu A, Yang Z (2019) Review of current strategies for delivering Alzheimer's disease drugs across the bloodbrain barrier. Int J Mol Sci 20:381
483. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K (2013) Liposome: classification, preparation, and applications. Nanoscale Res Lett 8:102
484. Bozzuto G, Molinari A (2015) Liposomes as nanomedical devices. Int J Nanomedicine 10:975
485. Çağdaş M, Sezer AD, Bucak S (2014) Liposomes as potential drug carrier systems for drug delivery. In: Application of nanotechnology in drug delivery. IntechOpen, London
486. Silva S, Almeida AJ, Vale N (2019) Combination of cell-penetrating peptides with nanoparticles for therapeutic application: a review. Biomol Ther 9:22
487. Spuch C, Navarro C (2011) Liposomes for targeted delivery of active agents against neurodegenerative diseases (Alzheimer's disease and Parkinson's disease). Journal Drug Del 2011:1–12
488. Nizri E, Irony-Tur-Sinai M, Faranesh N, Lavon I, Lavi E, Weinstock M, Brenner T (2008) Suppression of neuroinflammation and immunomodulation by the acetylcholinesterase inhibitor rivastigmine. J Neuroimmunol 203:12–22
489. Ravi G, Gupta NV (2017) Development of Solid lipid Nanoparticles of Rivastigmine Tartrate by using full factorial design for the treatment of Alzheimer's disease. J Pharm Sci Res 9:2447–2452

# Role of Heat Shock Factor 1 in HIV


**Xinfeng Xu, Xiaoyan Pan, and Shuwen Liu**

## Abstract

**Introduction** Viral infection is one of the triggers to heat shock response which is an evolutionarily conserved cell protection mechanism in eukaryotic cells and is closely related to the key transcriptional factor heat shock factor 1 (HSF1). It is generally recognized that HSF1 and its downstream factors especially heat shock proteins play extensive roles in viral infection-associated immune response, cell death, and tissue injury. They work constructively in cell development, differentiation and aging in non-pathological conditions, and work inductively in cell stresses from external environment, cancer development, drug resistance and neurodegenerative diseases. In addition, HSF1 and its downstream factors were reported to extensively participate in HIV infection, propagation, latency reversing, and associated inflammatory response. Understanding the mechanism of HSF1 in both physiological and pathological conditions is important to elucidate the role of proteostasis in the action of protein machine in eukaryotic cells.

**Methods** Systematic PubMed retrieval was conducted for all real publications on HSF1, Hsp and HIV. The literature on the role of HSF1 in HIV infection and latent activation was comprehensively investigated, and the searching information was sorted out and analyzed.

**Results** HSF1 and Heat shock proteins are widely involved in the entire life cycle of HIV. HSF1 acts directly on HIV LTR through autophosphorylation to promote HIV transcription activation, and its downstream heat shock proteins hsp90 and

X. Xu · S. Liu (✉)
Guangdong Provincial Key Laboratory of New Drug Screening, Guangzhou Key Laboratory of Drug Research for Emerging Virus Prevention and Treatment, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, China
e-mail: liusw@smu.edu.cn

X. Pan
State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Science, Wuhan, China

Heat Shock Proteins (2021) 22: 147–175
https://doi.org/10.1007/7515_2020_11

Published Online: 2 September 2020

hsp60 play active role in promoting HIV transcription, infection, and latent activation. In contrast, some heat shock proteins like HSP70 exert inhibitory effects.

**Conclusions** This article reviews the role of HSF1 and its downstream Hsp in HIV infection. Realizing the role of HSF1 in HIV is in favor of developing new drugs and therapies targeting HSF1 or associated factors for being acquired by immune deficiency syndrome.

**Keywords** Heat shock factor 1 · Heat shock protein 90 · Heat shock response · HIV · Inflammatory response · Latency reactivation

## Abbreviations

| | |
|---|---|
| AIDS | acquired immune deficiency syndrome |
| AP-1 | activator protein 1 |
| APOBEC3G | apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G |
| BRG1 | brahma related gene 1 |
| CaMK II | calcium/calmodulin-dependent protein kinase II |
| CBP | CREB-binding protein |
| CHIP | carboxy terminus of Hsp70-binding protein |
| CK2 | casein kinase 2 |
| CREB | cyclic AMP response element-binding protein |
| DBD | deoxyribonucleic acid binding domain |
| ELL2 | eleven nineteen lysine-rich leukemia gene |
| ER stress | endoplasmic reticulum stress |
| FACT | facilitates chromatin transcription |
| GM-CSF | granulocyte-macrophage colony-stimulating factor |
| HAART | highly active antiretroviral therapy |
| HADCi | histone deacetylase inhibitors |
| HIV | human immunodeficiency virus |
| HO-1 | heme oxygenase-1 |
| HR | heptad repeats |
| HSE | heat shock element |
| HSF1 | heat shock factor 1 |
| HSFs | heat shock factors |
| Hsp | heat shock proteins |
| Hsp90 | heat shock protein 90 |
| HSR | heat shock response |
| IKKγ | IκB phosphorylation kinase γ |
| LEDGF | lens epithelial-derived growth factor |
| LEF-1 | lymphoid enhancer-binding factor 1 |
| LPS | lipopolysaccharide |
| LRAs | latency reversing agents |
| LTR | long terminal repeat |

| | |
|---|---|
| MAPK | mitogen-activated protein kinase |
| MNSF-β | monoclonal nonspecific suppressor factor β |
| mTOR | mammalian target of rapamycin |
| NF-AT | nuclear factor of activated T cells |
| NF-κB | nuclear factor kappa-B |
| PARP1 | poly (ADP-ribose) polymerase 1 |
| PIC | preintegration complex |
| PIs | proteasome inhibitors |
| PKA | protein kinase A |
| PKC | protein kinase C |
| PLK1 | polo-like kinase 1 |
| PPARγ | peroxisome proliferation activated receptor γ |
| PSMD | 26S proteasome non-ATPase regulatory subunit |
| p-TEFb | positive transcription elongation factor b |
| REACT | reiterative enrichment and authentication of CRISPRi targets |
| RPA | replication protein A |
| SIRT1 | sirtuin1 |
| SIV | simian immunodeficiency virus |
| Sp1 | specificity protein 1 |
| STAT5 | signal transducer and activator of transcription 5 |
| TAD | transcriptional activation domain |

## 1 Introduction

Heat shock response (HSR) is an extensively and highly conserved stress response [121], which is especially important to cell stresses from external environment, including physical (e.g. high temperature or radiation), chemical (e.g. toxic chemicals), and biological (e.g. bacteria, virus or parasite) stresses [70, 108, 115, 134]. Once eukaryotes contact with harmful external environment, protein misfolding and aggregation even cell death may occur. The activation of HSR can protect eukaryotes from fatal factors [32, 91]. Therefore, HSR is an important eukaryotes to survive in harmful environments, which is one of the indispensable mechanism for life continuation.

Heat shock factors (HSFs), a group of structural and functional homologous transcription factors, affects the expression of some specific genes at transcriptional level, producing a series of proteins to resist stresses in eukaryotes during HSR [136]. HSF forms a trimer and undergoes various post-translational modifications which enables it to interact with DNA, thereby binding heat shock element (HSE) to modulate the transcription of heat shock proteins (Hsp) and some other related genes [60, 217]. Hsp, as important molecular chaperone, involves in protein folding, transport, aggregation, depolymerization, and renaturation [54, 66, 90]. They were firstly discovered in the salivary glands of Drosophila in 1962 [164]. Subsequent studies have found that most organisms contain Hsp, from bacteria and yeast to human [59].

Viral infection disease is one of the major threats to human health. Viral infection induced activation of HSR which affects viral infection, replication, and budding [94, 199]. HSF is the transcriptional regulator of HSR in eukaryotic, mediating the activation or inhibition of downstream target genes, and producing Hsp that are positively or negatively correlated with viral infection and propagation [3]. Study has shown that dengue virus infection activated HSF1 by activating $Ca^{2+}$ and protein kinase A (PKA)-dependent pathways to promote viral replication [193]. Similarly, HSF1 promoted the spread of Human cytomegalovirus (HCMV)-infected monocytes [154], and is closely related to the infection of orthopoxviruses including monkeypox, smallpox, and vaccinia [62]. What's more, through inducing the expression of heat shock protein 40 (Hsp40) and the binding of HSF1 to HIV-1 long terminal repeat (LTR), HSF1 positively regulates HIV gene expression and replication [162]. However, Hsp70 showed same antiviral effect against both HIV and Zika [101, 110].

HSF1 is closely related to HIV infection. Cellular HSR was activated after HIV infection, then HSF1 binds to HIV LTR to promote HIV infection and replication [162] meanwhile modulating the expression of downstream Hsp90, Hsp60, and Hsp40 [38, 127, 203]. HSF1 can also directly bind to HSE on HIV LTR and recruit positive transcription elongation factor b (p-TEFb) to reactivate latent HIV, or inhibit HIV infection induced-inflammatory response [146–148]. Thus, HSF1 plays an important role in HIV infection, replication, latency reversing, and inflammation. In this chapter, we will review the mechanism of HSF in HIV infection, especially HSF1 in latent HIV-1.

## 2 Biological Characteristics of HSF

### *2.1 HSF in Vertebrate*

HSF family mainly includes four classical members, HSF1-4 [140, 141, 159, 174]. HSF3 is an avian-specific heat shock factor, and the other three are in mammalian cells. Among them, HSF1 is the major regulatory factor of Hsp and highly conserved among different species, despite that HSF2 and HSF4 also regulate gene expression in mammals [48, 66, 197]. Compared with HSF1, HSF3 is more important than HSF1 in birds in resisting severe heat stress, because it has a higher thermal threshold for activation [189]. HSF2 is mainly related to cell development especially in reduction mitosis and neurodegeneration [57, 191]. However, recent studies showed that HSF2 is involved in cancer development, such as hepatocellular carcinoma [210], breast cancer [209], and lung cancer [216]. While HSF4 is mainly associated with the occurrence of cataract in human [9, 33], it preferentially expresses in the heat, brain, skeletal muscle, and pancreas [141]. The four HSFs are unique features of vertebrate to date. However, a new study confirmed that HSF5 which predominantly expresses in testis is the fifth member of HSF family in vertebrate and it is required for proper spermatogenesis and fertility in male zebrafish [166].

## 2.2 Biological Activity of HSF

HSF is composed of multiple functional domains, among which the DNA binding domain (DBD) is the most conserved. DBD locates at the N-terminus of HSF and has a DNA binding protein characteristic with the helix-turn-helix motif, which determines the binding of HSF to HSE of the target genes [27, 76, 198]. The trimerization domain of HSF consists of three hydrophobic aliphatic heptad repeats (HR), including HR-A, HR-B, and HR-C. The first and fourth hydrophobic residues of each heptavalent amino acid repeat are unique to the coiled-coil structure, and can form a leucine zipper to facilitate the formation of homologous or heterologous trimer [92, 160, 170]. At the C-terminus of HSF, an extra HR-C which interacts with HR-A or HR-B to form three coiled helices, maintains HSF in an inactive monomer state [135, 160]. However, human HSF4 (hHSF4) can maintain as a trimer state [188] without HR-C domain. The transcriptional activation domain (TAD), lies in the C-terminus of HSF, is an activated region that promotes transcription of Hsp genes [37]. The TAD of HSF1 is composed of two units, TAD-1 and TAD-2, which are regulated by a central regulatory domain between trimer domain and TAD [26, 48, 72]. Similar with other HSFs, HSF5 has a helix-turn-helix DBD at the N-terminal region in zebrafish, showing 37-39% similarity with other HSFs, while HSF5 in zebrafish lacks structural domains except DBD mentioned above ([166]; Fig. 1a).

The binding site of HSFs is heat shock element (HSE), which is bound by DBD of HSF. In the front of the transcription initiation site of heat shock genes, there are hundreds of base-pairs with multiple copies of HSE. These copies of HSE are sequenced as repeated units of 5 bases, 5′-nGAAn-3′, which is called GAA boxes [53, 144]. HSE structure usually has three inverted repeat, GAA boxes [122], and HSFs bind to HSE in the form of trimer, which possesses the greatest affinity thus guaranteeing efficient transcription of the genes of Hsp [114]. However, the member of HSFs has different binding affinities for HSE in mammal [168]. Yamamoto et al. analyzed different types of HSE and characteristics of binding to human HSF1 (hHSF1), hHSF2, and hHSF4 [208]. The study suggested that it is preferential for hHSF1 to combine with continuous HSEs rather than discontinuous HSEs, while hHSF2 has a higher binding affinity for discontinuous HSEs than HSF1. hHSF4 shows a significantly different binding characteristic from hHSF1 and hHSF2, which has high affinity for discontinuous nGAAn sequences [208].

## 2.3 Post-translational Modifications of HSF

In non-stress state, different HSFs exist in different forms. HSF1 mainly rests in cytoplasm in the form of inactive monomers [23], while HSF2 exists in the form of homodimers [3, 125]. When stimulated by stresses, HSF1 forms into active trimers and enter into the nucleus, functioning with various post-translational modifications

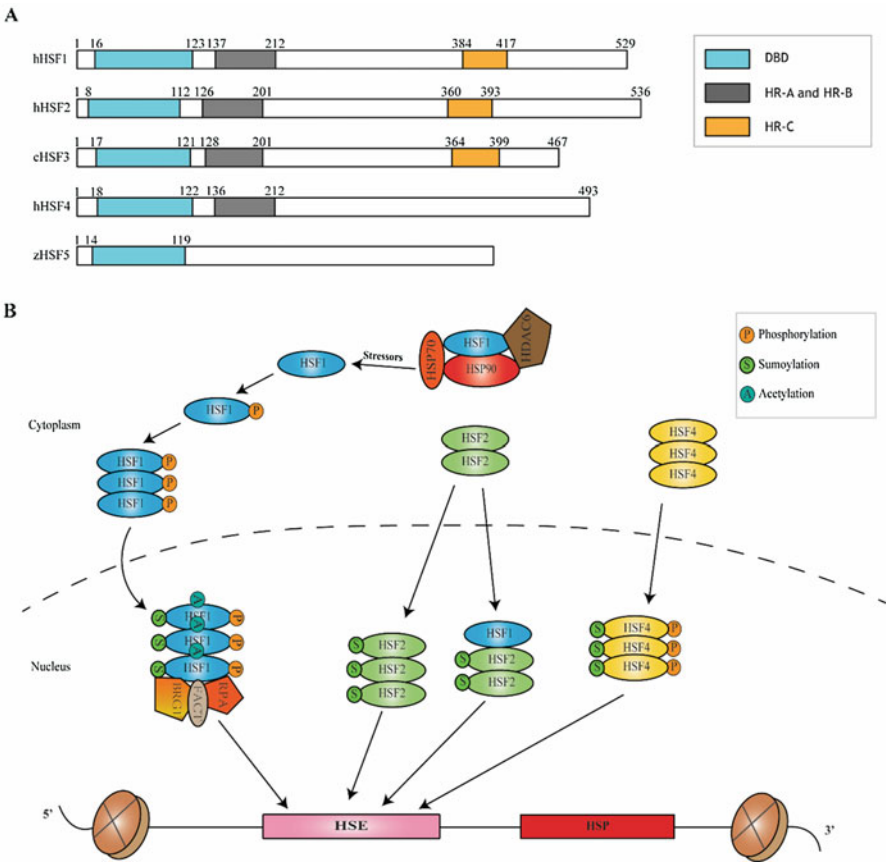



**Fig. 1 The biological characteristics of HSFs in vertebrate.** (**a**) Diagrammatic representation of HSF family members. The number of amino acids in different regions of each HSF is shown in the end of the amino acid of corresponding regions. HSF family have a DBD domain, and human HSF1 (hHSF1), hHSF2, and chicken HSF3 (cHSF3) have HR-A, HR-B, and HR-C domains. While hHSF4 only have HR-A and HR-B. (**b**) The schematic of HSFs post-translational modifications. HSFs are required to be in the form of trimer binding to HSE to promote the expression of Hsp gene. HSF1 (blue ellipse) exists as monomer, which undergoes phosphorylation, acetylation, and/or sumoylation after a trimer is formed. HSF2 (green ellipse) exists as homodimers, and forms into a homotrimer with itself or a heterotrimer with HSF1, which is mainly undergoes sumoylation. HSF4 exists as a trimer mainly experiencing phosphorylation and sumoylation

simultaneously (phosphorylation, sumoylation, and acetylation) [65, 82, 204]. Under the mediation with replication protein A (RPA), brahma related gene 1 (BRG1) facilitates chromatin transcription (FACT) and Poly (ADP-ribose) polymerase 1 (PARP1) [16, 66, 67, 186], HSF binds to the HSE at the 5′ terminal of target genes and then initiates transcription. HSF2 forms into a homotrimer with itself or forms a heterotrimer with HSF1 in the nucleus thereby inducing the expression of related genes, which is always accompanied by post-translational

modification, sumoylation [170, 214]. Moreover, studies had shown that the activity of HSF2 depends on HSF1 when HSF2 works [145], and HSF4 obtains biological activity after phosphorylation and sumoylation [84]. So far, it is still unclear how HSF5 is post-translationally modified in fever (Fig. 1b).

### 2.3.1 HSF Phosphorylation

Phosphorylation of HSF1 is important to the function of HSF1. The phosphorylation of HSF1 mainly includes Calcium/calmodulin-dependent protein kinase II (CaMK II)-mediated phosphorylation at Ser230 [152], PKA-mediated phosphorylation at Ser320 [138], casein kinase 2 (CK2)-mediated phosphorylation at Thr142 [180], and polo-like kinase 1 (PLK1)-mediated phosphorylation at Ser419 [104]. In addition, phosphorylation of HSF1 at Ser326 plays an important role in stress-induced activation of HSF1, which is primarily associated with mammalian target of rapamycin (mTOR) pathway or rat sarcoma/mitogen-activated protein kinase (RAS/MAPK) pathway [39, 49]. Phosphorylation at the following sites of HSF1 negatively regulates the activation of HSF1, which include Ser121, Ser303, Ser307, and Ser363 [46, 73, 178]. Among them, phosphorylation at Ser303 is the premise of sumoylation of HSF1 at K298 [78]. Additionally, phosphorylation of HSF4b at Ser299 can downregulate the transcription activity of itself by MAPKinase [44].

### 2.3.2 HSF Sumoylation

Sumoylation, a post-translational modification modulated by ubiquitin-like proteins, primarily regulates protein modification without promoting protein hydrolysis [80]. Sumoylation can regulate the transcriptional activation of HSF1 moderately under stress responses [29, 119]. Heat shock stress can induce the termination of HSF1 through labelling small ubiquitin-related modifier, thereby inhibiting the transcriptional activation of HSF1 [10]. Sumoylation of HSF4b at K293 suppresses the transcription activity of HSF1 [206]. However, sumoylation of HSF2 at K82 enhances the binding of HSF2 to DNA in both human cell and Xenopus [71, 81, 86, 206].

### 2.3.3 HSF Acetylation

Compared to phosphorylation and sumoylation, acetylation of HSF1 is relatively latter, which often occurs during the recovery phase after the activation of HSF1. Acetylation of HSF1 mainly at K80, K118, K208, K298 and K524, is regulated by the balance between acetyltransferase (p300/CBP) and deacetylase (SIRT1) [163, 200, 205]. Acetylation at K80 prolongs the binding time of HSF1 on DNA, and acetylation at K118 negatively regulates the function of HSF1 [163, 200]. Studies suggested that activation of SIRT1 can prolong the transcriptional activity of HSF1,

while reducing the expression of SIRT1 to accelerate HSR into recovery phase [107, 200].

## 3 Participation of HSF1 in HIV Reactivation

Stanley et al. are the first to report that HSR (supraphysiologic temperature) induced HIV gene expression in promonocytic (U1 cells) and T cell lines (ACH2 cells) [183]. Specifically, HIV reverse transcriptase, viral proteins and RNA produced after treatment with hyperthemia in both U1 and ACH2 cells. Furthermore, NF-κB conserved sequences in HIV LTR are essentialunder the induction of hyperthemia. By contrast, it might be difficult to induce the expression of HIV by physiological temperature alone. However, it synergizes with interleukin 6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF) to promote viral production in U1 cells, and this phenomenon was not observed in other cytokines [183]. Pawat P et al. showed that HSF1 mediates the transcription of HIV gene positively, and they also found the HSE sites in the HIV 5′-LTR region (69–91 region, GGGACTTTCCAGGGAGGTGTGGC). These data highlight that HSF1 plays a crucial role in latent HIV reactivation [162].

### *3.1 Latent HIV Reactivation*

Acquired immune deficiency syndrome (AIDS) is a harmful infected disease to human health, which is caused by HIV infection [158]. The main transmission route is directly contact with bloods or fluids. Upon infection, HIV attacks $CD4^+$ T cells, an important member of the host immune system [172]. With the replication and spread of HIV, the number of host $CD4^+$ T cells in blood decreases sharply, and the immune system gradually disintegrates and collapses. As a result, host is disabled to recognize and remove cancerous cells and pathogens or control the normal growth of bacteria in the body, eventually dies from various opportunistic infections or cancers [47]. With the application of highly active antiretroviral therapy (HAART) which combines drugs targeting viral reverse transcriptase, protease, and integrase, in different stages of viral propagation [105], AIDS progression can be controlled. Once the therapy is administrated, the viral load in blood will be decreased apparently, survival time of patients has been greatly extended, and quality of life has also been improved [14, 181]. Once the therapy is withdrew, viremia will restrike rapidly [195].

Thus, the main obstacle to HIV cure becomes the presence of latent HIV in patients [40, 64]. As is known to all, the integration of HIV proviral DNA to host genome is a necessary step to HIV multiplication [45]. After integration, few cells reverted from activated state to static state with a relatively longer half-life period. Transcriptionally silenced latent HIV reservoir, however, could escape surveillance

of immune system and HAART suppression [41, 173]. The stable and integrated HIV proviral DNA become part of the genetic material and it is inherited with the transcription of host cells [155]. When stimulus signal appears, it would be transcribed and as a new source of infection [28]. HIV proviral DNA not only rests in HIV-infected monocytes, macrophages, resting memory $CD4^+$ T cells, and microglia, in blood, but also in tissues as lymph nodes, visceral associated lymphoid tissues, genital tract, marrow, lungs, kidneys, and central nervous system [4, 7, 8, 85].

At present, the mechanism of the establishment of HIV reservoir is still not clear. Recent studies have found that transcriptional repression is critical for establishment and maintenance of HIV latency [35]. The formation and maintenance of HIV reservoir is contributed by co-regulation of multiple factors, which work on both transcription and post-transcription [52, 102, 103]. In spite of that, transcriptional intervention, transcriptional regulation, and epigenetic silencing are known to be in correlation with HIV latency [35].

### 3.1.1 Transcriptional Intervention in HIV Latency

HIV proviral DNA is apt to integrate into active regions in host gene pool. Lens epithelial-derived growth factor (LEDGF/p75), which locates in the intron of active gene, integrates HIV proviral DNA by binding to chromosomal DNA and HIV integrase [112, 128, 177]. LEDGF/p75 is a highly efficient chromatin-binding protein required for lentiviral integration, and it is mediated in actively transcribed genes [126]. However, HIV provirus remains a state of transcriptional silence due to transcriptional interventions by enhancer trapping, promoter blockade, or steric hindrance [102, 103]. Enhancer trapping means that HIV 5′ LTR enhances the ability of weak cell promoter while suppressing the transcription of itself [102, 103]. Promoter blockade refers to the collision of RNA polymerase II complex (Pol II) extended by virus and cell promoter when HIV provirus is reversely integrated into host genes, leading to an early termination on one or both of the promoters [35, 177]. However, steric hindrance may occur when HIV proviral DNA has the same transcription direction with host genes and integrates into downstream host genes ([35]; Fig. 2).

### 3.1.2 Transcriptional Regulation in HIV Latency

HIV LTR contains binding sites of positive transcription factors, such as nuclear factor of activated T cells (NF-AT), nuclear factor kappa-B (NF-κB), activator protein 1 (AP-1), specificity protein 1 (Sp1), lymphoid enhancer-binding factor 1 (LEF-1), and so on [123, 175]. They are slightly active in the nucleus of HIV reservoir cells, but their activity will be increased under external stimuluses which promotes the transcription of HIV [83, 93]. At the stage of transcriptional elongation,

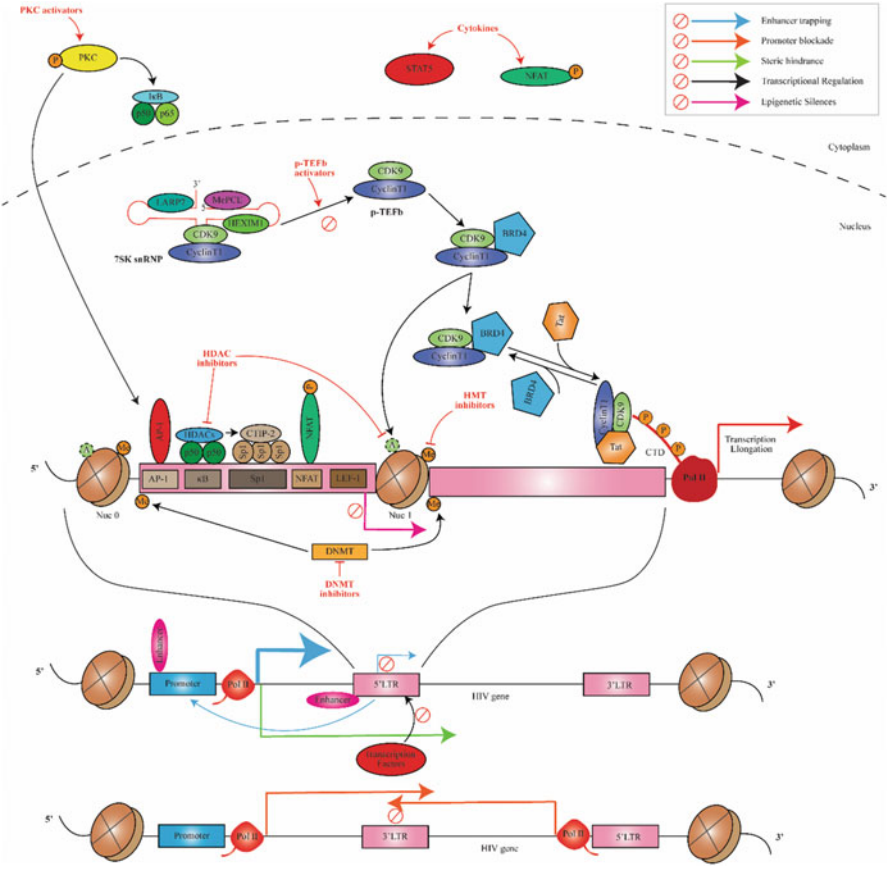



**Fig. 2 The diagrammatic of latent HIV reactivation.** The mechanism of HIV reservoir establishment as: (1) enhancer trapping (prohibition symbol with blue arrow), HIV 5'LTR enhances the ability of host promoter to suppress self-transcription; (2) promoter blockade (prohibition symbol with orange arrow), the opposite transcriptional direction of both host gene and HIV gene, the collision between transcription complex and extension complex causes the promoter prematurely terminate; (3) steric hindrance (prohibition symbol with green arrow), host transcription prevents the combination of HIV 5′ LTR with transcription factors; (4) transcriptional regulation (prohibition symbol with black arrow), 7SK snRNP binds to p-TEFb and the decrease in free p-TEFb restricts the transcriptional elongation of HIV; (5) epigenetic silences (prohibition symbol with pink arrow), epigenetic modification associated with transcriptional repression lead to HIV maintaining the state as nucleosome. Different LRAs activated latent HIV through different ways (red font with red lines) which are involved with host factors as PKC, protein kinase C; p-TEFb, positive transcription elongation factor b; HDAC, histone deacetylase; HMT, histone methyltransferase; DNMT, DNA methylase

low expression of Tat protein and p-TEFb in HIV reservoir cells may be involved in the establishment and maintenance of HIV latency [118, 194]. In addition, p-TEFb binds to small nuclear RNA 7SK ribonucleoprotein complex (7SK snRNP), resulting in fewer free p-TEFb to maintain HIV latency ([56]; Fig. 2).

### 3.1.3 Epigenetic Silences in HIV Latency

DNA is entrapped around by nucleosome and histone tails formed by histone octamers [171]. Experiencing multiple post-translational modifications like epigenetic modification, histone tail becomes heritable and ables to alter the expression of genes without changing DNA sequences [106]. Epigenetics affect the condensation of chromatin and determine whether transcription factors can bind to the corresponding sites. HIV LTR is encapsulated in Nuc-0 and Nuc-1 nucleosomes [196]. Thereby, Nuc-0 is in HIV 5′ LTR, and Nuc-1 covers +10 bp to +55 bp downstream of transcription start site to block transcriptional elongation [196]. The nucleosomes on HIV 5′ LTR of the latent provirus usually have epigenetic markers related to transcriptional repression [1, 133]. A series of epigenetic modifications contribute to the maintenance of HIV latency [75]. Stress responses potentially alter the genetic markers and reconstitute Nuc-1, thus providing the possibility of reactivating HIV reservoirs ([17, 207]; Fig. 2).

## *3.2 Mechanism of Latent HIV Reactivation*

To achieve the goal of HIV cure, researchers have put forward a series of strategies. (1) The first one is gene editing, which aims at deleting HIV co-receptor to disable the entry of invasive or reactivated virus [6, 150]. (2) The second is developing neutralizing antibodies to antagonize HIV and control disease progression [34]. (3) The third is 'Block and Lock' strategy, which is combined HAART with Tat inhibitors to inhibit the reactivation of HIV reservoir [99]. (4) The fourth is 'Shock and Kill' strategy, which employs latency reversing agents (LRAs) to activate latent HIV, combining with HAART to clear HIV reservoir [51]. Among them, the 'Shock and Kill' strategy is studied and accepted widely due to its high feasibility and low risk.

On the basis of the knowledge on establishment and maintenance of HIV reservoir, a series of LRAs that reactivate latent HIV through different pathways have been discovered. (1) Immunomodulatory LRAs, including cytokines as IL-2, IL-7, IL-15, and antibodies against CD3 and CD28, mediats cell activation and promotes latent HIV reactivation [201]. (2) LRAs targeting transcription factor act via following pathways: (a) promoting PKC phosphorylation via activating PKC thereby facilitating the binding of its downstream transcription factors NF-κB and AP-1 to LTR and mediating viral transcription initiation [132, 202]; (b) activating the p-TEFb transcriptional complex, promoting the binding of Tat protein to facilitate HIV transcriptional elongation [42, 116]. (3) LRAs targeting epigenetic modifications mainly includes following: (a) histone deacetylase inhibitors (HADCi) loose the nucleosomes in chromosomes, exposing viral DNA entangled by nucleosomes and promoting the extension of HIV transcription [20, 161, 187]; (b) DNA methylase inhibitor [61]; and (c) histone methyltransferase inhibitor ([22]; Fig. 2).

### 3.3 HSF1 Plays Important Part in Latent HIV-1 Reactivation

Based on the finding of Pawat P et al., we found that latent HIV is reactivated effectively by stress responses including high temperature, Poly I:C, STA-4783, and Thapsigargin, which induce the phosphorylation of α subunit of eukaryotic translation initiation factors 2 (eIF2α) in J-Lat 10.6 cell line, a cell model of HIV latency [146, 147]. Latent HIV is confirmed to be reactivated by Salubrinal, which is called an inhibitor of eIF2α dephosphorylation, in cell models (J-Lat 10.6 cells, ACH2 cells, U1 cells) and primary $CD4^+$ T cells from HIV infected individuals after HAART. Further research revealed that healthy physical mechanism is closely related to HSF1, and HSF1 is activated by stresses. HSF1 is further found to recruit acetylase (p300) to promote self-modification, and its downstream factors (Hsp27, Hsp70, and Hsp90) are involved widely in this process.

Anderson et al. suggested that Hsp90 assists IκB phosphorylation kinase IKKγ to promote NF-κB activation and nuclear translocation, thereby reactivating latent HIV [11]. Additional studies showed that heat shock-induced Hsp90 accelerates HIV transcription and activates transcription factors as NF-κB, NF-AT, and signal transducer and activator of transcription 5 (STAT5) [97]. Moreover, Hsp90 specific inhibitors, Tanespimycin (17-AAG) and Luminespib (AUY-922), prevent viral rebound in HIV-infected humanized mice. In conclusion, these studies demonstrated that (1) activated HSF1 occurs in latent HIV reactivation and (2) Hsp90 plays an important role in HIV reservoir reactivation. Pan et al. found that proteasome inhibitors (PIs)-induced endoplasmic reticulum stress (ER stress) can reactivate latent HIV through HSF1. They investigated the function of PIs (Carfilzomib, Bortezomib, and MG132) in latent HIV reactivation and HIV gene transcription [146, 147]. HSF1 is demonstrated to closely relate to PIs-stimulated latent HIV reactivation and promote transcriptional elongation by recruiting p-TEFb. More importantly, Hsp90 interacts with CDK9 to protect CDK9 from proteasomal degradation during latent HIV reactivation, which provides a positive feedback to HSF1-induced latent HIV reactivation [146, 147]. Besides, recent study suggested that PIs like Bortezomib and Carfilzomib can stabilize the transcription elongation factor 1,119 lysine-rich leukemia gene (ELL2) to reverse HIV latency by inhibiting proteasome [117]. This study has illustrated the role of proteasome in HIV latency reversing from another perspective, in which they used the reiterative enrichment and authentication of CRISPRi targets (REACT) to discover new HIV-1 restriction factors in a HIV latency model. They found that proteasomal subunits (PSMD1, PSMD3, and PSMD8) are closely related to Tat-dependent HIV-1 transcription, and the inhibition of proteasome increases ELL2-containing super elongation complexes (ELL2-SECs) to reverse HIV latency.

Above all, HSF1 is considered as a target for screening small molecule compounds against latent HIV. Natural polyphenolic compound, resveratrol, was demonstrated to target HSF1 for promoting latent HIV transcription through phosphorylation HSF1 and facilitating its binding to HIV LTR [213]. Further research found that resveratrol is a potent LRA drug candidate that induces latent

HIV reactivation on a series of cell models without causing extensive T cell activation. In addition, resveratrol in combination with the LRAs (Prostratin, JQ1, and SAHA) has a synergistic effect on reactivating latent HIV [213]. The immunoproteasome inhibitor, PR-957, is also demonstrated to be able to reverse HIV latency effectively both in latent HIV cell lines and primary $CD4^+$ T cells from HIV infected individuals after HAART [120]. Most importantly, PR-957 reduces the expression of HIV receptors and co-receptors, causing no extensive T cell activation. PR-957 also synergizes with Prostratin to activate latent HIV and decreases the extensive T cell activation caused by Prostratin. The mechanism of PR-957 reversing latent HIV is revealed as activating HSF1 pathway and up-regulating the expression of p-TEFb [120].

To sum up, HSF1 plays an important role in latent HIV-1 reactivation. Specifically, it is phosphorylated and translocated into the nucleus, then recruits p300 to promote auto-acetylation and binds HIV LTR to induce transcription initiation of HIV genes meanwhile recruiting p-TEFb to facilitate transcription elongation. Beyond that, Hsp90 downstream of HSF1 is able to protect the p-TEFb subunit CDK9 from ubiquitination degradation thus giving a positive feedback on HSF1. Based on these findings, two LRAs, resveratrol and PR-957, were found to activate both HSF1 pathway and p-TEFb to reverse HIV latency (Fig. 3).

## 4 HSF1 Downstream Hsp in HIV

### *4.1 Biological Functions of Hsp*

Hsp is important effector in HSR. When cells are under stress, HSF binds to upstream genes of Hsp and then mediates the production of Hsp, protecting cells from stresses [139]. Hsp, known as molecular chaperone, engages widely in cellular stress, inflammation, tumors, and infections [25, 91, 113, 131]. Particularly, Hsp not only plays an important role in the process of viral infection including virus entry, nuclear translocation, replication, viral protein folding and assembly, but also participates in DNA repair, apoptosis and immuno-regulation [25, 54].

The protein molecular of Hsp is ranging from 10 kDa to 110 kDa, which is classified into Hsp110, Hsp90, Hsp70, Hsp60, Hsp40, and small molecules Hsp, according to their molecular weight [63, 111]. Their main biological function in eukaryotic cells is regarded as molecular chaperone to play roles in the conditions of both physiological and stressful conditions. In physiological condition, Hsp regulates the folding and translocation of nascent proteins to prevent their accumulation [55]. Under stress, they promote the renaturation of denatured proteins or promote the degradation of damaged proteins. Moreover, Hsp has other functions as following: (a) Hsp60 enhances the function of $CD4^+$ $CD25^+$ regulatory T cells and plays a part in innate immunity [212]; (b) Hsp32, known as heme oxygenase-1 (HO-1), removes the oxidant heme to produce CO and biliverdin which plays a key role in diabetes, vascular diseases, and kidney diseases [167, 169]; (c) Hsp20 mediates adipocyte function through forming Hsp20-FBXO4 axis to regulate peroxisome

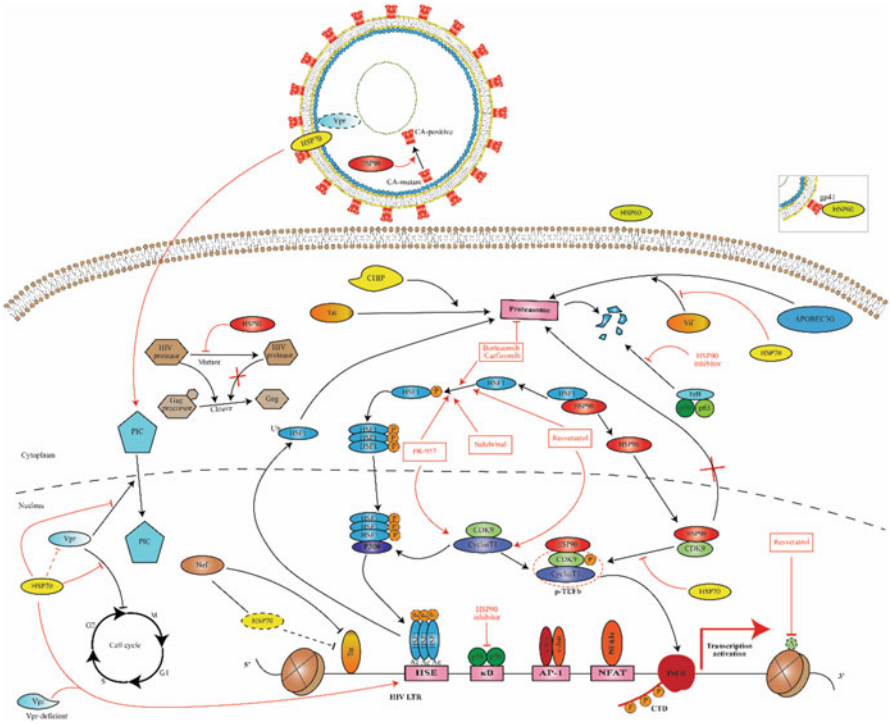



**Fig. 3 The role of HSF1 and Hsp in HIV infection, replication, integration, and latent reversing.** (1) HSF1 mediates latent HIV reversing. Phosphorylated HSF1 forms a trimer and enters the nucleus, recruits p300 to promote self-acetylation, further binds to HIV LTR to promote the initiation of HIV DNA transcription. Meanwhile, HSF1 recruits p-TEFb to generate transcriptional elongation. Moreover, HSF1 promotes the expression of Hsp90 to protect p-TEFb subunit CDK9 from ubiquitination degradation and gives positive feedback to HSF1 regulation. PR-957, Salubrinal, Resveratrol, Bortezomib/Carfilzomib (red box with red arrow) act on this process. (2) Hsp90 (red ellipse) positively regulates HIV infection and transcription. Hsp90 reverses mutations in HIV protease in the cytoplasm and protects CDK9 from degradation. Hsp90 inhibition (red latter with red line) inhibits IκBα degradation thus suppress NF-κB pathway. Meanwhile, Hsp90 reverses the CA-mutant and protects the infectivity of HIV, which occurs between the viral membrane and core. (3) Hsp70 (bright yellow ellipse) inhibits HIV replication by interacting with viral proteins. Hsp70 inhibits Vpr-mediated PIC (light blue pentagon) nuclear translocation and host $G_2$ cycle arrest, maintains negative regulation of Nef, inhibits Vif-mediated APOBEC3G (deep blue ellipse) ubiquitination and degradation, and inhibits CDK9 phosphorylation to downregulate p-TEFb activation. All these approaches suppress HIV transcription and replication. However, Hsp70 promotes nuclear translocation of viral PIC and accelerates the replication of HIV when Vpr protein is deficient or deleted. (4) Hsp60 (deep yellow ellipse) exists in both intracellular and extracellular matrices and binds to HIV gp41 (at the upper right corner)

proliferation activated receptor γ (PPARγ)-ubiquitination pathway [153]; (d) Hsp70 modulates ATPase activity and its C-terminal helix subdomain [69]. In addition, Hsp60 has a negative function on mediating the ubiquitin-like protein monoclonal nonspecific suppressor factor β (MNSF-β) in macrophages [142], which was confirmed in other reports [98].

## 4.2 Roles of Hsp in HIV

Hsp is multifunctional in diseases such as cancer and virus infection. Actually, not all Hsp are associated with the infection, replication and reactivation of HIV, except for Hsp90, Hsp70 and Hsp60 [137]. Among these, Hsp90 is involved in HIV replication and reactivation by positively regulating p-TEFb, NF-κB activation and viral gene repair, while Hsp70 inhibits HIV transcription and replication through interacting with viral associated factors (Vpr, Nef, and p-TEFb etc.), and Hsp60 mainly participates in HIV membrane fusion and integration.

### 4.2.1 Hsp90 Is a Positive Regulator for HIV-1 Transcription

Studies have shown that hyperthermia can promote HIV replication in primary $CD4^+$ T lymphocytes and cell lines, through increasing Tat transactivation on LTR instead of facilitating viral entry or reverse transcription. This may be mainly attributed to Hsp90, because Hsp90 inhibitor can reverse the HIV replication induced by hyperthermia [165]. Another two studies suggested that hyperthermia activates HIV gene expression in HIV latency cell models effectively, which appears to be related to the binding of NF-κB to HIV LTR [77, 183]. Further studies have found that the regulation of Hsp90 in HIV also has something to do with p-TEFb complex, NF-κB and capsid protein.

Hsp90 functions in HIV mainly by interacting with p-TEFb complexes. P-TEFb is the central regulator in transcriptional elongation in cells. However, HIV Tat protein recruits p-TEFb to HIV promoter [24, 68]. Hsp90 binds CDK9 to stabilize it until assembling of active CDK9/CyclinT1 dimers [19, 143]. As previously mentioned, Hsp90 acts as a molecular chaperone of CDK9 to protect it from proteasomal degradation, and activates p-TEFb through sending positive feedback to HSF1 in modulating HIV reservoir [146, 147].

Hsp90 modulates latent HIV reactivation associated with NF-κB pathway. NF-κB pathway is one of the classical pathways for latent HIV reactivation [129]. After investigating the relationship between Hsp90 inhibitors and classical latent HIV reactivation pathway, inhibition of Hsp90 was found to reduce IκBα degradation and block nuclear translocation of transcription factor, thereby inhibiting NF-κB pathway [11]. Surprisingly, NF-κB and Hsp90 are essential for HIV protein expression [211]. But a study found that the mRNA level of Hsp90 in AIDS individuals was 1.43 fold lower than that in healthy individuals through comparative genomics [176].

Hsp90 promotes HIV survival through maintaining the core function of viral capsid. Mutations in HIV protease lead to the reduction of its enzyme activity, which finally results in the accumulation of uncleaved Gag precursor. This is the reason why HIV has drug resistance to Ritonavir [95, 130]. However, the persistent inhibition on HIV can be reversed in targeted cells, since Hsp90 reverses these mutations to improve viral adaptability and replication [95]. Accumulating

evidences revealed that Hsp90 locates between viral membrane and core, thereby reversing capsid-mutant (CA-mutant) virus from loss of infectivity [96]. In general, Hsp90 may be a turning point for HIV survival and drug resistance, which may provide new ideas to solve the problem of HAART resistance.

### 4.2.2 Hsp70 Is a Repressor for HIV Replication

During HIV infection, viral proteins modulate life cycle of both virus and host cell. Vpr which is highly conserved in HIV-1 meditates the entry of pre-integration complex (PIC) into the nucleus and induces host cell cycle G2 arrest [192, 215]. Definitely, Hsp70 can reverse G2 arrest and apoptosis of host cells caused by Vpr-induced HIV replication [31, 88, 89], and it also inhibits nuclear translocation of Vpr [88, 89]. These co-regulations make Hsp70 suppress HIV in the presence of Vpr. Hsp70 is instantly induced after HIV infection in macrophages and highly express in HIV-infected individuals after HAART [58, 88, 89]. These data suggested that Hsp70 may be an innate antiviral factor. Interestingly, Hsp70 can promote replication of Vpr-deficient HIV, while the replication increases in Vpr-positive HIV instead of in Vpr-deficient HIV when Hsp70 is inhibited [88, 89]. Studies have shown that Hsp70 can be incorporated into HIV viral membranes [74], and competes with Vpr to bind karyopherin α to facilitate nuclear translocation of PICs [2]. These evidences collectively proved that (a) Vpr has similar biological activity as Hsp70, guaranteeing HIV survival with mutant Vpr; (b) Vpr competes with Hsp70 to bind the same site which is high affinity to Vpr than Hsp70; (c) Hsp70 may directly interacts with Vpr to inactivate Vpr.

Hsp70 suppresses HIV by inhibiting HIV transcription. HIV Nef extensively expresses in the early stage of HIV infection, and promotes HIV replication through maintaining a host environment in favor of viral replication and pathopoiesis, such as changing the expression of cell membrane proteins and destroying host immunity [30, 100]. HIV Tat is responsible for viral propagation, which mediates the transcription of viral genes by binding LTR [15]. Nef inhibits LTR-driven transcription only in the presence of Tat, while it promotes proteasomal degradation of Tat in the cytoplasm [184, 185]. And the host cell E3 ubiquitin ligase protein CHIP which is the carboxy terminus of Hsp70-binding protein can mediate Tat degradation by proteasome [5]. However, the depletion of Hsp70 relieves the suppression of Nef on HIV-1 replication [184, 185]. While Hsp40, the co-chaperone of Hsp70 [124], is required for Nef-mediated viral gene expression and replication [109]. Furthermore, Hsp70 inhibits the phosphorylation of CDK9 in p-TEFb complex to suppress HIV transcription [19, 110]. Another study suggested that Hsp70 protects apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G (APOBEC3G), which is an innate restriction factor for HIV [87, 149], from being ubiquitinated and degraded by HIV-1 Vif [184, 185]. In fact, it is demonstrated that HIV can be inhibited by Hsp70 which is from human cells or microbials. [18, 36].

### 4.2.3 Hsp60 Participates in Membrane Fusion and Integration in HIV Infection

HIV is a member of the family Retroviridae and belongs to the lentivirus genus. When HIV attached to host cell, HIV gp120 recognized CD4 and co-receptor (CCR5 or CXCR4) as targets and membrane fusion occurs upon structural clues from gp41, thereby viral genome is released into the cytoplasm. As previously mentioned, HIV provrial DNA integration is essential step for HIV propagation. Hsp60 is a molecular chaperone that mainly locates in the mitochondria, cytoplasm, cell surface, and even extracellular space and plasma [156, 179].

Bartz et al. firstly observed that Hsp60 is induced in HIV or SIV infected cells [21]. A study on 295 sera from 132 HIV-infected individuals showed that C1qAb and Hsp60 autoantibodies exist in some of the sera, and C1q levels are strongly correlated with that of Hsp60 [157]. Meanwhile, C1q has been reported to combine with gp41 [190]. These data suggested that Hsp60 may be related to the function of gp41. Speth et al. provided the evidence for this hypothesis [182], and they demonstrated that Hsp60 tightly binds p18 which is a soluble fragment of gp41, whereas, Hsp60 hardly binds gp120. In spite of that, there is no definitive evidence to prove where the complex of Hsp60 and gp41 locate in during protein synthesis, cell membrane or endoplasmic reticulum [203].

Researches on Hsp60 in HIV integration have shown that Hsp60 interacts with HIV-1 integrase. Besides, Hsp60 can stimulate the processing and ligation activity of integrase in vitro, as well as protecting the enzyme from thermal denaturation. The presence of Hsp60-Hsp10 complex in ATP is able to identify the HIV-1 integrase as a substrate [151]. What's more, the level of circulating Hsp60 in HIV-infected individuals is higher than that in HIV-negative individuals, while the level of Hsp10 seems no differences between these two groups. In particular, circulating Hsp60 is significantly correlated with viral load, CD4 counts, soluble CD14, and lipopolysaccharide (LPS) levels [12]. These data imply that: (a) Hsp60 serves as an important target in the process of HIV infection, and it is necessary to target precisely when combining with gp41; (b) Hsp60 may be a potential HIV reservoir or a potential target for LRAs.

## 5 HSF1 and Inflammation Caused by HIV-1

HIV infection is accompanied by HSR. Although HIV is restrained by HAART effectively, HIV-infected individuals still suffer from chronic inflammation [79]. Chronic inflammation can lead to chronic immune activation, which in turn leads to impairment on the function of innate immune, T cell, and B cells [43]. In recent years, many studies have shown that HIV-induced inflammation is a major cause of the depletion for $CD4^+$ T cell in lymphoid tissues [13]. Therefore, understanding the activation mechanism of HIV-induced inflammation is critical to control the progression of AIDS. As we know, HIV infection accompanied with HSR [50] is closely related to HSF1 [162]. Pan et al. found that HIV infection

induces the production of inflammatory cytokines in THP-1 accompanied by activation of HSF1. And activated HSF1 inhibits the production of inflammatory cytokines, while HSF1 deficiency augments that. [148]. Furthermore, HSF1 is demonstrated to suppress the inflammatory response via competing with NF-κB to bind HIV LTR in the nuclear [148].

## 6 Conclusions

In summary, HSF1 as well as its downstream Hsp engaged in the infection significantly, integration, replication, and reactivation, throughout the whole life cycle of HIV. HSF1 reverses HIV reservoir under stress response through promoting Hsp90 to facilitate self-activation meanwhile inhibiting HIV-induced inflammatory response. Furthermore, Hsp90 and Hsp60 positively regulate HIV-1 transcription and infection through protecting CDK9 from proteasomal degradation thus maintaining p-TEFb complex, promoting NF-κB function, reversing viral capsids to improve viral adaptability and replication, and binding gp41 to protect integrase. By contrast, Hsp70 regulates negatively HIV-1 through inhibiting nuclear translocation, arresting cell cycle, suppressing p-TEFb activity to block transcriptional elongation, and enhancing inhibition of Nef on HIV transcription. Although these information highlight HSF1 as important role in HIV infection, the underlying impact of HSR on HIV and viral infection is complex and it is deserved to be further investigated (Fig. 3).

**Acknowledgements** Authors thank Dr. Shibo Jiang at Fudan University, China, for providing the essential cell lines used in the experiment. This study was funded by Natural Science of China (grant number 31370781 and 81773787), the National S&T Key Special foundation (2014ZX09509001-004), the Natural Science Foundation of Guangdong Province (S201102005207) and National Science and Technology Major Project (2018ZX10301101).

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Abdel-Hameed EA, Ji H, Shata MT (2016) HIV-induced epigenetic alterations in host cells. Adv Exp Med Biol 879:27–38
2. Agostini I, Popov S, Li J, Dubrovsky L, Hao T, Bukrinsky M (2000) Heat-shock protein 70 can replace viral protein R of HIV-1 during nuclear import of the viral preintegration complex. Exp Cell Res 259(2):398–403

3. Akerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 11(8):545–555
4. Al-Harti L, Joseph J, Nath A (2018) Astrocytes as an HIV CNS reservoir: highlights and reflections of an NIMH-sponsored symposium. J Neurovirol 24(6):665–669
5. Ali A, Farooqui SR, Banerjea AC (2019) The host cell ubiquitin ligase protein CHIP is a potent suppressor of HIV-1 replication. J Biol Chem 294(18):7283–7295
6. Allen AG, Chung CH, Atkins A, Dampier W, Khalili K, Nonnemacher MR, Wigdahl B (2018) Gene editing of HIV-1 co-receptors to prevent and/or cure virus infection. Front Microbiol 9:2940
7. Alvarez-Carbonell D, Garcia-Mesa Y, Milne S, Das B, Dobrowolski C, Rojas R, Karn J (2017) Toll-like receptor 3 activation selectively reverses HIV latency in microglial cells. Retrovirology 14(1):9
8. Alvarez-Carbonell D, Ye FC, Ramanath N, Dobrowolski C, Karn J (2019) The glucocorticoid receptor is a critical regulator of HIV latency in human microglial cells. J Neuroimmune Pharmacol 14(1):94–109
9. Anand D, Agrawal SA, Slavotinek A, Lachke SA (2018) Mutation update of transcription factor genes FOXE3, HSF4, MAF, and PITX3 causing cataracts and other developmental ocular defects. Hum Mutat 39(4):471–494
10. Anckar J, Hietakangas V, Denessiouk K, Thiele DJ, Johnson MS, Sistonen L (2006) Inhibition of DNA binding by differential sumoylation of heat shock factors. Mol Cell Biol 26 (3):955–964
11. Anderson I, Low JS, Weston S, Weinberger M, Zhyvoloup A, Labokha AA, Corazza G, Kitson RA, Moody CJ, Marcello A, Fassati A (2014) Heat shock protein 90 controls HIV-1 reactivation from latency. Proc Natl Acad Sci U S A 111(15):E1528–E1537
12. Anraku I, Rajasuriar R, Dobbin C, Brown R, Lewin SR, Suhrbier A (2012) Circulating heat shock protein 60 levels are elevated in HIV patients and are reduced by anti-retroviral therapy. PLoS One 7(9):e45291
13. Aounallah M, Dagenais-Lussier X, El-Far M, Mehraj V, Jenabian MA, Routy JP, van Grevenynghe J (2016) Current topics in HIV pathogenesis, part 2: inflammation drives a Warburg-like effect on the metabolism of HIV-infected subjects. Cytokine Growth Factor Rev 28:1–10
14. Arrizabalaga J, Arazo P, Aguirrebengoa K, Garcia-Palomo D, Chocarro A, Labarga P, Munoz-Sanchez MJ, Echevarria S, Oteo JA, Uriz J, Letona S, Farinas MC, Peralta G, Pinilla J, Ferrer P, Alvarez ML, Iribarren JA (2007) Effectiveness and safety of simplification therapy with once-daily tenofovir, lamivudine, and efavirenz in HIV-1-infected patients with undetectable plasma viral load on HAART. HIV Clin Trials 8(5):328–336
15. Asamitsu K, Fujinaga K, Okamoto T (2018) HIV tat/P-TEFb interaction: a potential target for novel anti-HIV therapies. Molecules 23(4)
16. Avvakumov N, Nourani A, Cote J (2011) Histone chaperones: modulators of chromatin marks. Mol Cell 41(5):502–514
17. Ay E, Banati F, Mezei M, Bakos A, Niller HH, Buzas K, Minarovits J (2013) Epigenetics of HIV infection: promising research areas and implications for therapy. AIDS Rev 15 (3):181–188
18. Babaahmady K, Oehlmann W, Singh M, Lehner T (2007) Inhibition of human immunodeficiency virus type 1 infection of human CD4+ T cells by microbial HSP70 and the peptide epitope 407-426. J Virol 81(7):3354–3360
19. Bacon CW, D'Orso I (2019) CDK9: a signaling hub for transcriptional control. Transcription 10(2):57–75
20. Bartholomeeusen K, Fujinaga K, Xiang Y, Peterlin BM (2013) Histone deacetylase inhibitors (HDACis) that release the positive transcription elongation factor b (P-TEFb) from its inhibitory complex also activate HIV transcription. J Biol Chem 288(20):14400–14407
21. Bartz SR, Pauza CD, Ivanyi J, Jindal S, Welch WJ, Malkovsky M (1994) An Hsp60 related protein is associated with purified HIV and SIV. J Med Primatol 23(2–3):151–154

22. Bernhard W, Barreto K, Saunders A, Dahabieh MS, Johnson P, Sadowski I (2011) The Suv39H1 methyltransferase inhibitor chaetocin causes induction of integrated HIV-1 without producing a T cell response. FEBS Lett 585(22):3549–3554
23. Bharadwaj S, Ali A, Ovsenek N (1999) Multiple components of the HSP90 chaperone complex function in regulation of heat shock factor 1 in vivo. Mol Cell Biol 19(12):8033–8041
24. Bieniasz PD, Grdina TA, Bogerd HP, Cullen BR (1998) Recruitment of a protein complex containing Tat and cyclin T1 to TAR governs the species specificity of HIV-1 Tat. EMBO J 17 (23):7056–7065
25. Bolhassani A, Agi E (2019) Heat shock proteins in infection. Clin Chim Acta 498:90–100
26. Bonner JJ, Heyward S, Fackenthal DL (1992) Temperature-dependent regulation of a heterologous transcriptional activation domain fused to yeast heat shock transcription factor. Mol Cell Biol 12(3):1021–1030
27. Bonner JJ, Chen D, Storey K, Tushan M, Lea K (2000) Structural analysis of yeast HSF by site-specific crosslinking. J Mol Biol 302(3):581–592
28. Bosque A, Planelles V (2009) Induction of HIV-1 latency and reactivation in primary memory CD4+ T cells. Blood 113(1):58–65
29. Brunet Simioni M, De Thonel A, Hammann A, Joly AL, Bossis G, Fourmaux E, Bouchot A, Landry J, Piechaczyk M, Garrido C (2009) Heat shock protein 27 is involved in SUMO-2/3 modification of heat shock factor 1 and thereby modulates the transcription factor activity. Oncogene 28(37):3332–3344
30. Buffalo CZ, Iwamoto Y, Hurley JH, Ren X (2019) How HIV Nef proteins hijack membrane traffic to promote infection. J Virol
31. Bukrinsky M, Zhao Y (2004) Heat-shock proteins reverse the G2 arrest caused by HIV-1 viral protein R. DNA Cell Biol 23(4):223–225
32. Burdon RH, Slater A, McMahon M, Cato AC (1982) Hyperthermia and the heat-shock proteins of HeLa cells. Br J Cancer 45(6):953–963
33. Cao Z, Zhu Y, Liu L, Wu S, Liu B, Zhuang J, Tong Y, Chen X, Xie Y, Nie K, Lu C, Ma X, Yang J (2018) Novel mutations in HSF4 cause congenital cataracts in Chinese families. BMC Med Genet 19(1):150
34. Carrillo J, Clotet B, Blanco J (2018) Antibodies and antibody derivatives: new partners in HIV eradication strategies. Front Immunol 9:2429
35. Cary DC, Fujinaga K, Peterlin BM (2016) Molecular mechanisms of HIV latency. J Clin Invest 126(2):448–454
36. Chaudhary P, Khan SZ, Rawat P, Augustine T, Raynes DA, Guerriero V, Mitra D (2016) HSP70 binding protein 1 (HspBP1) suppresses HIV-1 replication by inhibiting NF-kappaB mediated activation of viral gene expression. Nucleic Acids Res 44(4):1613–1629
37. Chen Y, Barlev NA, Westergaard O, Jakobsen BK (1993) Identification of the C-terminal activator domain in yeast heat shock factor: independent control of transient and sustained transcriptional activity. EMBO J 12(13):5007–5018
38. Cheng X, Belshan M, Ratner L (2008) Hsp40 facilitates nuclear import of the human immunodeficiency virus type 2 Vpx-mediated preintegration complex. J Virol 82 (3):1229–1237
39. Chou SD, Prince T, Gong J, Calderwood SK (2012) mTOR is essential for the proteotoxic stress response, HSF1 activation and heat shock protein synthesis. PLoS One 7(6):e39679
40. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, Lloyd AL, Nowak MA, Fauci AS (1997) Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci U S A 94(24):13193–13197
41. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS (1998) Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proc Natl Acad Sci U S A 95(15):8869–8873
42. Contreras X, Barboric M, Lenasi T, Peterlin BM (2007) HMBA releases P-TEFb from HEXIM1 and 7SK snRNA via PI3K/Akt and activates HIV transcription. PLoS Pathog 3 (10):1459–1469

43. Cribbs SK, Crothers K, Morris A (2019) Pathogenesis of HIV-related lung disease: immunity, infection, and inflammation. Physiol Rev
44. Cui X, Han J, Li J, Cui WW, Wu DD, Liu S, Xue W, Wang XX, Ma Y, Zhang J, Zhang J, Mu H, Zhang F, Hu Y (2018) Downregulation of heat shock factor 4 transcription activity via MAPKinase phosphorylation at serine 299. Int J Biochem Cell Biol 105:61–69
45. Cunningham AL, Dwyer DE, Mills J, Montagnier L (1996) Managing HIV. Part 3: mechanisms of disease. 3.1 structure and function of HIV. Med J Aust 164(3):161–165
46. Dai S, Tang Z, Cao J, Zhou W, Li H, Sampson S, Dai C (2015) Suppression of the HSF1-mediated proteotoxic stress response by the metabolic stress sensor AMPK. EMBO J 34 (3):275–293
47. Daul CB, deShazo RD (1983) Acquired immune deficiency syndrome: an update and interpretation. Ann Allergy 51(3):351–361
48. Dayalan Naidu S, Dinkova-Kostova AT (2017) Regulation of the mammalian heat shock factor 1. FEBS J 284(11):1606–1627
49. Dayalan Naidu S, Sutherland C, Zhang Y, Risco A, de la Vega L, Caunt CJ, Hastie CJ, Lamont DJ, Torrente L, Chowdhry S, Benjamin IJ, Keyse SM, Cuenda A, Dinkova-Kostova AT (2016) Heat shock factor 1 is a substrate for p38 mitogen-activated protein kinases. Mol Cell Biol 36(18):2403–2417
50. de Marco A, Carattoli A, Rozera C, Fortini D, Giorgi C, Belardo G, Amici C, Santoro MG (1998) Induction of the heat-shock response by antiviral prostaglandins in human cells infected with human immunodeficiency virus type 1. Eur J Biochem 256(2):334–341
51. Deeks SG (2012) HIV: shock and kill. Nature 487(7408):439–440
52. Donahue DA, Wainberg MA (2013) Cellular and molecular mechanisms involved in the establishment of HIV-1 latency. Retrovirology 10:11
53. Drees BL, Grotkopp EK, Nelson HC (1997) The GCN4 leucine zipper can functionally substitute for the heat shock transcription factor's trimerization domain. J Mol Biol 273 (1):61–74
54. Dubrez L, Causse S, Borges Bonan N, Dumetier B, Garrido C (2019) Heat-shock proteins: chaperoning DNA repair. Oncogene
55. Dukay B, Csoboz B, Toth ME (2019) Heat-shock proteins in neuroinflammation. Front Pharmacol 10:920
56. Eilebrecht S, Benecke BJ, Benecke AG (2017) Latent HIV-1 TAR regulates 7SK-responsive P-TEFb target genes and targets cellular immune responses in the absence of tat. Genomics Proteomics Bioinformatics 15(5):313–323
57. Elsing AN, Aspelin C, Bjork JK, Bergman HA, Himanen SV, Kallio MJ, Roos-Mattjus P, Sistonen L (2014) Expression of HSF2 decreases in mitosis to enable stress-inducible transcription and cell survival. J Cell Biol 206(6):735–749
58. Espigares E, Bueno A, Hernandez J, Garcia F, Luna JD, Espigares M, Galvez R (2006) Levels of HSP70 in HIV(+) patients in different viroimmunological states. J Med Virol 78 (3):318–323
59. Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243–282
60. Feng H, Wang S, Guo L, Punekar AS, Ladenstein R, Wang DC, Liu W (2016) MD simulation of high-resolution X-ray structures reveals post-translational modification dependent conformational changes in HSF-DNA interaction. Protein Cell 7(12):916–920
61. Fernandez G, Zeichner SL (2010) Cell line-dependent variability in HIV activation employing DNMT inhibitors. Virol J 7:266
62. Filone CM, Caballero IS, Dower K, Mendillo ML, Cowley GS, Santagata S, Rozelle DK, Yen J, Rubins KH, Hacohen N, Root DE, Hensley LE, Connor J (2014) The master regulator of the cellular stress response (HSF1) is critical for orthopoxvirus infection. PLoS Pathog 10 (2):e1003904

63. Finlayson-Trick E, Connors J, Stadnyk A, Limbergen JV (2018) Regulation of antimicrobial pathways by endogenous heat shock proteins in gastrointestinal disorders. Gastrointest Dis 1 (1):39–56
64. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF (1997) Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 278(5341):1295–1300
65. Fritah S, Col E, Boyault C, Govin J, Sadoul K, Chiocca S, Christians E, Khochbin S, Jolly C, Vourc'h C (2009) Heat-shock factor 1 controls genome-wide acetylation in heat-shocked cells. Mol Biol Cell 20(23):4976–4984
66. Fujimoto M, Nakai A (2010) The heat shock factor family and adaptation to proteotoxic stress. FEBS J 277(20):4112–4125
67. Fujimoto M, Takaki E, Takii R, Tan K, Prakasam R, Hayashida N, Iemura S, Natsume T, Nakai A (2012) RPA assists HSF1 access to nucleosomal DNA by recruiting histone chaperone FACT. Mol Cell 48(2):182–194
68. Fujinaga K, Taube R, Wimmer J, Cujec TP, Peterlin BM (1999) Interactions between human cyclin T, Tat, and the transactivation response element (TAR) are disrupted by a cysteine to tyrosine substitution found in mouse cyclin T. Proc Natl Acad Sci U S A 96(4):1285–1290
69. Gao XC, Zhou CJ, Zhou ZR, Wu M, Cao CY, Hu HY (2012) The C-terminal helices of heat shock protein 70 are essential for J-domain binding and ATPase activation. J Biol Chem 287 (8):6044–6052
70. Gelmedin V, Delaney A, Jennelle L, Hawdon JM (2015) Expression profile of heat shock response factors during hookworm larval activation and parasitic development. Mol Biochem Parasitol 202(1):1–14
71. Goodson ML, Hong Y, Rogers R, Matunis MJ, Park-Sarge OK, Sarge KD (2001) Sumo-1 modification regulates the DNA binding activity of heat shock transcription factor 2, a promyelocytic leukemia nuclear body associated transcription factor. J Biol Chem 276 (21):18513–18518
72. Green M, Schuetz TJ, Sullivan EK, Kingston RE (1995) A heat shock-responsive domain of human HSF1 that regulates transcription activation domain function. Mol Cell Biol 15 (6):3354–3362
73. Guettouche T, Boellmann F, Lane WS, Voellmy R (2005) Analysis of phosphorylation of human heat shock factor 1 in cells experiencing a stress. BMC Biochem 6:4
74. Gurer C, Cimarelli A, Luban J (2002) Specific incorporation of heat shock protein 70 family members into primate lentiviral virions. J Virol 76(9):4666–4670
75. Hakre S, Chavez L, Shirakawa K, Verdin E (2011) Epigenetic regulation of HIV latency. Curr Opin HIV AIDS 6(1):19–24
76. Harrison CJ, Bohm AA, Nelson HC (1994) Crystal structure of the DNA binding domain of the heat shock transcription factor. Science 263(5144):224–227
77. Hashimoto K, Baba M, Gohnai K, Sato M, Shigeta S (1996) Heat shock induces HIV-1 replication in chronically infected promyelocyte cell line OM10.1. Arch Virol 141 (3–4):439–447
78. Hietakangas V, Ahlskog JK, Jakobsson AM, Hellesuo M, Sahlberg NM, Holmberg CI, Mikhailov A, Palvimo JJ, Pirkkala L, Sistonen L (2003) Phosphorylation of serine 303 is a prerequisite for the stress-inducible SUMO modification of heat shock factor 1. Mol Cell Biol 23(8):2953–2968
79. Hileman CO, Funderburg NT (2017) Inflammation, immune activation, and antiretroviral therapy in HIV. Curr HIV/AIDS Rep 14(3):93–100
80. Hilgarth RS, Sarge KD (2005) Detection of sumoylated proteins. Methods Mol Biol 301:329–338
81. Hilgarth RS, Murphy LA, O'Connor CM, Clark JA, Park-Sarge OK, Sarge KD (2004) Identification of Xenopus heat shock transcription factor-2: conserved role of sumoylation in

regulating deoxyribonucleic acid-binding activity of heat shock transcription factor-2 proteins. Cell Stress Chaperones 9(2):214–220
82. Hong Y, Rogers R, Matunis MJ, Mayhew CN, Goodson ML, Park-Sarge OK, Sarge KD (2001) Regulation of heat shock transcription factor 1 by stress-induced SUMO-1 modification. J Biol Chem 276(43):40263–40267
83. Hotter D, Bosso M, Jonsson KL, Krapp C, Sturzel CM, Das A, Littwitz-Salomon E, Berkhout B, Russ A, Wittmann S, Gramberg T, Zheng Y, Martins LJ, Planelles V, Jakobsen MR, Hahn BH, Dittmer U, Sauter D, Kirchhoff F (2019) IFI16 targets the transcription factor Sp1 to suppress HIV-1 transcription and latency reactivation. Cell Host Microbe 25 (6):858–872 e813
84. Hu YZ, Zhang J, Li S, Wang C, Chu L, Zhang Z, Ma Z, Wang M, Jiang Q, Liu G, Qi Y, Ma Y (2013) The transcription activity of heat shock factor 4b is regulated by FGF2. Int J Biochem Cell Biol 45(2):317–325
85. Huang CB, Alimova YV, Strange S, Ebersole JL (2011) Polybacterial challenge enhances HIV reactivation in latently infected macrophages and dendritic cells. Immunology 132 (3):401–409
86. Huang CY, Kuo CH, Pai PY, Ho TJ, Lin YM, Chen RJ, Tsai FJ, Vijaya Padma V, Kuo WW, Huang CY (2018) Inhibition of HSF2 SUMOylation via MEL18 upregulates IGF-IIR and leads to hypertension-induced cardiac hypertrophy. Int J Cardiol 257:283–290
87. Ikeda T, Molan AM, Jarvis MC, Carpenter MA, Salamango DJ, Brown WL, Harris RS (2019) HIV-1 restriction by endogenous APOBEC3G in the myeloid cell line THP-1. J Gen Virol 100 (7):1140–1152
88. Iordanskiy S, Zhao Y, Dubrovsky L, Iordanskaya T, Chen M, Liang D, Bukrinsky M (2004a) Heat shock protein 70 protects cells from cell cycle arrest and apoptosis induced by human immunodeficiency virus type 1 viral protein R. J Virol 78(18):9697–9704
89. Iordanskiy S, Zhao Y, DiMarzio P, Agostini I, Dubrovsky L, Bukrinsky M (2004b) Heat-shock protein 70 exerts opposing effects on Vpr-dependent and Vpr-independent HIV-1 replication in macrophages. Blood 104(6):1867–1872
90. Izumi M (2019) Heat shock proteins support refolding and shredding of misfolded proteins. Plant Physiol 180(4):1777–1778
91. Jacob P, Hirt H, Bendahmane A (2017) The heat-shock protein/chaperone network and multiple stress resistance. Plant Biotechnol J 15(4):405–414
92. Jaeger AM, Pemble CW t, Sistonen L, Thiele DJ (2016) Structures of HSF2 reveal mechanisms for differential regulation of human heat-shock factors. Nat Struct Mol Biol 23 (2):147–154
93. Jiang G, Dandekar S (2015) Targeting NF-kappaB signaling with protein kinase C agonists as an emerging strategy for combating HIV latency. AIDS Res Hum Retrovir 31(1):4–12
94. Jiang S, Wu B, Jiang L, Zhang M, Lu Y, Wang S, Yan F, Xin X (2019) Triticum aestivum heat shock protein 23.6 interacts with the coat protein of wheat yellow mosaic virus. Virus Genes 55(2):209–217
95. Joshi P, Stoddart CA (2011) Impaired infectivity of ritonavir-resistant HIV is rescued by heat shock protein 90AB1. J Biol Chem 286(28):24581–24592
96. Joshi P, Sloan B, Torbett BE, Stoddart CA (2013) Heat shock protein 90AB1 and hyperthermia rescue infectivity of HIV with defective cores. Virology 436(1):162–172
97. Joshi P, Maidji E, Stoddart CA (2016) Inhibition of heat shock protein 90 prevents HIV rebound. J Biol Chem 291(19):10332–10346
98. Kalderon B, Kogan G, Bubis E, Pines O (2015) Cytosolic Hsp60 can modulate proteasome activity in yeast. J Biol Chem 290(6):3542–3551
99. Kessing CF, Nixon CC, Li C, Tsai P, Takata H, Mousseau G, Ho PT, Honeycutt JB, Fallahi M, Trautmann L, Garcia JV, Valente ST (2017) In vivo suppression of HIV rebound by didehydro-cortistatin A, a "block-and-lock" strategy for HIV-1 treatment. Cell Rep 21 (3):600–611

100. Kestler HW 3rd, Ringler DJ, Mori K, Panicali DL, Sehgal PK, Daniel MD, Desrosiers RC (1991) Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell 65(4):651–662
101. Khachatoorian R, Cohn W, Buzzanco A, Riahi R, Arumugaswami V, Dasgupta A, Whitelegge JP, French SW (2018) HSP70 Copurifies with Zika virus particles. Virology 522:228–233
102. Khoury G, Mota TM, Li S, Tumpach C, Lee MY, Jacobson J, Harty L, Anderson JL, Lewin SR, Purcell DFJ (2018a) HIV latency reversing agents act through tat post translational modifications. Retrovirology 15(1):36
103. Khoury G, Darcis G, Lee MY, Bouchat S, Van Driessche B, Purcell DFJ, Van Lint C (2018b) The molecular biology of HIV latency. Adv Exp Med Biol 1075:187–212
104. Kim SA, Yoon JH, Lee SH, Ahn SG (2005) Polo-like kinase 1 phosphorylates heat shock transcription factor 1 and mediates its nuclear translocation during heat stress. J Biol Chem 280 (13):12653–12657
105. Klaus BD, Grodesky MJ (1997) HIV and HAART in 1997. Highly active antiretroviral therapy. Nurse Pract 22(8):139–142
106. Kong FC, Ma CL, Zhong MK (2019) Epigenetic effects mediated by antiepileptic drugs and their potential application. Curr Neuropharmacol
107. Kourtis N, Moubarak RS, Aranda-Orgilles B, Lui K, Aydin IT, Trimarchi T, Darvishian F, Salvaggio C, Zhong J, Bhatt K, Chen EI, Celebi JT, Lazaris C, Tsirigos A, Osman I, Hernando E, Aifantis I (2015) FBXW7 modulates cellular stress response and metastatic potential through HSF1 post-translational modification. Nat Cell Biol 17(3):322–332
108. Kuhnel A, Schilling D, Combs SE, Haller B, Schwab M, Multhoff G (2019) Radiosensitization of HSF-1 knockdown lung cancer cells by low concentrations of Hsp90 Inhibitor NVP-AUY922. Cells 8(10)
109. Kumar M, Mitra D (2005) Heat shock protein 40 is necessary for human immunodeficiency virus-1 Nef-mediated enhancement of viral gene expression and replication. J Biol Chem 280 (48):40041–40050
110. Kumar M, Rawat P, Khan SZ, Dhamija N, Chaudhary P, Ravi DS, Mitra D (2011) Reciprocal regulation of human immunodeficiency virus-1 gene expression and replication by heat shock proteins 40 and 70. J Mol Biol 410(5):944–958
111. Lahaye X, Vidy A, Fouquet B, Blondel D (2012) Hsp70 protein positively regulates rabies virus infection. J Virol 86(9):4743–4751
112. Lampi Y, Van Looveren D, Vranckx LS, Thiry I, Bornschein S, Debyser Z, Gijsbers R (2019) Targeted editing of the PSIP1 gene encoding LEDGF/p75 protects cells against HIV infection. Sci Rep 9(1):2389
113. Lang BJ, Guerrero-Gimenez ME, Prince TL, Ackerman A, Bonorino C, Calderwood SK (2019) Heat shock proteins are essential components in transformation and tumor progression: cancer cell intrinsic pathways and beyond. Int J Mol Sci 20(18)
114. Larson JS, Schuetz TJ, Kingston RE (1995) In vitro activation of purified human heat shock factor by heat. Biochemistry 34(6):1902–1911
115. Li GC (1983) Induction of thermotolerance and enhanced heat shock protein synthesis in Chinese hamster fibroblasts by sodium arsenite and by ethanol. (0021-9541 (Print)): 115 (112):116–122
116. Li Z, Guo J, Wu Y, Zhou Q (2013) The BET bromodomain inhibitor JQ1 activates HIV latency through antagonizing Brd4 inhibition of Tat-transactivation. Nucleic Acids Res 41 (1):277–287
117. Li Z, Wu J, Chavez L, Hoh R, Deeks SG, Pillai SK, Zhou Q (2019) Reiterative enrichment and authentication of CRISPRi targets (REACT) identifies the proteasome as a key contributor to HIV-1 latency. PLoS Pathog 15(1):e1007498
118. Liang T, Zhang X, Lai F, Lin J, Zhou C, Xu X, Tan X, Liu S, Li L (2019) A novel bromodomain inhibitor, CPI-203, serves as an HIV-1 latency-reversing agent by activating positive transcription elongation factor b. Biochem Pharmacol 164:237–251

119. Liebelt F, Sebastian RM, Moore CL, Mulder MPC, Ovaa H, Shoulders MD, Vertegaal ACO (2019) SUMOylation and the HSF1-regulated chaperone network converge to promote proteostasis in response to heat shock. Cell Rep 26(1):236–249 e234
120. Lin J, Zhang X, Lu W, Xu X, Pan X, Liang T, Duan S, Chen Y, Li L, Liu S (2018) PR-957, a selective immunoproteasome inhibitor, reactivates latent HIV-1 through p-TEFb activation mediated by HSF-1. Biochem Pharmacol 156:511–523
121. Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191
122. Littlefield O, Nelson HC (1999) A new use for the 'wing' of the 'winged' helix-turn-helix motif in the HSF-DNA cocrystal. Nat Struct Biol 6(5):464–470
123. Liu R, Lin Y, Jia R, Geng Y, Liang C, Tan J, Qiao W (2014) HIV-1 Vpr stimulates NF-kappaB and AP-1 signaling by activating TAK1. Retrovirology 11:45
124. Liu Q, Liang C, Zhou L (2019) Structural and functional analysis of the Hsp70/Hsp40 chaperone system. Protein Sci
125. Loison F, Debure L, Nizard P, le Goff P, Michel D, le Drean Y (2006) Up-regulation of the clusterin gene after proteotoxic stress: implication of HSF1-HSF2 heterocomplexes. Biochem J 395(1):223–231
126. Lopez AP, Kugelman JR, Garcia-Rivera J, Urias E, Salinas SA, Fernandez-Zapico ME, Llano M (2016) The structure-specific recognition protein 1 associates with lens epithelium-derived growth factor proteins and modulates HIV-1 replication. J Mol Biol 428(14):2814–2831
127. Low JS, Fassati A (2014) Hsp90: a chaperone for HIV-1. Parasitology 141(9):1192–1202
128. Madlala P, Gijsbers R, Christ F, Hombrouck A, Werner L, Mlisana K, An P, Abdool Karim SS, Winkler CA, Debyser Z, Ndung'u T (2011) Association of polymorphisms in the LEDGF/p75 gene (PSIP1) with susceptibility to HIV-1 infection and disease progression. AIDS 25 (14):1711–1719
129. Madrid-Elena N, Garcia-Bermejo ML, Serrano-Villar S, Diaz-de Santiago A, Sastre B, Gutierrez C, Dronda F, Coronel Diaz M, Dominguez E, Lopez-Huertas MR, Hernandez-Novoa B, Moreno S (2018) Maraviroc is associated with latent HIV-1 reactivation through NF-kappaB activation in resting CD4(+) T cells from HIV-infected individuals on suppressive antiretroviral therapy. J Virol 92(9)
130. Mammano F, Petit C, Clavel F (1998) Resistance-associated loss of viral fitness in human immunodeficiency virus type 1: phenotypic analysis of protease and gag coevolution in protease inhibitor-treated patients. J Virol 72(9):7632–7637
131. Martine P, Rebe C (2019) Heat shock proteins and inflammasomes. Int J Mol Sci 20(18)
132. Martinez-Bonet M, Clemente MI, Alvarez S, Diaz L, Garcia-Alonso D, Munoz E, Moreno S, Munoz-Fernandez MA (2015) Antiretroviral drugs do not interfere with bryostatin-mediated HIV-1 latency reversal. Antivir Res 123:163–171
133. Mbonye U, Karn J (2014) Transcriptional control of HIV latency: cellular signaling pathways, epigenetics, happenstance and the hope for a cure. Virology 454–455:328–339
134. Merkling SH, Overheul GJ, van Mierlo JT, Arends D, Gilissen C, van Rij RP (2015) The heat shock response restricts virus infection in Drosophila. Sci Rep 5:12758
135. Morimoto RI (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev 12 (24):3788–3796
136. Morimoto RI, Kline MP, Bimston DN, Cotto JJ (1997) The heat-shock response: regulation and function of heat-shock proteins and molecular chaperones. Essays Biochem 32:17–29
137. Multhoff G (2006) Heat shock proteins in immunity. Handb Exp Pharmacol (172):279–304
138. Murshid A, Chou SD, Prince T, Zhang Y, Bharti A, Calderwood SK (2010) Protein kinase a binds and activates heat shock factor 1. PLoS One 5(11):e13830
139. Murshid A, Prince TL, Lang B, Calderwood SK (2018) Role of heat shock factors in stress-induced transcription. Methods Mol Biol 1709:23–34
140. Nakai A, Morimoto RI (1993) Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggests a new regulatory pathway. Mol Cell Biol 13 (4):1983–1997

141. Nakai A, Tanabe M, Kawazoe Y, Inazawa J, Morimoto RI, Nagata K (1997) HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator. Mol Cell Biol 17(1):469–481
142. Nakamura M, Notsu K, Nakagawa M (2019) Heat shock protein 60 negatively regulates the biological functions of ubiquitin-like protein MNSFbeta in macrophages. Mol Cell Biochem 456(1-2):29–39
143. O'Keeffe B, Fong Y, Chen D, Zhou S, Zhou Q (2000) Requirement for a kinase-specific chaperone pathway in the production of a Cdk9/cyclin T1 heterodimer responsible for P-TEFb-mediated tat stimulation of HIV-1 transcription. J Biol Chem 275(1):279–287
144. Ortner V, Ludwig A, Riegel E, Dunzinger S, Czerny T (2015) An artificial HSE promoter for efficient and selective detection of heat shock pathway activity. Cell Stress Chaperones 20 (2):277–288
145. Ostling P, Bjork JK, Roos-Mattjus P, Mezger V, Sistonen L (2007) Heat shock factor 2 (HSF2) contributes to inducible expression of hsp genes through interplay with HSF1. J Biol Chem 282(10):7077–7086
146. Pan XY, Zhao W, Zeng XY, Lin J, Li MM, Shen XT, Liu SW (2016a) Heat shock factor 1 mediates latent HIV reactivation. Sci Rep 6:26294
147. Pan XY, Zhao W, Wang CY, Lin J, Zeng XY, Ren RX, Wang K, Xun TR, Shai Y, Liu SW (2016b) Heat shock protein 90 facilitates latent HIV reactivation through maintaining the function of positive transcriptional elongation factor b (p-TEFb) under proteasome inhibition. J Biol Chem 291(50):26177–26187
148. Pan X, Lin J, Zeng X, Li W, Wu W, Lu WZ, Liu J, Liu S (2018) Heat shock factor 1 suppresses the HIV-induced inflammatory response by inhibiting nuclear factor-kappaB. Cell Immunol 327:26–35
149. Pan T, Song Z, Wu L, Liu G, Ma X, Peng Z, Zhou M, Liang L, Liu B, Liu J, Zhang J, Zhang X, Huang R, Zhao J, Li Y, Ling X, Luo Y, Tang X, Cai W, Deng K, Li L, Zhang H (2019) USP49 potently stabilizes APOBEC3G protein by removing ubiquitin and inhibits HIV-1 replication. elife 8
150. Panfil AR, London JA, Green PL, Yoder KE (2018) CRISPR/Cas9 genome editing to disable the latent HIV-1 provirus. Front Microbiol 9:3107
151. Parissi V, Calmels C, De Soultrait VR, Caumont A, Fournier M, Chaignepain S, Litvak S (2001) Functional interactions of human immunodeficiency virus type 1 integrase with human and yeast HSP60. J Virol 75(23):11344–11353
152. Peng W, Zhang Y, Zheng M, Cheng H, Zhu W, Cao CM, Xiao RP (2010) Cardioprotection by CaMKII-deltaB is mediated by phosphorylation of heat shock factor 1 and subsequent expression of inducible heat shock protein 70. Circ Res 106(1):102–110
153. Peng J, Li Y, Wang X, Deng S, Holland J, Yates E, Chen J, Gu H, Essandoh K, Mu X, Wang B, McNamara RK, Peng T, Jegga AG, Liu T, Nakamura T, Huang K, Perez-Tilve D, Fan GC (2018) An Hsp20-FBXO4 axis regulates adipocyte function through modulating PPARgamma ubiquitination. Cell Rep 23(12):3607–3620
154. Peppenelli MA, Miller MJ, Altman AM, Cojohari O, Chan GC (2018) Aberrant regulation of the Akt signaling network by human cytomegalovirus allows for targeting of infected monocytes. Antivir Res 158:13–24
155. Pierson T, McArthur J, Siliciano RF (2000) Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. Annu Rev Immunol 18:665–708
156. Poccia F, Piselli P, Vendetti S, Bach S, Amendola A, Placido R, Colizzi V (1996) Heat-shock protein expression on the membrane of T cells undergoing apoptosis. Immunology 88(1):6–12
157. Prohaszka Z, Daha MR, Susal C, Daniel V, Szlavik J, Banhegyi D, Nagy K, Varkonyi V, Horvath A, Ujhelyi E, Toth FD, Uray K, Hudecz F, Fust G (1999) C1q autoantibodies in HIV infection: correlation to elevated levels of autoantibodies against 60-kDa heat-shock proteins. Clin Immunol 90(2):247–255

158. Quagliarello V (1982) The acquired immunodeficiency syndrome: current status. Yale J Biol Med 55(5-6):443–452
159. Rabindran SK, Giorgi G, Clos J, Wu C (1991) Molecular cloning and expression of a human heat shock factor, HSF1. Proc Natl Acad Sci U S A 88(16):6906–6910
160. Rabindran SK, Haroun RI, Clos J, Wisniewski J, Wu C (1993) Regulation of heat shock factor trimer formation: role of a conserved leucine zipper. Science 259(5092):230–234
161. Rasmussen TA, Tolstrup M, Moller HJ, Brinkmann CR, Olesen R, Erikstrup C, Laursen AL, Ostergaard L, Sogaard OS (2015) Activation of latent human immunodeficiency virus by the histone deacetylase inhibitor panobinostat: a pilot study to assess effects on the central nervous system. Open Forum Infect Dis 2(1):ofv037
162. Rawat P, Mitra D (2011) Cellular heat shock factor 1 positively regulates human immunodeficiency virus-1 gene expression and replication by two distinct pathways. Nucleic Acids Res 39(14):5879–5892
163. Raychaudhuri S, Loew C, Korner R, Pinkert S, Theis M, Hayer-Hartl M, Buchholz F, Hartl FU (2014) Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1. Cell 156(5):975–985
164. Ritossa FM, Vonborstel RC (1964) Chromosome puffs in Drosophila induced by Ribonuclease. Science 145(3631):513–514
165. Roesch F, Meziane O, Kula A, Nisole S, Porrot F, Anderson I, Mammano F, Fassati A, Marcello A, Benkirane M, Schwartz O (2012) Hyperthermia stimulates HIV-1 replication. PLoS Pathog 8(7):e1002792
166. Saju JM, Hossain MS, Liew WC, Pradhan A, Thevasagayam NM, Tan LSE, Anand A, Olsson PE, Orban L (2018) Heat shock factor 5 is essential for spermatogenesis in Zebrafish. Cell Rep 25(12):3252–3261. e3254
167. Sakamoto N, Kokura S, Okuda T, Hattori T, Katada K, Isozaki Y, Nakabe N, Handa O, Takagi T, Ishikawa T, Naito Y, Yoshida N, Yoshikawa T (2005) Heme oxygenase-1 (Hsp32) is involved in the protection of small intestine by whole body mild hyperthermia from ischemia/reperfusion injury in rat. Int J Hyperth 21(7):603–614
168. Sakurai H, Takemori Y (2007) Interaction between heat shock transcription factors (HSFs) and divergent binding sequences: binding specificities of yeast HSFs and human HSF1. J Biol Chem 282(18):13334–13341
169. Salerno L, Floresta G, Ciaffaglione V, Gentile D, Margani F, Turnaturi R, Rescifina A, Pittala V (2019) Progress in the development of selective heme oxygenase-1 inhibitors and their potential therapeutic application. Eur J Med Chem 167:439–453
170. Sandqvist A, Bjork JK, Akerfelt M, Chitikova Z, Grichine A, Vourc'h C, Jolly C, Salminen TA, Nymalm Y, Sistonen L (2009) Heterotrimerization of heat-shock factors 1 and 2 provides a transcriptional switch in response to distinct stimuli. Mol Biol Cell 20(5):1340–1347
171. Saotome M, Horikoshi N, Urano K, Kujirai T, Yuzurihara H, Kurumizaka H, Kagawa W (2019) Structure determination of the nucleosome core particle by selenium SAD phasing. Acta Crystallogr D Struct Biol 75(Pt 10):930–936
172. Sattentau QJ, Dalgleish AG, Weiss RA, Beverley PC (1986) Epitopes of the CD4 antigen and HIV infection. Science 234(4780):1120–1123
173. Schrager LK, D'Souza MP (1998) Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. JAMA 280(1):67–71
174. Schuetz TJ, Gallo GJ, Sheldon L, Tempst P, Kingston RE (1991) Isolation of a cDNA for HSF2: evidence for two heat shock factor genes in humans. Proc Natl Acad Sci U S A 88 (16):6911–6915
175. Sen S, Deshmane SL, Kaminski R, Amini S, Datta PK (2017) Non-metabolic role of PKM2 in regulation of the HIV-1 LTR. J Cell Physiol 232(3):517–525
176. Serrao E, Wang CH, Frederick T, Lee CL, Anthony P, Arribas-Layton D, Baker K, Millstein J, Kovacs A, Neamati N (2014) Alteration of select gene expression patterns in individuals infected with HIV-1. J Med Virol 86(4):678–686

177. Shan L, Yang HC, Rabi SA, Bravo HC, Shroff NS, Irizarry RA, Zhang H, Margolick JB, Siliciano JD, Siliciano RF (2011) Influence of host gene transcription level and orientation on HIV-1 latency in a primary-cell model. J Virol 85(11):5384–5393
178. Shao J, Han B, Cao P, Zhang B, Liu M, Li D, Zhou N, Hao Q, Duan X, Chang Y, Nakai A, Fan Y, Tan K (2019) HSF1 phosphorylation by cyclosporin A confers hyperthermia sensitivity through suppression of HSP expression. Biochim Biophys Acta Gene Regul Mech 1862 (8):846–857
179. Soltys BJ, Gupta RS (1996) Immunoelectron microscopic localization of the 60-kDa heat shock chaperonin protein (Hsp60) in mammalian cells. Exp Cell Res 222(1):16–27
180. Soncin F, Zhang X, Chu B, Wang X, Asea A, Ann Stevenson M, Sacks DB, Calderwood SK (2003) Transcriptional activity and DNA binding of heat shock factor-1 involve phosphorylation on threonine 142 by CK2. Biochem Biophys Res Commun 303(2):700–706
181. Sousa AI, Pinto VLJ (2016) Community viral load of HIV in Brazil, 2007 – 2011: potential impact of highly active antiretroviral therapy (HAART) in reducing new infections. Rev Bras Epidemiol 19(3):582–593
182. Speth C, Prohaszka Z, Mair M, Stockl G, Zhu X, Jobstl B, Fust G, Dierich MP (1999) A 60 kD heat-shock protein-like molecule interacts with the HIV transmembrane glycoprotein gp41. Mol Immunol 36(9):619–628
183. Stanley SK, Bressler PB, Poli G, Fauci AS (1990) Heat shock induction of HIV production from chronically infected promonocytic and T cell lines. J Immunol 145(4):1120–1126
184. Sugiyama R, Naganuma H, Nishitsuji H, Takaku H (2011a) Human immunodeficiency virus-1 Nef suppresses Hsp70-mediated Tat activation. FEBS Lett 585(21):3367–3371
185. Sugiyama R, Nishitsuji H, Furukawa A, Katahira M, Habu Y, Takeuchi H, Ryo A, Takaku H (2011b) Heat shock protein 70 inhibits HIV-1 Vif-mediated ubiquitination and degradation of APOBEC3G. J Biol Chem 286(12):10051–10057
186. Sullivan EK, Weirich CS, Guyon JR, Sif S, Kingston RE (2001) Transcriptional activation domains of human heat shock factor 1 recruit human SWI/SNF. Mol Cell Biol 21 (17):5826–5837
187. Sung JA, Sholtis K, Kirchherr J, Kuruc JD, Gay CL, Nordstrom JL, Bollard CM, Archin NM, Margolis DM (2017) Vorinostat renders the replication-competent latent reservoir of human immunodeficiency virus (HIV) vulnerable to clearance by CD8 T cells. EBioMedicine 23:52–58
188. Swan CL, Evans TG, Sylvain N, Krone PH (2012) Zebrafish HSF4: a novel protein that shares features of both HSF1 and HSF4 of mammals. Cell Stress Chaperones 17(5):623–637
189. Tanabe M, Nakai A, Kawazoe Y, Nagata K (1997) Different thresholds in the responses of two heat shock transcription factors, HSF1 and HSF3. J Biol Chem 272(24):15389–15395
190. Thielens NM, Bally IM, Ebenbichler CF, Dierich MP, Arlaud GJ (1993) Further characterization of the interaction between the C1q subcomponent of human C1 and the transmembrane envelope glycoprotein gp41 of HIV-1. J Immunol 151(11):6583–6592
191. Tonkiss J, Calderwood SK (2005) Regulation of heat shock gene transcription in neuronal cells. Int J Hyperth 21(5):433–444
192. Tristem M, Purvis A, Quicke DL (1998) Complex evolutionary history of primate lentiviral vpr genes. Virology 240(2):232–237
193. Tsai TT, Chen CL, Tsai CC, Lin CF (2017) Targeting heat shock factor 1 as an antiviral strategy against dengue virus replication in vitro and in vivo. Antivir Res 145:44–53
194. Tyagi M, Pearson RJ, Karn J (2010) Establishment of HIV latency in primary CD4+ cells is due to epigenetic transcriptional silencing and P-TEFb restriction. J Virol 84(13):6425–6437
195. Vandamme AM, Van Vaerenbergh K, De Clercq E (1998) Anti-human immunodeficiency virus drug combination strategies. Antivir Chem Chemother 9(3):187–203
196. Verdin E, Paras P Jr, Van Lint C (1993) Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. EMBO J 12(8):3249–3259
197. Vihervaara A, Sistonen L (2014) HSF1 at a glance. J Cell Sci 127(Pt 2):261–266

198. Vuister GW, Kim SJ, Orosz A, Marquardt J, Wu C, Bax A (1994) Solution structure of the DNA-binding domain of Drosophila heat shock transcription factor. Nat Struct Biol 1 (9):605–614
199. Wang TY, Chen YM, Chen TY (2016) Molecular cloning of orange-spotted grouper (Epinephelus coioides) heat shock transcription factor 1 isoforms and characterization of their expressions in response to nodavirus. Fish Shellfish Immunol 59:123–136
200. Westerheide SD, Anckar J, Stevens SM Jr, Sistonen L, Morimoto RI (2009) Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. Science 323(5917):1063–1066
201. Williams SA, Greene WC (2007) Regulation of HIV-1 latency by T-cell activation. Cytokine 39(1):63–74
202. Williams SA, Chen LF, Kwon H, Fenard D, Bisgrove D, Verdin E, Greene WC (2004) Prostratin antagonizes HIV latency by activating NF-kappaB. J Biol Chem 279 (40):42008–42017
203. Wyzewski Z, Gregorczyk KP, Szczepanowska J, Szulc-Dabrowska L (2018) Functional role of Hsp60 as a positive regulator of human viral infection progression. Acta Virol 62(1):33–40
204. Xia W, Voellmy R (1997) Hyperphosphorylation of heat shock transcription factor 1 is correlated with transcriptional competence and slow dissociation of active factor trimers. J Biol Chem 272(7):4094–4102
205. Xu D, Zalmas LP, La Thangue NB (2008) A transcription cofactor required for the heat-shock response. EMBO Rep 9(7):662–669
206. Xu YM, Huang DY, Chiu JF, Lau AT (2012) Post-translational modification of human heat shock factors and their functions: a recent update by proteomic approach. J Proteome Res 11 (5):2625–2634
207. Xue Y, Acar M (2018) Mechanisms for the epigenetic inheritance of stress response in single cells. Curr Genet 64(6):1221–1228
208. Yamamoto N, Takemori Y, Sakurai M, Sugiyama K, Sakurai H (2009) Differential recognition of heat shock elements by members of the heat shock transcription factor family. FEBS J 276(7):1962–1974
209. Yang Y, Zhou Y, Xiong X, Huang M, Ying X, Wang M (2018) ALG3 is activated by heat shock factor 2 and promotes breast cancer growth. Med Sci Monit 24:3479–3487
210. Yang LN, Ning ZY, Wang L, Yan X, Meng ZQ (2019) HSF2 regulates aerobic glycolysis by suppression of FBP1 in hepatocellular carcinoma. Am J Cancer Res 9(8):1607–1621
211. Zaikos TD, Painter MM, Sebastian Kettinger NT, Terry VH, Collins KL (2018) Class 1-selective histone deacetylase (HDAC) inhibitors enhance HIV latency reversal while preserving the activity of HDAC isoforms necessary for maximal HIV gene expression. J Virol 92 (6)
212. Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR (2018) Heat shock protein 60 enhances CD4+CD25+ regulatory T cell function via innate TLR2 signaling. J Clin Invest 128(6):2651
213. Zeng X, Pan X, Xu X, Lin J, Que F, Tian Y, Li L, Liu S (2017) Resveratrol reactivates latent HIV through increasing histone acetylation and activating heat shock factor 1. J Agric Food Chem 65(22):4384–4394
214. Zhang J, Goodson ML, Hong Y, Sarge KD (2008) MEL-18 interacts with HSF2 and the SUMO E2 UBC9 to inhibit HSF2 sumoylation. J Biol Chem 283(12):7464–7469
215. Zhao RY, Bukrinsky MI (2014) HIV-1 accessory proteins: VpR. Methods Mol Biol 1087:125–134
216. Zhong YH, Cheng HZ, Peng H, Tang SC, Wang P (2016) Heat shock factor 2 is associated with the occurrence of lung cancer by enhancing the expression of heat shock proteins. Oncol Lett 12(6):5106–5112
217. Zou J, Salminen WF, Roberts SM, Voellmy R (1998) Correlation between glutathione oxidation and trimerization of heat shock factor 1, an early step in stress induction of the Hsp response. Cell Stress Chaperones 3(2):130–141

# Heat Shock Protein and Cancer Based Therapies


**Lütfi Tutar, Ezgi Nurdan Yenilmez Tunoglu, Berçem Yeman Kiyak, and Yusuf Tutar**

## Abstract

**Introduction** Tumor cells have higher metabolic rates compared to normal cells. Biochemical reactions require properly folded proteins to perform pathway specific functions. Nascent substrate proteins specially signaling and cell cycle molecules must be folded in shorter times. Tumor cells overexpress Heat Shock Proteins (HSP) to help client proteins folding. Most of the HSP have anti-apoptotic functions and help tumor cell survival. HSP90 and HSP70 are at the center of heat shock response mechanism and drug designers target HSP to inhibit these two essential functions to drive tumor cells to apoptosis.

**Methods** Small molecule inhibitors inhibited HSP by competitive inhibition with nucleotides, by perturbing protein-protein interactions through interface inhibitors, and by binding to allosteric sites and disrupting conformational changes. Major databases (Pubmed, Scopus, and WOS) were surveyed with the keywords “Hsp90”, “Hsp70”, “anti-cancer agent”, and “inhibitor”.

L. Tutar
Faculty of Science and Letters, Department of Molecular Biology and Genetics, Kırşehir Ahi Evran University, Kırşehir, Turkey

E. N. Yenilmez Tunoglu
Turkey Health Sciences Institutes, Division of Molecular Medicine, University of Health Sciences, İstanbul, Turkey

B. Yeman Kiyak
Turkey, Hamidiye Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Division of Biochemistry, University of Health Sciences, İstanbul, Turkey

Y. Tutar (✉)
Turkey Health Sciences Institutes, Division of Molecular Medicine, University of Health Sciences, İstanbul, Turkey

Turkey, Hamidiye Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Division of Biochemistry, University of Health Sciences, İstanbul, Turkey
e-mail: yusuf.tutar@sbu.edu.tr

Heat Shock Proteins (2021) 22: 177–201
https://doi.org/10.1007/7515_2020_14

Published Online: 9 September 2020

**Results** One major challenge for HSP inhibitor design is complementing function of this protein family. HSP70 can complement HSP90 function or alternatively, redundant isoforms may complement each other. Further, different isoforms have similar structures and inhibition of specific HSP may be difficult. In spite of these critical problems, several agents are designed for HSP inhibition. These inhibitors and their derivatives provide templates for drug design.

**Conclusions** HSP90 inhibitors have been widely studied and displayed promising anticancer activity for variety of cancer. This work explains current efforts on cancer treatment through HSP inhibition mechanisms.

**Keywords** DNAJ · Heat shock protein · HSP60 · HSP70 · HSP90 · Nucleotide exchange factor

## Abbreviations

| | |
|---|---|
| Hsc | Hsp70 constitutive form |
| HSP | heat shock proteins |
| Hsp70 | Hsp70 inductive form |
| NEF | nucleotide exchange factor |
| SBD | substrate binding domain |
| sHSP | small HSP |

## 1 Introduction

Drug targeting through key targets can modulate biochemical functions. HSP family control protein homeostasis and function therefore, manipulation of HSP related pathways are interest to drug designers. However, redundant isoforms and complex interaction of coordinating HSP molecules make specific design more difficult. Further, HSP isoforms add another layer of complexity to drug design, not only with distinct interaction partners but also with structural similarities. Elucidating HSP interaction network and protein-protein interaction can help designing precise inhibitor.

### *1.1 HSP: Structure and Function*

Hsp are evolutionarily conserved proteins that properly fold many other proteins to their three-dimensional structures to keep their functional native forms. Many species have structurally related but functionally different Hsp. A cell may have a variety of Hsp within its compartments such as endoplasmic reticulum,

mitochondrion, and cytosol. Further, functionally different but related Hsp may function in the same compartment, for example six different Hsp70 chaperones found in *S. cerevisiae* cytosol, Ssa1-4 and Ssb1-2. Some chaperone proteins are expressed constitutively in the cell and some others interact with different macromolecules to perform specific functions [124]. Hsp works with co-chaperones coordinately in cellular processes such as proper folding of nascent chains, protein translocation, protein disaggregation, preservation and restructuring of the cytoskeleton and targeting damaged proteins. Hsp70, Hsp40, Hsp90, Hsp100, Hsp60, nucleotide exchange factors, and small Hsp are the key Hsp families.

### 1.1.1 HSP70 Family

Hsp70s play key roles in substrate protein folding and in several essential biochemical functions. Basic action mechanism is based on its functional domains. Hsp70 ATPase domain hydrolyses ATP and transmits this energy to the substrate binding domain (SBD). The energy regulates lid closure of SBD. Hsp70 binds substrate weakly at ATP bound state and bind more strongly at ADP state. When the lid closes in the presence of ADP, SBD provides a hydrophobic environment to the unfolded substrate protein and lead proper folding. On the other hand, this energy transfer may work in reverse order. Substrate binding to SBD may trigger ATP hydrolyses however, kinetic studies yet to be done to reveal the mechanism. Hsp70 has several subcellular forms and can be either constitutively or inducibly expressed [125–127]. It is believed that inducing Hsp70 isoform help Hsc70 thermotolerans related stress. Hsp70 is the key player of the folding mechanism and interacts with partner proteins. Hsp40, Hsp100, and nucleotide exchange factors are the main partners of Hsp70 [39, 50]. Hsp70s are universally conserved and SBD has broader substrate specificity among species. Existence of redundant isoforms suggested possible roles of distinct functions. Hsp70s can substitute each other nonetheless constitutively expressed form (Hsc70) can only substitute constitutive form but not the inductive form (Hsp70) [123]. This action may depend on Hsp70 interaction partners as Hsc70 and Hsp70 coordinates and cooperates with different Hsp members. Hsp70 not only fold proteins but serves for different functions; nascent chain folding, translocation across membranes, targeting proteins for degradation, and apoptosis are the cellular processes that Hsp70s perform [126].

### 1.1.2 HSP40 Family

One key partner for Hsp70 is Hsp40. The protein family, also called DnaJ, is classified into DnaJA, DnaJB, and DnaJC depending on difference in domain structure. Hsp40s help substrates to form right orientation to be processed by Hsp70 [99]. Hsp40s has over 100 members and introduce the substrate to Hsp70. Hsp40s have a J domain and the domain connects with ATPase domain of Hsp70. This interaction induces ATPase activity of Hsp70 since Hsp70-Hsp40 pair folds

substrate proteins or send them to degradation [32]. And distinct Hsp70-Hsp40 isoform pairs form different cellular function. Besides its folding/refolding activity, Hsp40 is involved in protein translocation, translation, proteosomal degredation. HSP40 associate with tumor growth and metastasis.

### 1.1.3 Nucleotide Exchange Factors (NEFs)

During Hsp70 folding mechanism, nucleotide must be removed from Hsp70 to start a new cycle. Nucleotide exchange factors (NEF) function to regenerate acceptor ATPases. NEF family include a variety of unrelated protein classes, namely GrpE homologs in prokaryotes and Bag1, Fes1p, Hsp110 in eukaryotes [51, 81]. NEF acts on Hsp70 as pliers and remove ADP from ATPase pocket. This process must be syncronised to client protein folding as Hsp70 process seven residues at a time. Hsp70-NEF interaction interface inhibitors are potential targets for drug designers.

### 1.1.4 HSP90 Family

Hsp90s are the abundant cytoplasmic proteins in unstressed cells. Hsp90s have a highly conserved N-terminal domain and highly conserved C-terminal domain [80]. Hsp90s are dimeric proteins and two cytosolic isoforms, called α and β, can be found in higher eukaryotes. Hsp90 acts with Hsp70 chaperones and co-chaperones (e.g. Hop; Hsp organizing protein) to fold the substrate proteins to be functionally active conformations. Hsp90s play key roles in some cellular processes such as cell cycle control, transcriptional regulation and signal transduction [23]. The folding process can be viewed as prefolded with Hsp70 and folded-honed with Hsp90.

### 1.1.5 HSP60 Family

Hsp60s mediate folding of many different proteins. *E. coli* Hsp60, GroEL is the most familiar among all chaperones [4]. The family functions with a slightly different mechanism compared to other families since substrate is totally entrapped into the protein. Hsp70 and Hsp60 have similar features but cannot substitute each other. During folding and assembly of substrate proteins, the two proteins works coordinately [8].

### 1.1.6 sHSP

Small Hsp (sHsp) family is not highly conserved in organisms during evolution and various among species. Members of this family share a conserved C-terminal region, called α-crystalline and located at different cellular compartments. sHsp bring

substrate proteins into folding competent state by preventing their irreversible aggregation, but cannot refold them [27]. They take roles in some cellular and biochemical processes such as RNA stabilization and cytoskeleton interaction. Since HSP27 increases tumor growth and metastatic potential, focus will be on HSP27 as sHSP in this work.

## *1.2 HSP90 and Cancer*

HSP90 has a unique structure and forms dimer structure. The cavity in the protein may hold substrate proteins up to 30 kDa. HSP90 receives partially folded structures from HSP70 and locally fold the substrate proteins. This process helps proper folding of multimeric substrate proteins. Therefore, HSP90 plays an essential role in folding. Because of the unique structure, several small molecule inhibitors designed to inhibit HSP90 function as HSP70. HSP90 forms from three domains: N-terminal ATPase for ATP hydrolysis, middle domain for substrate protein binding, and C-terminal domain for dimerization. Cochaperones can bind to middle domain and C-terminal domain. HSP90 has more than 400 substrate/client proteins (https://www.picard.ch/Hsp90Int/index.php). These proteins are involved in signal transduction pathways, apoptotic evasion, differentiation, and metastasis [29, 103].

Like HSP70, HSP90 cooperates with HSP family proteins. HSP70 and HSP40 (DnaJ) co-chaperones submit client proteins to HSP90 for further folding process. HOP/STIP1 act as an adaptor and transfer substrate proteins from HSP90/HSP40 complex to HSP90. STIP1 also increases ATPase activity of HSP70 but inhibits HSP90 activity [43, 95] Cdc37/p50 is a signal transduction protein and form complex with several kinases and direct HSP90 to its target kinases. Cdc37 inhibits HSP90 ATPase activity [111] In contrast, Aha1 increases HSP90 ATPase activity [95] Sba-1/p23 cochaperone function as substrate release factor for HSP90 [149].

HSP90 substrate proteins involve (i.e. Cdk-4, eRBB-2, Raf-1, eNOS, N-Ras, B-Raf) signaling kinases, regulators of cell proliferation and apoptosis. HSP90 also interacts with tumor suppressor p53 transcription factor that regulate apoptosis. Overall, HSP90 provides an adaptive strategy by favoring survival signals over proapoptic signals. HSP90 resists apoptosis and promote angiogenesis and metastasis by folding substrate proteins (i.e. VEGF, NOS, and MMP-2) [117, 132] Thus, blockage of HSP90 through triggering apoptosis and by suppressing pro-cancerous signaling cascades. Thus, inhibition of HSP90 has been an attractive model for drug designers. This strategy seems elegant in design however, HSP proteins complement each other. Inhibition of HSP90 induces HSP70 expression and cancer cells use HSP70 in substrate protein folding. Current efforts employ dual HSP inhibitors to prevent cancer cell bypass mechanisms.

Extracellular HSP90 coordinates with HSP70, HSP40, HIP, HOP, and p23 to promote Matrix Metallopeptidase 2 (MMP-2) proteolytic activity. Thus, degradation of extracellular matrix with MMP-2 is a signal for the beginning of invasion as well

as metastasis. HSP90 further activates MMP-9 to promote cell invasion. Therefore, HSP90 has an essential role in tumor invasion and metastasis [112, 116, 62]. Small molecule inhibitors have been designed for HSP90: 17-AAG, 17-DMAG, Geldanamycin, Herbimycin A, Radicicol, Coumermycin A1, NVP-AUY922, and Novobiocin. 17-AAG is an HSP90 inhibitor. It destabilizes multiple oncogenic client proteins in cancer cells and is a derivative of geldanamycin. Since geldanamycin displayed solubility and toxicology problems, 17-AAG was synthesized [52]. 17-AAG display synergetic action with clinical drugs; taxol, cisplatin [128] 17-DMAG is semi-synthetic derivative of geldanamaycin and has advantages over 17-AAG such as higher water solubility, good bioavailability, and greater activity and it degrades Hsp90 client proteins [82] Herbimycin A is benzoquinoid ansamycin class of natural products of HSP90 inhibitor, but the compound has hepatotoxicity caused by the benzoquinone moiety [154]. Despite Radicicol promising *in vitro* activity (isolated from the fungus *Monosporium bonorden*), it is not efficient *in vivo* and stable derivatives-oxime displayed promising results but have not advanced into clinical trials [104].

Pyrimidinyl acyl thioureas designed by our group bound to Hsp90 ATP binding pocket and inhibit ATPase function effectively [59]. Coumermycin work with a different mechanism of action. It perturbs HSP90 dimerization rather than interfering with HSP90 client proteins [11]. NVP-AUY922 reduces cell proliferation and viability but lead HSP70 upregulation. This inhibitor also depletes HSP90 client proteins. Overall the molecule has promising effect *in vivo* [5]. Novobiocin, coumarin derivative, bind weakly to Hsp90 C-terminal ATP binding site and works similarly by degrading Hsp90 client proteins [28]. Novobiocin is different than the rest of the small molecule inhibitors since it acts on C-terminal domain of HSP90. Therefore, our lab designed novel compounds and combined thiazole with coumarine [58]. These structures displayed promising inhibitory activity. The inhibitors prevented HSP90 dimer formation as Coumermycin A1 and disrupted HSP90 interaction with HSP70/HSP40 through HOP. Compared to the other inhibition mechanism, this method is unique and may not lead potential off-target effects as N-terminal inhibitors that targets ATPase domain.

HSP90 has more than 400 client proteins and these proteins involve in signaling and cell cycle regulation (Table 1). HSP90 involvement with non-small cell lung cancer, breast cancer, hepatocellular carcinoma, and colorectal cancer is through two main categories. Inhibitors either block HSP90 or degrade client proteins. However, inhibitors can block HSP90 by preventing its dimerization, allosteric inhibition, and nucleotide analogs. These strategies provide highly effective anticancer treatment.

## *1.3 HSP70 and Cancer*

HSP70 structure consists of N-terminal ATPase domain, substrate binding domain, and C-terminal domain. HSP70 has a central role in HSP response and this protein

**Table 1** HSP90 associated cancers

| HSP90 | Cancer type | Association | References |
|---|---|---|---|
| HSP90AA1 (HSPC1) | Non-small cell lung cancer | As an elevated expression of **HSP90AA1**, HSP90AB1, HSP90B1 and TRAP1 was associated with poorer overall survival outcomes in patients with NSCLC, these HSP90 members may be potential prognostic biomarkers and drug targets for the treatment of NSCLC | [72] |
| Hsp90α, Hsp90β (HSPC2, HSPC3) | Invasive and metastatic breast cancer | The secreted form of **Hsp90α**, but not **Hsp90β**, is responsible the tumour cells' abilities to migrate, invade and form tumours in mice | [157] |
| HSP90B1 (HSPC4) | Hepatocellular carcinoma | High BCLC staging scores, advanced cirrhosis and the overexpression of HSPA12A and HSP90B1 might be associated with poor survival from HCC, whereas high levels of HSPA4, HSPA5 and HSPA6 might be associated with earlier recurrence of HCC | [146] |
| TRAP1 (HSPC5) | Colorectal Cancer | Overexpression of **TRAP1** might contribute to tumor cell local invasion of colorectal cancer | [92] |

coordinates and cooperates with other members of the family. These interactions regulate HSP70 chaperone activity as well. BAG1 and CHIP binding lead HSP70 to substrate protein degradation and HIP and HOP binding drives HSP70 to substrate protein folding. HSP70 has several isoforms located in different part of the cell. HSP70 isoforms interaction with different HSP family members serves to form unique functions. Often these functions contain signaling proteins, apoptotic and transcription factors. Therefore, HSP70 isoforms are associated with cancer. As HSP70 isoforms characterized, a variety of nomenclature used. To prevent confusion, HSP70 classified nomenclature "**HSPA**" but not common names are used throughout this work. **HSPA1**-HSP72, **HSPA6**-HSP70B, **HSPA8**-HSC70 (cytosolic HSP70s), **HSPA9**-mortalin (mitochondrial HSP70), **HSPA5**-BIP (ER HSP70), **HSPA2**-HSP70/2 [86, 124, 137]. Several cancer mechanisms employ HSP70 to survive because of HSP70's antiapoptotic function. Therefore, HSP70 is often over expressed in cancer. Further, cancer cells grow faster than normal cells but key proteins in cell cycle and signaling pathways must be properly folded for proper function. HSP70 overexpression help folding of these substrate proteins in cancer development [47, 91] These HSP70 functions increase oncogenic potential of some cancer cells. Therefore, HSP70 is a target for anticancer drug development. Several small molecule inhibitors developed to lead HSP70 to drive cancer cells to apoptosis. HSP70 inhibitors not only perturb antiapoptotic and folding function but also HSP70 interactions with other proteins. Recently designed HSP70 inhibitors are VER-155008, Apoptozole, MAL3-101, JG-231, YK5, HS-72, Novolactone, MKT-077, PES.

HSP70 inhibitor design initially started with natural product spergualin and its synthetic derivative DSG (15-deoxyspergualin). The molecule binds to the regulatory

C-terminal 'EEVD' motif [87, 100]. Two inhibitors geranylger and anylacetone designed to block substrate binding to HSP70 with low potency [90, 142]. Since HSP70 sites other than ATP and substrate binding cavity are more druggable, myricetin compound employed to inhibit HSP40 J-domain stimulated HSP70 ATPase activity. And in a similar analogy an analog of 15-deoxyspergualin MAL3-101 allosterically inhibited HSP70 ATPase function and perturb HSP70-HSP40 function through binding to the interaction interface. The inhibitor has anti-proliferative and apoptotic activity. The inhibitor displayed *in vivo* antitumor activity in xenograft model. [3, 13, 33, 135]. Another protein-protein interaction inhibitor is JG-231. This allosteric inhibitor binds HSP70-BAG3 interface and inhibits breast cancer cells MCF-7 and MDA-MB-231 [106].

VER-155008 is an ATP analogue that destabilizes oncoproteins and leads caspase-dependent and independent apoptosis in colon cancer cells. The inhibitor binds to different HSP70 isoforms (HSPA5, HSPA8) as well as HSP90 isoform (HSP90AB1). [79, 134] Apoptozole acts with a different mechanism, it binds to HSP70 ATPase domain and inhibits ATP hydrolysis and induce apoptosis [19, 133]. YK5 induces degradation of HER2, Raf-1, and Akt kinases, and triggers apoptosis in the SKBr3 breast cancer cells [67]. HS-72 just like YK5 induces substrate protein degradation and growth inhibition. HS-72 specifically target inducible HSP70 by allosterically inhibiting its ATP affinity [44]. Novalactone binds HSP70 ATPase and substrate binding domain interface. The inhibitor is specific to HSP70 ER and cytosolic isoforms [40].

MKT-077 interact with HSP70 just like myricetin but only in the ADP-bound state [101] (Pifithrin-m is different than other HSP70 inhibiors as it targets substrate binding, but specific saturable binding site was not monitored and like MKT-077, it works in HSP70-ADP but not in HSP70-ATP state [64, 104] Our group developed new generation inhibitors to prevent protein-protein interaction with interface inhibitors. These inhibitors also bind HSP70 in the presence of ATP at nM concentration, but the inhibitors also inhibit HSP90. *In vivo* testing of these dual inhibitors is underway (Unpublished results).

HSP70 involvement with bladder, lung, colorectal, ovarian, endometrial, cervical, head and neck, papillary thyroid cancer, breast, non-small cell lung, pancreatic, hepatocellular, nasopharyngeal esophageal squamous cell carcinoma shown in Table 2. HSP70 has three domains and interacts with variety of cochaperones. Domain specific and interface associated inhibitors designed for anticancer treatment. Drug designers further developed dual inhibitors that effect both HSP70 and HSP90.

## *1.4 HSP60 and Cancer*

Chaperonin-HSP60 interacts with HSP10 and located mainly in mitochondria. It is also located in cytosol, cell membrane, extracellular space. It has three domains: theapical, intermediate, andequatorial [137] HSP60:HSP10 is required for newly imported protein folding into mitochondria but substrate proteins of HSP60 are mostly unknown. However, HSP60 interacts with several factors in apoptosis and

**Table 2** HSP70 associated cancers

| HSP70 | Cancer Type | Association | References |
|---|---|---|---|
| HSPA1B | Bladder Urothelial Carcinoma | HSPA1B expression is associated with early spread and progression of urothelial carcinoma of bladder cancer | [36] |
| HSPA1B | Colorectal Cancer | Down regulation of HSPA1B expression reduces cellular proliferation and tumor growth | [48] |
| HSPA1B | Ovarian Cancer | Promote cellular growth and invasion of epithelial ovarian cancer cells | [48] |
| HSPA1 and HSPA2 | Non-small cell lung carcinoma | HSPA1 and HSPA2 gene expression reduced growth and chemoresistance of NSCLC cells | [115] |
| HSPA2 | Pancreatic carcinoma | HSPA2 is correlated with tumor angiogenesis and poor prognosis in pancreatic carcinoma | [150] |
| HSPA2 | Hepatocellular carcinoma | HSPA2 expression was an independent predictor of overall survival. Positive expression of HSPA2 in hepatocellular carcinoma is important in the acquisition of an aggressive phenotype and it is an independent biomarker for poor prognosis of patients with HCC | [34] |
| HSPA2 | Esophageal squamous cell carcinoma (ESCC) | HSPA2 contribute to the malignant progression of ESCC and present a novel prognostic indicator for ESCC patients | [152] |
| HSPA2 | Bladder Cancer | HSPA2 was identified as epigenetic biomarkers for bladder Cancer | [22] |
| HSPA5 | Lung cancer | Inhibition of SIRT1/2 induces pro-survival autophagy via acetylation of HSPA5 | [85] |
| HSPA5 | Breast cancer | HSPA5 is a marker for poor prognosis in breast cancer patients and has an important role in cancer progression, including promoting drug resistance and metastasis | [14] |
| HSPA5 | Papillary thyroid cancer | HSPA5 play a pivotal role in papillary thyroid cancer relapse. | [151]. |
| HSPA5 | Nasopharyngeal carcinoma | rs3216733 polymorphism in the GRP78 gene promoter may correlate with NPC susceptibility | [131] |
| HSPA5 | Lymph node metastases | HSPA5 significantly correlating with poor prognosis, whose high expression was in part confirmed in bioptic samples of lymph node metastases | [6] |
| HSPA5 | Head and neck cancer | HSPA5 contributes to head and neck cancer survival via maintenance of lysosomal activity | [56] |
| HSPA5 | Cervical cancer | High expression of HSPA5 is a tumor-promoting factor in cervical cancer and is thus a potential target for novel treatment | [75] |
| HSPA5 | Hepatocellular carcinoma | GRP78 and granulin-epithelin precursor (GEP) are interacting protein partners in liver cancer cells and may play a role in | [147] |

(continued)

**Table 2** (continued)

| HSP70 | Cancer Type | Association | References |
|---|---|---|---|
| | | GEP-mediated cancer progression in hepatocellular carcinoma | |
| HSPA6 | Hepatocellular carcinoma | High levels of HSPA4, HSPA5 and HSPA6 associated with earlier recurrence of hepatocellular carcinoma | [146] |
| HSPA8 | Endometrial cancer | The depletion of **HSPA8** siRNAs significantly reduced cell proliferation, promoted cell apoptosis, and suppressed cell growth in endometrial **cancer** cells RL-95-2 and HEC-1B | [105] |
| HSPA9 | Invasive breast cancer | Amplifications in the **HSPA9**gene lead to lower survivability rates for the patient samples, while missense mutations in **HSPA9** led to higher | [53] |
| HSPA13 | Hepatocellular carcinoma | HSPH1, HSPBP1, HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA4, HSPA5, HSPA8, HSPA9, HSPAA1, HSPAB1, HSPA14, HSPB11, HSPA13, HSP90B1 and HSPBAP1, were all overexpressed in hepatitis B virus (HBV)-related HCC tissues | [146] |
| HSPA12B | Lung cancer | **HSPA12B** stimulates lung tumor growth via a Cox-2-dependent mechanism | [76] |

cell cycle regulation [9] The expression of HSP60 is increased in various cancer due to its antiapoptotic function. HSP60 levels alter during carcinogenesis. HSP60 has a direct role in tumor transformation and progression. HSP60 protects transformed cells from apoptosis and binds surviving to stabilize it and control p53 function. Elimination of HSP60 leads loss of surviving (inhibitor of apoptosis) and p53 dependent apoptosis in cancer cells [9] HSP60 mediates drug resistance and overexpressed in cisplatin resistant cancer cells. HSP60 regulate apoptosis through cyclophilin D and inhibition of HSP60 activates caspase-dependent apoptosis and tumor suppression [37] HSP60 modulates growth factors and down regulation of HSP60 enhances insulin like growth factor (IGFBP7) on colorectal cancer cell tumor suppression [21, 102] Further, overexpression of HSP60 induces metastasis in different cancers through interacting with β-catenin [122] Surface HSP60 coordinates with α3β1-integrin in breast cancer and the integrin activity associate with tumor progression, differentiation, invasion and metastasis. Therefore, inhibition of HSP60 may inhibit tumor progression by perturbing the integrin activity [9].

Only a few researches have been conducted to develop inhibitors of cancer. Inhibition of HSP60 reverses drug resistance and therefore several small molecule inhibitors designed for HSP60 inhibition [1, 136] Geldanamycin dependent cell death led mitochondrial HSP60 in osteosarcoma cells and sinularin inhibition effected HSP60 expression in melonama cells [137] Although anticancer inhibitors that target HSP60 is low, several small molecule inhibitors exist that directly bind HSP60. Bortezomib is a proteasome inhibitor for chemotherapeutic drug that is used in

**Table 3** Chaperonins associated cancers

| Chaperonins | Cancer Type | Association | References |
|---|---|---|---|
| HSPD1 | Pancreatic cell | Knockdown of HSP60 attenuated pancreatic ductal cancer cell proliferation and migration/ invasion, whereas ectopic expression of HSP60 increased tumorigenesis | [156] |
| CCT1 | Breast cancer | Overexpressing HSPA2 and DNAJC20 exhibited longer survival, whereas overexpression of HSP90AA1, CCT1, CCT2, CCT6A resulted in unfavorable prognosis for breast cancer patients | [57] |
| CCT3 | Hepatocellular carcinoma | CCT3 and IQGAP3 protein level correlated well with HCC etiology, tumor size, TNM stage, and child-pugh classification | [96] |
| CCT5 | Non-small cell lung cancer | CCT5 is a potential tumor marker and may be useful in the diagnosis of NSCLC in an early stage | [35] |
| CCT7 | Endometrial carcinoma | The mRNA expressions of CCT7, HSPA8, PCBP2, LONP1, PFN1, and EEF2 in endometrial carcinoma tissues were upregulated. CCT7, HSPA8, PCBP2, LONP1, PFN1, and EEF2 are potential biomarkers for endo-metrial carcinoma tissues | [105] |
| CCT8 | Glioblastoma | High CCT8 protein expression might be related to poor outcome of glioma, and that CCT8 regulates the proliferation and invasion of glioblastomas | [98] |

tumor treatment by modulating cell surface [12] BF844 targets mainly with HSP60 [A small molecule mitigates hearing loss in a mouse model of Usher syndrome III]. BF844 also binds HSP90 but characterization studies to identify drugs for cancer treatment underway. Phenoxyacetanilide inhibits HIF transcriptional activity and HSP60 [31] KHS-101 inhibits uncontrolled cell growth of glioblastoma multiforme (GBM) by specifically binding to mitochondrial HSP60 (HSPD1). Thus, targeting HSPD1 dependent oncometabolic pathways by inducing bioenergetic dysfunction through inhibition of HSPD1 is a promising method [94]. Epolactaene inhibits HSP60 by binding to cys442 of HSP60 and suvanine also sulfanate cyteine residues however the compounds are not tested in cancer cells yet [88, 141] A recent work summarize several HSP60 inhibitors (mizoribine, epolactaene, myrtucommulone, stephacidin B, o-carboranylphenoxyacetanilides) but non of them tested on cancer pathways yet [83]. HSP60 and HSP10 associated with pancreatic, breast, non-small cell lung cancer, endometrial hepatocellular carcinoma, and glioblastoma (Table 3). However, inhibitors of these family members are not extensively studied, therefore researches limited with coordination of other HSP members.

## *1.5 Hsp27 (HSPB) and Cancer*

HSP27 belongs to the family of small HSP with only 11 members in total. Among all the HSP, this family is the only one that is ATP-independent. HSP are usually

categorized according to their molecular weight and all members of the small HSP family are less than 43 kDa. HSP27 consists of 205 amino acids and has three domains (N-terminal domain, α-crystallin domain and C-terminal domain) of which N-terminal domain is responsible for chaperone activity and multimer formation and C-terminal forms the compatible structure essential for chaperone activity. In order to accomplish its function, HSP27 goes through phosphorylation under stress at different sites (Ser-15, Ser-78, and Ser-82) with catalysis carried out by MAPKAPK (mitogen-activated protein kinase-activated protein kinases) that is activated by MAPK (mitogen-activated protein kinase) [137].

Studies on HSP27 and various cancer types have demonstrated the significance of this protein in regulating important features of cancer cells including but not limited to their growth, invasion, metastasis, and apoptosis. HSP27 also contributes to resistance of cells against chemotherapeutic drugs. It has enhanced the migration in breast and bladder cancer cells by increasing vascular endothelial growth factor and interfering with integrin-linked kinase, respectively [119] Angiogenesis in breast cancer and cell invasion in prostate cancer are also enhanced with the involvement of HSP27 [140] Knockdown and overexpression experiments have also shown the regulation of migration, invasion, proliferation, and metastasis of cancer cells [155].

A study on gastric adenocarcinoma has revealed an association between cancer development and HSP27. C-X-C chemokine receptor type 1 (CXCR1) has a role in the development and progression of cancer, and its knockout has decreased HSP27 expression hinting at its regulatory role along with CXCR1 [45]. When the activity of this protein is inhibited or downregulated, findings have shown that it induces the expression of phosphatase and tensin homolog (PTEN; a tumor-suppressor) in breast cancer [10] increases caspase-3 activity and therefore causes apoptosis in glioblastoma cells [65], and also induces apoptosis due to serum depletion and hypoxia in CD133+ colorectal cancer stem cells [71]. Other studies report on its inhibition of caspase activation though binding to cytochrome c [7] prevention of cellular senescence and regulation of p53 signaling [89], ncreased cell proliferation of lung cancer cells through activator protein-1 [153].

The expression of HSP27 is increased in several cancers including breast cancer stem cells, thyroid cancer, hepatocellular carcinoma, acute myeloid leukemia, and several others; thus, it can be used as a diagnosis biomarker in these cancer types [137]. As mentioned earlier, HSP27 contributes to resistance of cells against chemotherapeutic drugs. Studies with human pancreatic cancer cells show that cells develop resistance to gemcitabine when HSP27 is overexpressed [60, 73, 74]. On the other side, sensitivity against chemotherapeutics is observed in cancer cells when the activity of HSP27 is supressed or blocked. Doxorubicin sensitivity is increa sed, and its resistance is found to be overcome in the absence of HSP27 activity in colon carcinoma and breast cancer cells [89, 113]. Other studies with chemotherapeutic agents like 5-fluorouracil, cisplatin, genistein have also supported these results [44, 114, 139]. Overall these findings indicate that HSP27 may be a target for chemotherapeutics since it is involved in regulating their sensitivity and resistance. HSPB or HSP27 associates with prostate, cervical, colorectal, breast, renal cell, lung, gastric, non small cell lung cancer, glioblastoma, nasopharyngeal, hepatocellular carcinoma, and TNBC, osteosarcoma glioma (Table 4).

**Table 4** HSPB associated cancers

| HSPB | Cancer Type | Association | References |
|---|---|---|---|
| HSPB1 | Lung tumorigenesis | The mesenchymal transition of vascular endothelial cells caused by endothelial HSPB1 deficiency contributes to lung fibrosis and tumorigenesis | [20] |
| HSPB1 (HSP27) | Lung cancer | The expression of Redd1, HSP27 and HSP70 was highly increased in lung cancer tissues compared with that in normal lung tissues. Overexpression of Redd1 led to HSP27 and HSP70 induction and AKT activation, which were involved in lung cancer cell survival and resistance to ionizing radiation | [49] |
| HSPB1 | Lung cancer | rs2868371 genotypes of HSPB1 might be associated with radiation-induced esophagus damage risk, especially in Caucasians | [67, 68] |
| HSPB1 (HSP27) | Prostate cancer | Hsp27 could regulate EMT in prostate cells. Hsp27 was stably overexpressed in the human androgen receptor (AR)-positive prostate cancer cell line | [110] |
| HSPB1 | Cervical cancer | A direct relationship between Cadmium-induced toxicity and peroxynitrite production and a role for recombinant HSP27 as a potential therapeutic agent that may counteract Cd toxicity | [25] |
| HSPB1 | Glioblastoma | Expression level of heat shock protein 27 discriminated glioblastoma presenting short and long survival | [38] |
| HSPB2 | Hepatocellular carcinoma | HSPB6, HSPB7, HSPA6, HSPB2 and HSPB3 were upregulated in non-tumour tissues | [146]. |
| HSPB5 | Non small cell lung cancer | CRYAB mRNA and protein expression levels were significantly higher in NSCLC than in matched non-cancerous tissues | [97] |
| HSPB5 | Colorectal cancer | HspB5 (Alpha B-crystallin) maybe trigger the EMT in CRC by activating the ERK signaling pathway. It is a potential tumor biomarker for CRC diagnosis and prognosis | [66] |
| HSPB5 | Colorectal cancer | The expression of CRYAB protein in CRC was significantly associated with distant metastasis and overall survival | [108] |
| HSPB5 | Colorectal cancer | CG/GG at CRYAB C-802G is correlated with CRC susceptibility | [138] |
| HSPB5 (CRYAB) | Breast cancer | The disruption of the interaction between CRYAB and VEGF165 can lead to inhibition of VEGF production in breast cancer cells | [17] |
| HSPB5 | TNBC | αB-crystallin expression in primary breast carcinomas was associated with poor overall survival and poor survival after brain metastasis, even among patients with TNBC | [78] |
| HSPB5 | Breast cancer | αB-crystallin expression significantly correlated with triple negativity and basal-like markers | [129] |

(continued)

**Table 4** (continued)

| HSPB | Cancer Type | Association | References |
|---|---|---|---|
| | | CK5/6 and SMA. A significant correlation was also observed with pERK1/2 expression | |
| HSPB5 | TNBC | Overexpression of alpha B crystallin was observed more frequently in triple negative cancer (9/20, 45%) than in luminal type cancer | [54] |
| HSPB5 | Osteosarcoma | The high level of CRYAB was associated with shorten survival and tumor recurrence for the postoperative osteosarcoma patients. | [109] |
| HSPB7 | Renal cell carcinoma | HSBP7 is likely to be a tumor suppressor gene regulated by p53 and its downregulation by hypermethylation may play a critical role in renal carcinogenesis | [70] |
| HSPB8 | Gastric cancer | HSPB8 promotes cancer cell growth by activating the ERK-CREB pathway and may serve as a potential prognostic factor in GC patients | [107] |
| HSPB10 | Nasopharyngeal carcinoma (NPC) | Positive expression of HSP10 and HSP70 associated with advanced clinical stages | [49] |
| HSPB11 | Glioma | HSPB11 expression was associated with poor prognosis and was independently correlated with overall survival (OS) in high-grade glioma | [18] |

## *1.6 Hsp40 and Cancer*

As the largest family of human HSP with at least 49 members, HSP40s are classified into three groups (DNAJA, DNAJB, and DNAJC). These proteins are homologs of DnaJ HSP in bacteria and are therefore named as DNAJ family proteins. Most members of this family have a 'J' domain which binds to nucleotide binding domain (NBD) of HSP70 leading to regulation of protein folding/unfolding, translation, translocation, and degradation [137]. Also known as the co-chaperone of Hsp70, they help increase the ATPase activity of Hsp70. Together with the co-factor NEF, they form a cytosolic chaperoning machinery. Concurrently, presence of HSP60 and HSP90 is also necessary for ideal functioning of HSP70.

HSP40 family proteins have both pro-cancer and anti-cancer roles in cancer development. For instance, many studies have been carried out with DNAJA3 (also known as Tid1) functioning as a tumor suppressor in various cancers. In breast cancer MCF-7 cells, it takes the lead to apoptosis through p53-mediation [121]. Another study with breast cancer cells shows that Tid1 prevents cell migration by interfering with the production of IL-8 [55]. Overexpression studies of Tid1 in head and neck squamous cell carcinoma show its inhibition effect on hallmarks of cancerous cells including migration and invasion in addition to cell proliferation and growth *in vitro*. Reduced tumor growth and relapse have also been observed *in vivo*. [15]. On the other hand, knockdown experiments have been supportive of the

outcomes of overexpression studies. Tid1 knockdown has resulted in decreased apoptosis and increased growth of osteosarcoma cancer cells [30].

Overexpression of several HSP40 family proteins is seen in a variety of cancers including colorectal, lung, cervical, breast, ovarian, and gastric. DNAJC12 (also known as JDP1) is increased in estrogen receptor (ER) positive breast cancer and can be used as a target for hormonal therapy as it helps pursue the transactivation of ER [26]. Simultaneously, DNAJB6 expression is reduced in aggressive breast cancer [84]. DNAJA4 methylation is suggested to be a therapeutic and diagnostic target in pediatric rhabdomyosarcoma [77]. DNAJA3 expression is increased in cancers such as lung, ovarian, colon, and breast, while DNAJC25 is decreased in hepatocellular carcinoma [120].

In the treatment of cancer, this family of proteins is engaged in assisting chemotherapeutic agent effectiveness. Expression of the DNAJ proteins, along with other HSP, is inhibited when the agent KNK437 shows its antitumor effect [148]. Angiogenesis is an important hallmark of cancer that is inhibited by the agent R115777 (also known as Tipifamib) in addition to tumor growth and survival pathways. R115777 is known to inhibit the expression of DNAJ family members. It is also known to be effective in radio-sensitization of glioblastoma multiforme cells possibly indicating employment of a more effective radiation therapy [46, 130]. Effectivity of another chemotherapeutic agent gefitinib is improved by inhibition of DNAJB1 in human lung epithelial adenocarcinoma cells [93]. Human renal cell carcinoma cells may develop resistance to the chemotherapeutic agent docetaxel; however, the situation may be overcome by the knockout of DNAJB8 protein, which is demonstrated to have a role in initiating tumors [143]. Curcumin is being explored as a cancer therapeutic and has been shown to inhibit traits of cancer cells such as invasion, migration, and metastasis. It is able to do so by activating a DNAJ family member protein, HLJ1 [16]. HSP40 or DNAJ family proteins associate with various cancers. DNAJ family has the highest member among HSP proteins. DNAJ isoforms coordination with other HSP form specific functions and therefore, the members involve in different cancers (Table 5).

## 2 Conclusions

HSP family proteins overexpressed in various cancers and elevated expression is indication of poor prognosis in these cancers. Cancer cells employs the protective effect of HSP for survival, thus elevated HSP expression are required for growth, survival and formation of secondary cancers. Further, HSP involve in cancer treatment resistance. Extracellular HSP released from cancer cells influence metastasis. HSP gene families have multiple members and function of each member is well defined however, complex formation among HSP isoforms form totally different functions and these functions must be elucidated. Therefore, drug designers synthesize novel compounds to intervene complex formation through interface inhibitors. Another strategy is to allosterically inhibit HSP function and employ competitive

**Table 5** HSP40 associated cancers

| HSP40 | Cancer Type | Association | References |
|---|---|---|---|
| DNAJA1 | Colorectal cancer | DNAJA1 was significantly upregulated in CRC tissues and positively correlated with serosa invasion, lymph node metastasis | [144] |
| DNAJB1 | Lung, Breast, Ovary Cancer | DNAJB1 suppresses p53 function via PDCD5 | [24] |
| DNAJB2 (HLJ1) | Breast Cancer | HLJ1 was found to be downregulated in breast cancers and epigenomic events such as CpG methylation and histone deacetylation could be responsible for this downregulation | [2] |
| DNAJB6 | Esophageal squamous cell carcinoma | Nuclear localization of DNAJB6 is associated with longer survival times of patients with ESCC. DNAJB6a reduces AKT signaling, and DNAJB6 expression in KYSE510, KYSE 30TSI, KYSE140, and KYSE70TS cells reduces their proliferation and growth of xenograft tumors in mice | [63] |
| DNAJB8 | | DNAJB8 is a reasonable target for CSC/CIC-targeting therapy | [61] |
| DNAJB9 | Acute myeloid leukemia | The inhibitory effect trigged by HNA was at least in part mediated through the induction of important regulatory genes for apoptosis, G0/G1 cell cycle arrest and downstream targets of XBP1, namely p53, PARP, p21cip1, p58IPK, CHOP and **DNAJB9** | [118] |
| DNAJC3-DT(AS1) | Osteosarcoma | High level of DNAJC3-AS1 was correlated with high differentiated degree and advanced Enneking stage. DNAJC3-AS1 enhanced cell proliferation, migration, and invasion in vitro and in vivo and reduced sensitivity of osteosarcoma to cisplatin | [69] |
| DNAJC6 | Hepatocellular carcinoma | There was an important function of **DNAJC6 in the** progression of HCC by induction of EMT, and they implicate **DNAJC6 as a** marker of poor outcome in HCC | [145] |
| DNAJC7 | Renal cell carcinoma | Increased serum level of DNAJC7-polyE protein was also associated with advanced RCC stage and grade | [67, 68] |
| DNAJC12 | Rectal cancer | High expression of **DNAJC12 was** found to be correlated with poor prognosis for overall survival, disease-free survival | [42] |
| DNAJC25 | Hepatocellular carcinoma | DNAJC25 was significantly downregulated in HCC, suggesting that DNAJC25 is involved in hepotocellular carcinogenesis and acts as a suppressor of HCC | [74] |

inhibitors. Currently HSP70 and HSP90 inhibitors widely employed and designed for specific HSP isoforms. Individual oncogenes drive progression of cancers and HSP70 isoforms interact distinctly with different oncogenes. Thus, these inhibitors

may be used with clinical drugs to prevent drug resistance. The HSP inhibitors provide promising results and insights from how HSP are regulated in cancer help designing novel drugs. A recent work analyzed HSP signature for the outcome prediction of breast cancer patients by analyzing databases [57]. The results indicated that patients overexpressing HSPA2 and DNAJC20 exhibited longer survival and overexpression of HSP90AA1, CCT1, CCT2, CCT6A resulted in unfavorable prognosis for breast cancer patients. Therefore, understanding HSP complex behavior and drug effect over distinct cancers may open new avenues in anticancer treatment.

**Acknowledgements** The authors are greatful for the research work in the HSP field and apologise for not citing all excellent research in the field.

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Abu-Hadid M, Wilkes JD, Elakawi Z, Pendyala L, Perez RP (1997) Relationship between heat shock protein 60 (HSP6O) mRNA expression and resistance to platinum analogues in human ovarian and bladder carcinoma cell lines. Cancer Lett 119:63–70
2. Acun T, Doberstein N, Habermann JK et al (2017) HLJ1 (DNAJB4) gene is a novel biomarker candidate in breast cancer. OMICS 21:257–265
3. Adam C, Baeurle A, Brodsky JL et al (2014) The HSP70 modulator MAL3-101 inhibits Merkel cell carcinoma. PLoS One 9:e92041
4. Arsène F, Tomoyasu T, Bukau B (2000) The heat shock response of Escherichia coli. Int J Food Microbiol 55:3–9
5. Augello G, Emma MR, Cusimano A, Azzolina A, Mongiovì S, Puleio R, Strom SC (2019) Targeting HSP90 with the small molecule inhibitor AUY922 (luminespib) as a treatment strategy against hepatocellular carcinoma. Int J Cancer 144(10):2613–2624
6. Boussadia Z, Lamberti J, Mattei F et al (2018) Acidic microenvironment plays a key role in human melanoma progression through a sustained exosome mediated transfer of clinically relevant metastatic molecules. J Exp Clin Cancer Res 37:245
7. Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo AP, Kroemer G, Solary EGC (2000) Hsp27 negatively regulates cell death by interacting with cytochrome c. Nat Cell Biol 2(9):645–652
8. Bukau B, Horwich AL (1998) The Hsp70 and Hsp60 chaperone machines. Cell 92:351–366
9. Cappello F, Gammazza AM, Piccionello AP et al (2014) Hsp60 chaperonopathies and chaperonotherapy: targets and agents. Expert Opin Ther Targets 18:185–208
10. Cayado-Gutiérrez N, Moncalero VL, Rosales EM et al (2013) Downregulation of Hsp27 (HSPB1) in MCF-7 human breast cancer cells induces upregulation of PTEN. Cell Stress Chaperones 18:243–249

11. Cele FN, Kumalo H, Soliman ME (2016) Mechanism of Inhibition of Hsp90 Dimerization by Gyrase B Inhibitor Coumermycin A1 (C–A1) Revealed by Molecular Dynamics Simulations and Thermodynamic Calculations. Cell Biochem Biophys 74(3):353–363
12. Chang C-L, Hsu Y-T, Wu C-C et al (2012) Immune mechanism of the antitumor effects generated by Bortezomib. J Immunol 189:3209–3220
13. Chang L, Miyata Y, Ung PMU et al (2011) Chemical screens against a reconstituted multiprotein complex: Myricetin blocks DnaJ regulation of DnaK through an allosteric mechanism. Chem Biol 18:210–221
14. Chang YW, Tseng CF, Wang MY et al (2016) Deacetylation of HSPA5 by HDAC6 leads to GP78-mediated HSPA5 ubiquitination at K447 and suppresses metastasis of breast cancer. Oncogene 35:1517–1528
15. Chen CY, Chiou SH, Huang CY et al (2009) Tid1 functions as a tumour suppressor in head and neck squamous cell carcinoma. J Pathol 219:347–355
16. Chen HW, Lee JY, Huang JY et al (2008) Curcumin inhibits lung cancer cell invasion and metastasis through the tumor suppressor HLJ1. Cancer Res 68:7428–7438
17. Chen Z, Ruan Q, Han S et al (2014) Discovery of structure-based small molecular inhibitor of αb-crystallin against basal-like/triple-negative breast cancer development in vitro and in vivo. Breast Cancer Res Treat 145:45–59
18. Cheng W, Li M, Jiang Y et al (2016) Association between small heat shock protein B11 and the prognostic value of MGMT promoter methylation in patients with high-grade glioma. J Neurosurg 125:7–16
19. Cho HJ, Gee HY, Baek KH et al (2011) A small molecule that binds to an ATPase domain of Hsc70 promotes membrane trafficking of mutant cystic fibrosis transmembrane conductance regulator. J Am Chem Soc 133:20267–20276
20. Choi SH, Nam JK, Kim BY et al (2016) HSPB1 inhibits the endothelial-to-mesenchymal transition to suppress pulmonary fibrosis and lung tumorigenesis. Cancer Res 76:1019–1030
21. Chun JN, Choi B, Lee KW et al (2010) Cytosolic Hsp60 is involved in the NF-kB-dependent survival of cancer cells via IKK regulation. PLoS One 5:e9422
22. Costa VL, Costa VL, Henrique R et al (2010) Three epigenetic biomarkers, GDF15, TMEFF2, and VIM, accurately predict bladder cancer from DNA-based analyses of urine samples. Clin Cancer Res 16:5842–5851
23. Csermely P, Schnaider T, Soti C, Prohászka Z, Nardai G (1998) The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. Pharmacol Ther 79:129–168
24. Cui X, Choi HK, Choi YS et al (2015) DNAJB1 destabilizes PDCD5 to suppress p53-mediated apoptosis. Cancer Lett 357:307–315
25. Daiana GA-O, Biaggio VS, Koumbadinga GA et al (2017) Recombinant heat shock protein 27 (HSP27/HSPB1) protects against cadmium-induced oxidative stress and toxicity in human cervical cancer cells. Cell Stress Chaperones 22(3):357–369
26. De Bessa SA, Salaorni S, Patrão DFC, Neto MM, Brentani MM, Nagai MA (2006) JDP1 (DNAJC12/Hsp40) expression in breast cancer and its association with estrogen receptor status. Int J Mol Med 17:363–367
27. de Jong WW, Leunissen JA, Voorter CE (1993) Evolution of the alpha-crystallin/small heat-shock protein family. Mol Biol Evol 10:103–126
28. Donnelly A, Blagg BS (2008) Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide- binding pocket. Curr Med Chem 15(26):2702–2717
29. Echeverría PC, Bernthaler A, Dupuis P, Mayer B, Picard D (2011) An interaction network predicted from public data as a discovery tool: application to the Hsp90 molecular chaperone machine. PLoS One 6:e26044
30. Edwards KM, Münger K (2004) Depletion of physiological levels of the human TID1 protein renders cancer cell lines resistant to apoptosis mediated by multiple exogenous stimuli. Oncogene 23:8419–8431

31. Evamarie Hey-Hawkins CVT (2018) Boron-based compounds: potential and emerging applications in medicine, p 496, Wiley, UK
32. Fan C-Y, Lee S, Cyr DM (2003) Mechanisms for regulation of Hsp70 function by Hsp40. Cell Stress Chaperones 8:309–316
33. Fewell SW, Smith CM, Lyon MA et al (2004) Small molecule modulators of endogenous and co-chaperone-stimulated Hsp70 ATPase activity. J Biol Chem 279:51131–51140
34. Fu Y, Zhao H, Li XS et al (2014) Expression of HSPA2 in human hepatocellular carcinoma and its clinical significance. Tumor Biol 35:11283–11287
35. Gao H, Zheng M, Sun S et al (2017) Chaperonin containing TCP1 subunit 5 is a tumor associated antigen of non-small cell lung cancer. Oncotarget 8:64170–64179
36. Garg M, Kanojia D, Seth A et al (2010) Heat-shock protein 70-2 (HSP70-2) expression in bladder urothelial carcinoma is associated with tumour progression and promotes migration and invasion. Eur J Cancer 46:207–215
37. Ghosh JC, Siegelin MD, Dohi T, Altieri DC (2010) Heat shock protein 60 regulation of the mitochondrial permeability transition pore in tumor cells. Cancer Res 70:8988–8993
38. Gimenez M, Marie SKN, Oba-Shinjo S et al (2015) Quantitative proteomic analysis shows differentially expressed HSPB1 in glioblastoma as a discriminating short from long survival factor and NOVA1 as a differentiation factor between low-grade astrocytoma and oligodendroglioma. BMC Cancer 15:481
39. Glover JR, Lindquist S (1998) Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell 94:73–82
40. Hassan AQ, Kirby CA, Zhou W et al (2015) The novolactone natural product disrupts the allosteric regulation of Hsp70. Chem Biol 22:87–97
41. Hayashi R, Ishii Y, Ochiai H et al (2012) Suppression of heat shock protein 27 expression promotes 5-fluorouracil sensitivity in colon cancer cells in a xenograft model. Oncol Rep 28:1269–1274
42. He HL, Lee YE, Chen HP et al (2015) Overexpression of DNAJC12 predicts poor response to neoadjuvant concurrent chemoradiotherapy in patients with rectal cancer. Exp Mol Pathol 98:338–345
43. Hernández MP, Sullivan WP, Toft DO, Herna MP, Sullivan WP, Toft DO (2002) PROTEIN STRUCTURE AND FOLDING: the assembly and intermolecular properties of the hsp70-Hop-hsp90 molecular chaperone complex the assembly and intermolecular properties of the hsp70-Hop-hsp90 molecular chaperone complex *. J Biol Chem 277:38294–38304
44. Howe MK, Bodoor K, Carlson DA et al (2014) Identification of an allosteric small-molecule inhibitor selective for the inducible form of heat shock protein 70. Chem Biol 21:1648–1659
45. Hu W, Wang J, Luo G et al (2013) Proteomics-based analysis of differentially expressed proteins in the CXCR1-knockdown gastric carcinoma MKN45 cell line and its parental cell. Acta Biochim Biophys Sin Shanghai 45:857–866
46. Izbicka E, Campos D, Carrizales G, Patnaik A (2005) Biomarkers of anticancer activity of R115777 (Tipifarnib, Zarnestra???) in human breast cancer models in vitro. Anticancer Res 25:3215–3223
47. Jäättelä M, Wissing D, Bauer PA, Li GC (1992) Major heat shock protein hsp70 protects tumor cells from tumor necrosis factor cytotoxicity. EMBO J 11:3507–3512
48. Jagadish N, Parashar D, Gupta N et al (2016) Heat shock protein 70-2 (HSP70-2) is a novel therapeutic target for colorectal cancer and is associated with tumor growth. BMC Cancer 16:561
49. Jin HO, Hong SE, Kim JY et al (2019) Induction of HSP27 and HSP70 by constitutive overexpression of Redd1 confers resistance of lung cancer cells to ionizing radiation. Oncol Rep 41:3119–3126
50. Kabani M, Beckerich J-M, Brodsky JL (2002a) Nucleotide exchange factor for the yeast Hsp70 molecular chaperone Ssa1p. Mol Cell Biol 22:4677–4689

51. Kabani M, McLellan C, Raynes DA, Guerriero V, Brodsky JL (2002b) HspBP1, a homologue of the yeast Fes1 and Sls1 proteins, is an Hsc70 nucleotide exchange factor. FEBS Lett 531 (2):339–342
52. Kamal A, Thao L, Sensintaffar J et al (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425:407–410
53. Khamit-Kush KK, Lillard JW, Bond VC, Huang M, Roth W (2018) Abstract A42: identifying relationships between high expression levels of the HSPA9 gene, putative HSPA9 alterations, and patient survivability in invasive breast carcinomas. A42–A42
54. Kim MS, Lee HW, Jun SY, Lee EH (2015) Expression of alpha B crystallin and BCL2 in patients with infiltrating ductal carcinoma. Int J Clin Exp Pathol 8:8842–8856
55. Kim SW, Hayashi M, Lo JF et al (2005) Tid1 negatively regulates the migratory potential of cancer cells by inhibiting the production of interleukin-8. Cancer Res 65:8784–8791
56. Kim SY, Kim HJ, Kim HJ et al (2018) HSPA5 negatively regulates lysosomal activity through ubiquitination of MUL1 in head and neck cancer. Autophagy 14:385–403
57. Klimczak M, Biecek P, Zylicz A, Zylicz M (2019) Heat shock proteins create a signature to predict the clinical outcome in breast cancer. Sci Rep 9:7507
58. Koca İ, Gumus M, Ozgur A, Disli A, Tutar Y (2015) A novel approach to inhibit heat shock response as anticancer strategy by coumarine compounds containing thiazole skeleton. Anti-Cancer Agents Med Chem (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 15 (7):916–930
59. Koca İ, Özgür A, Er M, Gümüş M, Coşkun KA, Tutar Y (2016) Design and synthesis of pyrimidinyl acyl thioureas as novel Hsp90 inhibitors in invasive ductal breast cancer and its bone metastasis. Eur J Med Chem 122:280–290
60. Kuramitsu Y, Suenaga S, Wang Y et al (2013) Up-regulation of DDX39 in human pancreatic cancer cells with acquired gemcitabine resistance compared to gemcitabine-sensitive parental cells. Anticancer Res 33:3133–3136
61. Kusumoto H, Hirohashi Y, Nishizawa S et al (2018) Cellular stress induces cancer stem-like cells through expression of DNAJB8 by activation of heat shock factor 1. Cancer Sci 109:741–750
62. Lagarrigue F, Dupuis-Coronas S, Ramel D et al (2010) Matrix metalloproteinase-9 is upregulated in nucleophosmin-anaplastic lymphoma kinase-positive anaplastic lymphomas and activated at the cell surface by the chaperone heat shock protein 90 to promote cell invasion. Cancer Res 70:6978–6987
63. Lee HJ, Kim JM, Kim KH, Heo JI, Kwak SJ, Han JA (2015) Genotoxic stress/p53-induced DNAJB9 inhibits the pro-apoptotic function of p53. Cell Death Differ 22:86–95
64. Leu JIJ, Pimkina J, Frank A, Murphy ME, George DL (2009) A small molecule inhibitor of inducible heat shock protein 70. Mol Cell 36:15–27
65. Li J, Hu W, Lan Q (2012) The apoptosis-resistance in t-AUCB-treated glioblastoma cells depends on activation of Hsp27. J Neuro-Oncol 110:187–194
66. Li Q, Wang Y, Lai Y, Xu P, Yang Z (2017) HspB5 correlates with poor prognosis in colorectal cancer and prompts epithelial-mesenchymal transition through ERK signaling. PLoS One 12: e0182588
67. Li X, Shao HR, Taylor IE, Gestwicki J (2016b) Targeting allosteric control mechanisms in heat shock protein 70 (Hsp70). Curr Top Med Chem 16:2729–2740
68. Li X, Xu S, Cheng Y, Shu J (2016c) HSPB1 polymorphisms might be associated with radiation-induced damage risk in lung cancer patients treated with radiotherapy. Tumor Biol 37:5743–5749
69. Liang R, Liu Z, Chen Z et al (2019) Long noncoding RNA DNAJC3-AS1 promotes osteosarcoma progression via its sense-cognate gene DNAJC3. Cancer Med 8:761–772
70. Lin J, Deng Z, Tanikawa C et al (2014) Downregulation of the tumor suppressor HSPB7, involved in the p53 pathway, in renal cell carcinoma by hypermethylation. Int J Oncol 44:1490–1498

71. Lin SP, Lee YT, Wang JY et al (2012) Survival of Cancer stem cells under hypoxia and serum depletion via decrease in PP2A activity and activation of p38-MAPKAPK2-Hsp27. PLoS One 7:e49605
72. Liu K, Kang M, Li J et al (2019) Prognostic value of the mRNA expression of members of the HSP90 family in non small cell lung cancer. Exp Ther Med 17(4):2657–2665
73. Liu QH, Zhao CY, Zhang J et al (2012a) Role of heat shock protein 27 in gemcitabine-resistant human pancreatic cancer: comparative proteomic analyses. Mol Med Rep 6:767–773
74. Liu T, Jiang W, Han D, Yu L (2012b) DNAJC25 is downregulated in hepatocellular carcinoma and is a novel tumor suppressor gene. Oncol Lett 4:1274–1280
75. Luo C, Qiu J (2017) miR-181a inhibits cervical cancer development via downregulating GRP78. Oncol Res 25:1341–1348
76. Ma H, Lu T, Zhang X et al (2015) HSPA12B: A novel facilitator of lung tumor growth. Oncotarget 6:9924–9936
77. Mahoney SE, Yao Z, Keyes CC, Tapscott SJ, Diede SJ (2012) Genome-wide DNA methylation studies suggest distinct DNA methylation patterns in pediatric embryonal and alveolar rhabdomyosarcomas. Epigenetics 7:400–408
78. Malin D, Strekalova E, Petrovic V et al (2014) αb-crystallin: A novel regulator of breast cancer metastasis to the brain. Clin Cancer Res 20:1–12
79. Massey AJ, Williamson DS, Browne H et al (2010) A novel, small molecule inhibitor of Hsc70/Hsp70 potentiates Hsp90 inhibitor induced apoptosis in HCT116 colon carcinoma cells. Cancer Chemother Pharmacol 66:535–545
80. Mayer MP, Bukau B (1999) Molecular chaperones: the busy life of Hsp90. Curr Biol 9:R322–R325
81. Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670–684
82. Mellatyar H, Talaei S, Pilehvar-Soltanahmadi Y et al (2018) Targeted cancer therapy through 17-DMAG as an Hsp90 inhibitor: overview and current state of the art. Biomed Pharmacother 102:608–617. Elsevier
83. Meng Q, Li BX, Xiao X (2018) Toward developing chemical modulators of Hsp60 as potential therapeutics. Front Mol Biosci 5:35
84. Mitra A, Fillmore RA, Metge BJ et al (2008) Large isoform of MRJ (DNAJB6) reduces malignant activity of breast cancer. Breast Cancer Res 10:R22
85. Mu N, Lei Y, Wang Y et al (2019) Inhibition of SIRT1/2 upregulates HSPA5 acetylation and induces pro-survival autophagy via ATF4-DDIT4-mTORC1 axis in human lung cancer cells. Apoptosis 24:798–811
86. Murphy ME (2013) The HSP70 family and cancer. Carcinogenesis 34:1181–1188
87. Nadler SG, Tepper MA, Schacter B, Mazzucco CE (1992) Interaction of the immunosuppressant deoxyspergualin with a member of the Hsp70 family of heat shock proteins. Science (80-) 258:484–486
88. Nagumo Y, Kakeya H, Yamaguchi J et al (2004) Structure-activity relationships of epolactaene derivatives: structural requirements for inhibition of Hsp60 chaperone activity. Bioorganic Med Chem Lett 14:4425–4429
89. O'Callaghan-Sunol C, Gabai VL, Sherman MY (2007) Hsp27 modulates p53 signaling and suppresses cellular senescence. Cancer Res 67:11779–11788
90. Otaka M, Yamamoto S, Ogasawara K et al (2007) The induction mechanism of the molecular chaperone HSP70 in the gastric mucosa by Geranylgeranylacetone (HSP-inducer). Biochem Biophys Res Commun 353:399–404
91. Özgür A, Bayram B, Tutar L, Tutar Y (2016) Recent developments in anticancer agents targeting heat shock proteins. Top Anti-Cancer Res 5:205–224
92. Pak MG, Koh HJ, Roh MS (2017) Clinicopathologic significance of TRAP1 expression in colorectal cancer: a large scale study of human colorectal adenocarcinoma tissues. Diagn Pathol 12:6

93. Park SY, Choi HK, Seo JS et al (2015) DNAJB1 negatively regulates MIG6 to promote epidermal growth factor receptor signaling. Biochim Biophys Acta, Mol Cell Res 1853:2722–2730
94. Polson E, Kuchler V, Abbosh C et al (2017) The small molecule KHS101 induces bioenergetic dysfunction in glioblastoma cells through inhibition of mitochondrial HSPD1. bioRxiv 205203
95. Prodromou C, Siligardi G, O'Brien R et al (1999) Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones. EMBO J 18:754–762
96. Qian EA, Han SY, Ding SZ, Lv X (2016) Expression and diagnostic value of CCT3 and IQGAP3 in hepatocellular carcinoma. Cancer Cell Int 16(1):55
97. Qin H, Ni Y, Tong J et al (2014) Elevated expression of CRYAB predicts unfavorable prognosis in non-small cell lung cancer. Med Oncol 31:142
98. Qiu X, He X, Huang Q et al (2015) Overexpression of CCT8 and its significance for tumor cell proliferation, migration and invasion in glioma. Pathol Res Pract 211(10):717–725
99. Ramos CHI, Oliveira CLP, Fan C-Y, Torriani IL, Cyr DM (2008) Conserved central domains control the quaternary structure of type I and type II Hsp40 molecular chaperones. J Mol Biol 383:155–166
100. Ramya TNC, Surolia N, Surolia A (2007) 15-Deoxyspergualin inhibits eukaryotic protein synthesis through eIF2α phosphorylation. Biochem J 401:411–420
101. Rousaki A, Miyata Y, Jinwal UK, Dickey CA, Gestwicki JE, Zuiderweg ERP (2011) Allosteric drugs: the interaction of antitumor compound MKT-077 with human Hsp70 chaperones. J Mol Biol 411:614–632
102. Ruan W, Wang Y, Ma Y et al (2010) HSP60, a protein downregulated by IGFBP7 in colorectal carcinoma. J Exp Clin Cancer Res 29:41
103. Samant RS, Clarke PA, Workman P (2012) The expanding proteome of the molecular chaperone HSP90. Cell Cycle 11:1301–1308
104. Schlecht R, Scholz SR, Dahmen H et al (2013) Functional analysis of Hsp70 inhibitors. PLoS One 8:e78443
105. Shan N, Zhou W, Zhang S, Zhang Y (2016) Identification of HSPA8 as a candidate biomarker for endometrial carcinoma by using iTRAQ-based proteomic analysis. Onco Targets Ther 9:2169–2179
106. Shao H, Li X, Moses MA et al (2018) Exploration of Benzothiazole Rhodacyanines as allosteric inhibitors of protein-protein interactions with heat shock protein 70 (Hsp70). J Med Chem 61:6163–6177
107. Shen J, Li M, Min L (2018) HSPB8 promotes cancer cell growth by activating the ERK-CREB pathway and is indicative of a poor prognosis in gastric cancer patients. Oncol Rep 39:2978–2986
108. Shi C, He Z, Hou N, Ni Y, Xiong L, Chen P (2014) Alpha B-crystallin correlates with poor survival in colorectal cancer. Int J Clin Exp Pathol 7:6056–6063
109. Shi QM, Luo J, Wu K, Yin M, Gu YR, Cheng XG (2016) High level of αB-crystallin contributes to the progression of osteosarcoma. Oncotarget 7:9007–9016
110. Shiota M, Bishop JL, Nip KM et al (2013) Hsp27 regulates epithelial mesenchymal transition, metastasis, and circulating tumor cells in prostate cancer. Cancer Res 73:3109–3119
111. Siligardi G, Panaretou B, Meyerc P et al (2002) Regulation of Hsp90 ATPase activity by the Co-chaperone Cdc37p/p50 cdc37. J Biol Chem 277:20151–20159
112. Sims JD, McCready J, Jay DG (2011) Extracellular heat shock protein (Hsp)70 and Hsp90α assist in matrix metalloproteinase-2 activation and breast cancer cell migration and invasion. PLoS One 6:e18848
113. Sims JT, Ganguly SS, Bennett H, Friend JW, Tepe J, Plattner R (2013) Imatinib reverses doxorubicin resistance by affecting activation of STAT3-dependent NF-κB and HSP27/p38/AKT pathways and by inhibiting ABCB1. PLoS One 8:e55509

114. Slotta-Huspenina J, Wolff C, Drecoll E et al (2013) A specific expression profile of heat-shock proteins and glucose-regulated proteins is associated with response to neoadjuvant chemotherapy in oesophageal adenocarcinomas. Br J Cancer 109:370–378
115. Sojka DR, Gogler-Pigłowska A, Vydra N et al (2019) Functional redundancy of HSPA1, HSPA2 and other HSPA proteins in non-small cell lung carcinoma (NSCLC); an implication for NSCLC treatment. Sci Rep 9:14394
116. Song X, Wang X, Zhuo W et al (2010) The regulatory mechanism of extracellular Hsp90α on matrix metalloproteinase-2 processing and tumor angiogenesis. J Biol Chem 285:40039–40049
117. Song X, Wang X, Zhuo W et al (2014) Heat shock proteins in cancer: chaperones of tumorigenesis. J Biol Chem 31:987–1001. Elsevier
118. Sun H, Lin DC, Guo X et al (2016) Inhibition of IRE1α-driven pro-survival pathways is a promising therapeutic application in acute myeloid leukemia. Oncotarget 7:18736–18749
119. Thuringer D, Jego G, Wettstein G et al (2013) Extracellular HSP27 mediates angiogenesis through toll-like receptor 3. FASEB J 27:4169–4183
120. Traicoff JL, Chung JY, Braunschweig T et al (2007) Expression of EIF3-p48/INT6, TID1 and patched in cancer, a profiling of multiple tumor types and correlation of expression. J Biomed Sci 14:395–405
121. Trinh DLN, Elwi AN, Kim SW (2010) Direct interaction between p53 and Tid1 proteins affects p53 mitochondrial localization and apoptosis. Oncotarget 1:396–404
122. Tsai YP, Yang MH, Huang CH et al (2009) Interaction between HSP60 and β-catenin promotes metastasis. Carcinogenesis 30:1049–1057
123. Tutar L, Tutar Y (2008) Ydj1 but not Sis1 stabilizes Hsp70 protein under prolonged stress in vitro. Biopolymers 89:171–174
124. Tutar L, Tutar Y (2010) Heat shock proteins: an overview. Curr Pharm Biotechnol 11:216–222
125. Tutar Y, Song Y, Masison DC (2006) Primate chaperones Hsc70 (constitutive) and Hsp70 (induced) differ functionally in supporting growth and prion propagation in Saccharomyces cerevisiae. Genetics 172:851–861
126. Tutar Y (2006a) Heat shock proteins, substrate specificity and modulation of function. Protein Pept Lett 13:699–705
127. Tutar Y (2006b) Key residues involved in Hsp70 regulatory activity and affect of co-chaperones on mechanism of action. Protein Pept Lett 13:693–698
128. Ui T, Morishima K, Saito S et al (2014) The HSP90 inhibitor 17-N-allylamino-17-demethoxy geldanamycin (17-AAG) synergizes with cisplatin and induces apoptosis in cisplatin-resistant esophageal squamous cell carcinoma cell lines via the Akt/XIAP pathway. Oncol Rep 31:619–624
129. van de Schootbrugge C, van Asten F, Nagtegaal ID et al (2013) αB-crystallin expression is correlated with phospho-ERK1/2 expression in human breast cancer. Int J Biol Markers 28: e365–e370
130. Wang CC, Liao YP, Mischel PS, Iwamoto KS, Cacalano NA, McBride WH (2006) HDJ-2 as a target for radiosensitization of glioblastoma multiforme cells by the farnesyltransferase inhibitor R115777 and the role of the p53/p21 pathway. Cancer Res 66:6756–6762
131. Wang R, Qin HM, Qin L, Wei JX, Wei YX, Wang JL (2019) Genetic association of promoter in GRP78 gene with nasopharyngeal carcinoma in a Chinese population. Int J Clin Oncol 24:359–365
132. Whitesell L, Lindquist SL (2005) HSP90 and the chaperoning of cancer. Nat Rev Cancer 5:761–772
133. Williams DR, Ko SK, Park S, Lee MR, Shin I (2008) An apoptosis-inducing small molecule that binds to heat shock protein 70. Angew Chem Int Ed 47:7466–7469
134. Williamson DS, Borgognoni J, Clay A et al (2009) Novel adenosine-derived inhibitors of 70 kDa heat shock protein, discovered through structure-based design. J Med Chem 52:1510–1513

135. Wisén S, Bertelsen EB, Thompson AD et al (2010) Binding of a small molecule at a protein-protein interface regulates the chaperone activity of Hsp70-Hsp40. ACS Chem Biol 5:611–622
136. Wong CSC, Wai-Ki Wong V, Ming-Lok Chan C et al (2008) Identification of 5-fluorouracil response proteins in colorectal carcinoma cell line SW480 by two-dimensional electrophoresis and MALDI-TOF mass spectrometry. Oncol Rep 20:89–98
137. Wu J, Liu T, Rios Z, Mei Q, Lin X, Cao S (2017) Heat shock proteins and Cancer. Trends Pharmacol Sci 38:226–256
138. Wu X, Zheng YZ, Han B, Wang K (2018) Alpha B-crystallin C-802G polymorphism and colorectal cancer susceptibility and clinical outcome in Chinese population. Sci Rep 8:11731
139. Xu L, Bergan RC (2006) Genistein inhibits matrix metalloproteinase type 2 activation and prostate cancer cell invasion by blocking the transforming growth factor β-mediated activation of mitogen-activated protein kinase-activated protein kinase 2-27-kDa heat shock protein pathw. Mol Pharmacol 70:869–877
140. Xu L, Chen S, Bergan RC (2006) MAPKAPK2 and HSP27 are downstream effectors of p38 MAP kinase-mediated matrix metalloproteinase type 2 activation and cell invasion in human prostate cancer. Oncogene 25:2987–2998
141. Yamaguchi J, Kakeya H, Uno T, Shoji M, Osada H, Hayashi Y (2005) Determination by asymmetric total synthesis of the absolute configuration of lucilactaene, a cell-cycle inhibitor in p53-transfected cancer cells. Angew Chem Int Ed 44:3110–3115
142. Yamamoto S, Nakano S, Owari K et al (2010) Gentamicin inhibits HSP70-assisted protein folding by interfering with substrate recognition. FEBS Lett 584:645–651
143. Yamashita M, Hirohashi Y, Torigoe T et al (2016) Dnajb8, a member of the heat shock protein 40 family has a role in the tumor initiation and resistance to docetaxel but is dispensable for stress response. PLoS One 11:e0146501
144. Yang S, Ren X, Liang Y et al (2019) KNK437 restricts the growth and metastasis of colorectal cancer via targeting DNAJA1/CDC45 axis. Oncogene 39(2):249–261
145. Yang T, Li XN, Li XG, Li M, Gao PZ (2014) DNAJC6 promotes hepatocellular carcinoma progression through induction of epithelial-mesenchymal transition. Biochem Biophys Res Commun 455:298–304
146. Yang Z, Zhuang L, Szatmary P et al (2015) Upregulation of heat shock proteins (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in tumour tissues is associated with poor outcomes from HBV-related early-stage hepatocellular carcinoma. Int J Med Sci 12:256–263
147. Yip CW, Lam CY, Poon TCW et al (2017) Granulin-epithelin precursor interacts with 78-kDa glucose-regulated protein in hepatocellular carcinoma. BMC Cancer 17:409
148. Yokota SI, Kitahara M, Nagata K (2000) Benzylidene lactam compound, KNK437, a novel inhibitor of acquisition of thermotolerance and heat shock protein induction in human colon carcinoma cells. Cancer Res 60:2942–2948
149. Young JC (2000) Polypeptide release by Hsp90 involves ATP hydrolysis and is enhanced by the co-chaperone p23. EMBO J 19:5930–5940
150. Zhai LL, Xie Q, Zhou CH, Huang DW, Tang ZG, Ju TF (2017) Overexpressed HSPA2 correlates with tumor angiogenesis and unfavorable prognosis in pancreatic carcinoma. Pancreatology 17:457–463
151. Zhai T, Muhanhali D, Jia X, Wu Z, Cai Z, Ling Y (2019) Identification of gene co-expression modules and hub genes associated with lymph node metastasis of papillary thyroid cancer. Endocrine 66(3):573–584
152. Zhang H, Chen W, Duan CJ, Zhang CF (2013) Overexpression of HSPA2 is correlated with poor prognosis in esophageal squamous cell carcinoma. World J Surg Oncol 11:141
153. Zhang S, Hu Y, Huang Y, Xu H, Wu G, Dai H (2015) Heat shock protein 27 promotes cell proliferation through activator protein-1 in lung cancer. Oncol Lett 9:2572–2576
154. Zhang Z, Xue N, Bian C, Yan R, Jin L, Chen X, Yu X (2016) C15-methoxyphenylated 18-deoxy-herbimycin A analogues, their in vitro anticancer activity and heat shock protein 90 binding affinity. Bioorg Med Chem Lett 26(17):4287–4291

155. Zhao M, Shen F, Yin YX, Yang YY, Xiang DJ, Chen Q (2012) Increased expression of heat shock protein 27 correlates with peritoneal metastasis in epithelial ovarian cancer. Reprod Sci 19:748–753
156. Zhou C, Sun H, Zheng C et al (2018) Oncogenic HSP60 regulates mitochondrial oxidative phosphorylation to support Erk1/2 activation during pancreatic cancer cell growth article. Cell Death Dis 9:161
157. Zou M, Bhatia A, Dong H et al (2017) Evolutionarily conserved dual lysine motif determines the non-chaperone function of secreted Hsp90alpha in tumour progression. Oncogene 36 (15):2160

# Effects of Heat Shock Protein 70 kDa in Allergic Airway Inflammation


**Marina A. Shevchenko, Natalia I. Troyanova, and Alexander M. Sapozhnikov**

## Abstract

**Introduction** Local elevation of extracellular heat shock protein 70 kDa (HSP70) concentration is an attribute of the acute phase of inflammation of different origins, particularly, allergic airway inflammation. The effects and the mechanisms of HSP70-mediated effects in allergic airway inflammation are still not completely understood. In this book chapter, we summarize the data regarding the effects of HSP70 on immune cells in the course of allergic airway inflammation. We discuss the role of ATPase and chaperone activity in the regulation of immune responses that cause inflammation resolution.

**Methods** Our rationale is based on *in vivo* studies to assess Hsp70 effects in the allergic airway inflammation mouse models and *in vitro* experiments tested direct Hsp70 impact on activated neutrophils yielded from the bone marrow of intact mice.

**Results** Here we provide the data regarding the implication of HSP70 in the allergic airway inflammation. In addition to the role of endogenous HSP70 in this immune process, we discuss some effects of allergens belonging to the HSP70 family that can induce a cross-reactive humoral response to self HSP70. Chaperone properties of the proteins support the hypothesis of the anti-inflammatory activities of HSP70. Indeed, the suppressive activity of HSP70 was reported for inflammatory processes in the respiratory tract. We provide the data indicating possible and established mechanisms of HSP70 effects on immune cells in the course of allergic airway inflammation.

**Conclusions** In this book chapter, we demonstrated the implication of HSP70 in the allergic airway inflammation. We also discussed the role of ATPase and chaperone activity of HSP70 in the regulation of immune responses that cause inflammation resolution.

M. A. Shevchenko · N. I. Troyanova · A. M. Sapozhnikov (✉)
Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia
e-mail: amsap@ibch.ru

Heat Shock Proteins (2021) 22: 203–212
https://doi.org/10.1007/7515_2020_8

Published Online: 23 July 2020

**Keywords** Allergens · Allergic airway inflammation · ATP · Granulocytes · HSP70 · Suppressive effect

## Abbreviations

| | |
|---|---|
| BSA | bovine serum albumin |
| fMLP | N-formylmethionine-leucyl-phenylalanine |
| HBSS | Hank's balanced salt solution |
| NETs | neutrophil extracellular traps |
| OVA | ovalbumin |
| PMA | phorbol 12-myristate 13-acetate |
| ROS | reactive oxygen species |

## 1 Introduction

HSP70 in some cases can act as a stimulator of immune inflammation and in other situations reveal an anti-inflammatory activity. Multiple factors influence the effects of HSP70 in inflammation, such as the origin of HSP70, the interaction of HSP70 with other proteins, particularly with receptors that are expressed by different cell populations. The local immune cell microenvironment is also of great importance in the context of HSP70 effects. Such a complex process as allergic airway inflammation is driven by different immune cell populations, in particular Th2, ILC2, and eosinophils. Antigen-presenting cells and neutrophils are also implicated in the regulation of allergic airway inflammation. In this chapter, we concern the allergenic and suppressive effects of HSP70 in the course of allergic airway inflammation. We discuss the anti-inflammatory potential of ATPase activity of HSP70 and summarize the reported HSP70-induced neutrophil-mediated regulation of allergic airway inflammation.

### *1.1 HSP70 in Allergic Airway Inflammation and Asthma*

Extracellular heat shock protein 70 kDa (HSP70) does not serve as a specific marker of asthma, but the elevation of extracellular HSP70 level was detected in samples of bronchopulmonary lavages in the model of induced allergic airway inflammation in mice [31]. Moreover, systemic and local elevation of extracellular HSP70 level was shown to correlate with the severity of airway inflammation in asthmatic patients [19]. It is important that in the course of inflammation, extracellular HSP70 can reveal both pro-inflammatory and anti-inflammatory activity [7, 10, 30–32, 36].

## 1.2 Allergenic Potential of HSP70

Several members of HSP70 family belong to allergens. Among them Alt a 3 [8] from mold *Alternaria alternate*, Mala s 10 [3] from yeast *Malacesia sympodialys*; mite protein Der f 28 [1, 2] from house dust mite *Dermatophagoides farina* and some others (the data are presented in accordance with database AllFam (http://www.meduniwien.ac.at/allfam/) [29]). Due to high homology between HSP70 family members, cross-reactivity of serum Mala s 10-specific IgE from patients with allergic eczema or dermatitis to self Hsp70 was described [3]. Elevation of self Hsp70-specific IgG1 was reported for patients with allergic bronchial asthma [38]. Later, Hou et al. [19] observed the associations of increased serum Hsp70 level and airway neutrophilia. Besides, correlation of sputum Hsp70 level and the number of lymphocytes was detected in asthmatics [19]. Thus, the level of the circulating in the organism extracellular HSP70 elevates in the course of allergic inflammation. Primary structure homology between allergens from HSP70 family and self, autologous HSP70 proteins under certain conditions can promote the inflammation progression.

## 1.3 Suppressive Effect of HSP70 in the Airway Inflammation of Different Etiology

As it was mentioned above, HSP70 can display both pro- and anti-inflammatory activity in the course of inflammation. The anti-inflammatory effects of HSP70 were demonstrated initially by the findings that the induction of increased Hsp70 expression protected lung tissues from the fibrosis caused by chronic inflammation [16, 33]. Regarding allergy and asthma, increased Hsp70 levels were detected in exosomes that were yielded from bronchoalveolar lavages of mice with induced tolerance to the allergen [28]. Moreover, preventive intranasal immunization of mice with Hsp70-rich exosomes blocked the allergic airway inflammation development in response to olive pollen allergen [28]. The direct protective effect of Hsp70 was demonstrated in the mouse model of allergic airway inflammation [31]. In particular, oropharyngeal application of Hsp70 in the acute phase of ovalbumin-induces allergic airway inflammation prevented airway eosinophilia and suppressed the total bronchoalveolar lavage cell number and pro-allergic cytokine IL-4, IL-5 и IL-13 levels (Fig. 1). Thus, the elevation of extracellular HSP70 level can be protective in the airway inflammation, particularly allergic airway inflammation, however, the mechanisms of the protective HSP70 effects are still not completely understand.

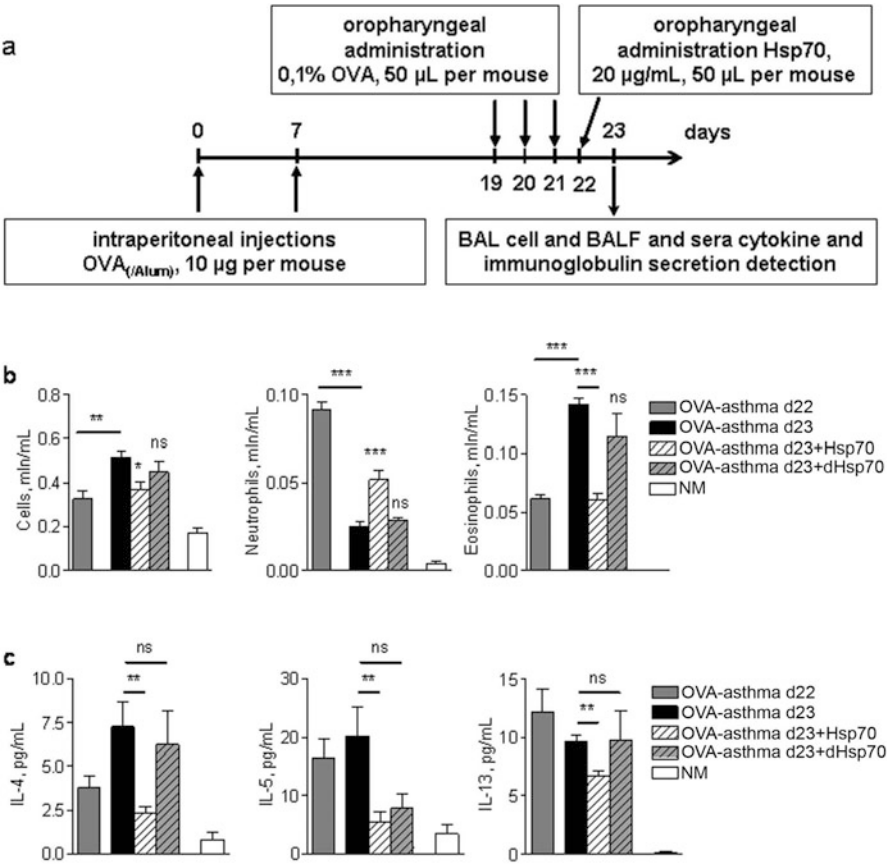



**Fig. 1 Protective effects of exogenous Hsp70 in mouse model of allergic airway inflammation.** (Adopted from [31]). Mice received Hsp70 24 h after the last allergen challenge (**a**). (**b**) Total cell number (left), neutrophil (middle), and eosinophil (right) counts in BAL of mice with induced allergic airway inflammation. (**c**) BAL fluid levels of IL-4, IL-5, and IL-13 of mice with induced allergic airway inflammation and intact mice. Representative data of three independent experiments. Data are presented as mean ± SEM for five mice per group. Significant difference is indicated; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant

## *1.4 ATPase Activity as Potentially Protective Mechanism of HSP70-Mediated Suppression in Allergic Airway Inflammation*

It is known that the main functions of HSP70 are connected with ATPase activity of the protein. We suggest that this activity can be implicated in the mechanisms of protective effects of HSP70 in the inflammation. ATP is known to be one of the danger signals. Elevation of extracellular ATP level was observed in asthmatics at the acute phase of inflammation [20]. Binding to purinergic receptors P2X and P2Y

on dendritic cells causes the pro-inflammatory activation that leads to skewing immune response towards Th2 proliferation that together with eosinophil recruitment drive allergic airway inflammation [21]. In non-inflamed steady-state conditions exoferments - ectonucleoside triphosphate diphosphohydrolases (E-NTPD) – regulate extracellular ATP level at physiological conditions [39]. E-NTPD, as well as HSP70, belongs to sugar kinase/heat shock protein 70/actin superfamily and possesses a similar conformation of ATP-binding domain that is sensitive to $Ca2^+$ or $Mg2^+$ [41]. E-NTPDs manifest ATPase activity, however, due to their membrane-bound location they can act only on the cell surface, but not in the extracellular matrix, like HSP70 [35, 39]. The implication of HSP70 in the regulation of extracellular ATP concentration was not yet detected *in vivo*, however, *in vitro* studies revealed the decrease of extracellular ATP level in presence of Hsp70 in culture filtrate of bone-marrow-derived dendritic cells [6]. Taken together, increased HSP70 concentration at the site of allergic airway inflammation and the ATPase activity of the protein support the hypothesis that HSP70 can downregulate the extracellular ATP levels and suppress the P2X activation by reduction of activation stimulus. Thus, the neutralization of extracellular ATP by extracellular pool of HSP70 can be considered as one of the potential molecular mechanisms of anti-inflammatory HSP70 potential and is to be further investigated.

## *1.5 Effects of HSP70 on Granulocytes in the Course of Allergic Airway Inflammation*

At the acute phase of allergic airway inflammation like in any inflammatory process both clinical and experimental studies observed IL-8 secretion and subsequent neutrophil attraction to the site of inflammation [23, 24]. However, multiple repeats of allergen exposure led to a decrease of neutrophil numbers and the establishment of Th2-mediated response [5, 18]. In accordance to the classical view on allergic airway inflammation, development of pro-allergic cytokines IL-4, IL-5 and IL-13 (that are secreted by ILC2s at the early stage of inflammation and in most extent by Th2 at the latter stage) induces the egression of eosinophils from the bone marrow, activate endothelium to retain the eosinophils in the sites of inflammation and promote eosinophil maturation [18, 22, 26]. The simultaneously rising concentration of IL-4 blocks the egression of neutrophils from bone marrow [37].

As it was shown above, the exogenous elevation of extracellular Hsp70 concentration in the site of inflammation decreased both the level of Il-4 and eosinophilia in the experimental mouse model of OVA-induced asthma (Fig. 1). Besides, exogenous Hsp70 application at the acute phase prevented subsequent decrease of bronchoalveolar lavage neutrophil numbers at the late phase of allergic airway inflammation (Fig. 1).

The potential of Hsp70 to stimulate chemotaxis of neutrophils was reported previously for *in vitro* models [27]. One possible mechanism of neutrophil attraction

is the interaction of ATP-bound form of Hsp70 to TLR4 [11] that can potentially activate subsequent immune responses [17, 25]. Another mechanism by which HSP70 can influence the neutrophil behavior was coming from the ability of Hsp70 to interact with TPR domain-containing proteins, so-called co-chaperones [4]. One of such proteins is Hsp interacting protein (Hip) that can also interact with CXCR2 expressing on mature neutrophils and responsible for neutrophil recruitment from bone marrow to the periphery [13]. In this way, the elevation of extracellular Hsp70 concentration in the site of inflammation may affect the chemotactic activity of neutrophils. Thus, the decrease of IL-4 level in bronchoalveolar lavage that was observed after Hsp70 application together with the neutrophil attractive potential of Hsp70 can be reasons for the retention of neutrophils in the airways. Recruited to the site of inflammation neutrophils can neutralize the pathogen by one of the following strategies: phagocytosis, reactive oxygen species (ROS) production and neutrophil extracellular traps (NETs) formation. ROS hyperproduction, as well as uncontrolled netosis, are the important factors of asthma complications in response to allergen provocation [9, 12].

At the same time, *in vitro* studies demonstrated that Hsp70 prevented bacterial antigen-induced ROS production and pro-inflammatory cytokine secretion by both human peripheral blood and mouse bone marrow neutrophils [34, 40]. In particular, the set of experiments was carried out with bone marrow-derived neutrophils (Fig. 2a). The observation that heat shock itself (Fig. 2b), as well as exogenously added Hsp70, decreased fMLP-induced ROS production (Fig. 2c) supported the hypothesis of Hsp70-mediated prevention of neutrophil hyperactivation. Besides, HSP70 was shown to be able to *in vitro* suppress PMA-induced NET formation by neutrophils (Fig. 2d). These data are in agreement with previously showed suppression of ROS production since the NET release was shown to be linked to the ROS-dependent cell death pathway in the inflammation [15]. Together with that, the percentage of spontaneous apoptosis in the primary culture of bone marrow-derived apoptosis significantly elevated in the presence of Hsp70 (Fig. 2e). Thus, Hsp70 prevented netosis and necrosis and facilitated spontaneous apoptosis of neutrophils. The demonstrated data support the hypothesis that extracellular HSP70 – neutrophil interaction affects the functional activity of neutrophils. In relation to allergic airway inflammation, neutrophil apoptosis was shown to be advantageous for resolution of inflammation [14]. Thus, the elevation of extracellular HSP70 concentration facilitates neutrophil recruitment, but prevents their hyperactivation and in this way stops the inflammation development.

## 2 Conclusions

In conclusion, we summarize some possible mechanisms of the demonstrated previously HSP70 protective effects in allergic airway inflammation. Based on ATPase and chaperone activity of HSP70 we can outline the points of potential HSP70 implication in suppression of allergic airway inflammation.

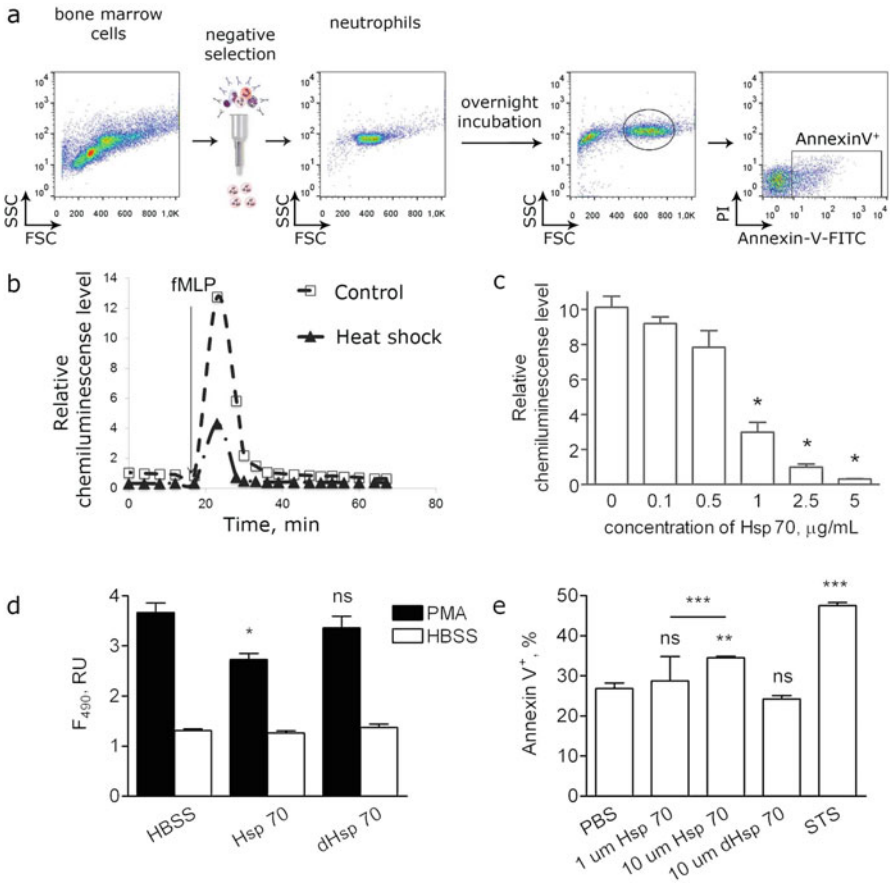



**Fig. 2 Hsp70 prevents hyperactivation of bone marrow-derived neutrophils *in vitro*** (adopted from [31, 34]. (**a**) Schematic representation of the neutrophil yielding from bone marrow cells. Representative images of flow cytometry dot plots of bone marrow cells (the first from left), neutrophils after negative selection using Miltenyi Biotech Kit for the mouse neutrophil negative enrichment by magnetic separation (the second from left), the same sample of neutrophils after overnight separation (the third from left), and the gating for apoptotic cells in accordance to Annexin-PI staining. (**b**) fMLP-induced ROS production of bone marrow-derived neutrophils after 10 min long heat shock at 43°C (white squares) and without heat shock (black triangles). (**c**) The dose-dependent effect of Hsp70 that was adding to the primary neutrophil culture on fMLP-induced ROS production. (**d**) Effect of Hsp70 on PMA-induced extracellular nucleotide secretion. (**e**) Hsp70-mediated dose-dependent elevation of Annexin$^{+}$ cells in the primary culture of bone marrow-derived neutrophils after overnight incubation

Dephosphorylization of extracellular ATP to ADP by HSP70 at the site of inflammation limits the massive migration of immature antigen-presenting cells and their subsequent activation and maturation toward inflammatory cells and in this way reduces the activation of allergen-induced Th2-mediated response. Reduced number of allergen-specific Th2 clones shows abate secretion of pro-allergic cytokines that

lead to attenuated eosinophilia. Together with that, in the absence of elevated IL-4 level neutrophils egress from bone marrow and migrate to the site of inflammation. In the presence of HSP70, activated neutrophils reveal a weak disposition towards hyperactivation. Non-activated neutrophils spontaneously dying by apoptosis (but not netosis or necrosis), facilitated by this way inflammation resolution. Thus, we suggest the protective rather than pro-inflammatory effect of the elevated extracellular HSP70 concentration at the site of inflammation, including allergic airway inflammation.

**Acknowledgements** This study was funded by the Russian Science Foundation (grant no. 19-75-00082).

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Approval was granted by the Institutional Animal Care and Use Committee of Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, protocol № 179/2015 from 21.07.2015.

## References

1. Aki T, Fujikawa A, Wada T, Jyo T, Shigeta S, Murooka Y, Oka S, Ono K (1994) Cloning and expression of cDNA coding for a new allergen from the house dust mite, Dermatophagoides farinae: homology with human heat shock cognate proteins in the heat shock protein 70 family. J Biochem 115:435–440
2. An S, Chen LL, Long CB, Liu XY, Xu XM, Lu XR, Rong MQ, Liu ZG, Lai R (2013) Dermatophagoides farinae allergens diversity identification by proteomics. Mol Cell Proteomics 12:1818–1828
3. Andersson A, Rasool O, Schmidt M, Kodzius R, Fluckiger S, Zargari A, Crameri R, Scheynius A (2004) Cloning, expression and characterization of two new IgE-binding proteins from the yeast Malassezia sympodialis with sequence similarities to heat shock proteins and manganese superoxide dismutase. Eur J Biochem 271:1885–1894
4. Assimon VA, Gillies AT, Rauch JN, Gestwicki JE (2013) Hsp70 protein complexes as drug targets. Curr Pharm Des 19:404–417
5. Bafadhel M, McCormick M, Saha S, McKenna S, Shelley M, Hargadon B, Mistry V, Reid C, Parker D, Dodson P, Jenkins M, Lloyd A, Rugman P, Newbold P, Brightling CE (2012) Profiling of sputum inflammatory mediators in asthma and chronic obstructive pulmonary disease. Respiration 83:36–44
6. Bolkhovitina EL, Sapozhnikov AM, shevchenko MA (2013) Effect of heat shock protein 70 on ATP-induced dendritic cell chemotaxis. Russ J Immunol 7(16):342–343
7. Borges TJ, Wieten L, van Herwijnen MJ, Broere F, van der Zee R, Bonorino C, van Eden W (2012) The anti-inflammatory mechanisms of Hsp70. Front Immunol 3:95
8. Breitenbach M, Simon-Nobbe B (2002) The allergens of Cladosporium herbarum and Alternaria alternata. Chem Immunol 81:48–72

9. Ciepiela O, Ostafin M, Demkow U (2015) Neutrophils in asthma--a review. Respir Physiol Neurobiol 209:13–16
10. De Maio A (2014) Extracellular Hsp70: export and function. Curr Protein Pept Sci 15:225–231
11. de Oliveira AA, Faustino J, de Lima ME, Menezes R, Nunes KP (2019) Unveiling the interplay between the TLR4/MD2 complex and HSP70 in the human cardiovascular system: A computational approach. Int J Mol Sci 20
12. Dworski R, Simon HU, Hoskins A, Yousefi S (2011) Eosinophil and neutrophil extracellular DNA traps in human allergic asthmatic airways. J Allergy Clin Immunol 127:1260–1266
13. Fan GH, Yang W, Sai J, Richmond A (2002) Hsc/Hsp70 interacting protein (hip) associates with CXCR2 and regulates the receptor signaling and trafficking. J Biol Chem 277:6590–6597
14. Fox S, Leitch AE, Duffin R, Haslett C, Rossi AG (2010) Neutrophil apoptosis: relevance to the innate immune response and inflammatory disease. J Innate Immun 2:216–227
15. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A (2007) Novel cell death program leads to neutrophil extracellular traps. J Cell Biol 176:231–241
16. Fujibayashi T, Hashimoto N, Jijiwa M, Hasegawa Y, Kojima T, Ishiguro N (2009) Protective effect of geranylgeranylacetone, an inducer of heat shock protein 70, against drug-induced lung injury/fibrosis in an animal model. BMC Pulm Med 9:45
17. Gao B, Tsan MF (2003) Endotoxin contamination in recombinant human heat shock protein 70 (Hsp70) preparation is responsible for the induction of tumor necrosis factor alpha release by murine macrophages. J Biol Chem 278:174–179
18. Holtzman MJ (2012) Asthma as a chronic disease of the innate and adaptive immune systems responding to viruses and allergens. J Clin Invest 122:2741–2748
19. Hou C, Zhao H, Li W, Liang Z, Zhang D, Liu L, Tong W, Cai SX, Zou F (2011) Increased heat shock protein 70 levels in induced sputum and plasma correlate with severity of asthma patients. Cell Stress Chaperones 16:663–671
20. Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MA, Muskens F, Hoogsteden HC, Luttmann W, Ferrari D, Di Virgilio F, Virchow JC Jr, Lambrecht BN (2007) Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. Nat Med 13:913–919
21. Kobayashi T, Soma T, Noguchi T, Nakagome K, Nakamoto H, Kita H, Nagata M (2015) ATP drives eosinophil effector responses through P2 purinergic receptors. Allergol Int 64:S30–S36
22. Lambrecht BN, Hammad H (2015) The immunology of asthma. Nat Immunol 16:45–56
23. Lommatzsch M, Julius P, Kuepper M, Garn H, Bratke K, Irmscher S, Luttmann W, Renz H, Braun A, Virchow JC (2006) The course of allergen-induced leukocyte infiltration in human and experimental asthma. J Allergy Clin Immunol 118:91–97
24. Lutfi R, Lewkowich IP, Zhou P, Ledford JR, Page K (2012) The role of protease-activated receptor-2 on pulmonary neutrophils in the innate immune response to cockroach allergen. J Inflamm (Lond) 9:32
25. Marincek BC, Kuhnle MC, Srokowski C, Schild H, Hammerling G, Momburg F (2008) Heat shock protein-antigen fusions lose their enhanced immunostimulatory capacity after endotoxin depletion. Mol Immunol 46:181–191
26. Nakagome K, Nagata M (2011) Pathogenesis of airway inflammation in bronchial asthma. Auris Nasus Larynx 38:555–563
27. Ortega E, Hinchado MD, Martin-Cordero L, Asea A (2009) The effect of stress-inducible extracellular Hsp72 on human neutrophil chemotaxis: a role during acute intense exercise. Stress 12:240–249
28. Prado N, Marazuela EG, Segura E, Fernandez-Garcia H, Villalba M, Thery C, Rodriguez R, Batanero E (2008) Exosomes from bronchoalveolar fluid of tolerized mice prevent allergic reaction. J Immunol 181:1519–1525
29. Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H (2008) Allergens are distributed into few protein families and possess a restricted number of biochemical functions. J Allergy Clin Immunol 121(847–52):e7

30. Satoh M, Shimoda Y, Akatsu T, Ishikawa Y, Minami Y, Nakamura M (2006) Elevated circulating levels of heat shock protein 70 are related to systemic inflammatory reaction through monocyte toll signal in patients with heart failure after acute myocardial infarction. Eur J Heart Fail 8:810–815
31. Shevchenko MA, Troyanova NI, Servuli EA, Bolkhovitina EL, Fedorina AS, Sapozhnikov AM (2016) Study of immunomodulatory effects of extracellular HSP70 in a mouse model of allergic airway inflammation. Biochemistry (Mosc) 81:1384–1395
32. Stocki P, Dickinson AM (2012) The immunosuppressive activity of heat shock protein 70. Autoimmune Dis 2012:617213
33. Tanaka K, Tanaka Y, Namba T, Azuma A, Mizushima T (2010) Heat shock protein 70 protects against bleomycin-induced pulmonary fibrosis in mice. Biochem Pharmacol 80:920–931
34. Troyanova NI, Shevchenko MA, Boyko AA, Mirzoyev RR, Pertseva MA, Kovalenko EI, Sapozhnikov AM (2015) Modulating effect of extracellular HSP70 on generation of reactive oxigen species in populations of phagocytes. Bioorg Khim 41:305–315
35. Vitiello L, Gorini S, Rosano G, la Sala A (2012) Immunoregulation through extracellular nucleotides. Blood 120:511–518
36. Wallin RP, Lundqvist A, More SH, von Bonin A, Kiessling R, Ljunggren HG (2002) Heat-shock proteins as activators of the innate immune system. Trends Immunol 23:130–135
37. Woytschak J, Keller N, Krieg C, Impellizzieri D, Thompson RW, Wynn TA, Zinkernagel AS, Boyman O (2016) Type 2 interleukin-4 receptor signaling in neutrophils antagonizes their expansion and migration during infection and inflammation. Immunity 45:172–184
38. Yang M, Wu T, Cheng L, Wang F, Wei Q, Tanguay RM (2005) Plasma antibodies against heat shock protein 70 correlate with the incidence and severity of asthma in a Chinese population. Respir Res 6:18
39. Yegutkin GG (2008) Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. Biochim Biophys Acta 1783:673–694
40. Yurinskaya MM, Evgen'ev MB, Antonova OY, Vinokurov MG (2010) Exogenous heat shock protein HSP70 suppresses bacterial pathogen-induced activation of human neutrophils. Dokl Biochem Biophys 435:316–319
41. Zimmermann H, Zebisch M, Strater N (2012) Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal 8:437–502

# Role of Heat Shock Factor 1 in Neural Development and Disorders


**Dipankar J. Dutta, Kazue Hashimoto-Torii, and Masaaki Torii**

## Abstract

**Introduction** The Heat Shock Response (HSR) pathway plays a pivotal role in regulating various aspects of fetal Central Nervous System (CNS) development under normal non-stressed conditions. Its disruption, by prenatal exposure to various environmental stressors, hampers fetal neurodevelopment resulting in deficits that are in some cases obvious, such as in Fetal Alcohol Spectrum Disorders (FASD), and in others pernicious, such as an increased predisposition for adult-onset psychiatric illnesses. This chapter explores how stressors, acting alone or with genetic predispositions, can impair the ability of the HSR pathway in facilitating normal fetal neurodevelopment.

**Methods** We searched PubMed with the keyword 'Heat Shock Factor' to find relevant peer-reviewed articles.

**Results** We focus primarily on Heat Shock Factor1 (HSF1), the predominant mammalian activator of the HSR pathway. Following a brief introduction on HSR and HSF1, the first section explores the burgeoning literature on the role of HSF1 and its downstream effectors, the molecular chaperones, in various aspects of embryonic neurodevelopment. The second section discusses the interaction of various environmental stressors with HSF1 expression and activity in the fetal CNS, and how such interactions; depending on dosage, duration, and time of exposure; can result in a spectrum of deficits within the fetal brain.

D. J. Dutta
Center for Neuroscience Research, Children's Research Institute, Children's National Hospital, Washington, DC, USA

K. Hashimoto-Torii · M. Torii (✉)
Center for Neuroscience Research, Children's Research Institute, Children's National Hospital, Washington, DC, USA

Department of Pediatrics, Pharmacology and Physiology, School of Medicine and Health Sciences, George Washington University, Washington, DC, USA
e-mail: mtorii@childrensnational.org

Heat Shock Proteins (2021) 22: 213–240
https://doi.org/10.1007/7515_2020_10

Published Online: 27 June 2020

**Conclusions** We conclude with our perspective on pressing and challenging outstanding questions in the field.

**Keywords** Chaperones · FASD · Heat shock factor 1 · Heat shock protein · Heat shock response · Neurodevelopment

## Abbreviations

| | |
|---|---|
| ADHD | attention deficit hyperactivity disorder |
| AMP | adenosine monophosphate |
| AMPK | adenosine monophosphate-activated protein kinase |
| ATP | adenosine triphosphate |
| BAG1 | BCL2 associated athanogene 1 |
| CK2 | casein kinase 2 |
| CNS | central nervous system |
| DCC | deleted in colorectal cancer |
| DISC1 | disrupted in schizophrenia 1 |
| DNA | deoxyribonucleic acid |
| FASD | fetal alcohol spectrum disorders |
| FRT | flippase recognition target |
| GEF | guanine nucleotide exchange factor |
| GTP | guanosine-5′-triphosphate |
| HOP | HSP70/90 heat shock organizing protein |
| HSC | heat shock cognate |
| HSE | heat shock element |
| HSF | heat shock factor |
| HSP | heat shock proteins |
| HSR | heat shock response |
| iPS | induced pluripotent stem |
| JNK | c-Jun N-terminal kinase |
| kDa | kilo Dalton |
| KO | knockout |
| PDZ | post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), Zonula occludens-1 protein (zo-1) |
| PSA-NCAM | polysialic acid neural cell adhesion molecule |
| RFP | red fluorescent protein |
| RNA | ribonucleic acid |
| siRNA | small interfering RNA |
| SIRT1 | silent mating type information regulation 2 homolog 1 |
| SVZ | subventricular zone |

## 1 Introduction

The staggering complexity of the human brain that fuels its seemingly unbounded abilities comes with a steep cost. Neurodevelopment is an intricate, elaborate, and protracted affair in humans [1]. We are born with a brain less than a third of its ultimate adult proportions [2]. Different brain regions mature at different rates [3], and different cognitive functions come with their own critical and sensitive developmental periods [4] that extend well into late adolescence [5]. Myelination, insulation that makes vertebrate neuronal communication zippy, only reaches its peak by 39 years of age [6]. By then, we already have one foot out the door [7]. Meanwhile, a lot of things can and do go awry. Even seemingly benign and minute alterations during fetal neurodevelopment, within the same genetic background, can have a disproportionate impact in later life. For instance, subtle variations in the in utero environment of identical twins, as indexed by mild birth-weight variation, are accompanied by significant alterations in brain anatomy and intelligence that persist at least into late adolescence [5]. Therefore, it is no wonder that heat shock factors and their target proteins, chaperones and otherwise, play such a pivotal role in brain development by both ensuring proper neurodevelopment, and insulating the fragile brain from internal and external stressors. In this chapter, we discuss these roles of various components of the HSR pathway on neural development and disorders, with an emphasis on HSF1 and its target genes.

## 2 The Heat Shock Response – A Brief History

The discovery of the HSR pathway was a happy accident. In the early 1960s, Ferruccio Ritossa, an Italian geneticist, was studying nucleic acid synthesis in puffs of polytene chromosomes in larval Drosophila salivary glands. Puffs, sites of vigorous transcription, are local enlargements in these chromosomes that can be spatially tagged and followed over time [8]. One eventful day, someone in Ritossa's lab inadvertently changed the temperature of his incubator. Ritossa later remarked, "I cannot remember whether it was John Pulitzer or Inge or Clara Ghini or Giordano who shifted the temperature of my incubator, but one day I noticed a different puffing pattern!" Entirely new puffs emerged in Drosophila polytene chromosomes in response to heat shock from elevated temperature inside his incubator. As is par for novel discoveries, Ritossa's early attempts to publish his findings were met with skepticism. The editor of an influential journal, who rejected the manuscript, remarked that "the observations lacked biological importance", implying that it was likely an artifact [9]. The seminal discovery was eventually published in *Experientia* in 1962 [10]. However, interest lagged for another dozen years, until 1974, when Tissieres et al. heralded the era of HSR research by showing that heat shock-induced puffs culminated with intense protein synthesis and, beyond the salivary gland, can be induced by a *heat shock* in various other tissues throughout the body [11].

Decades of research have since demonstrated two fundamental aspects of the HSR; its universality across all life forms, and the plethora of stimuli, primarily stressful but also non-stressful developmental cues, which can lead to its activation. The HSR has been observed in every living context it has been sought in; from archaebacteria to *Arabidopsis* to humans [12]. The HSR is activated in a multitude of stressful contexts including changes to various properties of the extracellular milieu, such as temperature [11] or hydration [13]; the presence of chemical stressors, such as ethanol [14], free radicals [15], or heavy metals [16]; and pathophysiological states associated with ischemia [17], pathogenic infections [18], or mutations resulting in protein misfolding and aggregation [19]. As we discuss later, components of the HSR pathway also regulate normal fetal neurodevelopment in the absence of external stressors, suggesting that their influences extend well beyond their stereotyped role as mere facilitators of protein folding.

There are two principal components of the HSR pathway, the activators, and the executors. The activators are heat shock factors (HSF) [20, 21], transcriptional factors that, upon proteotoxic stress, activate the transcription of the executors, the heat shock proteins (HSP) [11]. HSP are chaperone proteins that help maintain protein homeostasis, or *proteostasis*, by facilitating proper protein folding [22]. Together, HSF and HSP represent some of the most evolutionarily conserved protein families and constitute a first line of defense against a diverse range of threats to protein stability [23].

## 3 Heat Shock Proteins – Chaperones that Execute the Heat Shock Response

The canonical role of the HSR pathway is to maintain optimal folding of native tertiary protein structures in the face of proteotoxic stressors [22]. This task is both critical and urgent during periods of cellular stress, and yet exceedingly difficult to accomplish, at least in theory. For example, a 101 amino acid long protein can fold in $3^{100}$ different ways, and if it were to sample each fold sequentially at a brisk rate of $10^{13}$ folds per second, it would take the protein up to $10^{27}$ years, much longer than the age of the universe, to find its native state [24]. However, in practice, proteins fold in sub-second timescales [25]. This paradox, formulated by Cyrus Levinthal in 1969 [26], underscores the key role of chaperones in facilitating proper protein folding via *hydrophobic collapse,* in which hydrophobic residues are excluded from the aqueous milieu and sequestered in the core of the protein [27]. Proteins are the ultimate manifestation of our genetic information, and cells have evolved an extensive arsenal of mechanisms to protect and preserve the fidelity of this information, both during the formation of proteins and after they have formed. Molecular chaperones are a key component of this arsenal [28].

The word *chaperone* harks back to an antiquated time, the 1700s in France, when young unmarried women were not allowed to appear in public without the company

of a married woman, the *chaperon*. It is the French synonym for *hood*, with the notion being to *cover* someone who is ostensibly *vulnerable* [29]. Biologists coopted the term for proteins that help other proteins fold properly and remain properly folded. Two main stressful events occur during the lifecycle of a protein; one when it is trying to fold *de novo* after ribosomal synthesis, and the other when its optimally folded native structure is under threat from proteotoxic stressors [30]. HSP are stress-activated chaperones that associate with proteins during cellular stress, and thereby both prevent protein misfolding and facilitate proper refolding of misfolded proteins. Co-chaperones and myriad other proteins aid HSP in this crucial task [22].

HSP are classified based on their molecular mass, and range in size from 10 to 110 kDa. The small HSP, up to 40 kDa in size, are adenosine triphosphate (ATP)-independent and include α-crystallins and HSPB1 among others. The large HSP range from 40 to 110 kDa, function by expending ATP, and predominantly contain the 40, 70, and 90 kDa HSP families. HSP70 and 90 also come in two flavors, a stress-inducible form (iHSP70, HSP90α) and curiously, a constitutively expressed heat shock cognate (HSC) variant (HSC70, HSP90β). HSP40, HSP100, and HSP110 are co-chaperones that interact with and regulate the activity of HSP70 and HSP90 [31]. Later in this chapter, we will discuss the contributions of individual HSP to neurodevelopment in the context of their transcriptional activation by HSF1.

## 4 Heat Shock Factors – Transcription Factors that Initiate the Heat Shock Response

Heat shock factors are transcription factors that activate the transcription of various genes, including HSP chaperones, in response to proteotoxic stress, thereby initiating the HSR. They do so by binding to conserved heat shock elements (HSE) in the promoters of target genes [32]. HSE consist of alternating inverted repeats with the sequence nGAAn, where n is any nucleotide [33]. The vertebrate genome encodes seven HSF (HSF 1–5 & X-Y), with unique and overlapping functions, where some are universally expressed, and others restricted to specific tissues and developmental periods [34]. Heat shock factor 1 (HSF1), the master regulator of this pathway in vertebrates, is necessary to mount an effective HSR [35–37]. HSF2 modulates, both positively and negatively, HSF1-mediated activation of the HSR [38, 39]. HSF2 also regulates neuronal migration during corticogenesis [40]. HSF4, working in concert with HSF1, plays a key role in the growth and development of sensory organs such as the eye lens [41, 42] and the olfactory epithelium [43]. Mutations in HSF4 cause cataracts [44, 45]. HSF5 is essential for spermatogenesis [46]. HSFX and HSFY are in the X and Y chromosomes respectively. While the function of HSFX is unknown, the deletion of HSF5 and HSFY results in male infertility [46–48]. HSF3, the most recent and exciting addition to vertebrate HSF, does not crosstalk with other members of the family, even HSF1, and can activate transcription of non-classical

heat shock genes [49]. However, a human orthologue of HSF3 remains to be identified.

## 5 Heat Shock Factor 1 – Expression, Activation and Regulation

The expression of each of these HSF varies depending on the developmental state of the cell and the tissue it is expressed in [50]. This is due to a multitude of factors that regulate the expression and stability of HSF, including long non-coding RNAs (ribonucleic acid) [51], splicing factors [52, 53], and proteasome-mediated degradation [54]. HSF1, the master regulator of the HSR pathway, is constitutively expressed [55, 56]. In the CNS, its activity varies depending on the type and maturation status of the neuron [57, 58]. For example, in contrast to developing fetal neurons, HSF1 response is muted in mature CNS neurons [59, 60], and this dichotomy exacerbates neuronal vulnerability to toxic protein aggregates in adult-onset neurodegenerative disorders [58, 61]. To compound these detriments, HSF1 levels and activity progressively decline with age [62], ironically during a period when they are needed dearly. This paradox is in stark contrast with the critical and active role of HSF1 in facilitating proper fetal neurodevelopment.

Although having enough levels of HSF1 is necessary for the HSR, it is by itself insufficient to mount an effective HSR, which also requires the transformation of HSF1 into a DNA (deoxyribonucleic acid)-binding transcriptionally competent form. HSF1 exists in an inactive monomeric state, either unbound or bound to repressive proteins, in the cytoplasm of unstressed cells [63]. In response to a stressor, HSF1 migrates to the nucleus, oligomerizes with itself to form homotrimers, and then binds to HSE in promoters of target genes [64]. This results in the upregulation of various target genes, primarily HSP chaperones; such as HSP40, HSP70, and HSP90; which mount an effective proteostatic response to the stressor. Once enough chaperones are generated to mount an appropriate HSR, they assemble into a repressive complex which then binds to and disrupts active homotrimeric HSF1 complexes resulting in the termination of HSF1 activation. In a classic example of feedback inhibition, this, in turn, represses further transcription of HSP chaperones themselves [65, 66].

Considering the critical importance of the HSF1 pathway in maintaining proteostasis, multiple steps in this parsimonious model of HSF1 activation-inactivation are actively regulated. The proteasomal machinery plays an important role in maintaining appropriate cellular levels of HSF1, by regulating its stability. Different post-translational modifications, such as phosphorylation and acetylation of various residues, determine whether HSF1 is degraded by proteasomes [67], but have no effect on its transcriptional capability [68]. For example, while phosphorylation of Serines 303 and 307 marks HSF1 for proteasomal degradation [61], acetylation of Lysines 208 and 298 prevents it [69]. HSF1 activity is also regulated

by cellular nutrient-sensing pathways. During nutrient deprivation, the ratio of adenosine monophosphate (AMP) to ATP increases. To ensure cellular survival, ATP-consuming cellular processes are then suppressed, and ATP-generating ones are prioritized. Since stress-responsive chaperones expend ATP to refold proteins, nutrient stress is accompanied by a downregulation of the HSF1 pathway. This is accomplished by phosphorylation of Serine 121 in HSF1 by 5′ Adenosine Monophosphate-activated Protein Kinase (AMPK), which represses HSF1 activity directly instead of marking it for proteasomal degradation [70]. Although beneficial in the short term, this paradoxically makes starved cells more vulnerable to proteotoxic stressors.

A comprehensive review of the regulation of HSF1 activity is beyond the scope of this chapter, and readers are instead encouraged to peruse these excellent topical reviews on the subject [34, 71]. Moreover, exact details for some of these mechanisms are unknown; such as what drives HSF1 transition between oligomeric activation states, and how HSF1 differentiates between different kinds of cellular stress and are therefore rich fodder for future scientific explorations. Interestingly, HSF1 can trimerize in isolation, in response to thermal or oxidative stress, and therefore is likely an *intrinsic thermo-redox sensor* [72, 73]. We will now dive into the contribution of HSF1 to fetal neurodevelopment and the detriments that result from its impairment.

## 6 Heat Shock Factor1 in Neurodevelopment

HSF1 is primarily responsible for inducing the transcription of effector chaperones, HSP, in vertebrates. However, HSF1 has also been shown to regulate the transcription of various non-chaperone genes involved in myriad neurodevelopmental functions [74]. Loss of HSF1, therefore, results in gross CNS abnormalities such as enlarged ventricles, demyelination, astrogliosis, accumulation of ubiquitinated proteins, and neurodegeneration [75–77]. Similarly, loss of the other prominent vertebrate heat shock factor, HSF2, results in various neurodevelopmental deficits, including lateral ventricle collapse, ventricular hemorrhages, third and lateral ventricle dilation, diminution of the hippocampus and striatum, and disruption of cortical layers due to impaired neuronal migration [40, 78, 79]. Since HSF2 can also regulate HSF1 function [38, 80], the deficits in HSF2 knockout (KO) mice are likely reflective of the disruption of both HSF1 and HSF2 activity.

The canonical function of the heat shock factor pathway in the CNS is to confer protection to neuronal cells from apoptosis upon exposure to internal [81] and external stressors [82]. As we discuss in our next section, this stress-responsive component of the heat shock factor pathway is relevant in a wide range of conditions, from fetal neurodevelopment disrupted by toxins [83] to adult-onset neurodegenerative disorders denominated by misfolded proteins [34, 61]. Both their activity as molecular chaperones and their modulation of anti-apoptotic signaling pathways help rescue neuronal apoptosis. For example, HSP27 prevents c-Jun N-Terminal

Kinase (JNK) induced apoptosis in stressed neurons via activation of the Akt pathway [84]. HSP70 similarly protects neurons from ischemia [85] by modulating both intrinsic and extrinsic apoptotic pathways [86].

However, apoptosis is an integral part of normal neurodevelopment [87]. The exuberant growth of neurons, glia, and their processes during early neurodevelopment must be appropriately trimmed to establish proper neuronal wiring and architecture [88]. Besides apoptosis, the heat shock factor pathway and its target genes have also been shown to execute various non-apoptotic functions fundamental to the proper development of the nervous system. In the following section, we explore such non-canonical roles of HSF1 and its transcriptional targets, both HSP, and non-chaperone proteins, during neurodevelopment.

## 7 Role of Heat Shock Factor1 on Neuronal Development and Behavior

Generally considered to be an inducer of transcription, HSF1 activity can also be transcriptionally repressive [74, 89]. This latter ability is important in the development of the olfactory epithelium. DNA binding activity of HSF1 is induced in the olfactory epithelium 4 weeks after birth, during which it binds to and represses the transcription of Leukemia Inhibitory Factor. Loss of HSF1 in HSF1 KO mice, therefore, results in an olfactory epithelium that is normal until 3 weeks post-birth, but is atrophied thereafter due to increased apoptosis of olfactory sensory neurons [43].

HSF1 activity is also important for proper neuronal development in the hippocampus with implications for behavior. Loss of HSF1 in HSF1 KO mice results in impaired neurogenesis in the dentate gyrus as well as an aberrant morphology of its resident granule neurons. The underlying molecular mechanism involves disruption of HSF1-mediated modulation of polysialyltransferase gene expression, which in turn modulates polysialic acid neural cell adhesion molecule (PSA-NCAM) expression in the hippocampus. PSA-NCAM is important for synaptogenesis and synaptic plasticity [90]. Loss of PSA-NCAM is accompanied by behavioral consequences with HSF1 KO mice exhibiting aberrant affective behavior, such as reduced anxiety and sociability, but increased depressive and aggressive behaviors, and an increased vulnerability to restraint stress. These cellular and behavioral deficits can be rescued by HSF1 expression in neonates but not in adults, indicating the existence of a critical developmental window during which HSF1 can mediate its effect. In summary, HSF1 plays a key role in spinogenesis and neurogenesis in the hippocampus by regulating the transcription of polysialyltransferases [91].

HSF1 has also been shown to play a central role in establishing and maintaining synaptic fidelity by promoting the transcription and preservation of synaptic proteins; with important consequences for cognitive processes such as memory formation, and their disruption in neurodegenerative disorders [92]. Although these studies

were mostly performed in the context of rescue from neurodegenerative pathologies, the underlying mechanisms are likely conserved during neurodevelopment.

## 8 Role of Heat Shock Factor1 and its Transcriptional Targets in Neurite Outgrowth

CNS development is accompanied by neuronal differentiation during which axonal and dendritic extensions, or *neurites*, emerge from the cell-body proper. These neurites go on to form about 150 trillion synapses in the cerebral cortex alone [93], or roughly a trillion synapses per cubic centimeter of the cortex [94]. To put this in perspective, as per our most recent estimates, there are about 2 trillion galaxies in the known universe [95]. Neurite outgrowth is also a precursor to subsequent neuronal endeavors; such as axon pathfinding and neuronal migration, in that similar cellular processes are involved. The transcriptional targets of HSF1, both HSP and non-chaperone proteins, play important roles in neurite development, by regulating cytoskeletal dynamics, as discussed below.

HSF1 activity is involved in the transcription of HSP27 [96], and HSP27 phosphorylation is a common target of signaling pathways that modulate cytoskeletal remodeling to facilitate neuronal differentiation [97] and neurite outgrowth [98]. Non-phosphorylated HSP27 monomers function as actin-capping proteins, thereby preventing actin polymerization. This ability is lost with their phosphorylation and subsequent aggregation into oligomers which then stabilize the actin filament network [99]. Mutations in HSP27 cause several forms of hereditary motor neuropathies including Charcot Marie Type 2 [100], underlining its importance in neuronal function.

Both HSP27 and its phosphorylated form, pHSP27, colocalize with actin and tubulin in lamellipodia and focal contacts in early neurites, as well as in processes, branch points, and growth cones in mature neurites [98]. Small interfering RNA (siRNA)-mediated downregulation of HSP27 results in diminished neuritic tree-length and complexity, while its upregulation accomplishes the opposite [101, 102]. HSP27 can also promote neurite outgrowth by regulating signaling pathways involved in modulating cytoskeletal dynamics. Activation of a small GTPase (guanosine triphosphatase), RhoA, prevents neurite outgrowth [103]. RhoA is activated by a specific Guanine Nucleotide Exchange Factor (GEF), PDZ-RhoGEF. HSP27 can promote neurite outgrowth by inhibiting the activator of RhoA, via upregulating expression of two microRNAs, miR-20a and miR-128, that inhibit translation of the Rho activator, PDZ-RhoGEF [104]. Inhibition of RhoA signaling to promote neurite outgrowth can also be accomplished with other small HSP such as HSPB5, another transcriptional target of HSF1 [105]. HSPB5 activation, in response to heat shock, increases dendritic complexity and preserves dendritic arbors in hippocampal neurons in vitro [106].

Larger chaperone transcriptional products of HSF1 activation, HSP70, and HSP90, also play important roles in neurite outgrowth during development. HSP70, acting in concert with its co-chaperone BAG1 (BCL2 associated athanogene 1), can regulate neurotrophin-mediated neurite outgrowth [107]. HSP90 promotes neurite outgrowth of chick telencephalic and spinal neurons in vitro [108]. Furthermore, inhibition of HSP90 activity in embryonic hippocampal neurons disrupts neuronal polarization and subsequent axon elongation [109].

## 9 Role of Heat Shock Factor1 and its Transcriptional Targets in Axon Pathfinding

Neural circuitry, communication among neurons close and far, underlie all brain functions. Axons, 0.1–10 μm ($10^{-3}$ mm) across [110], must navigate their way through a fetal brain with up to 86 billion neuronal and 85 billion non-neuronal cells [111], packed into a relatively vast volume of about 350,000 cubic millimeters ($mm^3$) [112], to connect with other neurons. Yet axons find their way repeatedly, billions of times over, within a relatively short gestational period, and with extraordinary precision and fidelity [113]. Disruptions do occur however, and often come with debilitating consequences, as a variety of neurodevelopmental and psychiatric disorders, including autism spectrum disorders and schizophrenia, will attest [114]. Loss of functional circuitry due to neuronal loss also underlies the familiar disabilities in neurodegenerative disorders such as Alzheimer's and Parkinson's.

In the developing CNS, axons navigate by expressing guidance receptors on their elongating tip, the growth cone, and by integrating attractive and repulsive cues present in their environment [115]. Netrin1 is one such cue that mediates its attractive action through the axonal receptor Deleted in Colorectal Cancer (DCC), during cortical and spinal cord development in vertebrates [116, 117]. Netrin1-DCC binding recruits Trio, a GEF, to DCC, which then activates Rac1, a Rho family GTPase. Rac1, in turn, facilitates cytoskeletal reorganization appropriate for axon outgrowth [118]. Small nucleotide polymorphisms in *DCC* and *netrin1* are associated with schizophrenia [119], Parkinson's disease, and Amyotrophic Lateral Sclerosis [120].

HSC70, a constitutive chaperone, is expressed in neurons during normal CNS development in unstressed conditions [121, 122]. HSC70 associates with the GEF Trio in the developing cerebral cortex and recruits it to DCC upon Netrin1-DCC binding, which then leads to Rac1 activation. HSC70 chaperone activity is required for Netrin1–induced activation of Rac1, surface enrichment of DCC at the growth cone, and axon outgrowth and guidance. HSC70-mediated regulation of Trio is therefore essential for the stability of the DCC/Trio signaling complex at the cell surface of growth cones to mediate Netrin1–induced axonal outgrowth and guidance [123].

## 10 Role of Heat Shock Factor1 and its Transcriptional Targets in Neuronal Migration

Neuronal migration, the process by which neurons migrate from their origin in the germinal ventricular and subventricular zones (SVZ) to their final destinations, is another major way appropriate neural circuitry and overall architecture are established in the developing brain [124]. For example, neuroblasts arising from the SVZ migrate through the rostral migratory stream to reach the olfactory bulb at the anterior tip of the brain [125]. Migrating neurons use similar strategies as pathfinding axons, such as molecular guidance cues, to navigate their trajectory [126]. Radial glia also facilitate neuronal migration by acting as a scaffold for neurons to migrate along, and are important in the development of multilayered structures such as the cortex, cerebellum, and hippocampus [127]. Cell-surface expression of ATP-dependent HSP, such as HSP90, have been shown to be important for neuronal migration during neurodevelopment [128]. Whether they act as chaperones for other guidance receptors, or act as receptors themselves, remains to be conclusively determined.

HSP90α is expressed on the cell surface of primary cerebellar neurons and its activity is associated with actin cytoskeletal organization and lamellipodia formation. Inhibition of HSP90α activity with an antibody impairs migration of cerebellar neurons in explant cultures [128]. Similarly, extracellular membrane-bound HSP90, along with its co-chaperone HOP (HSP70/90 Heat-shock Organizing Protein), is expressed in the anterior SVZ and on migrating neuroblasts along the rostral migratory stream. This HSP90 complex is involved in facilitating neuroblast migration from the SVZ. Inhibition of HSP90 activity, with antibodies and chemical inhibitors, disrupts neuroblast migration in SVZ explant cultures [129]. Theoretically, membrane-bound extracellular HSP90 can promote migration either by acting as a receptor itself or via chaperoning appropriate conformation of guidance receptors on its host neurons. There is some evidence in support of the former. Extracellular HSP90-mediated activation of the epidermal growth factor receptor via Toll-like receptor 4 stimulation has been shown to promote the migration of glioblastoma cells [130].

## 11 Heat Shock Factor1 in Neurodevelopmental Disorders Resulting from Prenatal Exposure to Environmental Stressors

Fetal neurodevelopment is sensitive to perturbation by extrinsic environmental stressors such as alcohol [131], heavy metals such as methylmercury [132], and adverse maternal health statuses such as infections [133], stroke [134] and epileptic seizures [135]. Fetal cerebral cortex development, in particular, is extremely sensitive to any external stressors that can potentially impair the highly intricate

neurogenesis, maturation, and migration programs that underlie the successful development of a multilayered gyrated cortex unique to higher-order vertebrates [82]. Here we discuss the role of HSF1 in counteracting external stressors during embryonic development of the brain, with an emphasis on the development of the cerebral cortex. We also discuss how HSF1 overactivation can be detrimental to cortical development by impairing neuronal migration, demonstrating that too much of a good thing can also be a handicap during the delicate period that is fetal neurodevelopment.

There are two interesting emergent themes from studies on the role of environmental stressors on in utero neurodevelopment. First, exposure to a diverse array of prenatal environmental challenges culminate in a set of similar pathological outcomes on brain development. Exposure to alcohol [136], cocaine [137], hypoxia [138], methylazoxymethanol (used for modeling schizophrenia and epilepsy) [139], methylmercury [132], and X-ray irradiation [140] cause similar cortical structural abnormalities such as heterotopias, which are abnormally located neuronal densities, and reduced cortical volume and thickness. These corticostructural changes are accompanied by a reduction in the seizure threshold of the offspring [141–145]. Heterotopias can also affect the cognitive function of distant brain regions, and are accompanied by various behavioral deficits [146]. This suggests there are common underlying molecular mechanisms that are perturbed by these diverse adverse environmental paradigms.

Secondly, although suprathreshold exposure to these environmental stressors results in obvious and striking developmental deficits, such as FASD in neonates exposed to alcohol in utero; subthreshold exposure to the same environmental toxins can also result in pernicious harm, such as an increased susceptibility to seizures after birth, and an increased predisposition to adult-onset neuropsychiatric disorders such as schizophrenia [83]. Interestingly, similar neurodevelopmental deficits underlie pathologies resulting from suprathreshold and subthreshold exposures to these environmental stressors, with differences being merely of scale and age of onset. For example, abnormalities in fetal brains of humans with FASD include heterotopia, microcephaly, hydrocephaly, and agenesis of the corpus callosum; and these gross structural perturbations have drastic chronic consequences for cognitive and other faculties in the newborn [147]. On the other hand, a fetus exposed to subthreshold levels of alcohol exhibits similar malformations, albeit on a smaller scale, such as the formation of leptomeningeal heterotopias, reduction of brain volume, and diminished cortical thickness accompanied by an increased susceptibility to postnatal seizures and an elevated risk for neuropsychiatric disorders in later life [83]. Moreover, phenotypes resulting from supra and subthreshold exposures can also coexist. For example, patients with FASD exhibit higher rates of comorbidity with attention deficit hyperactivity disorder (ADHD) [148] and epilepsy [149]. Here we discuss the contribution of HSF1 to these two unifying themes in disruption of fetal neurodevelopment due to exposure to environmental stressors in utero.

The HSF1 pathway plays a major role in the integration of pathophysiology across a diverse range of environmental insults. Embryonic exposure to a wide array of environmental factors such as alcohol, methylmercury, and maternal seizure, even

at subthreshold levels, activate HSF1 in cerebral cortical cells. This activation reduces the risk of cortical malfunction and susceptibility to postnatal seizures. Conversely, deficiency of HSF1 during such exposures, even at the subthreshold level, results in structural cortical abnormalities accompanied by increased susceptibility to adverse postnatal neurological disorders such as seizures and neuropsychiatric disorders. Such effects are traced to perturbations of normal neuronal development such as the premature cell-cycle exit of neural progenitors, and an increase in apoptosis in the absence of the protective effects of HSF1 [83]. Recent genome-wide exploration of HSF1 binding via ChIP-on-chip has uncovered a diverse range of target genes, well beyond the usual suspects, the stress-responsive chaperone genes [150]. For example, many cortical genes involved in neuronal proliferation and migration are under the transcriptional control of HSF1 [151]. Thus, the pleiotropic effects of HSF1 activation could be due to context-dependent activation of different transcriptional targets based on the nature, duration, and intensity of the environmental stressor, the characterization of which will pave the way for novel therapeutics targeted to the unique characteristics of each stressor.

Another important consideration for pathophysiology due to environmental stressors is whether HSF1 expression and activation are homogeneous across all cells exposed to an environmental insult. Repetitive prenatal exposure to identical or similar doses of noxious environmental stressors can result in highly variable and unpredictable negative outcomes on fetal brain development [152, 153]. FASD, for example, constitute a spectrum of four different clinical diagnoses arranged from the least to the most dysmorphic [154]. While a modest amount of HSF1 activation is essential for protecting the embryonic brain from these insults, excessive HSF1 activation can disrupt other essential developmental programs such as neuronal migration [155]. Variability in HSF1 expression could also be the underlying reason for cortical malformations like heterotopias involving clusters of aberrantly dense neuronal populations. Although some of the factors contributing to these variabilities are known, the molecular and cellular basis for this differential vulnerability of exposed cells is not well understood. The duration and intensity of exposure to the stressor play a large role in the variability of the response [152]. A sustained suprathreshold exposure will have a much higher penetrance than say an intermittent subthreshold exposure [153]. The gender of the affected fetus also has been shown to be an important variable in determining the penetrance of various symptoms. In the case of FASD, for example, males are more than twice as likely to be diagnosed with ADHD than females [156]. HSF1 activity also varies depending on the maturation status and type of the neuron involved [58, 157].

Of even greater significance is whether there is variability in HSF1 activation among neurons of the same developmental stage. Such epigenomic variabilities, in interaction with the underlying genetic factors, could be the driving force behind the etiologies of various adult-onset neuropsychiatric disorders such as schizophrenia. Recent studies have begun to shed light on this important question. Induced pluripotent stem (iPS) cells from schizophrenia patients were differentiated into neural progenitor cells and exposed to alcohol, methylmercury, and hydrogen peroxide.

Single-cell RNA sequencing revealed a highly heterogeneous HSF1-mediated HSR response to these environmental stressors in these schizophrenic induced pluripotent neural stem cells [83]. Such highly variable HSF1 activity in response to cell-extrinsic stressors, in concert with underlying genetic mutations, can be an important component of the etiology of these complex neuropsychiatric disorders.

Other prenatal environmental challenges such as viral infection [158], inflammation [158], hypoxia [138], irradiation [159], and heat [160], all known epidemiological risk factors for adult-onset neuropsychiatric disorders such as schizophrenia, also induce the expression of HSF1 target genes [10, 161–164]. The aberrant differential activation of HSF1 could also be due to underlying but unidentified genetic mutations in schizophrenic patients. Thus, genetic mutations underlying schizophrenia that dysregulate HSF1 activity can further contribute to neural progenitor cell vulnerability to various environmental risk factors that are in turn epidemiologically linked to an increased risk for schizophrenia. Consistent with this interesting hypothesis, HSP70 polymorphism is observed in patients with schizophrenia among a Korean population [165, 166]. The production of antibodies against HSP, including HSP70, has been reported in never-medicated schizophrenic patients, and HSP70 overexpression was found in the prefrontal cortex of schizophrenic patients [167]. As discussed above, overactivation of the HSF1 pathway can hinder neuronal migration and thereby detrimentally affect cortical development [155]. Pathogenic aggregates of HSP70 with DISC1 (disrupted in schizophrenia 1), one of the genes linked to abnormal neural development in schizophrenia, has also been reported [168]. Cortical thinning, one of the structural abnormalities in HSF1 KO mice, also strongly correlates with behavioral attributes of schizophrenia [169]. Interestingly, cell-to-cell transcriptional variability in aged cardiomyocytes has been traced to oxidative damage of the genome [170]. This could also be a contributor to schizophrenia, as cellular samples procured from schizophrenic patients exhibit increased oxidative DNA damage [171], and HSF1 activity is strongly implicated in mitochondrial vitality and function [172, 173].

These observations raise other tantalizing questions. Is the variability in the HSF1 response in schizophrenic iPS cell lines caused by the underlying genetic deficits characteristic of schizophrenia? Or is it something intrinsic and unique to the mode of HSF1 activation? Or in other words, is there variability in the HSF1 response in healthy neurons and brains to adverse environmental stressors alone? A recent study answered this question by showing that exposing mouse and human embryonic brain tissue to equal doses of noxious stimuli, such as ethanol, methylmercury, and hydrogen peroxide, activates HSF1 in a highly variable and stochastic manner among cortical neural progenitor cells and immature neurons [174]. Such mosaic and probabilistic activation of HSF1 among neuronal cells at similar developmental stages may contribute to the phenotypic variations in complex congenital disorders like FASD, and thereby account for variations in cortical malformations among fetuses exposed to similar stressful events. It might also be important in selective neuronal vulnerability prevalent in adult-onset neurodegenerative disorders. Deleterious epigenetic mosaicism in a small population of neurons can be selected by these environmental stressors, resulting in a disproportionately mosaic brain with

vulnerable pockets in psychiatric disorders. Interestingly, this mechanism of mosaic gene activation might be an evolutionary vestigial remnant [175]. Unicellular microbial populations, such as yeasts, rely on this epigenetic cell-to-cell variability in gene expression for the survival of a clonal population in response to abrupt environmental changes [176]. When extrapolated to the development of the single most sophisticated entity in the known universe, the human brain, such rudimentary unicellular adaptations can have lasting detrimental effects, including an increased risk for developing adult-onset psychiatric disorders. Differential cell fates of neural progenitor cells (glial vs neuronal, for example) and uneven tissue distribution of the environmental stressor could also be non-trivial contributors to the inherent propensity for differential HSF1 activation among stressed neurons.

New techniques and tools are being developed to help fully appreciate the significance and consequences of HSF1 activation on fetal neurodevelopment. For instance, an HSE-RFP reporter system has been developed in which HSF1 binds to a heat shock response element (HSE) containing the mouse *Hsp70* promoter to transcribe a Red Fluorescent Protein (RFP) reporter [177]. This reporter system can, depending on the context of its expression, detect differential levels of HSF1 activation in vivo. In an intra-utero electroporation-mediated reporter assay, the HSE-RFP system can detect low levels of HSF1 activation, thereby enabling early identification of neurons stressed by various environmental insults, well before the manifestation of symptoms. Such early vulnerable neurons have hitherto eluded detection [178]. In contrast, upon genetically engineering it into mice, the HSE-RFP reporter is only activated with high levels of HSF1 activation in these transgenic mice, thereby enabling the detection of highly vulnerable cells. The functionality of the HSE-RFP reporter system can be further extended, with the help of a Flippase-FRT (flippase recognition target) recombination system [179], to allow tracing of the descendants of these stressed cells with varying levels of HSF1 activation. Therefore, it is now possible to identify neurons that survive prenatal exposure to noxious agents but remain vulnerable in postnatal life. The HSE-RFP reporter system has already been used to identify neurons with primary and secondary damage in models of spinal cord and sciatic nerve injury [180]. Interestingly, injured but surviving secondarily damaged neurons, have altered physiological properties which likely hamper their functioning, and can also be found at locations distant from the primary site of injury. The development of other novel tools will go a long way in elucidating the critical role of HSF1 in neurodevelopment interrupted by environmental stressors.

## 12 Heat Shock Factor1 in Aging and Adult-Onset Neurodegenerative Disorders

Stress-induced HSF1 activation progressively declines with age [51, 181]. This is unintuitive because HSF1-mediated protein homeostasis should become more relevant as cells age, especially in terminally differentiated cells like neurons [59]. The

underlying mechanism is not fully appreciated, but possibly involves increased proteasomal degradation of HSF1. The activity of histone acetyltransferase p300, which acetylates HSF1 at Lysines 208 and 298, and thereby prevents its proteasomal degradation, diminishes with aging [182]. Also, neuronal differentiation, and cellular aging in general, is accompanied by a decrease in the deacetylase, SIRT1 (silent mating type information regulation 2 homolog 1), which plays a crucial role in preventing proteasomal degradation of HSF1, via deacetylation of its Lysine 80 [183]. Intriguingly, SIRT1 has also been suggested to be neuroprotective via a trimerization and HSP-independent mode of HSF1 activation [184].

Both HSF1 levels and activity are severely depleted in various neurodegenerative disorders characterized by toxic aggregation of mutant proteins such as Alzheimer's, Parkinson's, and Huntington's [34]. The underlying cause of this paradox, protein misfolding diseases characterized by reduced expression and activity of the protein quality control machinery, is unknown. Since HSF1 levels generally decline with age, the adult-onset nature of these debilitating disorders is a likely contributor to their diminished HSF1 levels. However, polyglutamine diseases such as Huntington's are relatively earlier onset, with clinical manifestations by the early-30s, suggesting that pathophysiological mechanisms unique to these disorders likely exacerbate the underlying age-related decline of HSF1 levels in these disorders. For example, the upregulation of casein kinase 2 (CK2) activity is a common denominator of these pathologies and CK2 marks HSF1 for proteasomal degradation via phosphorylation of its Serines at 303 and 307 [61]. Another possibility is that the magnitude of the cellular toxic load, with rampant aggregation of mutant proteins, leads to indiscriminate targeting of proteins, including HSF1, for proteasomal degradation.

Irrespective of the mechanism, reduction in HSF1 levels further encumbers afflicted neurons, already saddled with toxic protein aggregations, from their ability to repair misfolded proteins, which in turn hastens their untimely dysfunction and demise. This is in stark contrast to the pivotal role of HSF1 in fetal neurodevelopment. Although impaired HSF1 activation is not the proximate cause of neurodegenerative disease; restoration of its expression and activity reduces toxic protein load, improves neuronal viability, rescues associated cognitive deficits, and increases lifespan in various animal models of these disorders [185]. Hopefully, these advances in animal models will translate to more effective clinical interventions for these neurodegenerative disorders, therapeutics for which have been remarkably recalcitrant.

## 13 Conclusions

There is an interesting context-dependent dichotomy in the function of heat shock factors, and its myriad downstream effectors, in regulating aspects of fetal neurodevelopment. Normally, in the absence of extrinsic stressors, components of the heat shock factor pathway execute non-stress related functions during fetal

neurodevelopment (Fig. 1a). For example, HSP play a pivotal role in the regulation of the neuronal cytoskeleton, either by interacting with it directly or by modulating signaling pathways that influence its stability. This has wide-ranging consequences on CNS development due to its impact on neuronal differentiation and migration, and axonal pathfinding [186]. In contrast, in the presence of extrinsic stressors, inhibition of neuronal apoptosis via upregulation of stress-responsive chaperones

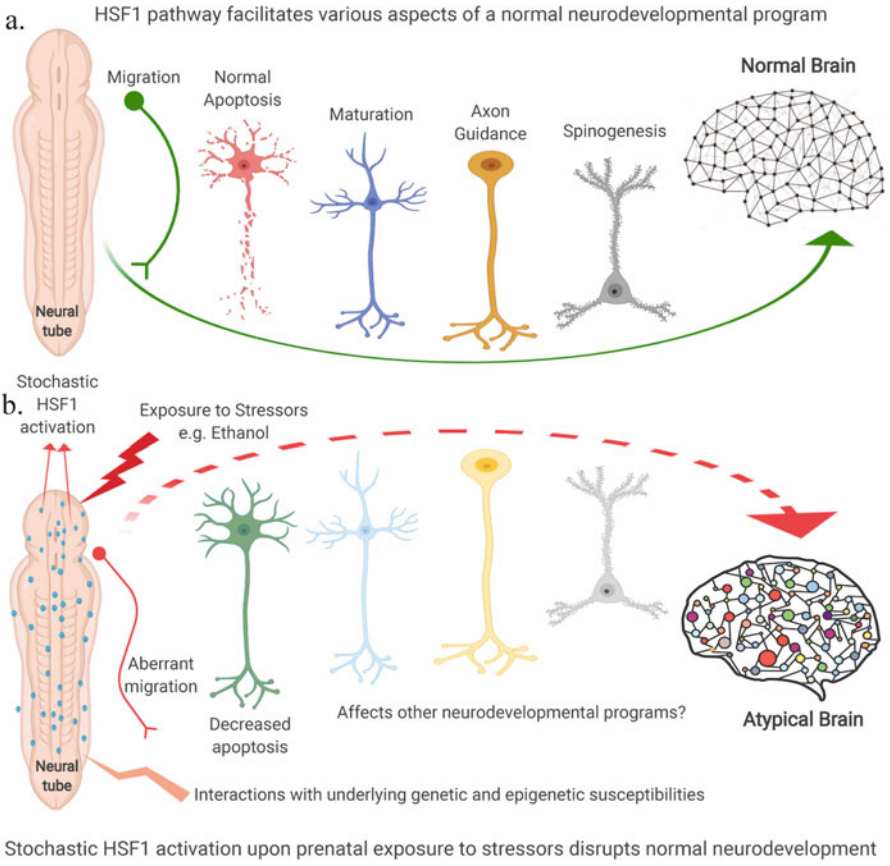



**Fig. 1** (**a**) **HSF1 pathway facilitates various aspects of a normal neurodevelopmental program.** In the absence of extrinsic stressors, activation of the HSF1 pathway facilitates various aspects of a normal neurodevelopmental program including adequate levels of neuronal apoptosis and proper migration, differentiation, axon guidance and spinogenesis of developing neurons, culminating in a normal healthy brain. (**b**) **Stochastic HSF1 activation due to prenatal stressors disrupts normal neurodevelopment.** In contrast, in the presence of various prenatal stressors, the HSF1 pathway is stochastically activated which, in interaction with underlying genetic and epigenetic susceptibilities, has been shown to disrupt neuronal migration and normal levels of neuronal apoptosis in the developing brain. It is possible other neurodevelopmental programs such as neuronal maturation, axon guidance and spinogenesis are also impacted, culminating in an atypical brain with a disrupted connectome

becomes the immediate priority of the HSF pathway [83] (Fig. 1b). This comes at a cost, however, to other essential functions; for example, neuronal migration is impaired by activation of the HSF pathway [155] (Fig. 1b). Moreover, apoptosis plays a central role in shaping the fetal CNS and is necessary for pruning exuberant neuronal and non-neuronal growth during development [87]. Therefore, inhibition of apoptosis, although necessary during periods of external stress, probably comes with a hidden burden. To summarize, while stress-mediated activation of the HSF pathway can be beneficial in the short term, its persistent activation can also have lasting detrimental consequences, both obvious and pernicious, on fetal neurodevelopment (Fig. 1b). New research is beginning to shed light on how dysregulation of the HSF1 pathway by extrinsic stressors, either acting alone or in concert with underlying genetic and epigenetic factors, can disrupt neurodevelopment. For example, the HSF1 response to extrinsic stressors was previously assumed to be homogeneous among all exposed cells. However, surprisingly, recent studies show that this is not the case. Instead, HSF1 is activated in a stochastic manner among stressed neurons [174]. This phenomenon can have a disproportionate impact during early neurodevelopment, as a small number of neural progenitor cells are responsible for generating a large majority of adult neurons. The field of HSF research has come a long way since Ritossa's landmark discovery in 1962 [10]. HSF1 is no longer ascribed a solely cytoprotective function during periods of cellular stress but is implicated in a wide range of diverse functions. Transcriptional targets of HSF1 have expanded well beyond the canonical stress-activated chaperones [34]. Multiple recent reports even describe HSF1-mediated transcription to be important for functions as diverse as regulation of metabolism [187] and mitochondrial energy homeostasis [172, 173]. HSP are no longer considered to be entirely intracellular and are found to be expressed on cell membranes where they participate in signal transduction pathways [188]. Whether they act as receptors themselves or chaperone other cell surface receptors remains unresolved. HSP can also be secreted into the extracellular milieu. As far back as 1986, Tytell and colleagues reported the transfer of glial heat shock proteins to the squid giant axon after a heat shock [189]. Interest lagged in this novel observation until the 2000s when secreted extracellular HSP were shown to confer thermotolerance to nearby neurons [190], and to exert a potent immunomodulatory effect [191]. Since then, multiple secreted HSP have been shown to perform a myriad of functions, both during CNS development [192] and in pathology [193]. Lately, HSP have also been characterized in glial exosomes, which have been hypothesized to play a key role in axonal vitality and function [194–196]. To conclude, there is a lot that we do not know about neurodevelopment, and a lot more is unknown about the role of HSF1 in neurodevelopment.

**Acknowledgements** We thank Stephen J. Page for his valuable feedback on the manuscript. This work was supported in part by NIH/NIMH (R01MH111674, to M.T.), NIH/NIAAA (R01AA026272, to K.H.-T. and M.T.), and Scott-Gentle Foundation (to K.H.-T. and M.T.).

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants, performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals, performed by any of the authors.

## References


1. Stiles J, Jernigan TL (2010 Dec) The basics of brain development. Neuropsychol Rev 20 (4):327–348
2. Sakai T, Hirata S, Fuwa K, Sugama K, Kusunoki K, Makishima H et al (2012 Sep) Fetal brain development in chimpanzees versus humans. Curr Biol 22(18):R791–R792
3. Jernigan TL, Baaré WFC, Stiles J, Madsen KS (2011) Postnatal brain development: structural imaging of dynamic neurodevelopmental processes. Prog Brain Res 189:77–92
4. Nelson CA, Zeanah CH, Fox NA (2019 Jan 14) How early experience shapes human development: the case of psychosocial deprivation. Neural Plast 2019:1676285
5. Raznahan A, Greenstein D, Lee NR, Clasen LS, Giedd JN (2012 Jul 10) Prenatal growth in humans and postnatal brain maturation into late adolescence. Proc Natl Acad Sci U S A 109 (28):11366–11371
6. Bartzokis G, Lu PH, Tingus K, Mendez MF, Richard A, Peters DG et al (2010 Sep) Lifespan trajectory of myelin integrity and maximum motor speed. Neurobiol Aging 31(9):1554–1562
7. WHO | Life expectancy [Internet]. [cited 2019 Dec 3]. Available from: https://www.who.int/gho/mortality_burden_disease/life_tables/situation_trends_text/en/
8. Bonner JJ, Pardue ML (1977 Sep) Polytene chromosome puffing and in situ hybridization measure different aspects of RNA metabolism. Cell 12(1):227–234
9. De Maio A, Santoro MG, Tanguay RM, Hightower LE (2012 Mar) Ferruccio Ritossa's scientific legacy 50 years after his discovery of the heat shock response: a new view of biology, a new society, and a new journal. Cell Stress Chaperones 17(2):139–143
10. Ritossa F (1962 Dec) A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia 18(12):571–573
11. Tissières A, Mitchell HK, Tracy UM (1974 Apr 15) Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. J Mol Biol 84(3):389–398
12. Richter K, Haslbeck M, Buchner J (2010 Oct 22) The heat shock response: life on the verge of death. Mol Cell 40(2):253–266
13. Luu BE, Wijenayake S, Malik AI, Storey KB (2018) The regulation of heat shock proteins in response to dehydration in Xenopus laevis. Cell Stress Chaperones 23(1):45–53
14. Pignataro L, Miller AN, Ma L, Midha S, Protiva P, Herrera DG et al (2007 Nov 21) Alcohol regulates gene expression in neurons via activation of heat shock factor 1. J Neurosci 27 (47):12957–12966
15. Omar R, Pappolla M (1993) Oxygen free radicals as inducers of heat shock protein synthesis in cultured human neuroblastoma cells: relevance to neurodegenerative disease. Eur Arch Psychiatry Clin Neurosci 242(5):262–267
16. Wagner M, Hermanns I, Bittinger F, Kirkpatrick CJ (1999) Induction of stress proteins in human endothelial cells by heavy metal ions and heat shock. Am J Phys 277(5):L1026–L1033
17. Knowlton AA, Brecher P, Apstein CS (1991 Jan) Rapid expression of heat shock protein in the rabbit after brief cardiac ischemia. J Clin Invest 87(1):139–147
18. Zügel U, Kaufmann SH (1999 Jan) Role of heat shock proteins in protection from and pathogenesis of infectious diseases. Clin Microbiol Rev 12(1):19–39

19. Webster JM, Darling AL, Uversky VN, Blair LJ (2019 Sep 18) Small heat shock proteins, big impact on protein aggregation in neurodegenerative disease. Front Pharmacol 10:1047
20. Wu C (1984 Sep 11) Activating protein factor binds in vitro to upstream control sequences in heat shock gene chromatin. Nature 311(5981):81–84
21. Parker CS, Topol J (1984 May) A Drosophila RNA polymerase II transcription factor binds to the regulatory site of an hsp 70 gene. Cell 37(1):273–283
22. Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243–282
23. Verghese J, Abrams J, Wang Y, Morano KA (2012 Jun) Biology of the heat shock response and protein chaperones: budding yeast (Saccharomyces cerevisiae) as a model system. Microbiol Mol Biol Rev 76(2):115–158
24. Zwanzig R, Szabo A, Bagchi B (1992 Jan 1) Levinthal's paradox. Proc Natl Acad Sci U S A 89 (1):20–22
25. Eaton WA, Muñoz V, Hagen SJ, Jas GS, Lapidus LJ, Henry ER et al (2000) Fast kinetics and mechanisms in protein folding. Annu Rev Biophys Biomol Struct 29:327–359
26. Levinthal C (1968) Are there pathways for protein folding? J Chim Phys 65:44–45
27. Koldewey P, Stull F, Horowitz S, Martin R, Bardwell JCA (2016 Jul 14) Forces driving chaperone action. Cell 166(2):369–379
28. Rothman JE, Schekman R (2011 Sep 16) Molecular mechanism of protein folding in the cell. Cell 146(6):851–854
29. chaperon | Origin and meaning of chaperon by Online Etymology Dictionary [Internet]. [cited 2019 Nov 24]. Available from: https://www.etymonline.com/word/chaperon
30. Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU (2013) Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 82:323–355
31. Jee H (2016 Aug 31) Size dependent classification of heat shock proteins: a mini-review. J Exerc Rehabil 12(4):255–259
32. Pelham HR (1982 Sep) A regulatory upstream promoter element in the Drosophila hsp 70 heat-shock gene. Cell 30(2):517–528
33. Amin J, Ananthan J, Voellmy R (1988 Sep) Key features of heat shock regulatory elements. Mol Cell Biol 8(9):3761–3769
34. Gomez-Pastor R, Burchfiel ET, Thiele DJ (2018 Jan) Regulation of heat shock transcription factors and their roles in physiology and disease. Nat Rev Mol Cell Biol 19(1):4–19
35. McMillan DR, Xiao X, Shao L, Graves K, Benjamin IJ (1998 Mar 27) Targeted disruption of heat shock transcription factor 1 abolishes thermotolerance and protection against heat-inducible apoptosis. J Biol Chem 273(13):7523–7528
36. Pirkkala L, Alastalo TP, Zuo X, Benjamin IJ, Sistonen L (2000 Apr) Disruption of heat shock factor 1 reveals an essential role in the ubiquitin proteolytic pathway. Mol Cell Biol 20 (8):2670–2675
37. Xiao X, Zuo X, Davis AA, McMillan DR, Curry BB, Richardson JA et al (1999 Nov 1) HSF1 is required for extra-embryonic development, postnatal growth and protection during inflammatory responses in mice. EMBO J 18(21):5943–5952
38. Ostling P, Björk JK, Roos-Mattjus P, Mezger V, Sistonen L (2007 Mar 9) Heat shock factor 2 (HSF2) contributes to inducible expression of hsp genes through interplay with HSF1. J Biol Chem 282(10):7077–7086
39. He H, Soncin F, Grammatikakis N, Li Y, Siganou A, Gong J et al (2003 Sep 12) Elevated expression of heat shock factor (HSF) 2A stimulates HSF1-induced transcription during stress. J Biol Chem 278(37):35465–35475
40. Chang Y, Ostling P, Akerfelt M, Trouillet D, Rallu M, Gitton Y et al (2006 Apr 1) Role of heat-shock factor 2 in cerebral cortex formation and as a regulator of p35 expression. Genes Dev 20(7):836–847
41. Fujimoto M, Izu H, Seki K, Fukuda K, Nishida T, Yamada S-I et al (2004 Oct 27) HSF4 is required for normal cell growth and differentiation during mouse lens development. EMBO J 23(21):4297–4306

42. Min J-N, Zhang Y, Moskophidis D, Mivechi NF (2004 Dec) Unique contribution of heat shock transcription factor 4 in ocular lens development and fiber cell differentiation. Genesis 40(4):205–217
43. Takaki E, Fujimoto M, Sugahara K, Nakahari T, Yonemura S, Tanaka Y et al (2006 Feb 24) Maintenance of olfactory neurogenesis requires HSF1, a major heat shock transcription factor in mice. J Biol Chem 281(8):4931–4937
44. Shi X, Cui B, Wang Z, Weng L, Xu Z, Ma J et al (2009 Feb 19) Removal of Hsf4 leads to cataract development in mice through down-regulation of gamma S-crystallin and Bfsp expression. BMC Mol Biol 10:10
45. Bu L, Jin Y, Shi Y, Chu R, Ban A, Eiberg H et al (2002 Jul) Mutant DNA-binding domain of HSF4 is associated with autosomal dominant lamellar and Marner cataract. Nat Genet 31 (3):276–278
46. Saju JM, Hossain MS, Liew WC, Pradhan A, Thevasagayam NM, Tan LSE et al (2018 Dec 18) Heat shock factor 5 is essential for spermatogenesis in zebrafish. Cell Rep 25 (12):3252–3261.e4
47. Bhowmick BK, Takahata N, Watanabe M, Satta Y (2006 Nov 30) Comparative analysis of human masculinity. Genet Mol Res 5(4):696–712
48. Shinka T, Sato Y, Chen G, Naroda T, Kinoshita K, Unemi Y et al (2004 Jul) Molecular characterization of heat shock-like factor encoded on the human Y chromosome, and implications for male infertility. Biol Reprod 71(1):297–306
49. Fujimoto M, Hayashida N, Katoh T, Oshima K, Shinkawa T, Prakasam R et al (2010 Jan 1) A novel mouse HSF3 has the potential to activate nonclassical heat-shock genes during heat shock. Mol Biol Cell 21(1):106–116
50. Fiorenza MT, Farkas T, Dissing M, Kolding D, Zimarino V (1995 Feb 11) Complex expression of murine heat shock transcription factors. Nucleic Acids Res 23(3):467–474
51. Shamovsky I, Gershon D (2004 Nov) Novel regulatory factors of HSF-1 activation: facts and perspectives regarding their involvement in the age-associated attenuation of the heat shock response. Mech Ageing Dev 125(10–11):767–775
52. Kim Guisbert KS, Guisbert E (2017 Apr 26) SF3B1 is a stress-sensitive splicing factor that regulates both HSF1 concentration and activity. PLoS One 12(4):e0176382
53. Fujikake N, Nagai Y, Popiel HA, Kano H, Yamaguchi M, Toda T (2005 Jul 4) Alternative splicing regulates the transcriptional activity of Drosophila heat shock transcription factor in response to heat/cold stress. FEBS Lett 579(17):3842–3848
54. Mathew A, Mathur SK, Morimoto RI (1998 Sep) Heat shock response and protein degradation: regulation of HSF2 by the ubiquitin-proteasome pathway. Mol Cell Biol 18 (9):5091–5098
55. Brown IR, Rush SJ (1999 Mar 13) Cellular localization of the heat shock transcription factors HSF1 and HSF2 in the rat brain during postnatal development and following hyperthermia. Brain Res 821(2):333–340
56. Mercier PA, Winegarden NA, Westwood JT (1999 Aug) Human heat shock factor 1 is predominantly a nuclear protein before and after heat stress. J Cell Sci 112(Pt 16):2765–2774
57. Stacchiotti A, Rezzani R, Rodella L, Tiberio L, Schiaffonati L, Bianchi R (1999 Jun 18) Cell-specific expression of heat shock transcription factors 1 and 2 in unstressed rat spinal cord. Neurosci Lett 268(2):73–76
58. Batulan Z, Shinder GA, Minotti S, He BP, Doroudchi MM, Nalbantoglu J et al (2003 Jul 2) High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1. J Neurosci 23(13):5789–5798
59. Yang J, Oza J, Bridges K, Chen KY, Liu AY-C (2008 Apr 8) Neural differentiation and the attenuated heat shock response. Brain Res 1203:39–50
60. Oza J, Yang J, Chen KY, Liu AY-C (2008 Feb 7) Changes in the regulation of heat shock gene expression in neuronal cell differentiation. Cell Stress Chaperones 13(1):73–84

61. Gomez-Pastor R, Burchfiel ET, Neef DW, Jaeger AM, Cabiscol E, McKinstry SU et al (2017 Feb 13) Abnormal degradation of the neuronal stress-protective transcription factor HSF1 in Huntington's disease. Nat Commun 8:14405
62. Shemesh N, Ben-Zvi A (2016) HSF1 regulation in aging and its role in longevity. In: Nakai A (ed) Heat shock factor. Springer Japan, Tokyo, pp 93–113
63. Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R (1998 Aug 21) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell 94(4):471–480
64. Baler R, Dahl G, Voellmy R (1993 Apr) Activation of human heat shock genes is accompanied by oligomerization, modification, and rapid translocation of heat shock transcription factor HSF1. Mol Cell Biol 13(4):2486–2496
65. Abravaya K, Myers MP, Murphy SP, Morimoto RI (1992 Jul) The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. Genes Dev 6(7):1153–1164
66. Shi Y, Mosser DD, Morimoto RI (1998 Mar 1) Molecular chaperones as HSF1-specific transcriptional repressors. Genes Dev 12(5):654–666
67. Anckar J, Sistonen L (2011) Regulation of HSF1 function in the heat stress response: implications in aging and disease. Annu Rev Biochem 80:1089–1115
68. Budzyński MA, Puustinen MC, Joutsen J, Sistonen L (2015 Jul) Uncoupling stress-inducible phosphorylation of heat shock factor 1 from its activation. Mol Cell Biol 35(14):2530–2540
69. Raychaudhuri S, Loew C, Körner R, Pinkert S, Theis M, Hayer-Hartl M et al (2014 Feb 27) Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1. Cell 156(5):975–985
70. Dai S, Tang Z, Cao J, Zhou W, Li H, Sampson S et al (2015 Feb 3) Suppression of the HSF1-mediated proteotoxic stress response by the metabolic stress sensor AMPK. EMBO J 34 (3):275–293
71. Morimoto RI (1998 Dec 15) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev 12(24):3788–3796
72. Hentze N, Le Breton L, Wiesner J, Kempf G, Mayer MP (2016 Jan) Molecular mechanism of thermosensory function of human heat shock transcription factor Hsf1. elife 19:5
73. Zhong M, Orosz A, Wu C (1998 Jul) Direct sensing of heat and oxidation by Drosophila heat shock transcription factor. Mol Cell 2(1):101–108
74. Mahat DB, Salamanca HH, Duarte FM, Danko CG, Lis JT (2016 Apr 7) Mammalian heat shock response and mechanisms underlying its genome-wide transcriptional regulation. Mol Cell 62(1):63–78
75. Homma S, Jin X, Wang G, Tu N, Min J, Yanasak N et al (2007 Jul 25) Demyelination, astrogliosis, and accumulation of ubiquitinated proteins, hallmarks of CNS disease in hsf1-deficient mice. J Neurosci 27(30):7974–7986
76. Fairfield DA, Lomax MI, Dootz GA, Chen S, Galecki AT, Benjamin IJ et al (2005 Aug 15) Heat shock factor 1-deficient mice exhibit decreased recovery of hearing following noise overstimulation. J Neurosci Res 81(4):589–596
77. Santos SD, Saraiva MJ (2004) Enlarged ventricles, astrogliosis and neurodegeneration in heat shock factor 1 null mouse brain. Neuroscience 126(3):657–663
78. Wang G, Zhang J, Moskophidis D, Mivechi NF (2003 May) Targeted disruption of the heat shock transcription factor (hsf)-2 gene results in increased embryonic lethality, neuronal defects, and reduced spermatogenesis. Genesis 36(1):48–61
79. Kallio M, Chang Y, Manuel M, Alastalo T-P, Rallu M, Gitton Y et al (2002 Jun 3) Brain abnormalities, defective meiotic chromosome synapsis and female subfertility in HSF2 null mice. EMBO J 21(11):2591–2601
80. Sandqvist A, Björk JK, Akerfelt M, Chitikova Z, Grichine A, Vourc'h C et al (2009 Mar) Heterotrimerization of heat-shock factors 1 and 2 provides a transcriptional switch in response to distinct stimuli. Mol Biol Cell 20(5):1340–1347

81. San Gil R, Ooi L, Yerbury JJ, Ecroyd H (2017 Sep 18) The heat shock response in neurons and astroglia and its role in neurodegenerative diseases. Mol Neurodegener 12(1):65
82. Ishii S, Hashimoto-Torii K (2015 May 27) Impact of prenatal environmental stress on cortical development. Front Cell Neurosci 9:207
83. Hashimoto-Torii K, Torii M, Fujimoto M, Nakai A, El Fatimy R, Mezger V et al (2014 May 7) Roles of heat shock factor 1 in neuronal response to fetal environmental risks and its relevance to brain disorders. Neuron 82(3):560–572
84. Nakagomi S, Suzuki Y, Namikawa K, Kiryu-Seo S, Kiyama H (2003 Jun 15) Expression of the activating transcription factor 3 prevents c-Jun N-terminal kinase-induced neuronal death by promoting heat shock protein 27 expression and Akt activation. J Neurosci 23 (12):5187–5196
85. Nishi S, Taki W, Uemura Y, Higashi T, Kikuchi H, Kudoh H et al (1993 Jul 2) Ischemic tolerance due to the induction of HSP70 in a rat ischemic recirculation model. Brain Res 615 (2):281–288
86. Kim JY, Han Y, Lee JE, Yenari MA (2018 Feb 15) The 70-kDa heat shock protein (Hsp70) as a therapeutic target for stroke. Expert Opin Ther Targets 22(3):191–199
87. Yamaguchi Y, Miura M (2015 Feb 23) Programmed cell death in neurodevelopment. Dev Cell 32(4):478–490
88. Innocenti GM, Price DJ (2005 Dec) Exuberance in the development of cortical networks. Nat Rev Neurosci 6(12):955–965
89. Xie Y, Chen C, Stevenson MA, Auron PE, Calderwood SK (2002 Apr 5) Heat shock factor 1 represses transcription of the IL-1beta gene through physical interaction with the nuclear factor of interleukin 6. J Biol Chem 277(14):11802–11810
90. Seki T, Rutishauser U (1998 May 15) Removal of polysialic acid-neural cell adhesion molecule induces aberrant mossy fiber innervation and ectopic synaptogenesis in the hippocampus. J Neurosci 18(10):3757–3766
91. Uchida S, Hara K, Kobayashi A, Fujimoto M, Otsuki K, Yamagata H et al (2011 Jan 25) Impaired hippocampal spinogenesis and neurogenesis and altered affective behavior in mice lacking heat shock factor 1. Proc Natl Acad Sci U S A 108(4):1681–1686
92. Hooper PL, Durham HD, Török Z, Hooper PL, Crul T, Vígh L (2016 Jun 9) The central role of heat shock factor 1 in synaptic fidelity and memory consolidation. Cell Stress Chaperones 21 (5):745–753
93. Pakkenberg B, Pelvig D, Marner L, Bundgaard MJ, Gundersen HJG, Nyengaard JR et al (2003 Feb) Aging and the human neocortex. Exp Gerontol 38(1–2):95–99
94. Drachman DA (2005 Jun 28) Do we have brain to spare? Neurology 64(12):2004–2005
95. Conselice CJ, Wilkinson A, Duncan K, Mortlock A (2016 Oct 13) The evolution of galaxy number density at $z < 8$ and its implications. Astrophys J 830(2):83
96. Trinklein ND, Chen WC, Kingston RE, Myers RM (2004 Mar) Transcriptional regulation and binding of heat shock factor 1 and heat shock factor 2 to 32 human heat shock genes during thermal stress and differentiation. Cell Stress Chaperones 9(1):21–28
97. Cheng Y-C, Huang C-J, Lee Y-J, Tien L-T, Ku W-C, Chien R et al (2016 Jul 22) Knocking down of heat-shock protein 27 directs differentiation of functional glutamatergic neurons from placenta-derived multipotent cells. Sci Rep 6:30314
98. Williams KL, Rahimtula M, Mearow KM (2005 Apr 8) Hsp27 and axonal growth in adult sensory neurons in vitro. BMC Neurosci 6:24
99. Lavoie JN, Hickey E, Weber LA, Landry J (1993 Nov 15) Modulation of actin microfilament dynamics and fluid phase pinocytosis by phosphorylation of heat shock protein 27. J Biol Chem 268(32):24210–24214
100. Evgrafov OV, Mersiyanova I, Irobi J, Van Den Bosch L, Dierick I, Leung CL et al (2004 Jun) Mutant small heat-shock protein 27 causes axonal Charcot-Marie-tooth disease and distal hereditary motor neuropathy. Nat Genet 36(6):602–606

101. Williams KL, Rahimtula M, Mearow KM (2006 Sep) Heat shock protein 27 is involved in neurite extension and branching of dorsal root ganglion neurons in vitro. J Neurosci Res 84 (4):716–723
102. Ma CHE, Omura T, Cobos EJ, Latrémolière A, Ghasemlou N, Brenner GJ et al (2011 Nov) Accelerating axonal growth promotes motor recovery after peripheral nerve injury in mice. J Clin Invest 121(11):4332–4347
103. Bito H, Furuyashiki T, Ishihara H, Shibasaki Y, Ohashi K, Mizuno K et al (2000 May) A critical role for a rho-associated kinase, p160ROCK, in determining axon outgrowth in mammalian CNS neurons. Neuron 26(2):431–441
104. Sun X, Zhou Z, Fink DJ, Mata M (2013 Nov) HspB1 silences translation of PDZ-RhoGEF by enhancing miR-20a and miR-128 expression to promote neurite extension. Mol Cell Neurosci 57:111–119
105. de Thonel A, Le Mouël A, Mezger V (2012 Oct) Transcriptional regulation of small HSP-HSF1 and beyond. Int J Biochem Cell Biol 44(10):1593–1612
106. Bartelt-Kirbach B, Moron M, Glomb M, Beck C-M, Weller M-P, Golenhofen N (2016 Apr 16) HspB5/αB-crystallin increases dendritic complexity and protects the dendritic arbor during heat shock in cultured rat hippocampal neurons. Cell Mol Life Sci 73(19):3761–3775
107. Frebel K, Wiese S, Funk N, Pühringer D, Sendtner M (2007) Differential modulation of neurite growth by the S- and the L-forms of bag1, a co-chaperone of Hsp70. Neurodegener Dis 4(2–3):261–269
108. Ishimoto T, Kamei A, Koyanagi S, Nishide N, Uyeda A, Kasai M et al (1998 Dec 18) HSP90 has neurite-promoting activity in vitro for telencephalic and spinal neurons of chick embryos. Biochem Biophys Res Commun 253(2):283–287
109. Benitez MJ, Sanchez-Ponce D, Garrido JJ, Wandosell F (2014 Feb) Hsp90 activity is necessary to acquire a proper neuronal polarization. Biochim Biophys Acta 1843(2):245–252
110. Perge JA, Niven JE, Mugnaini E, Balasubramanian V, Sterling P (2012 Jan 11) Why do axons differ in caliber? J Neurosci 32(2):626–638
111. Azevedo FAC, Carvalho LRB, Grinberg LT, Farfel JM, Ferretti REL, Leite REP et al (2009 Apr 10) Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol 513(5):532–541
112. Holland D, Chang L, Ernst TM, Curran M, Buchthal SD, Alicata D et al (2014 Oct) Structural growth trajectories and rates of change in the first 3 months of infant brain development. JAMA Neurol 71(10):1266–1274
113. Seiradake E, Jones EY, Klein R (2016 Oct 6) Structural perspectives on axon guidance. Annu Rev Cell Dev Biol 32:577–608
114. Engle EC (2010 Mar) Human genetic disorders of axon guidance. Cold Spring Harb Perspect Biol 2(3):a001784
115. Stoeckli ET (2018 May) Understanding axon guidance: are we nearly there yet? Development 14:145(10)
116. Kennedy TE, Serafini T, de la Torre JR, Tessier-Lavigne M (1994 Aug 12) Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. Cell 78 (3):425–435
117. Richards LJ, Koester SE, Tuttle R, O'Leary DD (1997 Apr 1) Directed growth of early cortical axons is influenced by a chemoattractant released from an intermediate target. J Neurosci 17 (7):2445–2458
118. Boyer NP, Gupton SL (2018 Jul 31) Revisiting Netrin-1: one who guides (axons). Front Cell Neurosci 12:221
119. Grant A, Fathalli F, Rouleau G, Joober R, Flores C (2012 May) Association between schizophrenia and genetic variation in DCC: a case-control study. Schizophr Res 137 (1–3):26–31
120. Lesnick TG, Sorenson EJ, Ahlskog JE, Henley JR, Shehadeh L, Papapetropoulos S et al (2008 Jan 16) Beyond Parkinson disease: amyotrophic lateral sclerosis and the axon guidance pathway. PLoS One 3(1):e1449

121. Loones MT, Chang Y, Morange M (2000 Oct) The distribution of heat shock proteins in the nervous system of the unstressed mouse embryo suggests a role in neuronal and non-neuronal differentiation. Cell Stress Chaperones 5(4):291–305
122. D'Souza SM, Brown IR (1998 Sep) Constitutive expression of heat shock proteins Hsp90, Hsc70, Hsp70 and Hsp60 in neural and non-neural tissues of the rat during postnatal development. Cell Stress Chaperones 3(3):188–199
123. DeGeer J, Kaplan A, Mattar P, Morabito M, Stochaj U, Kennedy TE et al (2015 Aug 31) Hsc70 chaperone activity underlies trio GEF function in axon growth and guidance induced by netrin-1. J Cell Biol 210(5):817–832
124. Buchsbaum IY, Cappello S (2019 Jan) Neuronal migration in the CNS during development and disease: insights from in vivo and in vitro models. Development 9:146(1)
125. Curtis MA, Monzo HJ, RLM F (2009) The rostral migratory stream and olfactory system: smell, disease and slippery cells. Neurotherapy: progress in restorative neuroscience and neurology. Elsevier:33–42
126. Valiente M, Marín O (2010 Feb) Neuronal migration mechanisms in development and disease. Curr Opin Neurobiol 20(1):68–78
127. Marín O, Valiente M, Ge X, Tsai L-H (2010 Feb) Guiding neuronal cell migrations. Cold Spring Harb Perspect Biol 2(2):a001834
128. Sidera K, Samiotaki M, Yfanti E, Panayotou G, Patsavoudi E (2004 Oct 29) Involvement of cell surface HSP90 in cell migration reveals a novel role in the developing nervous system. J Biol Chem 279(44):45379–45388
129. Miyakoshi LM, Marques-Coelho D, De Souza LER, Lima FRS, Martins VR, Zanata SM et al (2017 May 17) Evidence of a cell surface role for hsp90 complex proteins mediating neuroblast migration in the subventricular zone. Front Cell Neurosci 11:138
130. Thuringer D, Hammann A, Benikhlef N, Fourmaux E, Bouchot A, Wettstein G et al (2011 Feb 4) Transactivation of the epidermal growth factor receptor by heat shock protein 90 via toll-like receptor 4 contributes to the migration of glioblastoma cells. J Biol Chem 286 (5):3418–3428
131. Hashimoto-Torii K, Kawasawa YI, Kuhn A, Rakic P (2011 Mar 8) Combined transcriptome analysis of fetal human and mouse cerebral cortex exposed to alcohol. Proc Natl Acad Sci U S A 108(10):4212–4217
132. Kakita A, Wakabayashi K, Su M, Piao YS, Takahashi H (2001 Aug) Experimentally induced leptomeningeal glioneuronal heterotopia and underlying cortical dysplasia of the lateral limbic area in rats treated transplacentally with methylmercury. J Neuropathol Exp Neurol 60 (8):768–777
133. Khandaker GM, Zimbron J, Lewis G, Jones PB (2013 Feb) Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population-based studies. Psychol Med 43(2):239–257
134. Lee J, Croen LA, Backstrand KH, Yoshida CK, Henning LH, Lindan C et al (2005 Feb 9) Maternal and infant characteristics associated with perinatal arterial stroke in the infant. JAMA 293(6):723–729
135. Sveberg L, Svalheim S, Taubøll E (2015 May) The impact of seizures on pregnancy and delivery. Seizure 28:35–38
136. Mooney SM, Siegenthaler JA, Miller MW (2004 Oct) Ethanol induces heterotopias in organotypic cultures of rat cerebral cortex. Cereb Cortex 14(10):1071–1080
137. Lidow MS (2003 Dec 30) Consequences of prenatal cocaine exposure in nonhuman primates. Brain Res Dev Brain Res 147(1–2):23–36
138. Cannon TD, van Erp TGM, Rosso IM, Huttunen M, Lönnqvist J, Pirkola T et al (2002 Jan) Fetal hypoxia and structural brain abnormalities in schizophrenic patients, their siblings, and controls. Arch Gen Psychiatry 59(1):35–41
139. Johnston MV, Coyle JT (1979 Jul 6) Histological and neurochemical effects of fetal treatment with methylazoxymethanol on rat neocortex in adulthood. Brain Res 170(1):135–155

140. Algan O, Rakic P (1997 May 12) Radiation-induced, lamina-specific deletion of neurons in the primate visual cortex. J Comp Neurol 381(3):335–352
141. de Feo MR, Del Priore D, Mecarelli O (1995 Feb) Prenatal cocaine: seizure susceptibility in rat offspring. Pharmacol Res 31(2):137–141
142. Russo E, Citraro R, De Fazio S, Torcasio G, De Sarro G, Di Paola ED (2008 Nov) Effects of ethanol on the development of genetically determined epilepsies in rats. Int J Dev Neurosci 26 (7):739–744
143. Szász A, Barna B, Szupera Z, De Visscher G, Galbács Z, Kirsch-Volders M et al (1999 Nov) Chronic low-dose maternal exposure to methylmercury enhances epileptogenicity in developing rats. Int J Dev Neurosci 17(7):733–742
144. Oghlakian RO, Tilelli CQ, Hiremath GK, Alexopoulos AV, Najm IM (2009 Apr) Single injection of a low dose of pentylenetetrazole leads to epileptogenesis in an animal model of cortical dysplasia. Epilepsia 50(4):801–810
145. De Riu PL, Mameli P, Becciu A, Simula ME, Mameli O (1995 Feb) Effect of fetal hypoxia on seizure susceptibility in rats. Physiol Behav 57(2):315–318
146. Ishii K, Kubo K, Endo T, Yoshida K, Benner S, Ito Y et al (2015 Sep 9) Neuronal heterotopias affect the activities of distant brain areas and lead to behavioral deficits. J Neurosci 35 (36):12432–12445
147. Guerri C, Bazinet A, Riley EP (2009 Apr) Foetal alcohol Spectrum disorders and alterations in brain and behaviour. Alcohol Alcohol 44(2):108–114
148. Peadon E, Elliott EJ (2010 Sep 7) Distinguishing between attention-deficit hyperactivity and fetal alcohol spectrum disorders in children: clinical guidelines. Neuropsychiatr Dis Treat 6:509–515
149. Bell SH, Stade B, Reynolds JN, Rasmussen C, Andrew G, Hwang PA et al (2010 Jun) The remarkably high prevalence of epilepsy and seizure history in fetal alcohol spectrum disorders. Alcohol Clin Exp Res 34(6):1084–1089
150. Hahn J-S, Hu Z, Thiele DJ, Iyer VR (2004 Jun) Genome-wide analysis of the biology of stress responses through heat shock transcription factor. Mol Cell Biol 24(12):5249–5256
151. Kawasawa YI, Mohammad S, Son AI, Morizono H, Basha A, Salzberg AC et al (2017 May 4) Genome-wide profiling of differentially spliced mRNAs in human fetal cortical tissue exposed to alcohol. Alcohol 62:1–9
152. Maier SE, West JR (2001) Drinking patterns and alcohol-related birth defects. Alcohol Res Health 25(3):168–174
153. Thomas JD, Wasserman EA, West JR, Goodlett CR (1996 Jul) Behavioral deficits induced by bingelike exposure to alcohol in neonatal rats: importance of developmental timing and number of episodes. Dev Psychobiol 29(5):433–452
154. Stratton K, Howe C, Battaglia FC (eds) (1996) Institute of Medicine CSFAS. Fetal alcohol syndrome: diagnosis, epidemiology, prevention, and treatment. National Academies Press, Washington, DC
155. Hwang HM, Ku RY, Hashimoto-Torii K (2019 Jul 17) Prenatal environment that affects neuronal migration. Front Cell Dev Biol 7:138
156. Herman LE, Acosta MC, Chang P-N (2008 Oct 24) Gender and attention deficits in children diagnosed with a Fetal alcohol Spectrum disorder. Can J Clin Pharmacol 15(3):e411–e419
157. Tagawa K, Marubuchi S, Qi M-L, Enokido Y, Tamura T, Inagaki R et al (2007 Jan 24) The induction levels of heat shock protein 70 differentiate the vulnerabilities to mutant huntingtin among neuronal subtypes. J Neurosci 27(4):868–880
158. Kneeland RE, Fatemi SH (2013 Apr 5) Viral infection, inflammation and schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry 42:35–48
159. Selemon LD, Wang L, Nebel MB, Csernansky JG, Goldman-Rakic PS, Rakic P (2005 Jan 1) Direct and indirect effects of fetal irradiation on cortical gray and white matter volume in the macaque. Biol Psychiatry 57(1):83–90

160. Lin M, Zhao D, Hrabovsky A, Pedrosa E, Zheng D, Lachman HM (2014 Apr 15) Heat shock alters the expression of schizophrenia and autism candidate genes in an induced pluripotent stem cell model of the human telencephalon. PLoS One 9(4):e94968
161. Zhou X, Tron VA, Li G, Trotter MJ (1998 Aug) Heat shock transcription factor-1 regulates heat shock protein-72 expression in human keratinocytes exposed to ultraviolet B light. J Invest Dermatol 111(2):194–198
162. Li Q, Martinez JD (2011 Jul) Loss of HSF1 results in defective radiation-induced G(2) arrest and DNA repair. Radiat Res 176(1):17–24
163. Ianaro A, Ialenti A, Maffia P, Pisano B, Di Rosa M (2001 Jun 22) HSF1/ *hsp72* pathway as an endogenous anti-inflammatory system. FEBS Lett 499(3):239–244
164. Filone CM, Caballero IS, Dower K, Mendillo ML, Cowley GS, Santagata S et al (2014 Feb 6) The master regulator of the cellular stress response (HSF1) is critical for orthopoxvirus infection. PLoS Pathog 10(2):e1003904
165. Pae C-U, Kim T-S, Kwon O-J, Artioli P, Serretti A, Lee C-U et al (2005 Sep) Polymorphisms of heat shock protein 70 gene (HSPA1A, HSPA1B and HSPA1L) and schizophrenia. Neurosci Res 53(1):8–13
166. Kim JJ, Lee SJ, Toh KY, Lee CU, Lee C, Paik IH (2001 Oct 1) Identification of antibodies to heat shock proteins 90 kDa and 70 kDa in patients with schizophrenia. Schizophr Res 52 (1–2):127–135
167. Schwarz MJ, Riedel M, Gruber R, Ackenheil M, Müller N (1999 Jul) Antibodies to heat shock proteins in schizophrenic patients: implications for the mechanism of the disease. Am J Psychiatry 156(7):1103–1104
168. Jaaro-Peled H, Hayashi-Takagi A, Seshadri S, Kamiya A, Brandon NJ, Sawa A (2009 Sep) Neurodevelopmental mechanisms of schizophrenia: understanding disturbed postnatal brain maturation through neuregulin-1-ErbB4 and DISC1. Trends Neurosci 32(9):485–495
169. Kuperberg GR, Broome MR, McGuire PK, David AS, Eddy M, Ozawa F et al (2003 Sep) Regionally localized thinning of the cerebral cortex in schizophrenia. Arch Gen Psychiatry 60 (9):878–888
170. Bahar R, Hartmann CH, Rodriguez KA, Denny AD, Busuttil RA, Dollé MET et al (2006 Jun 22) Increased cell-to-cell variation in gene expression in ageing mouse heart. Nature 441 (7096):1011–1014
171. Clay HB, Sillivan S, Konradi C (2011 May) Mitochondrial dysfunction and pathology in bipolar disorder and schizophrenia. Int J Dev Neurosci 29(3):311–324
172. Bierkamp C, Luxey M, Metchat A, Audouard C, Dumollard R, Christians E (2010 Mar 15) Lack of maternal heat shock factor 1 results in multiple cellular and developmental defects, including mitochondrial damage and altered redox homeostasis, and leads to reduced survival of mammalian oocytes and embryos. Dev Biol 339(2):338–353
173. Yan L-J, Christians ES, Liu L, Xiao X, Sohal RS, Benjamin IJ (2002 Oct 1) Mouse heat shock transcription factor 1 deficiency alters cardiac redox homeostasis and increases mitochondrial oxidative damage. EMBO J 21(19):5164–5172
174. Ishii S, Torii M, Son AI, Rajendraprasad M, Morozov YM, Kawasawa YI et al (2017 May 2) Variations in brain defects result from cellular mosaicism in the activation of heat shock signalling. Nat Commun 8:15157
175. Fraser D, Kaern M (2009 Mar) A chance at survival: gene expression noise and phenotypic diversification strategies. Mol Microbiol 71(6):1333–1340
176. Bishop AL, Rab FA, Sumner ER, Avery SV (2007 Jan) Phenotypic heterogeneity can enhance rare-cell survival in “stress-sensitive” yeast populations. Mol Microbiol 63(2):507–520
177. Torii M, Sasaki M, Chang Y-W, Ishii S, Waxman SG, Kocsis JD et al (2017 Feb 28) Detection of vulnerable neurons damaged by environmental insults in utero. Proc Natl Acad Sci U S A 114(9):2367–2372
178. Mehler MF (2017 Feb 28) Shining a light on early stress responses and late-onset disease vulnerability. Proc Natl Acad Sci U S A 114(9):2109–2111

179. Turan S, Kuehle J, Schambach A, Baum C, Bode J (2010 Sep 10) Multiplexing RMCE: versatile extensions of the Flp-recombinase-mediated cassette-exchange technology. J Mol Biol 402(1):52–69
180. Hashimoto-Torii K, Sasaki M, Chang Y-W, Hwang H, Waxman SG, Kocsis JD et al (2018 Dec) Detection of local and remote cellular damage caused by spinal cord and peripheral nerve injury using a heat shock signaling reporter system. IBRO Rep 5:91–98
181. Morimoto RI (2008 Jun 1) Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. Genes Dev 22(11):1427–1438
182. Li Q, Xiao H, Isobe K (2002 Mar) Histone acetyltransferase activities of cAMP-regulated enhancer-binding protein and p300 in tissues of fetal, young, and old mice. J Gerontol A Biol Sci Med Sci 57(3):B93–B98
183. Liu DJ, Hammer D, Komlos D, Chen KY, Firestein BL, Liu AY-C (2014 Sep) SIRT1 knockdown promotes neural differentiation and attenuates the heat shock response. J Cell Physiol 229(9):1224–1235
184. Verma P, Pfister JA, Mallick S, D'Mello SR (2014 Jan 29) HSF1 protects neurons through a novel trimerization- and HSP-independent mechanism. J Neurosci 34(5):1599–1612
185. Neef DW, Jaeger AM, Thiele DJ (2011 Dec 1) Heat shock transcription factor 1 as a therapeutic target in neurodegenerative diseases. Nat Rev Drug Discov 10(12):930–944
186. Herbert KR, Samali A, Gorman A (2009) The role of hsps in neuronal differentiation and development. Heat shock proteins in neural cells. Springer, New York, pp 25–37
187. Jin X, Qiao A, Moskophidis D, Mivechi NF (2018 Sep) Modulation of heat shock factor 1 activity through silencing of Ser303/Ser307 phosphorylation supports a metabolic program leading to age-related obesity and insulin resistance. Mol Cell Biol 15:38(18)
188. Horváth I, Multhoff G, Sonnleitner A, Vígh L (2008 Aug) Membrane-associated stress proteins: more than simply chaperones. Biochim Biophys Acta 1778(7–8):1653–1664
189. Tytell M, Greenberg SG, Lasek RJ (1986 Jan 15) Heat shock-like protein is transferred from glia to axon. Brain Res 363(1):161–164
190. Guzhova I, Kislyakova K, Moskaliova O, Fridlanskaya I, Tytell M, Cheetham M et al (2001 Sep 28) In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance. Brain Res 914(1–2):66–73
191. Calderwood SK, Gong J, Murshid A (2016 Apr 25) Extracellular hsps: the complicated roles of extracellular hsps in immunity. Front Immunol 7:159
192. Robinson MB, Tidwell JL, Gould T, Taylor AR, Newbern JM, Graves J et al (2005 Oct 19) Extracellular heat shock protein 70: a critical component for motoneuron survival. J Neurosci 25(42):9735–9745
193. Lyon MS, Milligan C (2019 Oct 15) Extracellular heat shock proteins in neurodegenerative diseases: new perspectives. Neurosci Lett 711:134462
194. Tytell M, Lasek RJ, Gainer H. Axonal maintenance, glia, exosomes, and heat shock proteins. [version 1; peer review: 3 approved]. F1000Res. 2016 Feb 22;5
195. Nafar F, Williams JB, Mearow KM (2016) Astrocytes release HspB1 in response to amyloid-β exposure in vitro. J Alzheimers Dis 49(1):251–263
196. Sreekumar PG, Kannan R, Kitamura M, Spee C, Barron E, Ryan SJ et al (2010 Oct 8) αB crystallin is apically secreted within exosomes by polarized human retinal pigment epithelium and provides neuroprotection to adjacent cells. PLoS One 5(10):e12578

# Small Heat Shock Proteins in Inflammatory Diseases


**V. Sudhakar Reddy, Trinath Jamma, and G. Bhanuprakash Reddy**

## Abstract

**Introduction** Small heat shock proteins (sHsp) are a group of proteins characterized by presence of a conserved α-crystallin domain that assist in proper folding and assembly of nascent protein in addition to protecting the cells from cell death, preserving the cytoskeletal architecture, and inhibiting the platelet aggregation. However, functions of sHsp in inflammation remain unexplored.

**Methods** An extensive literature review has been carried out to gather the available information on role of sHsp in inflammatory diseases.

**Results** Intriguingly, residues 73–92 in sHsp have been shown to possess chaperone and therapeutic activity in neurodegenerative, inflammatory and metabolic diseases in addition to full length proteins. In the last two decades, remarkable work has been done on secretion, therapeutic effect of sHsp, and their implication in inflammatory diseases as well. Secretion of sHsp into extracellular milieu, found to generate not only the auto-antibodies, but also acts as immune modulators at the site of inflammation, tissues, body fluids including blood and cerebrospinal fluid in inflammatory diseases. Mechanistically, sHsp were found to interact with the toll-like receptors and suppresses the NF-κB signaling and resultant inflammatory gene expression.

**Conclusions** Even though the mechanistic insights into anti-inflammatory function of sHsp remain elusive, the clinical implications of these sHsp as therapeutic molecules are being explored at a great pace. However, a great deal of work still remains to be carried out to decipher the physiological impact of sHsp.

V. S. Reddy (✉) · G. B. Reddy (✉)
Biochemistry Division, National Institute of Nutrition, Hyderabad, India
e-mail: sudhakarr.v@icmr.gov.in; sudhakarnin@gmail.com; Reddyg.bp@icmr.gov.in; geereddy@yahoo.com

T. Jamma
Department of Biological Sciences, BITS-Pilani, Hyderabad, Telangana, India

Heat Shock Proteins (2021) 22: 241–269
https://doi.org/10.1007/7515_2020_9

Published Online: 23 July 2020

**Keywords** Auto-antibodies · Experimental autoimmune encephalomyelitis · Fibrosis · Inflammation · Small heat shock proteins · Toll-like receptors

## Abbreviations

| | |
|---|---|
| AGE | advanced glycation end products |
| BMDC | bone marrow-derived dendritic cells |
| CBP | CREB binding protein |
| COPD | chronic obstructive pulmonary disease |
| CSF | cerebrospinal fluid |
| DRD2 | dopamine D2 receptors |
| DSS | dextran sodium sulfate |
| EAU | experimental autoimmune uveitis |
| EMT | epithelial to mesenchymal transition |
| GBS | Guillain-Barre syndrome |
| HCC | hepatocellular carcinoma |
| HERC | human retinal endothelial cells |
| HSP | heat shock proteins |
| IBD | inflammatory bowel disease |
| IDO | indoleamine 2,3-dioxygenase |
| IL | interleukin |
| iNOS | inducible nitric oxide synthase |
| IPF | idiopathic pulmonary fibrosis |
| LPS | lipopolysaccharide |
| MAPK | mitogen-activated protein kinase |
| MAPKAPK-2 | mitogen-activated protein kinase (MAPK)-activated protein kinase 2 |
| MARCO | macrophage scavenger receptor with collagenous structure |
| MCRA | macrophage scavenger receptor A |
| MKBP | myotonic dystrophy protein kinase |
| NBD | Neuro-Behçet's disease |
| ODF1 | outer dense fiber protein 1 |
| PGE2 | prostaglandin E2 |
| PLGA | poly (lactic-co-glycolic acid) |
| ROS | reactive oxygen species |
| sHsp | small heat shock proteins |
| SR-A | scavenger receptor-A |
| STZ | streptozotocin |
| TLRs | toll like receptors |
| TNFα | tumor necrosis factor-α |
| UUO | unilateral ureteral obstruction |
| αAC | alpha A crystallin |
| αBC | alpha B crystallin |

## 1 Introduction

Small heat shock proteins (sHsp) are a group of proteins characterized by presence of a conserved C terminal α-crystallin domain [33, 77]. International Human Genome Sequencing Consortium and the Celera Human Genome Project results showed retrieval of ten sHsp proteins [67] with different classifications [7, 40, 141]. These 10 sHsp have been formally named as, Hsp27/Hsp25 of rodents (HSPB1), MKBP (myotonic dystrophy protein kinase; HSPB2), HSPB3, αA-crystallin (αAC; HSPB4), αB-crystallin (αBC; HSPB5), Hsp20 (HSPB6), cvHsp (HSPB7), Hsp22/H11/H2IG1 (HSPB8), HSPB9, and ODF1 (sperm outer dense fiber protein 1; HSPB10). The 10 sHsp genes are dispersed over 9 different chromosomes which again reveal the ancient duplications that have generated the human sHsp family. The molecular mass of sub units comprising from 12 to 43 kDa [48] and they can exist in the form of monomers, dimers and assemble into reversible and polydispersed homo or heterooligomeric complexes ranging from 2 to 40 subunits which in turn regulated by post translational modifications such as phosphorylation and glycation. The names of the sHsp, their expression, molecular weight, response

**Table 1** Characteristics of small heat shock proteins

| Common name | Nomenclature | Heat induction | Expression | Sub unit molecular mass (kDa) | Human gene ID | Mouse gene ID |
|---|---|---|---|---|---|---|
| Hsp25 (m), Hsp27 (h and r) | HSPB1 | Yes | Ubiquitous | 22.3 | 3315 | 15507 |
| Myotonic Dystrophy protein kinase binding protein (MKBP) | HSPB2 | No | Heart and muscle | 20.2 | 3316 | 69253 |
| HspB3 | HSPB3 | No | Heart and muscle | 17.0 | 8988 | 56534 |
| αA-Crystallin (αAC) | HSPB4 | No | Lens and retina | 19.9 | 1409 | 12954 |
| αB-Crystallin (αBC) | HSPB5 | Yes | Ubiquitous | 20.2 | 1410 | 12955 |
| Hsp20, p20 | HSPB6 | No | Ubiquitous | 17.1 | 126393 | 243912 |
| Cardiovascular heat shock protein (cvHsp) | HSPB7 | ? | Heart and muscle | 18.6 | 27129 | 29818 |
| H11 protein kinase, product of E2IG1 gene, CMT2L, Hsp22 | HSPB8 | Cell type dependent | Ubiquitous | 21.6 | 26353 | 80888 |
| CT51 | HSPB9 | ? | Testis | 17.5 | 94086 | 75482 |
| Outer dense fiber proteins (ODF1) | HSPB10 | ? | Testis | 28.4 | 4956 | 18285 |

to heat induction, human and mouse gene IDs are listed in Table 1. Some of the sHsp including Hsp27, and αBC are heat-inducible in contrast to MKBP, HspB3, αAC. Additionally, some of the sHsp (Hsp27, αBC, Hsp20, and Hsp22) are ubiquitously expressed whereas MKBP, HspB3, αAC, cvHsp, HspB9, and ODF1 are restricted to specific tissues. For instance, MKBP, HspB3, and cvHsp are abundantly expressed in heart, and skeletal muscle while HspB9, and ODF1 are expressed in testis. The main function of sHsp is prevention of partially unfolded or aggregated proteins through their chaperone-like activity under normal and stressful conditions by way of hydrophobic interactions [56, 112]. sHsp are not true chaperones but exhibit chaperone-like activity by binding to the partially unfolded proteins, forms intermediate complex, then the complex is transferred to Hsp70 and Hsp90 for appropriate protein refolding. Therefore, sHsp are called holders since they hold the partially unfolded proteins and their activity is referred to as holdase activity. In addition to holdase activity, sHsp are also involved in diverse physiological functions of the cell. It has been shown that sHsp protect the cells from multiple stressful events such as heat stress, $H_2O_2$ induced-oxidative stress, osmotic stress, and UV-light induced stress. sHsp promote cell survival and prevent the cells from programmed cell death through mitochondria-mediated, and extrinsic apoptotic pathways. Further, sHsp preserve the cytoskeletal architecture, and prevent the platelet aggregation, Interestingly, sHsp act as anti-inflammatory agents and inhibit the proinflammatory response. In addition, sHsp involve in fertilization and development [139, 162], angiogenesis and vascular remodeling, cell differentiation [106, 132], cell growth, cell development, cell movement and transport of proteins across membrane. Furthermore, sHsp assist protein degradation pathway by regulating the ubiquitin-proteasome system [34, 51], and autophagy [21]. Another interesting property of sHsp is their ability to bind the metal ions such as $Cu^{2+}$ thereby provide cytoprotection by binding to $Cu^{2+}$ and inhibiting the $Cu^{2+}$-ascorbate induced reactive oxygen species (ROS) [1]. sHsp interact with a wide variety of client proteins which results in pleiotropic functions [4]. Mutations in sHsp lead to several pathological conditions including cataracts [88], neuropathies [41], cardiomyopathies [125], and myopathies [41, 152]. sHsp undergo post-translational modifications such as glycation [79] and phosphorylation [115] under stress conditions. For instance, αAC and αBC undergo non-enzymatic glycation and forms advanced glycation end products (AGEs) under hyperglycemia while Hsp27, αAC, αBC and Hsp20 undergo phosphorylation in many pathological conditions [115, 116]. It has been shown that Hsp27 is phosphorylated on three serine residues S15, S78, and S82 in humans while S15 and S86 in rodents [75, 82] mediated by Mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MAPKAPK-2) [82]. Several previous studies reported the secretion of sHsp into the blood and cerebrospinal fluid suggesting their extracellular functions [117]. Later, recombinant proteins of full length sHsp are found to show protective and therapeutic functions upon treatment in cell culture and experimental animal models [19, 96, 111, 120]. Intriguingly, 19–20 amino acid sequences from sHsp (residues 73–92 of sHsp) found to show chaperone activity *in vitro* [15, 128]. These sequences referred to as mini peptides or chaperone peptides or mini chaperone peptides. Subsequently, the

administration of mini chaperone peptides into disease animal models ameliorated the disease symptoms suggesting the therapeutic functions of sHsp-derived mini chaperone peptides [80, 98]. Adding to this, intracellular and extracellular sHsp found to be elevated in acute and chronic inflammatory diseases including neuroinflammatory diseases, fibrosis, intestinal bowel disease, cancers, and diabetic complications. The following properties of the sHsp can be ascribed to their implication in inflammatory diseases (i). the sHsp are elevated in tissues, blood, cerebrospinal fluid, and at sites of inflammation in inflammatory diseases (ii). The secreted sHsp acts as auto-antigens to elicit the auto-antibody response (iii). sHsp are involved in signaling by binding to the receptors of immune and endothelial cells (iv). amelioration of inflammatory response upon administration of full-length sHsp and sHsp-derived mini chaperone peptides into experimental disease animal models and in humans. Several previous studies including research articles, review articles, and book chapters extensively discussed the structure, mechanisms of action [49, 65, 95], functions [95] of sHsp and their implication in many pathological conditions including neurodegenerative diseases [76], ageing [26], cardiovascular diseases [57], and cancers [22, 23, 169]. In addition, role of large Hsp in inflammatory diseases such as neuroinflammation [36, 90], auto-immune diseases [70, 109, 144], cancers [27, 160], fibrotic diseases [11] have been reported. In this context, this book chapter deals with the expression, function, and therapeutic effects of sHsp in inflammatory diseases.

## *1.1 Hsp27*

Hsp27 is the most abundant and extensively studied protein in sHsp family. Several studies reported the secretion of Hsp27 into the blood and cerebrospinal fluid under pathological conditions. Increased intracellular Hsp27 and its secretion into plasma and interstitial fluid (tumor microenvironment) has been reported in breast cancer patients [9]. It is found that secreted Hsp27 mediates the immune escape and tumor progression. Hsp27 induces the differentiation of monocytes to macrophages by increasing the levels of immunosuppressive and proangiogenic factors such as interleukin-6 (IL-6), IL-10, tumor necrosis factor α (TNF-α), IL1β, IL-8, MCP1, VEGF-A, and prostaglandin E2. It has been shown that Hsp27 differentiated macrophages induce tolerance in T-cells and also make macrophages more proangiogenic suggesting the role for Hsp27 in tumor progression [9]. Furthermore, Hsp27 is elevated in many cancers including hepatocellular carcinoma [38], pancreatic cancer [99], colon cancer [143], prostate cancer [154], and ovarian cancer [136]. It has been shown that Hsp27 induces IL-6 mediated epithelial to mesenchymal transition (EMT) in prostate cancer cells through STAT3/Twist signaling *in vitro* and *in vivo* [131]. These studies indicate that Hsp27 has a deleterious role in cancer and promotes tumor progression. Targeting the secretion of Hsp27 paves a way for therapeutic approach in developing the strategies in cancer. In addition to cancer, Hsp27 is also implicated in progression of fibrotic diseases. Fibrosis is an

abnormal wound healing process where injury to tissue epithelial cells results in recruitment of immune cells such as macrophages and lymphocytes. These macrophages secrete multiple pro-inflammatory mediators such as TGF-β1 that promote the release of pro-fibrotic mediators. The recruitment and differentiation of myofibroblasts/fibroblasts by pro-fibrotic mediators cause the injury to heal and myofibroblasts undergo apoptosis. Nevertheless, chronic injury to tissue causes constant activation of myofibroblasts and inhibition of apoptosis that resulting in excessive deposition of extracellular matrix proteins like collagen. The increased expression of Hsp27 is found in cells around the fibrotic foci in pulmonary fibrosis patients [74]. It has been shown that the Hsp27 is upregulated in kidney fibrosis and found protective against fibrosis [153]. The same group found the decreased levels of α-SMA, Collagen-III, and phospho-p38MAPK in Hsp27 overexpressed mice after induction of kidney fibrosis. Adding to this, another group reported the overexpression of Hsp27 in fibroblastic foci in pulmonary and pleural fibrosis. In their study, Hsp27 stabilized the important transcription factor snail and prevented its degradation, thereby inducing the fibrosis through activation of EMT [157]. Intriguingly, Hsp27 antisense oligonucleotide (OGX-427) used in cancer therapy rescued the rats from TGFβ1 induced pleural fibrosis. Further, the repression of Hsp27 using OGX-427 stalled the migration of pleural myofibroblasts [157]. So, it is imperative to study the tissue-specific effects of Hsp27 in the fibrosis due to its contrasting effects. For instance, inhibition of Hsp27 protects the mice from pulmonary fibrosis while overexpression of Hsp27 protects from kidney fibrosis. Furthermore, Hsp27 and Noxa complex reduces the allergen-induced inflammation by delaying the degradation of ubiquitinylated Iκ-B in airway epithelial cells [167]. Elevated levels of plasma Hsp27 has been reported in coal workers [155], young smokers [63], and chronic obstructive pulmonary disease (COPD) patients [46] revealing the potential of Hsp27 to develop as a biomarker for COPD. Recently, several studies reported the implication of Hsp27 in chronic inflammatory diseases such as diabetes, obesity and their complications. It has been reported that intracellular and extracellular Hsp27 is elevated in Streptozotocin (STZ)-induced diabetes in experimental animal models and in human patients [43, 89, 113, 117], and insulin resistance [20, 129]. In one study Hsp27 mRNA levels were increased while protein levels decreased in retina of STZ-induced diabetic rats [113]. Losicweiz et al. also reported the decreased levels of Hsp25 protein in the retina of STZ-induced diabetic mice [89]. Hyperglycemia promotes the insolubilization of Hsp27 by inducing the aggregation of Hsp27 in retina [113]. Intriguingly, pro-inflammatory cytokines decreased the expression of Hsp27 in human retinal endothelial cells (HREC) while hyperglycemia promoted the cytokine-induced downregulation of Hsp27 and induced the apoptosis of HREC by activating indoleamine 2,3-dioxygenase (IDO), ROS, inducible-nitric oxide synthase-2 (iNOS) and subsequently nitric oxide and peroxynitrite [87]. Adding to this, hyperglycemia promotes the inflammation by activating the pro-inflammatory mediators in the retina. All these studies indicate that inflammation trigger the development of diabetic retinopathy by decreasing the Hsp27 levels. Recent studies from our lab reported the elevated circulatory Hsp27 levels in diabetic nephropathy and found that Hsp27 can be

developed as a biomarker for diabetic nephropathy [62]. In addition to increasing in extracellular Hsp27, intracellular Hsp27 and its phosphorylated forms were also increased in kidney of diabetic rats and podocytes exposed to high glucose [10]. The increased levels of Hsp27 also reported in dorsal root ganglia of diabetic rats and in circulation from diabetic neuropathy patients and it has been proposed the development of Hsp27 as a biomarker for diabetic neuropathy [43]. Another study reported the elevated levels of Hsp27 antigen and its antibodies in serum of insulin-resistant subjects [20]. Of note, it is interesting to mention that Hsp27 provides cytoprotection against lipopolysaccharide (LPS)-induced inflammation. Hsp27 ameliorates the LPS-induced acute kidney injury in renal tubular epithelial cells by modulating NF-κB and JNK pathways, and programmed cell death [85]. In contrast, another study found that LPS pretreatment protects the renal ischemia/reperfusion injury via increasing the levels of Hsp27 by decreasing inflammation, oxidative stress, and apoptosis [50]. Similarly, overexpression of Hsp27 in rat liver aggravated the ischemia/reperfusion injury by decreasing T regulatory cells, antioxidant mediators, and increasing the pro-inflammatory mediators [163]. However, the differential effects of Hsp27 might be attributed to the duration of pathology (early and late stages) and tissue dependent manner in the context of ischemia/reperfusion injury. The majority of the studies demonstrated that anti-inflammatory effect of Hsp27 against LPS-induced inflammation is mediated through modulating the p38MAPK and NF-κB. In support to this, one study revealed that Hsp27 ameliorated the LPS-induced inflammation by regulating the NF-κB through the p38MAPK-MK2-pHsp27 signaling pathway via modulating the CREB binding protein (CBP) in THP-1 human monocyte derived macrophage cells [17]. It has been found that pHsp27 reduce ROS accumulation and inhibit the LPS-induced increase in CBP [17]. Intriguingly, one study has found that overexpression of Hsp27 decreased the pro-inflammatory mediators including IL-1β, IL-6, TNF-α and repress the activation of toll-like receptor-4 (TLR4) after LPS stimulation in THP-1 macrophages. Besides, overexpression of Hsp27 promoted the TLR4 endocytosis, ubiquitination and degradation through phosphorylation of Hsp27 [86]. The LPS stimulation phosphorylated the Hsp27 and pHsp27 can only be able to interact with TLR4 and suppress the activity of TLR4 through ubiquitination. These studies reveal that pHsp27 is a critical regulator of NF-κB via modulation of TLR4, p38MAPK, MK2 in LPS-induced inflammation. Interestingly, Hsp27 is a major player in differential regulation of IL-1β secretion by modulating ARE-mediated mRNA decay, which is a major component in chronic inflammatory diseases [47]. Sur et al. found that knockdown of Hsp27 in keratinocytes results in increased production of prostaglandin E2 (PGE2) and pro-inflammatory cytokine IL-8, increased NF-κB activity and degradation of IκBα after stimulation with TNF-α indicating the beneficial effects of Hsp27 in regulating inflammatory responses in the skin [137]. Mouse embryonic fibroblasts from Hsp27 deficient mice show increased levels of proinflammatory cytokine IL-6, reduced proliferation, increased expression of p27 and p21, impaired wound healing, and increased chemokine (C-X-C motif) ligand 1 driven infiltration of neutrophils to the site of wound indicating that Hsp27 is instrumental for wound healing [31]. Interestingly, Hsp27 is involved in dextran

sodium sulfate (DSS)-induced inflammation in inflammatory bowel disease (IBD). Hsp27 is one of the players along with the ROS, IKKβ in mediation of DSS-induced activation of NF-κB, IκB, and IL-8 [16]. Hsp27 mediates the migration of colonic myofibroblasts through p38MAPK along with Cyclooxygenase in TNFα treated human colonic MFB cell line 18Co [123]. These studies confirm that Hsp27 is a key player in mediation of inflammation in a group of IBD. The role of Hsp27 in cardiovascular diseases is established after it was found in the serum samples of several cardiovascular diseases including atherosclerosis [91], reperfusion after ischemic clamping during heart bypass surgery [66], and acute coronary syndrome [102]. The extracellular Hsp27 plays a significant role in immune signaling by interacting with cell surface receptors including TLRs, scavenger receptor-A (SR-A) and regulate the cytokine production, cell proliferation, and cell migration. The treatment of THP-1 macrophages with recombinant Hsp27 resulted in nuclear translocation of NF-κB, increased activity of NF-κB, and increased degradation of IκB. In addition, treatment of THP-1 macrophages with recombinant Hsp27 increased the expression of both pro-inflammatory and anti-inflammatory mediators including TNF-α, IL-1β, IL-10, and GM-CSF [124] suggesting that Hsp27 is a critical regulator in maintaining the balance between pro-inflammatory and anti-inflammatory agents. Another interesting study demonstrated the elevated levels of Hsp27 in human and mouse coronary sinus blood after global ischemia and that extracellular Hsp27 increases the cardiac inflammatory response and injury by increasing the NF-κB activity and IL-6 levels after ischemia/reperfusion in mouse hearts [66]. Furthermore, treatment of human coronary vascular endothelial cells with recombinant Hsp27 increased the levels of ICAM-1 in cells and IL-6, IL-8, TNF-α and MCP-1 in the culture medium. However, the receptors and signaling pathways involved in the stimulation of inflammation by Hsp27 remains elusive. In continuation, the researchers found that TLR2 and TLR4 are the major receptors that intervene the pro-inflammatory effect by extracellular Hsp27 via activation of NF-κB [66]. In contrast to this, extracellular Hsp27 activated the NF-κB followed by VEGF-mediated migration and angiogenesis by interacting with TLR3 receptor [142]. Hsp27 along with TLR3 internalized to the endosomal compartment in short span to activate the NF-κB. In another study, treatment of dendritic cells with Hsp27 increased the secretion of wide range of cytokines including TNF-α, IL-6, IL-1β, IL-12p40 and, IL-12p70 but not IL-23p19 and mediates the DNFB contact hypersensitivity [166]. In support to the TLR4 mediated inflammation by Hsp27, bone marrow-derived dendritic cells (BMDC) from TLR4 deficient C3H/HeJ mice did not augment the cytokine production when compared to BMDC from C3H/HeN mice, having normal TLR4 signaling. Further, levels of IL-6, IL-1β, and TNF-α are lower in BMDC from TLR4 deficient mice compared to BMDC from C3H/HeN mice upon treatment with Hsp27 confirming the major role of TLR4 mediated inflammation by Hsp27 [166]. One study found that Hsp27 shows a protective effect in atherosclerosis by competitively inhibiting the acetylated LDL binding to SR-A [110]. The treatment of human U937 macrophages with estrogen and acetylated LDL stimulates the secretion of Hsp27 into culture medium and thereby secreted Hsp27 binds to the SR-A and inhibits the lipid uptake. The addition of Hsp27 to macrophage culture

media decreased the acetylated LDL-induced proinflammatory cytokine IL-1β and increased the secretion of anti-inflammatory cytokine IL-10 [110]. Overexpression of Hsp27 protects the mice from atherosclerosis by reducing the atherosclerotic lesion area [32]. It has been shown that Hsp27 is released from THP-1 macrophages into the culture medium via extracellular vesicles and into plasma via exosomes [130]. Furthermore, Hsp27 has been detected in exosomal membrane by transmission electron microscope. It has been shown that Hsp27 laden exosomes exert anti-inflammatory activity by activating NF-κB and generate the IL-10 in THP-1 and HEK-293 cells [130]. Taken together, all the reported data shows that Hsp27 has differential effects. For instance, it shows protective effect against inflammation, and diabetes while the deleterious effect in fibrosis, cancer, and inflammation. For instance, few studies reported the anti-inflammatory effects while others pro-inflammatory effects. The differential effects might be due to many factors including duration of the disease, type of cell line/animal model, and treatment with exogenous/endogenous Hsp27. These effects should be carefully monitored in clinical trials from the patient point of view.

## 2 Alpha-Crystallin (αAC and αBC)

The expression of αAC is abundantly found in lens and retina and absent in other tissues. It occurs in 3:1 M ratio with αBC in lens. It is involved in maintenance of transparency of the lens and prevents the aggregation of substrate proteins including β and γ crystallins. Mutations in *cryAA* gene results in development of several types of cataracts in animals and humans. It has been shown that αAC is phosphorylated on S122, and S177 positions. The role of αAC in inflammatory diseases is least studied compared to Hsp27 and αBC. αAC has been implicated in inflammatory diseases including diabetic retinopathy [68, 72, 89, 113], sympathetic ophthalmia [69] and experimental autoimmune uveitis (EAU) [107, 108, 126]. Studies from our laboratory as well as others found several folds upregulation of αAC in STZ-induced diabetic animal models and corroborated with the findings from human diabetic patients both at mRNA and protein level [68, 89, 113]. Hyperglycemia impairs the chaperone activity of αAC by increasing the insolubilization and impeding the interaction with apoptotic mediators. It has also been found that expression of αAC is upregulated in the retina in early EAU and protects the photoreceptor cells from mitochondria dependent oxidative stress-induced apoptosis [107] in association with TLR4 [126]. It has been shown that upregulation of αAC is not found in TLR4$^{-/-}$, iNOS$^{-/-}$, TNF-α$^{-/-}$, MyD88$^{-/-}$ mice with EAU demonstrating that TLR4, iNOS, TNF-α, and MyD88 essential for upregulation of αAC. Transfection of recombinant adenovirus expressing αAC reduced the pericyte apoptosis, and vascular leakage in experimental diabetic retinopathy mice revealing the protective effects of αAC [72]. αBC is an abundant protein expressed in lens, retina, heart, skeletal muscle, brain, kidney, and liver. It occurs in one portion with three portions of αAC (3:1) in lens. It is susceptible to extensive post-translational modifications

(glycation, and phosphorylation) in pathological conditions including diabetes. It has been shown that αBC is phosphorylated on serine residues S19, S45 and S59. MAPKAPK-2 is known to phosphorylate S59 while p44/42MAP is known to phosphorylate S45. However, the kinase responsible for phosphorylation of S19 still remains elusive. The role of αBC is implicated in several inflammatory diseases including brain stroke, EAE, pulmonary fibrosis, COPD, cancer, diabetes and associated complications, obesity, optic neuropathy, spinal cord injury, experimental pneumococcal meningitis, cardiac ischemia/reperfusion and IBD. The elevated levels of either intracellular or extracellular αBC has been found in inflammatory diseases and shown both beneficial and deleterious effects. It has been found that αBC levels in the blood are increased in renal cell carcinoma [55], obesity [84], multiple sclerosis [25, 120, 146], and brain stroke [3] while its tissue levels are elevated in diabetes and its complications [113, 114, 116], pulmonary fibrosis [11–13], EAE [120], autoimmune optic neuritis [135], and obesity [84]. Auto-antibodies for αBC has been found in multiple sclerosis [148], Chronic obstructive pulmonary disease (COPD) [30], Guillain-Barré syndrome [25, 52], and neuro-Bechet's disease [25]. In the last two decades, remarkable work has been done on the functional role of αBC in multiple sclerosis, which is an autoimmune demyelinating disease. It has been shown that the αBC is increased in blood [120], cerebrospinal fluid (CSF) [134], white matter of brain, astrocytes in spinal cord lesions [42], exosomes secreted from astrocytes [44] in MS patients, EAE mice and/or in vitro cell lines. Tremendous efforts have been made to understand the role of αBC in MS pathogenesis. It has been proposed that αBC serves as a candidate autoantigen to human T cells since it's expression is increased in the active lesions in MS patients [146, 148] and its presentation to T cells is an early event after demyelination [6]. In lieu, proinflammatory $CD4^+$ T-cells against αBC from human peripheral blood are reported in MS patients [122, 147]. Later, it has been proposed that EAE triggered by αBC specific T-cells depend on post viral infection in brain. However, the conception of αBC binding to antibody as an antigen remains puzzled due to other studies. Rothbard et al. proposed that αBC binds to the antibodies as a molecular chaperone with high affinity at multiple sites instead of being identified as an antigen [119, 120]. It has been shown that ablation of αBC exacerbates the EAE with increased levels of Th1 cytokines (IFNγ, TNF-α, IL-2, IL-12p40), Th17 cytokines (IL-17), severe central nervous system (CNS) inflammation compared to wild type mice [100]. In support to this, intravenous administration of recombinant αBC ameliorated the clinical disease scores, reduced the proliferation and decreased the secretion of IFNγ, TNF-α, IL-2, IL-12p40, and IL-17 in EAE mice model. Adding to this, increased levels of plasma αBC have been reported to be elevated after stroke in human patients and after experimental stroke in mice. It has been shown that intraperitoneal administration of αBC 12 h after experimental stroke reduced the lesion size, proinflammatory cytokines and increased the anti-inflammatory cytokines [3]. In another study, daily intravitreous or intravenous administration of αBC reduced astrocyte and microglial activation in anterior ischemic optic neuropathy mice model [101]. Interestingly, short term treatment for 3 days did not improve visually evoked potential as compared to that of the long-term treatment (every other

day for 3 days) and also improved the survival of oligodendrocytes [101]. Intraperitoneal injection of 50 μg of αBC every other day for 40 days improved the left ventricular ejection fraction in myocardial ischemia-reperfusion mice model revealing the therapeutic effect of αBC in improving the cardiac function after ischemia-reperfusion [151]. αBC is able to bind and precipitate approximately 70 proteins from plasma of rheumatoid arthritis, amyloidosis, MS, and mice experimental allergic encephalomyelitis. The binding of these proteins is temperature-dependent and found to be members of acute-phase proteins/coagulation/complement cascades [80]. In another study, intravenous administration of αBC ameliorated the spinal cord injury by reducing recruitment of inflammatory macrophages, decreasing the secondary tissue damage, and increasing the locomotor skills in mice [73]. The injected αBC is able to enter the site of inflammation in spinal cord injury and showed anti-inflammatory activity. It has been reported that the intravenously injected αBC entered into the white matter at the site of inflammation and into the grey and white matter in the surrounding areas and it has been taken up by the oligodendrocytes (30.7%), neurons (45%), astrocytes (38.8%) but did not enter into granulocytes and microglial cells [73]. However, the underlying mechanism by which αBC induces the anti-inflammatory activity remain elusive despite few studies reported the receptors, co-receptors, and players of signaling pathways. Previous studies described that most of the large Hsp induce innate immune response via TLR-mediated signaling. In lieu, Van noort and co-workers have shown that TLR1/2 receptors along with CD14 co-receptor are minimally required for αBC-mediated cellular activation [149]. However, only macrophages and macrophage like cells express these receptors. In addition to TLR1/2 and CD14 receptors, the macrophage scavenger receptor A (MCRA), and the macrophage scavenger receptor with collagenous structure (MARCO) are also important for αBC-mediated cellular activation [149]. Another study has shown that astrocytic dopamine D2 receptors (DRD2) suppress the neuroinflammation through αBC in astrocytes [127]. The ablation of DRD2 augmented the inflammation response in several regions of CNS, decreased the αBC, increased the susceptibility of dopaminergic neurons to MPTP (inducer of Parkinson disease) neurotoxin and activated the astrocytes. For the first time, Masilmoni et al. have shown the protective effects of α-crystallin in silver nitrate-induced acute inflammation in mice [92]. In addition to this, it has been shown that αBC acts as anti-inflammatory agent by suppressing the activation of NF-κB, p38 MAPK and reduced the degradation of IκB. The intraperitoneal administration of α-crystallin (50 μg/100 g body weight) restored the antioxidant defense machinery including glutathione, glutathione peroxidase, superoxide dismutase, and catalase, suppressed the rise in lipid peroxidation in silver nitrate-induced inflammation in mice [92]. In addition to restoring the antioxidant defense machinery, studies from the same lab found that α-crystallin administration provides neuroprotection by reducing the inflammation-induced cytokines such as TNF-α, and IL-1α, release of nitric oxide, neurotransmitters dopamine, norepinephrine, and 5-hydroxytryptamine in inflammation-induced mice [93]. Adding to this, same group further found α-crystallin pretreatment reduced the increase in acetylcholine esterase activity, decreased the surge in expression of NF-κB, GFAP (marker for reactive astrogliosis)

in inflammation-induced primary astrocytes and experimental animals [93]. It has been shown that administration of αBC ameliorated the optic nerve crush injury by inhibiting the retinal microglia, decreasing the expression of inducible nitric oxide synthase, and TNF-α [159]. As earlier discussed, phosphorylation of αBC plays pivotal role in development of inflammatory diseases including autoimmune demyelination. It is interesting to mention the activation of ERK, p38MAPK signaling molecules, phosphorylation of αBC on ser59 is increased in astrocytes of cuprizone-induced lesions, and active regions of demyelinating MS tissue [78]. The phosphorylation of αBC enables the reactive astrocytes in demyelinating disease and also exerts differential binding of proteins and subsequently regulates the intracellular signaling. Of note, αBC is also instrumental in infection-induced inflammation. In support to this, αBC ameliorated the inner ear damage and improved the hearing capacity in experimental pneumococcal meningitis in infant rat model where increased infiltration of neutrophil and leukocyte in perilymphatic spaces of inner ear, increased secretion of proinflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) in CSF are features of the infection [37]. Intracisternal injection of αBC diminished the proinflammatory cytokines and improved the hearing capacity. A series of studies reported the secretion of αBC via exosomes rather than classical secretory pathway [14, 39]. It has been shown that secretion of αBC via exosomes plays vital role in suppression of neuroinflammation. For instance, αBC levels are increased in exosomes secreted from astrocytes after treatment with LPS. Moreover, treatment of αBC diminished the expression of proinflammatory cytokines in U251 cells after stimulation with LPS revealing that αBC may act in an autocrine manner [44]. Further, it has been shown that αBC may act in a paracrine manner in suppressing the inflammation in microglial cells since they did not express αBC. It has been shown that αBC acts as a pro-fibrotic factor in fibrotic diseases and shows deleterious effects [11–13]. It is upregulated in fibrotic foci, hyperplastic alveolar epithelial cells in lungs from pulmonary fibrotic patients, and in fibrosis-induced mouse models [13], after hepatic stellate cell activation in liver fibrosis [83, 145]. It has been shown that αBC forms complex with 14–3-3ζ protein thereby protects it from proteasomal degradation. This complex activates a series of signaling molecules and transcription factors including ERK, and Snail (inducer of EMT) in hepatic carcinoma [58]. It has been shown that αBC favors fibrosis by promoting TGFβ pathway via enhancing the nuclear localization of Smad proteins. The ablation of αBC reduced the TGFβ activation by abrogating the localization of Smad4, thereby triggered the fibrosis [12, 13]. Similarly, αBC regulates choroidal neovascularization-induced sub-retinal fibrosis by modulating EMT through TGFβ and Smad4 [59]. Our previous work suggested an important role for αBC in STZ-induced diabetes and its complications. We have shown the increased expression of αBC in retina [113], heart [114], skeletal muscle [116], and lens [115] of STZ-induced diabetic rats. Multiple other studies have also shown the increased expression of αBC in experimental diabetic animal models [53, 89] and in diabetic patients [35]. Hyperglycemia induces the aggregation of αBC by enhancing the insolubilization. Moreover, we have reported that hyperglycemia increases cell death by disrupting the interaction between apoptotic mediator bax. Furthermore, it has

been shown that αBC levels are increased in serum, adipose tissue of obese patients and in primary human adipocytes [84]. Of note, αBC shows the deleterious effect in cancer by enhancing the survival of the cells. It has been shown that αBC is overexpressed in several cancers including retinoblastomas [105], neuroectodermal tumors [60], prostate cancer [138], renal cell carcinoma [54, 55], breast cancer [133], and brain tumors [2]. αBC plays an important role in IBD by suppressing the mucosal inflammation and maintaining the barrier integrity. It has been shown that αBC expression is decreased in the inflamed colon of IBD patients and negatively correlated with the inflammation. The overexpression of αBC in HT29 and Caco-2 cells diminished the proinflammatory cytokines TNF-α, IL-6, IL-1β, and IL-8 in response to TNF-α-induced inflammation. Adding to this, treatment of recombinant αBC fused to cell penetration peptide TAT to HT29, Caco-2 cells (treated with TNF-α) and DSS-induced colitis mice model decreased the inflammatory response by decreasing the TNF-α, IL-6, IL-1β, and IL-8 via inhibiting the IKKβ activity [161, 168]. Furthermore, it has been shown that increased expression of αBC promotes the migration and metastasis of gastric cancer via NF-κB-induced EMT [28]. Very recently, it has been shown that tumor-associated macrophages (M2 macrophages) promote non-small cell lung carcinoma through EMT by increasing the expression of αBC mediated by ERK1/2-slug pathway [45]. The findings on the role of αBC in cancer suggest that it contributes to the progression of cancer cells by promoting EMT. These steps open avenues for developing therapeutic strategies (Tables 2 and 3).

## *2.1 Hsp20*

The role of Hsp20 is implicated in inflammatory diseases including diabetes [113, 114], cancer [97] and asthma [8]. We and others reported the decreased expression of Hsp20 in diabetic retina [113], and heart [114, 156]. It has been shown that lower levels of Hsp20 in exosomes secreted into the circulation in STZ-induced diabetes. Intriguingly, mice overexpressing Hsp20 exhibited increased secretion of exosomes containing Hsp20, SOD1, pAKT, survivin and alleviated hyperglycemia-induced cardiac dysfunction, fibrosis, cell death revealing that exosomal secretion of Hsp20 offers protection from diabetes [156]. Similarly, increased generation of exosomes in Hsp20 overexpressing mice attenuated the myocardial infarction by activating Akt, reducing proinflammatory cytokines such as TNF-α, IL-1β [164]. Interestingly, abrogation of Hsp20 improved glucose metabolism including insulin sensitivity despite of gain in body weight and fat mass. Intriguingly, ablation of Hsp20 ameliorated the meta-inflammation (chemokines and proinflammatory cytokines), insulin resistance, and liver steatosis associated with increased obesity in mice [104]. It has been shown that Hsp20 plays an instrumental role in cancer including hepatocellular carcinoma (HCC). The overexpression of Hsp20 in HCC-derived HuH7 cells suppressed the TNF-α-induced NF-κB activation

**Table 2** Expression and secretion of small heat shock proteins in inflammatory diseases

| sHsp | Model | Secretion/Expression | Tissue/blood | Disease | References |
|---|---|---|---|---|---|
| Hsp27 | Breast cancer patients, monocytes, T cells | Increased expression and secretion | Tumor cells and serum | Breast cancer pulmonary fibrosis, pleural/sub pleural fibrosis | [9] |
| | Human Idiopathic pulmonary fibrosis (IPF) patients, intraplueral injection of adenovirus expressing TGF-β1 (AdTGF-β1) to mice, | Increased | Lung | Pulmonary fibrosis | [18, 74, 157] |
| | Unilateral ureteral obstruction (UUO) model of kidney fibrosis in rats | Increased | Kidney | Kidney tubulointerstitial fibrosis. | [153] |
| | Chronic Obstructive Pulmonary Disease (COPD) patients | Elevated | Serum | COPD | [46] |
| | Young smokers | Elevated | Serum | Marker for early signs of COPD | [63] |
| | Diabetic neuropathy patients | Increased | Serum | Diabetic neuropathy | [43] |
| | Diabetic nephropathy patients | Increased | Plasma | Diabetic nephropathy | [62] |
| | Streptozotocin (STZ)-induced diabetic rat | mRNA increased while protein levels decreased | Retina | Diabetic retinopathy | [113] |
| | Humans with glucose intolerance | Hsp27 and its antibodies were elevated in plasma | Serum | Insulin resistance | [20] |
| | Myasthenia gravis patients | Increased | Serum | Myasthenia gravis | [64] |
| | Chronic pancreatitis patients | Increased | Serum | Chronic pancreatitis | [87] |
| | Multiple sclerosis (MS) patients | Increased | Serum | Relapsing and remitting MS | [24] |
| | Human and mice | Increased | Plasma | Global ischemia | [66] |
| | Acute coronary syndrome patients | Increased | Atherosclerotic plaque and plasma | Atherosclerosis | [102] |
| | Rat | Increased | Spinal cord | Experimental autoimmune encephalomyelitis | [71] |

| | | | | | |
|---|---|---|---|---|---|
| αAC | STZ-induced diabetic rat and Ins2 (Akita) mice, diabetic patients | Increased | Retina | Diabetic retinopathy | [68, 89, 113] |
| | Mice with experimental autoimmune uvetis | Increased | Retina (photo-receptor cells) | Experimental autoim-mune uvetis | [107] |
| αBC | Human idiopathic pulmonary fibrosis (IPF) patients, Bleomycin-induced pulmonary fibrosis in mice, intrapleural injection of AdTGF-β1 to rat. | Increased | Fibroblastic lung tissue | Pulmonary fibrosis | [12, 13] |
| | Healthy non-COPD smokers, COPD smokers and inflammatory lung disease | Anti-αBC antibodies were elevated in COPD smokers and inflammatory lung disease compared to non-COPD smokers. | Serum | COPD and inflamma-tory lung disease. | [30] |
| | Laser-induced choroidal neovascularisation in mice | Increased | Retina | Subretinal fibrosis | [59] |
| | MS patients | Increased | Brain | MS | [5, 61] |
| | Guillain-Barré syndrome (GBS), neuro-Behçet's disease (NBD) patients | Anti-αBC antibodies were increased | Cerebrospinal fluid, and serum | GBS, and NBD | [25] |
| | MS patients | Increased | Cerebrospinal fluid | Multiple sclerosis | [134] |
| | Experimental stroke in mice and stroke in human patients | Increased | Plasma | Brain stroke | [3] |
| | STZ-induced diabetic rat | Increased | Retina | Diabetic retinopathy | [113] |
| | STZ-induced diabetic rat and mice | Increased | Heart | Diabetic cardiomyopathy | [114, 156] |
| | STZ-induced diabetic rat | Increased | Skeletal muscle | Diabetic myopathy | [116] |
| | Intestinal bowel disease (IBD) patients and DSS-induced colitis mice model | Decreased | Intestinal mucosa | IBD | [161] |
| | Obese patients and primary human adipocyte culture | Increased | Plasma, adipose tissue, Culture medium | Obesity | [84] |
| Hsp20 | STZ-induced diabetic rat and mice | Decreased | Heart | Diabetic cardiomyopathy | [114, 156] |
| Hsp22 | STZ-induced diabetic rat | Increased | Retina | Diabetic retinopathy | [113] |

**Table 3** Protective and therapeutic effects of sHsp in inflammatory diseases

| sHsp | Model | Administration route | Protective or therapeutic effect | Disease | References |
|---|---|---|---|---|---|
| Hsp27 | DNFB-induced contact hypersensitivity in mice and dendritic cell cultures treated with recombinant Hsp27 | Anti-Hsp27 antibodies topically applied to abdominal skin. | Inhibited the Hsp27 expression and proinflammatory mediators IL-1β, TNF-α, IL-6, IL-12p70 and IL-12p40. | Contact hypersensitivity | [166] |
| | Bleomycin-induced pulmonary fibrosis | Intranasal delivery of Hsp27 siRNA | Suppressed lung fibrosis | Pulmonary fibrosis | [103] |
| | LPS treated renal epithelial HK-2 cells | Overexpression of Hsp27 | Decreased the proinflammatory cytokines including IL-1β, IL-6, TNF-α, MCP1, NF-κB and JNK pathways, apoptosis | LPS-induced kidney injury | [85] |
| | LPS-treated THP1 cells | Overexpression of Hsp27 | Phosphorylated Hsp27 reduced the proinflammatory cytokines including IL-1β, IL-6, TNF-α, inhibited the activation of TLR4 signaling by promoting its endocytosis, ubiquitination and degradation | LPS-induced inflammation | [29] |
| αAC | Mouse | Adenoviral mediated transfection of αAC | Reduced the vascular leakage and pericyte apoptosis | Diabetic retinopathy | [72] |
| αBC | CRY $AB^{-/-}$ mice | Intraperitoneum | Showed neuroprotection by reducing the pro-inflammatory mediators and lesion volume | Stroke | [3] |
| | Spinal cord contusion injury in mice | Intravenous injection of 10 μg of recombinant αBC | Improved locomotor skills, modulated the immune response by increased infiltration of granulocytes and reduced inflammatory macrophages. | Spinal cord injury | [73] |
| | Experimental autoimmune encephalomyelitis (EAE) mice model of multiple sclerosis (MS) | Intravenous injection of 10 μg of recombinant αBC | Reduced the clinical symptoms by decreasing the proliferation and production of pro-inflammatory mediators. | MS | [100] |

| | | | | | |
|---|---|---|---|---|---|
| | EAE mice model of MS | Intraperitoneal injection of 10 μg of αBC mini-peptide | αBC derived mini-peptide showed therapeutic effect equivalent to full length αBC | MS | [120] |
| | Cigarette smoke-induced COPD in mice | Intratracheal administration of porous αBC- Polylactic-co-glycolic acid (PLGA) microparticles | Encapsulated αBC with PLGA particles 100-fold more effective in activating macrophages than free protein | COPD | [149] |
| | Silver nitrate-induced neuroinflammation in mouse and astrocyte culture | Intraperitoneum | Reduced the NO, LPO, calcium and suppressed the GFAP, NF-κB expression | Neuroinflammation | [92, 94] |
| | Mouse and microglia culture | Intravitreous | Alleviated the inflammatory mediators iNOS, and TNF-α | Optic neuropathy | [158] |
| | Infant rat model of pneumococcal meningitis | Intraperitoneal and intracisternal administration of 10 μg of recombinant αBC | Intracisternal administration of αBC prevented the ear damage by reducing the levels of TNF-α, IL-10 IL-6, and IFN-γ. | Pneumococcal meningitis | [37] |
| | Patients with remitting-relapsing MS | Intravenous administration of 10, 12.5, 17.5 mg of αBC protein for 48 weeks | The volume and number of active lesions in MS reduced to 76% | Remitting-relapsing MS | [150] |

and IKKα revealing that increased expression of Hsp20 in cancer cells may suppress the progression of HCC [97].

## 2.2 Hsp22

The implication of Hsp22 in inflammatory diseases is least studied. We have shown the increased expression of Hsp22 in the retina of STZ-induced diabetic rats [113]. Yu et al. has shown the increased expression of Hsp22 in high fat diet and STZ-induced type-2 diabetes. Moreover, it has been shown that Hsp22 protects the vascular endothelium from high glucose-induced insult by attenuating the mitochondrial ROS generation [165]. Interestingly, it has been shown that Hsp22 is increased in synovial fluid from rheumatoid arthritis patients. Hsp22 is also able to activate monocyte-derived dendritic cells through TLR4 revealing that Hsp22 plays a pivotal role in the inflammatory process during rheumatoid arthritis [118].

## 2.3 Role of sHsp Derived Peptides in Inflammatory Diseases

Sharma and co-workers first identified the residues 73–92 in sHsp possessing chaperone activity *in vitro* and termed it as mini chaperone peptides/mini peptides/mini chaperones [15, 128]. Later, it has been shown that mini chaperone peptides are able to show protective and therapeutic effects equivalent to that of full length protein. Kurnellas and his coworkers have shown that residues 73–92 of sHsp are able to show therapeutic effect in EAE mice. Intraperitoneal administration of 1 μg of peptides of Hsp27, αAC, and αBC reduced the paralytic symptoms in EAE mice and also reduced the production of inflammatory cytokines IL-2, IL-6, and IL-17 in splenocytes [80]. Peptide treatment also reduced the inflammatory foci in meninges and parenchyma in the CNS of EAE mice. Peptides reduced the disease symptoms by suppressing the immune system and interestingly, cessation of administration of these peptides resulted in the recurrence of the disease revealing that peptides act as a biological inhibitor. The residues 73–92 in sHsp are important for amyloid formation and thus explain how a short peptide shows therapeutic activity equivalent to full length protein. In support to this, Tanaka and his colleagues reported that residues 73–92 from αAC show chaperone activity due to their ability to form amyloid fibrils [140]. Furthermore, it has been shown that 1 μg of amyloidogenic hexameric peptides of αBC (residues from 76 to 81, and 89 to 94) ameliorated the paralytic symptoms in EAE mice and reduced the levels of IL-6 [81]. In addition, it has been shown that the therapeutic activity of amyloidogenic peptides of sHsp correlated with their chaperone activity. They bind to α7 nicotinic acetyl choline receptor on B lymphocytes and peritoneal macrophages, changing the macrophages to an immune suppressive phenotype and promote the migration of macrophages and B lymphocytes from the peritoneum to secondary lymph organs [121]. Another interesting

study has shown that, phosphopeptide (YARAAARQARAWLRRAS($PO_3$) APLPGLK) mimetic of Hsp20 reduced the airway smooth muscle contraction via regulation of actin cytoskeleton downstream of β2-adrenergic receptor in humans and porcine [8].

## 3 Conclusions

sHsp are a group of proteins that share α-crystallin domain and respond to multiple external stimuli. They show chaperone-like function and prevent aggregation of denatured proteins. Intracellular and extracellular levels of sHsp are elevated in several inflammatory diseases including EAE, renal and cardiac ischemia, cancer, fibrosis, diabetes and its complications and so on. The elevated levels of sHsp diminish the inflammation by reducing proinflammatory cytokines and increasing the anti-inflammatory cytokines. However, the receptors involved in the interaction with sHsp, their binding partners and activation of downstream signaling cascade in mediating the anti-inflammatory action is not elucidated even though TLR1/2/4, α7 nicotinic acetylcholine receptor, MCRA, and MACRO have been proposed during the interplay. Therefore, in-depth studies are required to address the specific receptors involved in the amelioration of the inflammation. It has been shown that sHsp trigger innate immune response in alleviating the inflammation and gives the scope to elucidate if there is a trigger of adaptive response. It is also interesting to elucidate the differential response in the roles of intracellular and extracellular sHsp in reducing the inflammation. Another exciting area is to study the role of exosomes packed with sHsp in alleviation of inflammation. In addition, the secretion of sHsp laden exosomes in inflammatory diseases could be studied. The autocrine and paracrine roles of sHsp laden exosomes could be studied in inflammatory diseases. Furthermore, the therapeutic roles of exosomes laden with sHsp in the inflammatory diseases needs to be appropriately evaluated. In addition, liposomal mediated carriers could be used for the delivery of sHsp. For efficient delivery strategies, carrier molecule approach including microparticles or nanoparticles could be employed. For instance, poly (lactic-co-glycolic acid) (PLGA) nanoparticles have been efficiently employed for delivery of αBC in cigarette smoke-induced COPD mice. The intratracheal administration αBC laden PLGA microparticles were selectively taken up by alveolar macrophages and suppressed the infiltration of neutrophils and lymphocytes in the lungs. Additionally, αBC laden PLGA microparticles are 100-fold more efficient than the free soluble protein in activating macrophages without altering their immune regulatory behavior. Therefore, carrier molecules such as PLGA, polycaprolactone micro or nanoparticles, cell penetration peptides like TAT, gC peptides, fusion peptides, protein polymers could be employed for efficient delivery of sHsp. The therapeutic roles of sHsp have been successfully utilized in cell lines and experimental animal models, therefore, these studies can be extended to human clinical trials. For instance, Van noort et al. conducted a Phase IIa randomized clinical trial using 7.5, 12.5 or 17.5 mg of αBC in relapsing-remitting

MS for 48 weeks [150]. These doses were found to be safe and well tolerated in patients and repeated intravenous injections of low doses of αBC significantly reduced the lesion size indicating the therapeutic role of sHsp for MS patients. Similarly, other sHsp and their peptides could be employed in clinical trials by taking a large sample size and required duration in inflammatory disease patients.

**Acknowledgements** Apologies to all those whose work in the field has not been cited due to lack of space. VSR acknowledges the Science and Engineering Research Board-Early Career Research (SERB-ECR) grant (ECR2017/000277/LS), Department of Biotechnology (DBT; BT/PR27607/PFN/20/1338/2018), Government of India for the support. GBR acknowledges the financial assistance from the Department of Science and Technology, Government of India (Grant # EMR/2016/001984) and Department of Health Research, Government of India (Grant # V.25011/287-HRD/2016-HR). TJ acknowledges the OPERA award from BITS-Pilani Hyderabad.

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Ahmad MF, Singh D, Taiyab A, Ramakrishna T, Raman B, Rao Ch M (2008) Selective Cu2+ binding, redox silencing, and cytoprotective effects of the small heat shock proteins alphaA- and alphaB-crystallin. J Mol Biol 382:812–824
2. Aoyama A, Steiger RH, Frohli E, Schafer R, von Deimling A, Wiestler OD, Klemenz R (1993) Expression of alpha B-crystallin in human brain tumors. Int J Cancer/Journal International du Cancer 55:760–764
3. Arac A, Brownell SE, Rothbard JB, Chen C, Ko RM, Pereira MP, Albers GW, Steinman L, Steinberg GK (2011) Systemic augmentation of alphaB-crystallin provides therapeutic benefit twelve hours post-stroke onset via immune modulation. Proc Natl Acad Sci U S A 108:13287–13292
4. Arrigo AP (2013) Human small heat shock proteins: protein interactomes of homo- and hetero-oligomeric complexes: an update. FEBS Lett 587:1959–1969
5. Bajramovic JJ, Lassmann H, van Noort JM (1997) Expression of alphaB-crystallin in glia cells during lesional development in multiple sclerosis. J Neuroimmunol 78:143–151
6. Bajramovic JJ, Plomp AC, Goes A, Koevoets C, Newcombe J, Cuzner ML, van Noort JM (2000) Presentation of alpha B-crystallin to T cells in active multiple sclerosis lesions: an early event following inflammatory demyelination. J Immunol 164:4359–4366
7. Bakthisaran R, Tangirala R, Rao Ch M (2015) Small heat shock proteins: role in cellular functions and pathology. Biochim Biophys Acta 1854:291–319
8. Banathy A, Cheung-Flynn J, Goleniewska K, Boyd KL, Newcomb DC, Peebles RS Jr, Komalavilas P (2016) Heat shock-related protein 20 peptide decreases human airway constriction downstream of beta2-adrenergic receptor. Am J Respir Cell Mol Biol 55:225–233
9. Banerjee S, Lin CF, Skinner KA, Schiffhauer LM, Peacock J, Hicks DG, Redmond EM, Morrow D, Huston A, Shayne M, Langstein HN, Miller-Graziano CL, Strickland J,

O'Donoghue L, De AK (2011) Heat shock protein 27 differentiates tolerogenic macrophages that may support human breast cancer progression. Cancer Res 71:318–327
10. Barutta F, Pinach S, Giunti S, Vittone F, Forbes JM, Chiarle R, Arnstein M, Perin PC, Camussi G, Cooper ME, Gruden G (2008) Heat shock protein expression in diabetic nephropathy. Am J Physiol Renal Physiol 295:F1817–F1824
11. Bellaye PS, Burgy O, Causse S, Garrido C, Bonniaud P (2014a) Heat shock proteins in fibrosis and wound healing: good or evil? Pharmacol Ther 143:119–132
12. Bellaye PS, Burgy O, Colas J, Fabre A, Marchal-Somme J, Crestani B, Kolb M, Camus P, Garrido C, Bonniaud P (2014b) Anti-fibrotic role of alphaB-crystallin inhibition in pleural and subpleural fibrosis. Am J Respir Cell Mol Biol
13. Bellaye PS, Wettstein G, Burgy O, Besnard V, Joannes A, Colas J, Causse S, Marchal-Somme J, Fabre A, Crestani B, Kolb M, Gauldie J, Camus P, Garrido C, Bonniaud P (2014c) The small heat-shock protein alphaB-crystallin is essential for the nuclear localization of Smad4: impact on pulmonary fibrosis. J Pathol 232:458–472
14. Bhat SP, Gangalum RK (2011) Secretion of alphaB-Crystallin via exosomes: new clues to the function of human retinal pigment epithelium. Commun Integr Biol 4:739–741
15. Bhattacharyya J, Padmanabha Udupa EG, Wang J, Sharma KK (2006) Mini-alphaB-crystallin: a functional element of alphaB-crystallin with chaperone-like activity. Biochemistry 45:3069–3076
16. Bhattacharyya S, Dudeja PK, Tobacman JK (2009) ROS, Hsp27, and IKKbeta mediate dextran sodium sulfate (DSS) activation of IkappaBa, NFkappaB, and IL-8. Inflamm Bowel Dis 15:673–683
17. Bi X, Jiang B, Zhou J, Luo L, Yin Z (2019) Phosphorylated Hsp27 prevents LPS-induced excessive inflammation in THP-1 cells via suppressing ROS-mediated upregulation of CBP. Cell Biol Int
18. Bonniaud P, Bellaye PS, Burgy O, Kolb M (2017) Heat shock protein: a hot topic in idiopathic pulmonary fibrosis. Eur Respir J 49
19. Brownell SE, Becker RA, Steinman L (2012) The protective and therapeutic function of small heat shock proteins in neurological diseases. Front Immunol 3:74
20. Burut DF, Borai A, Livingstone C, Ferns G (2010) Serum heat shock protein 27 antigen and antibody levels appear to be related to the macrovascular complications associated with insulin resistance: a pilot study. Cell Stress Chaperones 15:379–386
21. Carra S, Seguin SJ, Landry J (2008) HspB8 and Bag3: a new chaperone complex targeting misfolded proteins to macroautophagy. Autophagy 4:237–239
22. Carra S, Alberti S, Arrigo PA, Benesch JL, Benjamin IJ, Boelens W, Bartelt-Kirbach B, Brundel B, Buchner J, Bukau B, Carver JA, Ecroyd H, Emanuelsson C, Finet S, Golenhofen N, Goloubinoff P, Gusev N, Haslbeck M, Hightower LE, Kampinga HH, Klevit RE, Liberek K, McHaourab HS, McMenimen KA, Poletti A, Quinlan R, Strelkov SV, Toth ME, Vierling E, Tanguay RM (2017) The growing world of small heat shock proteins: from structure to functions. Cell Stress Chaperones 22:601–611
23. Carra S, Alberti S, Benesch JLP, Boelens W, Buchner J, Carver JA, Cecconi C, Ecroyd H, Gusev N, Hightower LE, Klevit RE, Lee HO, Liberek K, Lockwood B, Poletti A, Timmerman V, Toth ME, Vierling E, Wu T, Tanguay RM (2019) Small heat shock proteins: multifaceted proteins with important implications for life. Cell Stress Chaperones 24:295–308
24. Ce P, Erkizan O, Gedizlioglu M (2011) Elevated HSP27 levels during attacks in patients with multiple sclerosis. Acta Neurol Scand 124:317–320
25. Celet B, Akman-Demir G, Serdaroglu P, Yentur SP, Tasci B, van Noort JM, Eraksoy M, Saruhan-Direskeneli G (2000) Anti-alpha B-crystallin immunoreactivity in inflammatory nervous system diseases. J Neurol 247:935–939
26. Charmpilas N, Kyriakakis E, Tavernarakis N (2017) Small heat shock proteins in ageing and age-related diseases. Cell Stress Chaperones 22:481–492
27. Chatterjee S, Burns TF (2017) Targeting heat shock proteins in cancer: a promising therapeutic approach. Int J Mol Sci 18

28. Chen D, Cao G, Qiao C, Liu G, Zhou H, Liu Q (2018) Alpha B-crystallin promotes the invasion and metastasis of gastric cancer via NF-kappaB-induced epithelial-mesenchymal transition. J Cell Mol Med 22:3215–3222
29. Chen QQ, Haikal C, Li W, Li JY (2019) Gut inflammation in association with pathogenesis of Parkinson's disease. Front Mol Neurosci 12:218
30. Cherneva RV, Georgiev OB, Petrova DS, Trifonova NL, Stamenova M, Ivanova V, Vlasov VI (2012) The role of small heat-shock protein alphaB-crystalline (HspB5) in COPD pathogenesis. Int J Chron Obstruct Pulmon Dis 7:633–640
31. Crowe J, Aubareda A, McNamee K, Przybycien PM, Lu X, Williams RO, Bou-Gharios G, Saklatvala J, Dean JL (2013) Heat shock protein B1-deficient mice display impaired wound healing. PLoS One 8:e77383
32. Cuerrier CM, Chen YX, Tremblay D, Rayner K, McNulty M, Zhao X, Kennedy CR, de BelleRoche J, Pelling AE, O'Brien ER (2013) Chronic over-expression of heat shock protein 27 attenuates atherogenesis and enhances plaque remodeling: a combined histological and mechanical assessment of aortic lesions. PLoS One 8:e55867
33. de Jong WW, Leunissen JA, Voorter CE (1993) Evolution of the alpha-crystallin/small heat-shock protein family. Mol Biol Evol 10:103–126
34. den Engelsman J, Keijsers V, de Jong WW, Boelens WC (2003) The small heat-shock protein alpha B-crystallin promotes FBX4-dependent ubiquitination. J Biol Chem 278:4699–4704
35. Dong Z, Kase S, Ando R, Fukuhara J, Saito W, Kanda A, Murata M, Noda K, Ishida S (2012) Alphab-crystallin expression in epiretinal membrane of human proliferative diabetic retinopathy. Retina 32:1190–1196
36. Dukay B, Csoboz B, Toth ME (2019) Heat-shock proteins in neuroinflammation. Front Pharmacol 10:920
37. Erni ST, Fernandes G, Buri M, Perny M, Rutten RJ, van Noort JM, Senn P, Grandgirard D, Roccio M, Leib SL (2019) Anti-inflammatory and Oto-protective effect of the small heat shock protein alpha B-Crystallin (HspB5) in experimental pneumococcal meningitis. Front Neurol 10:570
38. Feng JT, Liu YK, Song HY, Dai Z, Qin LX, Almofti MR, Fang CY, Lu HJ, Yang PY, Tang ZY (2005) Heat-shock protein 27: a potential biomarker for hepatocellular carcinoma identified by serum proteome analysis. Proteomics 5:4581–4588
39. Gangalum RK, Bhat AM, Kohan SA, Bhat SP (2016) Inhibition of the expression of the small heat shock protein alphaB-Crystallin inhibits exosome secretion in human retinal pigment epithelial cells in culture. J Biol Chem 291:12930–12942
40. Garrido C, Paul C, Seigneuric R, Kampinga HH (2012) The small heat shock proteins family: the long forgotten chaperones. Int J Biochem Cell Biol 44:1588–1592
41. Ghaoui R, Palmio J, Brewer J, Lek M, Needham M, Evila A, Hackman P, Jonson PH, Penttila S, Vihola A, Huovinen S, Lindfors M, Davis RL, Waddell L, Kaur S, Yiannikas C, North K, Clarke N, MacArthur DG, Sue CM, Udd B (2016) Mutations in HSPB8 causing a new phenotype of distal myopathy and motor neuropathy. Neurology 86:391–398
42. Gorter RP, Nutma E, Jahrei MC, de Jonge JC, Quinlan RA, van der Valk P, van Noort JM, Baron W, Amor S (2018) Heat shock proteins are differentially expressed in brain and spinal cord: implications for multiple sclerosis. Clin Exp Immunol 194:137–152
43. Gruden G, Bruno G, Chaturvedi N, Burt D, Schalkwijk C, Pinach S, Stehouwer CD, Witte DR, Fuller JH, Perin PC (2008) Serum heat shock protein 27 and diabetes complications in the EURODIAB prospective complications study: a novel circulating marker for diabetic neuropathy. Diabetes 57:1966–1970
44. Guo YS, Liang PZ, Lu SZ, Chen R, Yin YQ, Zhou JW (2019a) Extracellular alphaB-crystallin modulates the inflammatory responses. Biochem Biophys Res Commun 508:282–288
45. Guo Z, Song J, Hao J, Zhao H, Du X, Li E, Kuang Y, Yang F, Wang W, Deng J, Wang Q (2019b) M2 macrophages promote NSCLC metastasis by upregulating CRYAB. Cell Death Dis 10:377

46. Hacker S, Lambers C, Hoetzenecker K, Pollreisz A, Aigner C, Lichtenauer M, Mangold A, Niederpold T, Zimmermann M, Taghavi S, Klepetko W, Ankersmit HJ (2009) Elevated HSP27, HSP70 and HSP90 alpha in chronic obstructive pulmonary disease: markers for immune activation and tissue destruction. Clin Lab 55:31–40
47. Hadadi E, Zhang B, Baidzajevas K, Yusof N, Puan KJ, Ong SM, Yeap WH, Rotzschke O, Kiss-Toth E, Wilson H, Wong SC (2016) Differential IL-1beta secretion by monocyte subsets is regulated by Hsp27 through modulating mRNA stability. Sci Rep 6:39035
48. Haslbeck M (2002) sHsp and their role in the chaperone network. Cell Mol Life Sci 59:1649–1657
49. Haslbeck M, Weinkauf S, Buchner J (2019) Small heat shock proteins: simplicity meets complexity. J Biol Chem 294:2121–2132
50. He K, Xia L, Zhang J (2018) LPS ameliorates renal ischemia/reperfusion injury via Hsp27 up-regulation. Int Urol Nephrol 50:571–580
51. Hedhli N, Wang L, Wang Q, Rashed E, Tian Y, Sui X, Madura K, Depre C (2008) Proteasome activation during cardiac hypertrophy by the chaperone H11 kinase/Hsp22. Cardiovasc Res 77:497–505
52. Hegen H, Wanschitz J, Ehling R, Deisenhammer F, Loscher WN, Reindl M, Berger T (2010) Anti-alpha B-crystallin immunoreactivity in Guillain-Barre syndrome and chronic inflammatory demyelinating polyneuropathy. J Peripheral Nervous Syst: JPNS 15:150–152
53. Heise EA, Marozas LM, Grafton SA, Green KM, Kirwin SJ, Fort PE (2013) Strain-independent increases of crystallin proteins in the retina of type 1 diabetic rats. PLoS One 8: e82520
54. Ho PY, Chueh SC, Chiou SH, Wang SM, Lin WC, Lee IL, Yang HY, Peng HC, Lai MK (2013) AlphaB-crystallin in clear cell renal cell carcinoma: tumor progression and prognostic significance. Urol Oncol 31:1367–1377
55. Holcakova J, Hernychova L, Bouchal P, Brozkova K, Zaloudik J, Valik D, Nenutil R, Vojtesek B (2008) Identification of alphaB-crystallin, a biomarker of renal cell carcinoma by SELDI-TOF MS. Int J Biol Markers 23:48–53
56. Horwitz J (1992) Alpha-crystallin can function as a molecular chaperone. Proc Natl Acad Sci U S A 89:10449–10453
57. Hu X, Van Marion DMS, Wiersma M, Zhang D, Brundel B (2017) The protective role of small heat shock proteins in cardiac diseases: key role in atrial fibrillation. Cell Stress Chaperones 22:665–674
58. Huang XY, Ke AW, Shi GM, Zhang X, Zhang C, Shi YH, Wang XY, Ding ZB, Xiao YS, Yan J, Qiu SJ, Fan J, Zhou J (2013) alphaB-crystallin complexes with 14-3-3zeta to induce epithelial-mesenchymal transition and resistance to sorafenib in hepatocellular carcinoma. Hepatology 57:2235–2247
59. Ishikawa K, Sreekumar PG, Spee C, Nazari H, Zhu D, Kannan R, Hinton DR (2016) alphaB-Crystallin regulates subretinal fibrosis by modulation of epithelial-mesenchymal transition. Am J Pathol 186:859–873
60. Iwaki T, Iwaki A, Miyazono M, Goldman JE (1991) Preferential expression of alpha B-crystallin in astrocytic elements of neuroectodermal tumors. Cancer 68:2230–2240
61. Iwaki T, Wisniewski T, Iwaki A, Corbin E, Tomokane N, Tateishi J, Goldman JE (1992) Accumulation of alpha B-crystallin in central nervous system glia and neurons in pathologic conditions. Am J Pathol 140:345–356
62. Jakhotia S, Sivaprasad M, Shalini T, Reddy PY, Viswanath K, Jakhotia K, Sahay R, Sahay M, Reddy GB (2018) Circulating levels of Hsp27 in microvascular complications of diabetes: prospects as a biomarker of diabetic nephropathy. J Diabetes Complicat 32:221–225
63. Jan Ankersmit H, Nickl S, Hoeltl E, Toepker M, Lambers C, Mitterbauer A, Kortuem B, Zimmermann M, Moser B, Bekos C, Steinlechner B, Hofbauer H, Klepetko W, Schenk P, Dome B (2012) Increased serum levels of HSP27 as a marker for incipient chronic obstructive pulmonary disease in young smokers. Respiration 83:391–399

64. Janik S, Schiefer AI, Bekos C, Hacker P, Haider T, Moser J, Klepetko W, Mullauer L, Ankersmit HJ, Moser B (2016) HSP27 and 70 expression in thymic epithelial tumors and benign thymic alterations: diagnostic, prognostic and physiologic implications. Sci Rep 6:24267
65. Janowska MK, Baughman HER, Woods CN, Klevit RE (2019) Mechanisms of small heat shock proteins. Cold Spring Harb Perspect Biol 11
66. Jin C, Cleveland JC, Ao L, Li J, Zeng Q, Fullerton DA, Meng X (2014) Human myocardium releases heat shock protein 27 (HSP27) after global ischemia: the proinflammatory effect of extracellular HSP27 through toll-like receptor (TLR)-2 and TLR4. Mol Med 20:280–289
67. Kappe G, Franck E, Verschuure P, Boelens WC, Leunissen JA, de Jong WW (2003) The human genome encodes 10 alpha-crystallin-related small heat shock proteins: HspB1-10. Cell Stress Chaperones 8:53–61
68. Kase S, Ishida S, Rao NA (2011) Increased expression of alphaA-crystallin in human diabetic eye. Int J Mol Med 28:505–511
69. Kase S, Meghpara BB, Ishida S, Rao NA (2012) Expression of alpha-crystallin in the retina of human sympathetic ophthalmia. Mol Med Rep 5:395–399
70. Keijzer C, Wieten L, van Herwijnen M, van der Zee R, Van Eden W, Broere F (2012) Heat shock proteins are therapeutic targets in autoimmune diseases and other chronic inflammatory conditions. Expert Opin Ther Targets 16:849–857
71. Kim H, Moon C, Ahn M, Byun J, Lee Y, Kim MD, Matsumoto Y, Koh CS, Shin T (2009) Heat shock protein 27 upregulation and phosphorylation in rat experimental autoimmune encephalomyelitis. Brain Res 1304:155–163
72. Kim YH, Park SY, Park J, Kim YS, Hwang EM, Park JY, Roh GS, Kim HJ, Kang SS, Cho GJ, Choi WS (2012) Reduction of experimental diabetic vascular leakage and pericyte apoptosis in mice by delivery of alphaA-crystallin with a recombinant adenovirus. Diabetologia 55:2835–2844
73. Klopstein A, Santos-Nogueira E, Francos-Quijorna I, Redensek A, David S, Navarro X, Lopez-Vales R (2012) Beneficial effects of alphaB-Crystallin in spinal cord contusion injury. J Neurosci Off J Soc Neurosci 32:14478–14488
74. Korfei M, Schmitt S, Ruppert C, Henneke I, Markart P, Loeh B, Mahavadi P, Wygrecka M, Klepetko W, Fink L, Bonniaud P, Preissner KT, Lochnit G, Schaefer L, Seeger W, Guenther A (2011) Comparative proteomic analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF) and lung transplant donor lungs. J Proteome Res 10:2185–2205
75. Kostenko S, Moens U (2009) Heat shock protein 27 phosphorylation: kinases, phosphatases, functions and pathology. Cell Mol Life Sci 66:3289–3307
76. Kourtis N, Tavernarakis N (2018) Small heat shock proteins and neurodegeneration: recent developments. Biomol Concepts 9:94–102
77. Kriehuber T, Rattei T, Weinmaier T, Bepperling A, Haslbeck M, Buchner J (2010) Independent evolution of the core domain and its flanking sequences in small heat shock proteins. FASEB J 24:3633–3642
78. Kuipers HF, Yoon J, van Horssen J, Han MH, Bollyky PL, Palmer TD, Steinman L (2017) Phosphorylation of alphaB-crystallin supports reactive astrogliosis in demyelination. Proc Natl Acad Sci U S A 114:E1745–E1754
79. Kumar PA, Kumar MS, Reddy GB (2007) Effect of glycation on alpha-crystallin structure and chaperone-like function. Biochem J 408:251–258
80. Kurnellas MP, Brownell SE, Su L, Malkovskiy AV, Rajadas J, Dolganov G, Chopra S, Schoolnik GK, Sobel RA, Webster J, Ousman SS, Becker RA, Steinman L, Rothbard JB (2012) Chaperone activity of small heat shock proteins underlies therapeutic efficacy in experimental autoimmune encephalomyelitis. J Biol Chem 287:36423–36434
81. Kurnellas MP, Adams CM, Sobel RA, Steinman L, Rothbard JB (2013) Amyloid fibrils composed of hexameric peptides attenuate neuroinflammation. Sci Transl Med 5:179ra142

82. Landry J, Lambert H, Zhou M, Lavoie JN, Hickey E, Weber LA, Anderson CW (1992) Human HSP27 is phosphorylated at serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II. J Biol Chem 267:794–803
83. Lang A, Schrum LW, Schoonhoven R, Tuvia S, Solis-Herruzo JA, Tsukamoto H, Brenner DA, Rippe RA (2000) Expression of small heat shock protein alphaB-crystallin is induced after hepatic stellate cell activation. Am J Physiol Gastrointest Liver Physiol 279:G1333–G1342
84. Lehr S, Hartwig S, Lamers D, Famulla S, Muller S, Hanisch FG, Cuvelier C, Ruige J, Eckardt K, Ouwens DM, Sell H, Eckel J (2012) Identification and validation of novel adipokines released from primary human adipocytes. Mol Cell Proteomics: MCP 11 (M111):010504
85. Li C, Wu J, Li Y, Xing G (2017) Cytoprotective effect of heat shock protein 27 against lipopolysaccharide-induced apoptosis of renal epithelial HK-2 cells. Cell Physiol Biochem 41:2211–2220
86. Li J, Qi X, Jiang B, Huang T, Luo L, Liu S, Yin Z (2019) Phosphorylated heat shock protein 27 inhibits lipopolysaccharide-induced inflammation in Thp1 cells by promoting TLR4 endocytosis, ubiquitination, and degradation. Inflammation 42:1788–1799
87. Liao WC, Wu MS, Wang HP, Tien YW, Lin JT (2009) Serum heat shock protein 27 is increased in chronic pancreatitis and pancreatic carcinoma. Pancreas 38:422–426
88. Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW, Weleber RG (1998) Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet 7:471–474
89. Losiewicz MK, Fort PE (2011) Diabetes impairs the neuroprotective properties of retinal alpha-crystallins. Invest Ophthalmol Vis Sci 52:5034–5042
90. Martine P, Rebe C (2019) Heat shock proteins and inflammasomes. Int J Mol Sci 20
91. Martin-Ventura JL, Duran MC, Blanco-Colio LM, Meilhac O, Leclercq A, Michel JB, Jensen ON, Hernandez-Merida S, Tunon J, Vivanco F, Egido J (2004) Identification by a differential proteomic approach of heat shock protein 27 as a potential marker of atherosclerosis. Circulation 110:2216–2219
92. Masilamoni JG, Jesudason EP, Bharathi SN, Jayakumar R (2005a) The protective effect of alpha-crystallin against acute inflammation in mice. Biochim Biophys Acta 1740:411–420
93. Masilamoni JG, Vignesh S, Kirubagaran R, Jesudason EP, Jayakumar R (2005b) The neuroprotective efficacy of alpha-crystallin against acute inflammation in mice. Brain Res Bull 67:235–241
94. Masilamoni JG, Jesudason EP, Baben B, Jebaraj CE, Dhandayuthapani S, Jayakumar R (2006) Molecular chaperone alpha-crystallin prevents detrimental effects of neuroinflammation. Biochim Biophys Acta 1762:284–293
95. Mogk A, Ruger-Herreros C, Bukau B (2019) Cellular functions and mechanisms of action of small heat shock proteins. Annu Rev Microbiol 73:89–110
96. Nagaraj RH, Nahomi RB, Mueller NH, Raghavan CT, Ammar DA, Petrash JM (2016) Therapeutic potential of alpha-crystallin. Biochim Biophys Acta 1860:252–257
97. Nagasawa T, Matsushima-Nishiwaki R, Yasuda E, Matsuura J, Toyoda H, Kaneoka Y, Kumada T, Kozawa O (2015) Heat shock protein 20 (HSPB6) regulates TNF-alpha-induced intracellular signaling pathway in human hepatocellular carcinoma cells. Arch Biochem Biophys 565:1–8
98. Nahomi RB, Wang B, Raghavan CT, Voss O, Doseff AI, Santhoshkumar P, Nagaraj RH (2013) Chaperone peptides of alpha-crystallin inhibit epithelial cell apoptosis, protein insolubilization, and opacification in experimental cataracts. J Biol Chem 288:13022–13035
99. Okuno M, Yasuda I, Adachi S, Nakashima M, Kawaguchi J, Doi S, Iwashita T, Hirose Y, Kozawa O, Yoshimi N, Shimizu M, Moriwaki H (2016) The significance of phosphorylated heat shock protein 27 on the prognosis of pancreatic cancer. Oncotarget 7:14291–14299

100. Ousman SS, Tomooka BH, van Noort JM, Wawrousek EF, O'Connor KC, Hafler DA, Sobel RA, Robinson WH, Steinman L (2007) Protective and therapeutic role for alphaB-crystallin in autoimmune demyelination. Nature 448:474–479
101. Pangratz-Fuehrer S, Kaur K, Ousman SS, Steinman L, Liao YJ (2011) Functional rescue of experimental ischemic optic neuropathy with alphaB-crystallin. Eye (Lond) 25:809–817
102. Park HK, Park EC, Bae SW, Park MY, Kim SW, Yoo HS, Tudev M, Ko YH, Choi YH, Kim S, Kim DI, Kim YW, Lee BB, Yoon JB, Park JE (2006) Expression of heat shock protein 27 in human atherosclerotic plaques and increased plasma level of heat shock protein 27 in patients with acute coronary syndrome. Circulation 114:886–893
103. Park AM, Kanai K, Itoh T, Sato T, Tsukui T, Inagaki Y, Selman M, Matsushima K, Yoshie O (2016) Heat shock protein 27 plays a pivotal role in myofibroblast differentiation and in the development of Bleomycin-induced pulmonary fibrosis. PLoS One 11:e0148998
104. Peng J, Li Y, Wang X, Deng S, Holland J, Yates E, Chen J, Gu H, Essandoh K, Mu X, Wang B, McNamara RK, Peng T, Jegga AG, Liu T, Nakamura T, Huang K, Perez-Tilve D, Fan GC (2018) An Hsp20-FBXO4 Axis regulates adipocyte function through modulating PPARgamma ubiquitination. Cell Rep 23:3607–3620
105. Pineda R 2nd, Chan CC, Ni M, Hayden BJ, Johnson MA, Nickerson J, Chader GJ (1993) Human retinoblastoma cells express alpha B-crystallin in vivo and in vitro. Curr Eye Res 12:239–245
106. Ramirez-Rodriguez G, Babu H, Klempin F, Krylyshkina O, Baekelandt V, Gijsbers R, Debyser Z, Overall RW, Nicola Z, Fabel K, Kempermann G (2013) The alpha crystallin domain of small heat shock protein b8 (Hspb8) acts as survival and differentiation factor in adult hippocampal neurogenesis. J Neurosci Off J Soc Neurosci 33:5785–5796
107. Rao NA, Saraswathy S, Wu GS, Katselis GS, Wawrousek EF, Bhat S (2008) Elevated retina-specific expression of the small heat shock protein, alphaA-crystallin, is associated with photoreceptor protection in experimental uveitis. Invest Ophthalmol Vis Sci 49:1161–1171
108. Rao NA, Saraswathy S, Pararajasegaram G, Bhat SP (2012) Small heat shock protein alphaA-crystallin prevents photoreceptor degeneration in experimental autoimmune uveitis. PLoS One 7:e33582
109. Raska M, Weigl E (2005) Heat shock proteins in autoimmune diseases. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia 149:243–249
110. Rayner K, Chen YX, McNulty M, Simard T, Zhao X, Wells DJ, de Belleroche J, O'Brien ER (2008) Extracellular release of the atheroprotective heat shock protein 27 is mediated by estrogen and competitively inhibits acLDL binding to scavenger receptor-a. Circ Res 103:133–141
111. Reddy VS, Reddy GB (2015) Emerging role for alphaB-crystallin as a therapeutic agent: pros and cons. Curr Mol Med 15:47–61
112. Reddy GB, Kumar PA, Kumar MS (2006) Chaperone-like activity and hydrophobicity of alpha-crystallin. IUBMB Life 58:632–641
113. Reddy VS, Raghu G, Reddy SS, Pasupulati AK, Suryanarayana P, Reddy GB (2013) Response of small heat shock proteins in diabetic rat retina. Invest Ophthalmol Vis Sci 54:7674–7682
114. Reddy VS, Kumar CU, Raghu G, Reddy GB (2014a) Expression and induction of small heat shock proteins in rat heart under chronic hyperglycemic conditions. Arch Biochem Biophys 558C:1–9
115. Reddy VS, Kumar CU, Reddy GB (2014b) Effect of chronic hyperglycemia on crystallin levels in rat lens. Biochem Biophys Res Commun 446:602–607
116. Reddy VS, Jakhotia S, Reddy PY, Reddy GB (2015) Hyperglycemia induced expression, phosphorylation, and translocation of alphaB-crystallin in rat skeletal muscle. IUBMB Life 67:291–299
117. Reddy VS, Madala SK, Trinath J, Reddy GB (2018) Extracellular small heat shock proteins: exosomal biogenesis and function. Cell Stress Chaperones 23:441–454

118. Roelofs MF, Boelens WC, Joosten LA, Abdollahi-Roodsaz S, Geurts J, Wunderink LU, Schreurs BW, van den Berg WB, Radstake TR (2006) Identification of small heat shock protein B8 (HSP22) as a novel TLR4 ligand and potential involvement in the pathogenesis of rheumatoid arthritis. J Immunol 176:7021–7027
119. Rothbard JB, Zhao X, Sharpe O, Strohman MJ, Kurnellas M, Mellins ED, Robinson WH, Steinman L (2011) Chaperone activity of alpha B-crystallin is responsible for its incorrect assignment as an autoantigen in multiple sclerosis. J Immunol 186:4263–4268
120. Rothbard JB, Kurnellas MP, Brownell S, Adams CM, Su L, Axtell RC, Chen R, Fathman CG, Robinson WH, Steinman L (2012) Therapeutic effects of systemic administration of chaperone alphaB-crystallin associated with binding proinflammatory plasma proteins. J Biol Chem 287:9708–9721
121. Rothbard JB, Kurnellas MP, Ousman SS, Brownell S, Rothbard JJ, Steinman L (2019) Small heat shock proteins, amyloid fibrils, and nicotine stimulate a common immune suppressive pathway with implications for future therapies. Cold Spring Harb Perspect Med 9
122. Saez-Torres I, Brieva L, Espejo C, Barrau MA, Montalban X, Martinez-Caceres EM (2002) Specific proliferation towards myelin antigens in patients with multiple sclerosis during a relapse. Autoimmunity 35:45–50
123. Saini S, Liu T, Yoo J (2016) TNF-alpha stimulates colonic myofibroblast migration via COX-2 and Hsp27. J Surg Res 204:145–152
124. Salari S, Seibert T, Chen YX, Hu T, Shi C, Zhao X, Cuerrier CM, Raizman JE, O'Brien ER (2013) Extracellular HSP27 acts as a signaling molecule to activate NF-kappaB in macrophages. Cell Stress Chaperones 18:53–63
125. Sanbe A, Marunouchi T, Abe T, Tezuka Y, Okada M, Aoki S, Tsumura H, Yamauchi J, Tanonaka K, Nishigori H, Tanoue A (2013) Phenotype of cardiomyopathy in cardiac-specific heat shock protein B8 K141N transgenic mouse. J Biol Chem 288:8910–8921
126. Saraswathy S, Nguyen AM, Rao NA (2010) The role of TLR4 in photoreceptor {alpha}a crystallin upregulation during early experimental autoimmune uveitis. Invest Ophthalmol Vis Sci 51:3680–3686
127. Shao W, Zhang SZ, Tang M, Zhang XH, Zhou Z, Yin YQ, Zhou QB, Huang YY, Liu YJ, Wawrousek E, Chen T, Li SB, Xu M, Zhou JN, Hu G, Zhou JW (2013) Suppression of neuroinflammation by astrocytic dopamine D2 receptors via alphaB-crystallin. Nature 494:90–94
128. Sharma KK, Kumar RS, Kumar GS, Quinn PT (2000) Synthesis and characterization of a peptide identified as a functional element in alphaA-crystallin. J Biol Chem 275:3767–3771
129. Sharma AK, Bharti S, Ojha S, Bhatia J, Kumar N, Ray R, Kumari S, Arya DS (2011) Up-regulation of PPARgamma, heat shock protein-27 and -72 by naringin attenuates insulin resistance, beta-cell dysfunction, hepatic steatosis and kidney damage in a rat model of type 2 diabetes. Br J Nutr 106:1713–1723
130. Shi C, Ulke-Lemee A, Deng J, Batulan Z, O'Brien ER (2019) Characterization of heat shock protein 27 in extracellular vesicles: a potential anti-inflammatory therapy. FASEB J 33:1617–1630
131. Shiota M, Bishop JL, Nip KM, Zardan A, Takeuchi A, Cordonnier T, Beraldi E, Bazov J, Fazli L, Chi K, Gleave M, Zoubeidi A (2013) Hsp27 regulates epithelial mesenchymal transition, metastasis, and circulating tumor cells in prostate cancer. Cancer Res 73:3109–3119
132. Singh BN, Rao KS, Rao Ch M (2010) Ubiquitin-proteasome-mediated degradation and synthesis of MyoD is modulated by alphaB-crystallin, a small heat shock protein, during muscle differentiation. Biochim Biophys Acta 1803:288–299
133. Sitterding SM, Wiseman WR, Schiller CL, Luan C, Chen F, Moyano JV, Watkin WG, Wiley EL, Cryns VL, Diaz LK (2008) AlphaB-crystallin: a novel marker of invasive basal-like and metaplastic breast carcinomas. Ann Diagn Pathol 12:33–40
134. Stoevring B, Vang O, Christiansen M (2005) (alpha)B-crystallin in cerebrospinal fluid of patients with multiple sclerosis. Clin Chim Acta; Int J Clin Chem 356:95–101

135. Stojic A, Bojcevski J, Williams SK, Bas-Orth C, Nessler S, Linington C, Diem R, Fairless R (2019) Preclinical stress originates in the rat optic nerve head during development of autoimmune optic neuritis. Glia 67:512–524
136. Stope MB, Klinkmann G, Diesing K, Koensgen D, Burchardt M, Mustea A (2017) Heat shock protein HSP27 secretion by ovarian cancer cells is linked to intracellular expression levels, occurs independently of the endoplasmic reticulum pathway and HSP27's phosphorylation status, and is mediated by exosome liberation. Dis Markers 2017:1575374
137. Sur R, Lyte PA, Southall MD (2008) Hsp27 regulates pro-inflammatory mediator release in keratinocytes by modulating NF-kappaB signaling. J Invest Dermatol 128:1116–1122
138. Takashi M, Katsuno S, Sakata T, Ohshima S, Kato K (1998) Different concentrations of two small stress proteins, alphaB crystallin and HSP27 in human urological tumor tissues. Urol Res 26:395–399
139. Tallot P, Grongnet JF, David JC (2003) Dual perinatal and developmental expression of the small heat shock proteins [FC12]aB-crystallin and Hsp27 in different tissues of the developing piglet. Biol Neonate 83:281–288
140. Tanaka N, Tanaka R, Tokuhara M, Kunugi S, Lee YF, Hamada D (2008) Amyloid fibril formation and chaperone-like activity of peptides from alphaA-crystallin. Biochemistry 47:2961–2967
141. Taylor RP, Benjamin IJ (2005) Small heat shock proteins: a new classification scheme in mammals. J Mol Cell Cardiol 38:433–444
142. Thuringer D, Jego G, Wettstein G, Terrier O, Cronier L, Yousfi N, Hebrard S, Bouchot A, Hazoume A, Joly AL, Gleave M, Rosa-Calatrava M, Solary E, Garrido C (2013) Extracellular HSP27 mediates angiogenesis through toll-like receptor 3. FASEB J 27:4169–4183
143. Thuringer D, Berthenet K, Cronier L, Solary E, Garrido C (2015) Primary tumor- and metastasis-derived colon cancer cells differently modulate connexin expression and function in human capillary endothelial cells. Oncotarget 6:28800–28815
144. Tukaj S, Kaminski M (2019) Heat shock proteins in the therapy of autoimmune diseases: too simple to be true? Cell Stress Chaperones 24:475–479
145. van de Bovenkamp M, Groothuis GMM, Meijer DKF, Slooff MJH, Olinga P (2006) Human liver slices as an in vitro model to study toxicity-induced hepatic stellate cell activation in a multicellular milieu. Chem Biol Interact 162:62–69
146. van Noort JM, van Sechel AC, Bajramovic JJ, el Ouagmiri M, Polman CH, Lassmann H, Ravid R (1995) The small heat-shock protein alpha B-crystallin as candidate autoantigen in multiple sclerosis. Nature 375:798–801
147. van Noort JM, Bajramovic JJ, Plomp AC, van Stipdonk MJ (2000) Mistaken self, a novel model that links microbial infections with myelin-directed autoimmunity in multiple sclerosis. J Neuroimmunol 105:46–57
148. van Noort JM, Verbeek R, Meilof JF, Polman CH, Amor S (2006) Autoantibodies against alpha B-crystallin, a candidate autoantigen in multiple sclerosis, are part of a normal human immune repertoire. Mult Scler 12:287–293
149. van Noort JM, Bsibsi M, Nacken PJ, Gerritsen WH, Amor S, Holtman IR, Boddeke E, van Ark I, Leusink-Muis T, Folkerts G, Hennink WE, Amidi M (2013) Activation of an immune-regulatory macrophage response and inhibition of lung inflammation in a mouse model of COPD using heat-shock protein alpha B-crystallin-loaded PLGA microparticles. Biomaterials 34:831–840
150. van Noort JM, Bsibsi M, Nacken PJ, Verbeek R, Venneker EH (2015) Therapeutic intervention in multiple sclerosis with alpha B-Crystallin: a randomized controlled phase IIa trial. PLoS One 10:e0143366
151. Velotta JB, Kimura N, Chang SH, Chung J, Itoh S, Rothbard J, Yang PC, Steinman L, Robbins RC, Fischbein MP (2011) alphaB-crystallin improves murine cardiac function and attenuates apoptosis in human endothelial cells exposed to ischemia-reperfusion. Ann Thorac Surg 91:1907–1913

152. Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A, Chateau D, Chapon F, Tome F, Dupret JM, Paulin D, Fardeau M (1998) A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet 20:92–95
153. Vidyasagar A, Reese S, Acun Z, Hullett D, Djamali A (2008) HSP27 is involved in the pathogenesis of kidney tubulointerstitial fibrosis. Am J Physiol Renal Physiol 295:F707–F716
154. Voll EA, Ogden IM, Pavese JM, Huang X, Xu L, Jovanovic BD, Bergan RC (2014) Heat shock protein 27 regulates human prostate cancer cell motility and metastatic progression. Oncotarget 5:2648–2663
155. Wang H, Xing J, Wang F, Han W, Ren H, Wu T, Chen W (2010) Expression of Hsp27 and Hsp70 in lymphocytes and plasma in healthy workers and coal miners with lung cancer. J Huazhong Univ Sci Technol Med Sci = Hua zhong ke ji da xue xue bao. Yi xue Ying De wen ban = Huazhong keji daxue xuebao Yixue Yingdewen ban 30:415–420
156. Wang X, Gu H, Huang W, Peng J, Li Y, Yang L, Qin D, Essandoh K, Wang Y, Peng T, Fan GC (2016) Hsp20-mediated activation of exosome biogenesis in cardiomyocytes improves cardiac function and angiogenesis in diabetic mice. Diabetes 65:3111–3128
157. Wettstein G, Bellaye PS, Kolb M, Hammann A, Crestani B, Soler P, Marchal-Somme J, Hazoume A, Gauldie J, Gunther A, Micheau O, Gleave M, Camus P, Garrido C, Bonniaud P (2013) Inhibition of HSP27 blocks fibrosis development and EMT features by promoting snail degradation. FASEB J 27:1549–1560
158. Wu N, Wang YH, Zhao HS, Liu DN, Ying X, Yin ZQ, Wang Y (2009) alpha-crystallin downregulates the expression of TNF-alpha and iNOS by activated rat retinal microglia in vitro and in vivo. Ophthalmic Res 42:21–28
159. Wu N, Yu J, Chen S, Xu J, Ying X, Ye M, Li Y, Wang Y (2014) Alpha-Crystallin protects RGC survival and inhibits microglial activation after optic nerve crush. Life Sci 94:17–23
160. Wu J, Liu T, Rios Z, Mei Q, Lin X, Cao S (2017) Heat shock proteins and cancer. Trends Pharmacol Sci 38:226–256
161. Xu W, Guo Y, Huang Z, Zhao H, Zhou M, Huang Y, Wen D, Song J, Zhu Z, Sun M, Liu CY, Chen Y, Cui L, Wang X, Liu Z, Yang Y, Du P (2019) Small heat shock protein CRYAB inhibits intestinal mucosal inflammatory responses and protects barrier integrity through suppressing IKKbeta activity. Mucosal Immunol
162. Yang K, Meinhardt A, Zhang B, Grzmil P, Adham IM, Hoyer-Fender S (2012) The small heat shock protein ODF1/HSPB10 is essential for tight linkage of sperm head to tail and male fertility in mice. Mol Cell Biol 32:216–225
163. Ye S, Zhang C, Zhou J, Cheng J, Lv Z, Zhou L, Xie H, Wu J, Zheng S (2014) Human heat shock protein 27 exacerbates ischemia reperfusion injury in rats by reducing the number of T regulatory cells. Mol Med Rep 9:1998–2002
164. Yu DW, Ge PP, Liu AL, Yu XY, Liu TT (2019a) HSP20-mediated cardiomyocyte exosomes improve cardiac function in mice with myocardial infarction by activating Akt signaling pathway. Eur Rev Med Pharmacol Sci 23:4873–4881
165. Yu L, Liang Q, Zhang W, Liao M, Wen M, Zhan B, Bao H, Cheng X (2019b) HSP22 suppresses diabetes-induced endothelial injury by inhibiting mitochondrial reactive oxygen species formation. Redox Biol 21:101095
166. Yusuf N, Nasti TH, Huang CM, Huber BS, Jaleel T, Lin HY, Xu H, Elmets CA (2009) Heat shock proteins HSP27 and HSP70 are present in the skin and are important mediators of allergic contact hypersensitivity. J Immunol 182:675–683
167. Zhang C, Jones JT, Chand HS, Wathelet MG, Evans CM, Dickey B, Xiang J, Mebratu YA, Tesfaigzi Y (2018) Noxa/HSP27 complex delays degradation of ubiquitylated IkBalpha in airway epithelial cells to reduce pulmonary inflammation. Mucosal Immunol 11:741–751
168. Zhang J, Liu J, Wu J, Li W, Chen Z, Yang L (2019) Progression of the role of CRYAB in signaling pathways and cancers. Onco Targets Ther 12:4129–4139
169. Zoubeidi A, Gleave M (2012) Small heat shock proteins in cancer therapy and prognosis. Int J Biochem Cell Biol 44:1646–1656

# Overview of Multifaceted Role and Significance of Heat Shock Proteins During Inflammation, Apoptosis and Other Diseases


**Boopathi Balasubramaniam and Krishnaswamy Balamurugan**

## Abstract

**Introduction** Almost all cells are frequently exposed to various stimuli in their environment which causes a wide range of stresses. Due to the elevated levels of stresses, cells often respond back by producing different types of proteins that protect the cells to remain in a healthy condition which phenomenon is called cellular homeostasis. The important family of proteins which play cytoprotective activities under pathological conditions are Hsp which acts through the initiation of repair mechanism, protein folding, degradation of irreversible proteins and refolding of misfolded proteins. The foremost objective of this chapter is to outline the significance of Hsp during inflammatory and other disease.

**Methods** Journals and data bases (PubMed, Scopus and Google Scholar) were surveyed with the keywords "Hsp and its functions", "Hsp and Inflammation" and "Hsp and Apoptosis". Pertinent works were chosen for discussion.

**Results** Hsp are known to have multifaceted functions in various instances including maintaining the homeostasis during pathogenic conditions and disease progression. In connection to this, a number of HIDs and other diseases such as cancer are well known to exhibit elevated ROS levels followed by excessive apoptosis. Hsp alters the inflammation modules through inhibition of cytokines, pro-inflammatory factors, by which playing the significant functions in the HIDs and its pathogenesis.

**Conclusions** In this chapter, we summarized the importance and characteristics of Hsp especially in inflammation which is expected to help future treatment of HIDs and other related diseases.

B. Balasubramaniam · K. Balamurugan (✉)
Department of Biotechnology, Science Campus, Alagappa University, Karaikudi, Tamil Nadu, India
e-mail: bsuryar@yahoo.com; balamurugank@alagappauniversity.ac.in

Heat Shock Proteins (2021) 22: 271–280
https://doi.org/10.1007/7515_2020_28

Published Online: 23 November 2020

**Keywords** Apoptosis · Cancer · Cytoprotection · Homeostasis · Molecular chaperones · Therapeutics

## Abbreviations

| | |
|---|---|
| APC | antigen presenting cell(s) |
| ATP | adenosine triphosphate |
| COX | cyclooxygenase |
| DC | dendritic cells |
| HID | human inflammatory disease(s) |
| HSF | heat shock factor(s) |
| Hsp | heat shock protein(s) |
| IBD | inflammatory bowel disease |
| IL | interleukin(s) |
| MHC I | major histocompatibility complex class I |
| MS | multiple sclerosis |
| NF-κ | Bnuclear factor kappa-light-chain-enhancer of activated B cells |
| NK | natural killer cells |
| NOD | non-obese diabetic mice |
| PGE2 | Prostaglandin E2 |
| PTM | post translational modifications |
| RNS | reactive nitrogen species |
| ROS | reactive oxygen species |
| RU | rheumatoid arthritis |
| SOD | superoxide dismutase |
| TLR | toll-like receptor(s) |
| TNF | tumor necrosis factor(s) |
| Tregs | regulatory T cells |

## 1 Introduction

Hsp of eukaryotes are evolutionarily conserved macromolecules present in almost all the major cellular organelles. Indeed, Hsp are constitutively expressed in all types of living organisms starting from prokaryote to eukaryote [21]. The nomenclature of Hsp originated from the examination of their deliberate stimulation generally triggered by thermal stress [23]. Hsp comprise about a high proportion (5–10%) of total cellular proteins under normal environmental conditions, and their concentration/abundance may raise several folds under stressed conditions when the aggregates or misfolded proteins remain. Although Hsp are constitutively synthesized, most of them act as molecular chaperones that are normally over-expressed in response to external stimuli only [41]. Majorly, the stress factors include heat, oxidative stress, nutrient deficiency, viral infections, acute or chronic inflammatory diseases,

ischemia, heavy metals, bacterial infections and exercise [3, 16]. These types of exposures enable the cells to respond quickly to bring back the normal conditions. Moreover, lack of cellular protection/cytoprotection against degradation of misfolded proteins and protein denaturation may results in protein aggregation and diseases. That is where the Hsp play a critical role to maintain the cellular homeostasis during stressed conditions.

Hsp are part of the immune system against both external and internal factors which are mainly categorized into 02 groups (High molecular weight Hsp and Low molecular Hsp) according to their molecular weight, amino acid composition, as well as their specific cellular functions [10, 22]. The high molecular weight Hsp (typically ranges from 60 to 110 kDa) are found to be ATP-dependent and their key cellular function usually includes folding the native proteins, transportation, organization and degradation of unstructured/disordered/unfolded proteins. Low molecular weight Hsp (generally ranges from 15 to 43 kDa) are ATP-independent chaperones molecules as well and are reported to be involved in development of respiratory organs such as cardiac muscles, embryonic developmental processes, exercise-induced stress, as biomarkers for tumor formation and also protein folding. In spite of their involvement in multiple molecular and cellular functions including pathophysiological conditions, they attain more attention to explore their mode of action in deeper. Therefore, this chapter summarizes the roles of Hsp under different disease conditions with special attention to inflammatory diseases in detail.

## *1.1 Hsp and Immune System*

Hsp are reported to be involved in adaptive and innate immunity during various stressed conditions [29, 34]. They can significantly stimulate NK cells, macrophages and DCs. As per the literature, both recombinant and autologous Hsp trigger the immunomodulatory functions and T lymphocyte proliferation during stressed conditions [38, 46]. Hsp are found to enhance the efficiency of cross-presentation of extracellular and intracellular antigens to APCs in the MHC I. In addition, CD91, a Hsp receptor found in the APCs is required in this immunologic process and enhances the T-cell mediated immune responses in coordination with T helper cells [18]. In contrast, Hsp typically induce the anti- or pro-inflammatory cytokines secretion thereby they involve in the immune response against various stimuli. Typically, extracellular Hsp appear to contain cytokine-related properties needed for immune responses through the molecular association with few receptors such as Pattern Recognition Receptors (PRR) including CD14 and TLRs [42, 47] and thus influences the release of various cytokines such as TNF-α, IL-1β, IL-12, IL-6, etc. These are some of the known function of Hsp in connection to immunity. However, their underlying mechanisms are yet to be explored in detail.

## 1.2 *Link Between Hsp and NF-κB Pathway in Regulating the Inflammatory Diseases*

Inflammatory disease is collectively described as a group of apparently discrete conditions with the involvement of various immune cells and inflammatory pathways which may end up with various organ damages [9, 44]. Even though each disease has its unique characteristic pathophysiology and epidemiology, the differentially regulated inflammatory reaction is considered to be crucial to the disease pathogenesis and progression. In addition to conventional inflammatory mediators and responses, other diseases were also shown to involve in inflammatory responses as well. Those diseases are diabetes, RA, IBD, MS, etc. Despite many types of Hsp have been associated with immunity-mediated and inflammatory diseases during the past few decades [15, 39], the underlying mechanisms and the roles of large Hsp in disease progression and pathogenesis remain unclear.

During the whole inflammatory response (including its initial phase), there is a slight coordinated expression of nuclear transcription factors triggered from the NF-κB (Fig. 1). At the commencement of an inflammatory response during stressed

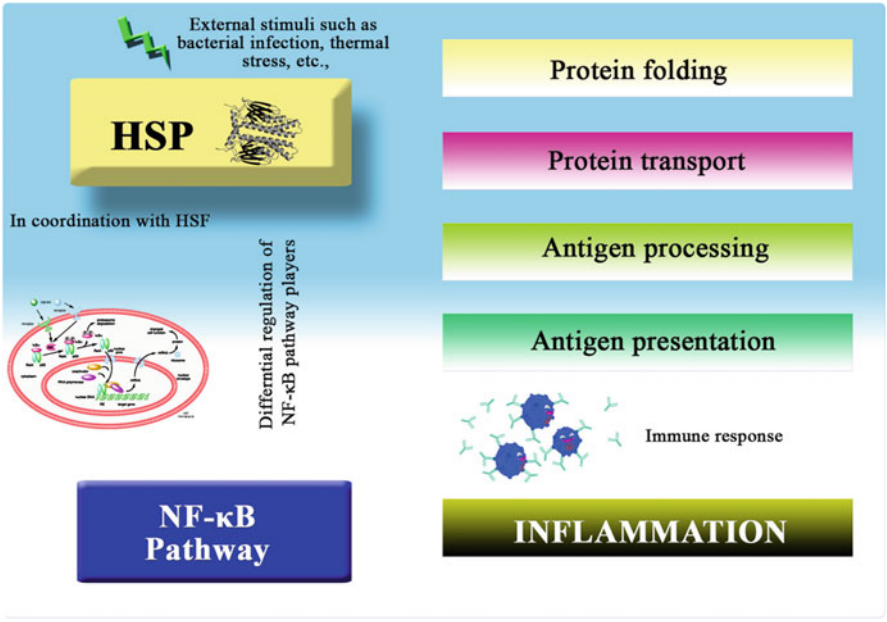



**Fig. 1 Role of Hsp in inflammatory diseases and immune system.** The image represents the importance of Hsp during stress condition. During exposure to external stimuli such as injury, bacterial infection, thermal stress, etc., Hsp regulates variety of functions including protein translocation, antigen processing, antigen presentation, etc. The immunity triggered due to external stimuli activates certain immune regulatory proteins and leads to the activation of inflammation through NF-κB pathway which is depicted in this image

conditions, the control of NF-κB transcription factors, inducible enzymes (such as COX-2) oblige the synthesis of PGE2, which further triggers fever. As a result of elevation in the temperature, the highly conserved heat shock response initiates the activation of transcriptional factors such as HSF1 [27]. By being a molecular chaperone, these HSF and Hsp work together to reduce the protein aggregates and protein denaturation to attain the cellular homeostasis. During that condition, a well characterized iHsp70 (inducer Hsp70) was found to be associated with the complex formed by NF-κB pathway with its inhibitor (IκB) to inhibit the NF-κB translocation to nucleus [6]. Overall, the Hsp and their involvement in inflammatory diseases are generally mediated by NF-κB pathway which could be considered as the primary target to treat the inflammatory diseases. Moreover, the identification of other associated players may deliver the basic understanding of underlying mechanisms in much deeper.

## *1.3 Relationship Between Hsp and Apoptosis, Oxidative Stress and Inflammatory Diseases*

Biological systems utilize a number of antioxidants (such as, reduced glutathione, catalase, etc.) to neutralize the negative effects of free radicals, in addition to prevent the ROS-mediated cellular and functional damages. However, in respect to non-cellular responses/external stimuli such as exercise, air pollutants, radiation, ozone, cigarette and cellular responses such as inflammatory reactions and phagocytosis, the host cells tend to generate excess ROS and RNS which interferes with the oxidant/antioxidant levels leading to fluctuation in cellular homeostasis [17, 24]. Conversely, inflammation response is said to be the component of first line defense mechanism of the innate immunity complex when there is a cellular damage triggered by xenobiotics of infectious agents [33]. Beginning of the stimuli/stress has been shown to accumulate several inflammatory responsive cells, such as macrophages, chemokines, and cytokines at the impaired site of stressed cells, which are generally mediated through TLRs activation. In contrast, interlink between oxidative stress and apoptosis with Hsp occur mostly due to the induction upon endogenous/exogenous stimuli. Therefore, the coordinated and induced HSP are highly expressed in the inflamed area, probably to refold the misfolded, denatured proteins/peptides, so that the oxidative stress condition can be reverted [32]. In addition, inhibition of the Hsp expression levels lead to severe inflammation cases and apoptosis by inducing the oxidative levels to many folds. Hence, the importance of Hsp is not only limited to inflammatory and other diseases; rather they have an association with other physiological condition such as oxidative stress, etc. to improve the condition.

## 1.4 Hsp in Autoimmune Diseases

Hsp are found to be a part of the autoimmune reactions also, which leads to the molecular imitation along with the origin relationship between endogenous and exogenous stressor molecules. For instance, Hsp of the invading pathogenic bacteria (molecular imitation) can also generate an immune response in the host system, which may result in the autoimmune reactions. A classic example is RA disease, a well-known autoimmune systemic disease that affects the connective tissues [36]. The development of heat shock response is considered as an earlier occurrence in pathogenesis and progression of the RA disease. In fact, the presence of many anti-Hsp antibodies and the presence of Hsp in the extracellular space were observed in patients affected with RA disease ([26]). Furthermore, immunization with recombinant HspD (Hsp60) in rats was found to protect them against the RA [7, 43]. Primarily, regulatory T cells recognised the HspD epitopes in a highly conserved manner and remarkably, transfer of these cells to the other animal with RA prohibited the disease onset [8, 20]. Additionally, both HspD and HspA (Hsp70) possess immunomodulatory effects by which they enhance the anti-inflammatory Tregs when used to treat arthritis. These reports suggest that the Hsp have a strong relationship with autoimmune diseases also. Exploring the involvement of other Hsp during autoimmune diseases may end up with the identification of precise drug targets.

## 1.5 Role of Hsp in Other Diseases

Hsp play an important role in Ischaemia/reperfusion-induced acute kidney injury which was characterized in mice model. Particularly, HspA was found to possess renoprotective effect, which may be partly mediated by the immunomodulatory effect exerted by Tregs. Also, Hsp play a critical role in atherosclerosis, which is defined to be a chronic inflammatory disease affects the arteries. During the inflammatory condition, Hsp released from damaged cells are considered to be the important autoantigens for the development of atherosclerotic plaques [11]. A classic example for the involvement of Hsp in diabetes (especially in Type 1 diabetes) is Hsp60 (an autoantigen) which was disclosed using NOD model [35]. There are many unexplored roles of Hsp in other diseases remain which could be studied well in near future.

## 1.6 Hsp and Future Perspectives

As discussed in this chapter, regulation of immune responses, inflammatory responses, endogenous generation of ROS and apoptosis by Hsp perhaps assist in

the management and treatment of inflammatory diseases. Hsp appear to be also play vital roles in the pathogenesis of inflammatory diseases and cancer due to their modulating properties in the inflammation cascades that possibly lead to the endogenous elevation of ROS levels and apoptosis [14, 25, 43]. These properties may be due to refolding of misfolded proteins, or through inhibition of anti- or pro-inflammatory cytokines during pathological conditions. Interestingly, HSF-1 was also upregulated during infection. HSF-1 enhances the protective Hsp resulting in inhibition of inflammatory reactions and substantial cellular damage via apoptosis. The elevated levels of ROS, possibly through inhibition of anti- or pro-inflammatory factors prevents the progression of acute and chronic inflammation, cancer and other diseases. Therefore, the Hsp are considered to be the natural enhancer which contains multiple roles during pathogenesis and autoimmune diseases. In this context, the therapeutic applications of active principles exploring Hsp may pave the way to treat variety of diseases with high potential are noticeably the hot topic in the recent years. Even though, Hsp and their crucial roles during diseases are known, the underlying mechanisms (PTMs, involvement of small molecules in regulating the Hsp, transcription factors, etc.,) are not fully studied. Therefore, Hsp and their associated factors can be studied in detail using various eukaryotic model systems such as *Mus musculus* [13], *Drosophila melanogaster* [5], *Caenorhabditis elegans* [1, 2, 4, 12, 19, 28, 30, 31, 37, 40, 45], etc., by exploring their importance during various stressed conditions.

## 2 Conclusions

The need for new therapeutics that will have minimal side effects for the better treatment of inflammatory diseases and cancer remains unclear. Recently, Hsp have attained a greater attention because of their undisputed roles in a number of infections and human diseases, including inflammatory disease, cancer, etc. Still, their mode of action in some of the inflammatory diseases is still unclear and yet to be identified, characterized and validated. In addition, the molecular insights about the mode of action (such as underlying PTMs, interacting partners, interacting metabolites and other molecules) could be explored well using suitable model systems. As discussed in this chapter, we suggest that targeting the Hsp in human inflammatory diseases and other diseases such as neurodegenerative diseases, cancer, etc., may end up with the identification of potential drug targets.

**Acknowledgements** The authors thankfully acknowledge the Editor(s) for inviting us to write the chapter. KB gratefully acknowledges the Department of Biotechnology (DBT), Government of India (GOI) [Ref. No. BT/PR17367/MED/122/44/2016] for the financial support. BB thankfully acknowledges UGC-BSR (University Grants commission – Basic Scientific Research, GOI) for the financial assistance in the form of UGC-BSR-Fellow and UGC-BSR-SRF [Ref. No. F.25-1/2014-15(BSR)/7-326/2011(BSR) dt. 13.03.2015] and Bioinformatics Infrastructure Facility (BIF), supported by the DBT, Ministry of Science and Technology, GOI for the financial aid in the form of DBT-BIF-Studentship [Ref. No. A13/DBF-BIF Studentship/5038/2014 dt. 10.09.2014]. Authors also acknowledge the Bioinformatics Facility (BIF) provided by Alagappa University

Bioinformatics Infrastructure Facility (funded by DBT, GOI; Ref. No. BT/BI/25/012/2012, BIF), DST-PURSE [Grant No. SR/PURSE Phase 2/38 (G)], DST-FIST [Grant No. SR/FST/LSI-639/2015(C)] and UGC-SAP [Grant No. F.5-1/2018/DRS-II(SAP-II)]. The Authors gratefully acknowledge the RUSA 2.0 [F. 24-51/2014-U, Policy (TN Multi-Gen), Dept of Education, GOI].

**Disclosure of Interests** All authors declare that they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Balasubramaniam B, Prithika U, Balamurugan K (2019a) Prevalence of bacterial infections in respiratory tract. In the Book: Pocket guide to bacterial infections, 79
2. Balasubramaniam B, Vinitha T, Deepika S, JebaMercy G, VenkataKrishna LM, Balamurugan K (2019b) Analysis of *Caenorhabditis elegans* phosphoproteome reveals the involvement of a molecular chaperone, HSP-90 protein during *Salmonella enterica* Serovar Typhi infection. Int J Biol Macromol 137:620–646
3. Balasubramaniam B, Alexpandi R, Darjily DR (2019c) Exploration of the optimized parameters for bioactive prodigiosin mass production and its biomedical applications *in vitro* as well as *in silico*. Biocat Agri Biotech:101385
4. Balasubramaniam B, VenkataKrishna LM, Vinitha T, JebaMercy G, Balamurugan K (2020) *Salmonella enterica* Serovar Typhi exposure elicits deliberate physiological alterations and triggers the involvement of ubiquitin mediated proteolysis pathway in *Caenorhabditis elegans*. Int J Biol Macromol 149:215–233
5. Beckingham KM, Armstrong JD, Texada MJ, Munjaal R, Baker DA (2007) *Drosophila melanogaster*-the model organism of choice for the complex biology of multi-cellular organisms. Gravit Space Res 18(2)
6. Bloemendal A, Van der Zee R, Rutten VP et al (1997) Experimental immunization with anti-rheumatic bacterial extract OM-89 induces T cell responses to heat shock protein (Hsp) 60 and Hsp70; modulation of peripheral immunological tolerance as its possible mode of action in the treatment of rheumatoid arthritis (RA). Clin Exp Immunol 110:72–78
7. de Kleer IM, Wedderburn LR, Taams LS et al (2004) CD4+CD25bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. J Immunol 172:6435–6443
8. DeMeester SL, Buchman TG, Cobb JP (2001) The heat shock paradox: does NF-kappaB determine cell fate? FASEB J 15:270–274
9. Edrey YH, Oddo S, Cornelius C, Caccamo A, Calabrese V, Buffenstein R (2014) Oxidative damage and amyloid-β metabolism in brain regions of the longest-lived rodents. J Neurosci Res 92(2):195–205
10. Garbuz DG, Astakhova LN, Zatsepina OG, Arkhipova IR, Nudler E, Evgen'ev MB (2011) Functional organization of hsp70 cluster in camel (Camelus dromedarius) and other mammals. PLoS One 6(11):e27205
11. Ghayour-Mobarhan M, Lamb DJ (2005) Heat shock protein antibody titers are reduced by statin therapy in dyslipidemic subjects: a pilot study. Angiology 56:61–68

12. Gowrishankar S, Pandian SK, Balasubramaniam B, Balamurugan K (2019) Quorum quelling efficacy of marine cyclic dipeptide-cyclo (L-leucyl-L-prolyl) against the uropathogen *Serratia marcescens*. Food Chem Toxicol 123:326–336
13. Han S, Yang A, Lee S, Lee HW, Park CB, Park HS (2017) Expanding the genetic code of Mus musculus. Nat Commun 8:14568
14. Hauet-Broere F, Wieten L, Guichelaar T, Berlo S, Van der Zee R, Van Eden W (2006) Heat shock proteins induce T cell regulation of chronic inflammation. Ann Rheum Dis 65(Suppl 3): iii65–iii68
15. Hoshino T, Murao N, Namba T, Takehara M, Adachi H, Katsuno M, Sobue G, Matsushima T, Suzuki T, Mizushima T (2011) Suppression of Alzheimer's disease-related phenotypes by expression of heat shock protein 70 in mice. J Neurosci 31(14):5225–5234
16. Jee H (2016) Size dependent classification of heat shock proteins: a mini-review. J Exercise Rehabil 12(4):255–259
17. Ji L (1995) Oxidative stress during exercise: implication of antioxidant nutrients. Free Radic Biol Med 18:1079–1086
18. Jin B, Sun T, Yu XH et al (2012) The effects of TLR activation on T-cell development and differentiation. Clin Dev Immunol 2012:836485
19. Kannappan A, Balasubramaniam B, Ranjitha R, Srinivasan R, Packiavathy IASV, Balamurugan K et al (2019) *In vitro* and *in vivo* biofilm inhibitory efficacy of geraniol-cefotaxime combination against *Staphylococcus* spp. Food Chem Toxicol 125:322–332
20. Kim MG, Jung Cho E, Won Lee J et al (2014) Tregs contribute to HSP70-induced renoprotective effect is partially mediated by CD4+CD25+Foxp3+ regulatory T cells in ischemia/reperfusion-induced acute kidney injury. Kidney Int 85:62–71
21. Koliński T, Marek-Trzonkowska N, Trzonkowski P, Siebert J (2016) Heat shock proteins (HSPs) in the homeostasis of regulatory T cells (Tregs). Cent Eur J Immunol 41(3):317
22. Lanneau D, Wettstein G, Bonniaud P, Garrido C (2010) Heat shock proteins: cell protection through protein triage. Sci World J 10:1543–1552
23. Lee AS (1992) Mammalian stress response: induction of the glucose-regulated protein family. Curr Opin Cell Biol 4(2):267–273
24. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4:118
25. Lowe SW, Lin AW (2000) Apoptosis in cancer. Carcinogenesis 21(3):485–495
26. Lundberg K, Wegner N, Yucel-Lindberg T, Venables PJ (2010) Periodontitis in RA-the citrullinated enolase connection. Nat Rev Rheumatol 6:727–730
27. Marcenaro E, Carlomagno S, Pesce S et al (2011) Bridging innate NK cell functions with adaptive immunity. Adv Exp Med Biol 780:45–55
28. Marudhupandiyan S, Prithika U, Balasubramaniam B, Balamurugan K (2017) RACK-1, a multifaceted regulator is required for *C. elegans* innate immunity against *S. flexneri* M9OT infection. Dev Comp Immunol 74:227–236
29. Miyara M, Sakaguch S (2007) Natural regulatory T cells mechanisms of suppression. Trends Mol Med 13:108–116
30. Muthamil S, Devi VA, Balasubramaniam B, Balamurugan K, Pandian SK (2018a) Green synthesized silver nanoparticles demonstrating enhanced *in vitro* and *in vivo* antibiofilm activity against *Candida* spp. J Basic Microbiol 58(4):343–357
31. Muthamil S, Balasubramaniam B, Balamurugan K, Pandian SK (2018b) Synergistic effect of quinic acid derived from *Syzygium cumini* and undecanoic acid against *Candida* spp. biofilm and virulence. Front Microbiol 9:2835
32. Nita M, Grzybowski A (2016) The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev:3164734
33. Oyinloye BE, Adenowo AF, Kappo AP (2015) Reactive oxygen species, apoptosis, antimicrobial peptides and human inflammatory diseases. Pharmaceuticals 8:151–175

34. Prithika U, Balamurugan K (2019) Dynamics of heat shock proteins in immunity and aging. In: Heat shock proteins in signaling pathways. Springer, Cham, pp 91–100
35. Pugliese A (2012) The multiple origins of type 1 diabetes. Diabet Med 10:12081
36. Rajagopal D, Bal V, Mayor S, George A, Rath S (2006) A role for the Hsp90 molecular chaperone family in antigen presentation to T lymphocytes via major histocompatibility complex class II molecules. Eur J Immunol 36(4):828–841
37. Sathya S, Shanmuganathan B, Balasubramaniam B, Balamurugan K, Devi KP (2019) Phytol loaded PLGA nanoparticles regulate the expression of Alzheimer's related genes and neuronal apoptosis against amyloid-β induced toxicity in Neuro-2a cells and transgenic *Caenorhabditis elegans*. Food Chem Toxicol 14:110962
38. Scheffold A, Hühn J, Höfer T (2005) Regulation of CD4+CD25+ regulatory T cell activity: it takes (IL-)two to tango. Eur J Immunol 35:1336–1341
39. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 14(8):837–842
40. Shanmuganathan B, Sathya S, Balasubramaniam B, Balamurugan K, Devi KP (2019) Amyloid-β induced neuropathological actions are suppressed by *Padina gymnospora* (Phaeophyceae) and its active constituent α-bisabolol in Neuro2a cells and transgenic *Caenorhabditis elegans* Alzheimer's model. Nitric Oxide 91:52–66
41. Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev Immumol 2(3):185–194
42. Stelter F (2000) Structure/function relationships of CD14. Chem Immunol 74:25–41
43. Van Eden W, Van der Zee R, Prakken B (2005) Heat-shock proteins induce T-cell regulation of chronic inflammation. Nat Rev Immunol 5(4):318–330
44. Verdile G, Keane KN, Cruzat VF, Medic S, Sabale M, Rowles J, Wijesekara N, Martins RN, Fraser PE, Newsholme P (2015) Inflammation and oxidative stress: the molecular connectivity between insulin resistance, obesity, and Alzheimer's disease. Mediat Inflamm 2015: 105828–105817. https://doi.org/10.1155/2015/105828
45. Vigneshwari L, Balasubramaniam B, Sethupathy S, Pandian SK, Balamurugan K (2018) O-GlcNAcylation confers protection against *Staphylococcus aureus* infection in *Caenorhabditis elegans* through ubiquitination. RSC Adv 8(41):23089–23100
46. Workman CJ, Szymczak-Workman AL, Collison LW, Pillai MR, Vignali DA (2009) The development and function of regulatory T cells. Cell Mol Life Sci 66(16):2603
47. Young JC, Agashe VR, Siegers K, Hartl FU (2004) Pathways of chaperone-mediated protein folding in the cytosol. Nat Rev Mol Cell Biol 5:781–791

# Heat Shock Proteins as Target Autoantigens in Autoimmune Rheumatic Diseases

**Georgios Efthymiou, Lazaros I. Sakkas, and Dimitrios P. Bogdanos**

## Abstract

**Introduction** Accumulative data emerged over the years on adaptive immune responses specific for heat shock proteins (Hsp) in murine and human studies assessing their role in immune-mediated inflammation. In here, we aim to highlight key findings of studies assessing heat shock protein (Hsp)-specific autoreactive responses in patients with autoimmune rheumatic diseases, focusing mainly on B-cell responses.

**Methods** Current literature was reviewed reporting of the prevalence of human Hsp-targeted autoantibody responses in systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis and Behçet's disease, as prototypic rheumatic diseases and assesses the diagnostic and clinical significance of these autoantibodies, as well as their pathogenic relevance in relation to the development of those diseases and their progression.

**Results** Most human Hsp are autoantigens. Anti-human hsp responses are noted in various patients with autoimmune rheumatic diseases, their prevalence varying amongst diseases and between studies. The titers of anti-Hsp antibodies vary amongst patients. It remains unclear whether autoantibody seropositivity or its concentration is of clinical importance. Also the triggering events responsible for their development are largely unexplored.

**Conclusions** Autoantibodies targeting hsp are present in patients with rheumatic diseases, but their diagnostic, clinical and pathogenic relevance is still undetermined.

G. Efthymiou · L. I. Sakkas · D. P. Bogdanos (✉)
Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece
e-mail: bogdanos@uth.gr; bogdanos@med.uth.gr

Heat Shock Proteins (2021) 22: 281–306
https://doi.org/10.1007/7515_2020_35

Published Online: 22 January 2021

**Keywords** Autoantibodies · Autoimmunity · Rheumatic diseases · Rheumatoid arthritis · Rheumatology · Systemic lupus erythematosus

## Abbreviations

| | |
|---|---|
| ACPA | anti-citrullinated peptide antibodies |
| AECA | anti-endothelial cell antibodies |
| aPL | anti-phospholipid |
| autoabs | autoantibodies |
| BD | Behçet's disease |
| BiP | immunoglobulin heavy-chain-binding protein |
| CCP | cyclic citrullinated peptide |
| Hsp | heat shock protein(s) |
| IL | interleukin |
| ILD | interstitial lung disease |
| PAH | pulmonary arterial hypertension |
| RA | rheumatoid arthritis |
| SjS | Sjogren's syndrome |
| SLE | Systemic lupus erythematosus |
| SSc | systemic sclerosis |
| TNF-α | tumor necrosis factor α |

## 1 Introduction

The human heat shock proteins (Hsp) are a group of proteins that play a pivotal role in folding of nascent and denatured cell proteins, assembly of polymeric complexes, transport and sorting of proteins into accurate cellular compartments and protection against stress and cell death [36, 59, 97]. Normally, they are constitutively expressed at low levels inside the cytosol, the endoplasmic reticulum or the mitochondria of eukaryotic cells [12, 36, 89] and are phylogenetically and functionally conserved across species [18, 32, 38, 42]. Under environmental stress conditions [3, 9, 68, 89], including heat shock, toxic chemicals, ultraviolet, as well as cell injury, hypoxia, inflammation and infections, Hsp participate in correct protein folding. In these cases, Hsp can be translocated in the nucleus, on the cell surface or in extracellular fluids [1, 2, 6, 44, 80].

In immunological terms, Hsp are highly immunogenic and antigen-specific T and B cells responses directed against Hsp have been reported in various inflammatory and autoimmune diseases. The exact mechanism by which Hsp become targets of autoreactive B and T cell responses, remains elusive [91], though mechanisms such as molecular mimicry between bacterial and human Hsp has been considered the most likely one to explain the induction of human Hsp-specific B and T cell responses and the development of human Hsp autoantibodies (autoabs).

## *1.1 Human Hsp Autoantibodies in Systemic Lupus Erythematosus*

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease of the connective tissue that can affect almost all organ systems, characterized by the presence of a plethora of autoabs that mainly target nuclear antigens [46].

### 1.1.1 Hsp60

In patients with SLE, studies reporting on the frequency of anti-human Hsp60 antibodies have produced inconsistent results (Table 1). A single study reported a higher frequency of human Hsp60 autoabs in SLE patients compared to healthy controls [40]. However, other studies reported no difference [21, 41] or even lower frequency of human Hsp60 autoabs in SLE compared to controls [22, 93]. A great variation of reported frequencies are noted ranging from 4.2% to 76%. These differences can be attributed mainly to differences in methodological approaches, including the assay used for antibody testing, the antigenic source and the cut off levels used for autoab seropositivity. Regarding anti-human Hsp60 antibodies serum levels, most studies present higher titres in SLE compared to healthy controls [37, 40, 55, 98, 99], although other reports fail to identify statistically significant differences [21, 33, 38]. Of interest, higher titres of human Hsp60 autoabs were detected in neuropsychiatric SLE patients compared to healthy and disease controls, suggesting that these autoabs or T cells specific for human Hsp60 may be involved in the development of this form of SLE [50, 51].

A potential role for anti-human Hsp60 antibodies was suggested in the pathogenesis of vascular manifestations of SLE. This autoimmune rheumatic disease carries an increased cardiovascular risk, including early or accelerated atherosclerosis [101]. Vascular involvement in autoimmune rheumatic diseases, including SLE, has been linked to the presence of anti-endothelial cell antibodies (AECA) [63, 73]. Dieude et al. using flow cytometry were able to detect AECA in the sera of SLE patients targeting Hsp60 localized on the surface of cultured endothelial cells [21]. The binding of SLE-affinity purified IgG anti-Hsp60 autoabs, but not that of control IgG antibodies, induced apoptosis of the epithelial cells. It is not clear, though, whether this pro-apoptotic feature ability is only induced by anti-human Hsp60 autoabs from SLE patients or also from those of IgG human anti-Hsp60 found in healthy individuals. In addition, high levels of anti-Hsp60 autoabs correlated with lupus anticoagulant levels and were associated with episodes of arterial, but not venous, thrombosis [21, 22]. Based on these findings, the authors suggested a "double-hit" model for vascular events in SLE patients in the presence of anti-human Hsp60 and anti-phospholipid (aPL) autoabs [22]. According to this model, the first hit is represented by the binding of anti-Hsp60 autoabs on Hsp60 molecules on the surface of endothelial cells. The expression of Hsp60 on the surface is upregulated following shear damage, an event more frequently noted in arterial

**Table 1** Cumulative findings of studies on human heat shock-specific autoantibodies in systemic lupus erythematosus

| Autoantibody | Reference(s) | IgG ab Prevalence compared to HC | IgG ab Prevalence in SLE | IgG ab Prevalence in HC | IgG ab Magnitude compared to HC | Other findings related to Hsp autoabs |
|---|---|---|---|---|---|---|
| Anti-Hsp60 | Jamin et al. [40] | Higher | 76% by Western and 39% by ELISA | <10% by Westen | | Hsp60 is a target antigen of AECA<br>Hsp60 is expressed on epithelial cell surface under stress<br>Binding of anti-Hsp60 autoabs purified from SLE patients induces epithelial apoptosis<br>Hsp60 autoabs are not the only AECA with apoptotic capacity<br>Association of Hsp60 with aPL and vascular events in SLE and neuroSLE<br>No correlation with disease activity |
| | Dieude et al. [21], Jarjour et al. [41] | No difference | 4.2–26.1% | 2.8–26.6% | | |
| | Dieude et al. [22], van Paassen et al. [93] | Lower | 10.7–19% | 24.4–35% | | |
| | Hirata et al. [37], Jamin et al. [40], Kimura et al. [50], Kimura et al. [51], Komiya et al. [55], Yokota et al. [99], Yokota et al. [98] | | | | Higher | |
| | Dieude et al. [21], Handley et al. [33], Horvath et al. [38] | | | | No difference | |
| Anti-Hsp70 | Jarjour et al. [41], Minota et al. [64] | Higher | 10.4–12.5% | 0–2.8% | | |
| | Kindas-Mugge et al. [52] | No difference | 21% | 23% | | |
| | Komiya et al. [55] | | | | Higher | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Anti-Hsp90 | Conroy et al. [16], Minota et al. [65] | Higher | 26–46.7% | 0–3.6% | | Association of Hsp90 autoabs with active lupus nephritis and Hsp90 immune deposits in glomeruli of SLE patients but not HC<br>Different binding properties of anti-Hsp90 autoabs from SLE and anti-Hsp90 autoabs from HC<br>Correlation with C3 but not anti-DNA autoabs<br>Anti-Hsp90 antibodies in SLE animal model and association with onset of the disease, appearing before anti-double stranded DNA antibodies. |
| | Jarjour et al. [41] | No difference | 2.1% | 0% | | |
| Anti-calreticulin | Eggleton et al. [26], Routsias et al. [75] | Higher | 33.3–37.5% | 0–5.8% | | Anti-calreticulin autoabs detected in SLE are not high affinity or are due to non-specific interactions<br>Autoabs recognized the N-terminal protein part, several neoepitopes |
| | Boehm et al. [8], Kishore et al. [53], Sanchez et al. [79] | | | | Higher | |
| | Sanchez et al. [79] | | | | No difference of IgM | |

than venous vascular walls. This could explain why anti-Hsp60 autoabs are associated with an increased risk of arterial and not venous vascular events. The anti-Hsp60 autoab-mediated apoptosis facilitates the exposure of membrane phospholipids and glycoproteins, rendering them as targets of aPL autoabs. This represents the second hit, which ultimately initiates a thrombosis cascade [22].

The complex group of AECA purified form SLE patients sera were later confirmed to contain antibodies that bound to Hsp60 on the surface of endothelial cells under stress and that their binding was sufficient to trigger apoptosis [40]. However, preincubation with Hsp60 did not completely abolish apoptosis, indicating that AECA contain more than one autoabs with apoptosis-inducing capacity [40]. The presence of human Hsp60 autoabs and their ability to promote endothelial dysfunction and impairment of microcirculation has also been implicated in the development of neuropsychiatric SLE [50, 51]. However, the role of human Hsp60 autoabs in inducing apoptosis was seriously questioned when SLE patients were found to be positive for vasculopathy-mediating AECA but lacked Hsp60 autoabs. This further supports that autoabs targeting autoantigens others than Hsp60 are also responsible for inducing endothelial cell apoptosis [93].

### 1.1.2 Hsp70

Anti-human Hsp70 antibody prevalence in SLE patients is higher compared to healthy and disease controls [41, 64] and their titres are more elevated compared to healthy controls [55] (Table 1). One study did not find any difference in their prevalence between SLE and controls [52]. Their presence ranged from 7.5% to 38.2%.

### 1.1.3 Hsp90

Inconsistencies amongst studies do really exist related to anti-human Hsp90 antibody detection rates in SLE and control cohorts (Table 1). These autoabs have been reported to be more frequent, less frequent or at the same frequency in SLE and healthy or disease controls, including rheumatoid arthritis (RA), systemic sclerosis (SSc) and Sjogren's syndrome (SjS) [16, 41, 65], with their prevalence ranging from 0% to 46.7%.

With regard to their clinical significance, a study reported that both IgG and IgM Hsp90 autoabs inversely correlated with complement C3 levels, but did not correlate with anti-DNA autoabs [16]. A potential role for anti-human Hsp90 autoabs in lupus nephritis was suggested when IgM anti-human Hsp90 autoabs were detected in elevated titres in SLE patients with active renal disease [16]. The presence of high-titre IgG anti-human Hsp90 autoabs in the sera of active lupus nephritis patients was also reported [48]. In that study, Hsp90 autoabs were associated to Hsp90 molecules deposited in the glomeruli of SLE patients, as detected by staining of renal biopsies. Such immune-complexes deposition was not observed in non-SLE

renal biopsies. In addition, human Hsp90 autoabs isolated from lupus nephritis patients displayed differential binding capacities compared to human Hsp90 autoabs of the natural repertoire of healthy individuals and could not be regulated by idiotypic interactions [48].

The role of Hsp90 autoabs in the pathogenesis of SLE was supported by experiments in an animal lupus model Mrl/lpr [27]. Anti-Hsp90 abs were detected in Mrl/lpr mice but not in BALB/c mice. Their generation was associated with the onset of the disease, occurring at a similar time or even earlier than the generation of double stranded DNA autoabs. In addition, their generation was attributed to high levels of Hsp90, which are observed in Mrl/lpr mice and in SLE patients [84].

Another potential mechanism of anti-human Hsp90 driven SLE pathogenesis is that attributed to the functional inhibition of Hsp90. Hsp90 inhibition has been shown to dysregulate the expression of several transposable elements in monocytes and lymphocytes from SLE patients [47]. Since these DNA elements have the capacity to stimulate type I interferons [61], a group of cytokines known to be involved in SLE pathogenesis [67], Kelly et al. suggested a link between Hsp90 inhibition mediated by the respective autoabs and SLE development [47]. Consequently, it would be of interest to determine whether neutralizing Hsp90 autoabs exert an effect on interferon production.

### 1.1.4 Calreticulin

Calreticulin, a high-affinity calcium binding protein of the endoplasmic reticulum that facilitates the loading of peptides onto major histocompatibility complex (MHC) class I molecules and the folding of glycoproteins [56], has also been identified as a potential autoantigen in SLE [74].

The prevalence of anti-calreticulin autoabs in SLE patients with active disease was higher compared to healthy controls, as detected by immunoabsorption [26, 75], but comparable between SLE patients with inactive disease and healthy controls [26] (Table 1). Of interest, none of these studies reporting on this topic used full-length human calreticulin as substrate. This is a point to be taken into account since Jorgensen et al. used the full-length human calreticulin in immunoblotting experiments and demonstrated that under high stringency conditions SLE sera were totally unreactive against calreticulin, suggesting that either anti-calreticulin autoabs are of low affinity or SLE patients sera contained abs against unspecific targets [43]. However, early experiments, where pre-absorption of SLE sera with human calreticulin could abolish reactivity against calreticulin, had already provided evidence in support of the thesis that SLE patients contain anti-human calreticulin abs and that these antibodies are not a result of non-specific interactions [8, 53].

The magnitude of IgG anti-calreticulin autoabs was elevated in SLE patients compared to healthy and disease controls [8, 53, 79], while IgM anti-calreticulin autoab levels were comparable between SLE patients and healthy controls [79]. Of pathogenic significance, linear epitope mapping studies showed that multiple, non-contiguous sequence regions of calreticulin are antigenic to sera from SLE

patients with active and inactive disease status [26]. Most of these antigenic regions were part of the N-terminal portion and some of them were cryptic neoepitopes, normally hidden in the correctly folded calreticulin [26, 53].

## *1.2 Human Hsp Autoantibodies in Rheumatoid Arthritis*

RA is a chronic, autoimmune disease characterized by inflammatory, destructive polyarthritis that may also affect extra-articular organs and tissues [14, 62]. The presence of anti-citrullinated peptide antibodies (ACPAs) is a disease-specific feature [78].

### 1.2.1 Hsp60

The majority of studies on the frequency of IgM and IgG anti-human Hsp60 autoabs in RA did not report significant differences when compared to healthy controls (Table 2), their presence ranging from 5% to 27%, as detected by either immunoblotting or immunoabsorption methods [40, 41, 71, 92]. However, the frequency of IgG Hsp60 autoabs was significantly increased in a group of 110 RA patients, being present in 25% of the patients compared to none of the healthy controls [31]. An earlier study reported a much higher frequency (73%) compared to just 25% prevalence in healthy controls [30]. Regarding the titres of anti-human Hsp60 autoabs, elevated titres in RA were reported by several studies [33, 37, 55, 60, 98, 99], although several others reported comparable or even lower levels in RA patients compared to healthy controls [21, 38, 40, 58].

Of clinical interest, the levels of anti-citrullinated Hsp60 abs were increased in RA patients compared to healthy controls and positively associated with joint damage [58]. Also, the levels of human Hsp60 autoabs significantly correlated with the levels of the rheumatoid factor [92] and interleukin 4 (IL-4) in sera of RA patients [60], but did not correlate with disease activity or the levels of IL-2, IL-6 or IL-10 [37, 60].

The clinical significance of human Hsp60 autoabs for cardiovascular disease in RA has been investigated, based on findings supporting that humoral immune responses against human Hsp60 have been linked with cardiovascular disease [104] and implicated in the development of atherosclerosis [49, 96]. Anti-human Hsp60 autoabs were detected at comparable frequencies in RA patients with and without cardiovascular disease, as well as in disease and healthy controls [92]. In addition, Hsp60 autoab positivity and levels were not related to carotid arterial cell wall thickness [71], a marker of atherosclerosis that is measured by ultrasonography. Hence, anti-human Hsp60 autoabs have not been considered as a marker of cardiovascular events in RA.

The pathogenic potential of anti-citrullinated Hsp60 autoabs in RA is supported by two studies demonstrating that ACPAs isolated from RA patients could bind to

**Table 2** Cumulative findings of human heat shock-specific autoantibodies in rheumatoid arthritis

| Autoantibody | Reference(s) | IgG ab prevalence compared to HC | IgG ab prevalence in RA | IgG ab prevalence in HC | IgG ab magnitude compared to HC | Other findings |
|---|---|---|---|---|---|---|
| Anti-Hsp60 | Girouard et al. [30], Goeb et al. [31] | Higher | 25–73% | 0–25% | | Identification of Hsp60 citrullination<br>Anti-citrullinated Hsp60 induces apoptosis in osteoblasts via TLR4<br>Role for *Escherichia coli* and Mycobacterium tuberculosis in anti-Hsp60 production via cross-reactivity<br>No correlation with atherosclerosis in RA<br>Correlation with rheumatoid factor<br>Anti-Hsp60 antibodies are involved in pathogenesis in murine disease model |
| | Jamin et al. [40], Jarjour et al. [41], Pereira et al. [71], van Halm et al. [92] | No difference of IgG or IgM | 5–27% | 2.8–13% | | |
| | Handley et al. [33], Hirata et al. [37], Komiya et al. [55], Mantej et al. [60], Yokota et al. [99], Yokota et al. [98] | | | | Higher | |
| | Dieude et al. [21], Horvath et al. [38], Jamin et al. [40], Lu et al. [58] | | | | Lower or no difference | |
| | Lu et al. [58] | | | | Anti-citHsp60 higher than HC | |
| Anti-Hsp70 | Jarjour et al. [41], Minota et al. [64] | No difference | 0–5% | 0–2.8% | | |
| | Komiya et al. [55], Mantej et al. [60] | | | | Higher | |
| Anti-Hsp90 | Harlow et al. [35] | Higher | 47.6% | 0% | | Citrullination of Hsp90 unfolds the protein and reveals cryptic epitopes<br>Local production in lungs<br>Conformational or cryptic epitopes<br>Useful biomarker for pulmonary manifestation in RA |
| | Conroy et al. [16], Jarjour et al. [41], Minota et al. [65] | No difference of IgG or IgM | 0% | 0–3.6% | | |
| | Mantej et al. [60] | | | | IgG, IgM and IgA higher | |

(continued)

**Table 2** (continued)

| Autoantibody | Reference(s) | IgG ab prevalence compared to HC | IgG ab prevalence in RA | IgG ab prevalence in HC | IgG ab magnitude compared to HC | Other findings |
|---|---|---|---|---|---|---|
| Anti-calreticulin | Clarke et al. [15], Goeb et al. [31], Routsias et al. [75] | No difference | 8.2–25% | 0–5.8% | | Citrullination<br>1/3 of patients were anti-citrullinated calreticulin (+)/rheumatoid factor (−)/ACPA (−) and had higher disease activity than triple positive patients |
| | Clarke et al. [15] | | | | No difference | |
| Anti-BiP | Blass et al. [6] | Higher | 63% | 0.7% | | Citrullination<br>BiP and anti-BiP together as diagnostic marker<br>Correlation of anti-CCP, anti-citrullinated BiP and anti-Bip<br>Identification of 3 citrullinated BiP and 5 BiP epitopes; epitopes do not match<br>Levels decreased with adalimumab<br>Higher titres in synovial fluid<br>Levels associated with disease onset<br>Correlation with anti-mycobacterial Hsp70<br>BiP can prevent the induction of experimental arthritis and anti-BiP are detected in it |
| | Goeb et al. [31], Yu et al. [100] | No difference | 6.1–8.2% | 10–12% | | |
| | Bodman-Smith et al. [7], Shoda et al. [81], Shoda et al. [82], Weber et al. [95] | | | | Higher | |
| | Yu et al. [100] | | | | No difference | |

citrullinated Hsp60 localized on the surface of human mature osteoblasts in vitro using mass spectrometry [31, 58]. Furthermore, ACPAs mediated osteoblast apoptosis via binding to cell surface-expressed citrullinated Hsp60 through Toll-like receptor 4 (TLR4) signaling and stimulate IL-6 and IL-8 gene expression [58]. Both interleukins promote osteoclast proliferation and contribute to bone erosion in RA [70]. As mentioned earlier, serum levels of anti-citrullinated Hsp60 autoabs were elevated in RA and correlated with joint damage, supporting an involvement of these autoabs in the pathogenesis of RA [58]. The homologue mycobacterial Hsp65 has been detected in the synovial membrane of arthritic joints both in RA and in animal models of arthritis [19, 45] and B cells specific for bacterial Hsp65 were locally produced and expanded within the affected joints of RA patients [76]. However, Hsp60 autoab levels were decreased in synovial fluid compared to sera in RA patients, arguing against local production of antibodies in arthritic joints [37].

A study investigating molecular mimicry between bacterial and endogenous Hsp60 reported the presence of cross-reactive responses [98]. Of pathogenic relevance, pre-absorption of RA sera with *Escherichia coli* GroEL abolishes reactivity to human Hsp60 [37]. Moreover, as cited above, the levels of human Hsp60 autoabs in the sera of RA patients was increased compared to the levels in the synovial fluids.

### 1.2.2 Hsp70

The frequency of human Hsp70 autoabs in sera from RA patients ranges from 0% to 21.7% (Table 2) and does not differ from that found in healthy controls [41, 64]. In contrast, the levels of Hsp70 autoabs were found elevated in RA patients compared to healthy controls [55, 60]. In addition, the levels of anti-Hsp70 antibodies inversely correlated with tumor necrosis factor α (TNF-α) levels, but not with disease activity, IL-2, IL-6 or IL-10 levels [60].

### 1.2.3 Hsp90

The prevalence of IgM and IgG anti-human Hsp90 autoabs in the sera of RA patients did not exceed 10% and did not differ from that of healthy controls [16, 41, 65] (Table 2). A study by Harlow et al. reported a high prevalence of 47.6% of IgG anti-Hsp90 autoabs in RA patients compared to healthy controls, where these autoabs were totally absent [35]. The magnitude of IgM, IgG and IgA human Hsp90 autoab titres were elevated in the sera of RA patients compared to healthy controls [60].

With regard to clinical significance, when the frequencies of autoabs against citrullinated or native recombinant human Hsp90 subunits α and β were determined by immunoabsorption in the sera of RA patients with and without interstitial lung disease (ILD), the frequencies were significantly higher in patients with ILD [34]. In these patients, anti-citrullinated Hsp90 autoab positivity associated with increased rheumatoid factor titres and anti-cyclic citrullinated peptide (anti-CCP) autoabs, while no associations were detected with disease activity or ILD severity

[34]. Despite a modest sensitivity of 30%, anti-citrullinated Hsp90 autoabs were highly specific for RA patients with ILD compared to RA patients without ILD, disease and healthy controls, indicating the significance of these autoabs as markers of co-current pulmonary manifestations in RA [34]. A positive correlation of anti-Hsp90 autoab levels with rheumatoid factor and interferon γ levels in the sera of RA patients, but not IL-2, IL-6 or IL-10 levels, has also been reported [60].

Of particular interest, the frequency of both IgM and IgG anti-citrullinated Hsp90 autoabs was significantly higher in the bronchoalveolar lavage fluid of RA patients compared to healthy controls, indicating an RA-specific, localized generation and action of these autoabs within the pulmonary mesenchyme [35]. These autoabs recognized conformational epitopes on the Hsp90 molecule, or cryptic linear epitopes, based on the finding that more than half of the seropositive RA patients recognized either the full length citrullinated Hsp90 protein or citrullinated Hsp90-derived peptides, but not both [35]. Using a proteomic and epitope-matching approach, Travers et al. supported the cryptic nature of Hsp90 epitopes by demonstrating that citrullination of Hsp90 in multiple arginine residues could induce the unfolding of this protein, thereby revealing cryptic, highly immunogenic epitopes [90]. The exposure of these pivotal immunogenic sites could potentially lead to the breakdown of B cell tolerance towards citrullinated Hsp90 and participate in the autoimmune pathogenesis of RA.

### 1.2.4 Calreticulin

The prevalence of anti-calreticulin autoabs in the sera of RA patients was moderate (Table 2), ranging from 0% to 25%, and was comparable to healthy controls [15, 31, 75]. The same applied to the levels of anti-calreticulin autoabs [15]. In contrast, the levels of anti-citrullinated calreticulin autoabs were elevated in RA patients compared to healthy controls [15].

Also, anti-citrullinated calreticulin autoabs were detected by immunoabsorption in 35–37% of RA patients or patients with bronchectasias alone, but this frequency increased to 58% in RA patients with bronchectasias, indicating that the citrullinated calreticulin could possibly be involved in pathogenic processes in this particular patient cohort [15]. One third of RA patients with bronchectasias were negative for both rheumatoid factor and ACPA antibodies and had higher disease activity scores compared to triple positive patients, clearly suggesting a diagnostic and clinical relevance of these autoabs [15]. In addition, three out of four patients with bronchectasias that were positive for anti-citrullinated calreticulin autoabs later developed RA, also suggesting their prognostic pathogenic role for these autoabs in RA [15].

### 1.2.5 BiP

Immunoglobulin heavy-chain-binding protein (BiP) or 78 kDa glucose-related protein (Grp78) is a heat shock protein of the endoplasmic reticulum, acting as an intracellular chaperone, correctly folding and assembly of nascent polypeptides, unfolded or denatured proteins [69]. BiP has been identified as an autoantigen in RA [17]. By immunoblotting using BiP purified from HeLa cells, Blass et al. reported high sensitivity (63%) and specificity (96%) of human BiP autoabs in the sera of 400 RA patients [6] (Table 2). However, two concomitant studies were not able to reproduce these findings and reported comparable frequencies of BiP autoabs in RA patients and healthy controls [31, 100]. With regards to the magnitude of BiP autoabs, several studies reported significantly elevated levels in RA patients compared to healthy controls [7, 81, 82, 95], while only one study found comparable levels between RA patients and controls [100].

The levels of human BiP autoab titres were stable over time and positively correlated with the levels of autoabs against other Hsp, namely calnexin and Grp94, but no correlation was found with rheumatoid factor levels, disease activity or human leukocyte antigen (HLA)-susceptibility alleles [95]. The data on the correlation of BiP autoab levels with anti-CCP levels are ambiguous, with one study reporting a positive correlation [100] and another no correlation [95].

Autoabs against two post-translational modifications on the BiP molecule, citrullination and carbamylation, have shown diagnostic and clinical significance. Anti-citrullinated BiP autoab levels were significantly elevated in RA patients compared to SLE patients and healthy controls and were associated with anti-CCP positivity [81]. Similarly, anti-carbamylated BiP autoab levels were also increased in RA patients compared to healthy controls [100].

Of pathogenic relevance, the binding of BiP autoabs to BiP localized on the surface of RA patients-derived macrophages elicited a pro-inflammatory TNF-α production and nuclear factor κB (NF-κB) activation [57]. BiP can be overexpressed in synovial tissue of RA patients under stress conditions, localized both in the nucleus and on the cell membrane, and ACPA isolated from RA patients can bind to it [6]. Also, both anti-BiP and anti-citrullinated levels positively correlated with the levels of anti-mycobacterial Hsp70 ab levels, suggesting humoral cross-reactivity and potential involvement of molecular mimicry [82]. Based on the aforementioned data and on the fact that administration of BiP can prevent the development of experimental arthritis as well as that BiP autoabs are detected at early stages of experimental arthritis [17], anti-BiP and/or citrullinated BiP autoabs, either cross-reactive or generated locally at arthritic joints, may have a prominent role in the pathogenesis of RA.

## *1.3 Human Hsp Autoantibodies in Systemic Sclerosis*

SSc is a complex connective tissues disease characterized by extensive fibrosis of skin and of internal organs, microvasculopathy and the production of a variety of disease-specific and disease-related autoabs [77].

### 1.3.1 Hsp60

The presence of human Hsp60 autoabs (Table 3), as detected both by immunoblotting and by immunoabsorption, has been reported infrequent in sera of SSc patients [40, 41] Their levels were elevated [37, 38, 55] or comparable to healthy controls [21, 40], although the three studies reporting higher magnitude in SSc patients either showed a trend for statistical difference or were performed in small disease cohorts. Their levels did not show any correlation with disease activity [37]. However, elevated levels of anti-human Hsp60 autoabs were detected in undifferentiated connective tissue disease (UCTD) [38], a systemic disorder with clinical characteristics from various autoimmune rheumatic diseases of the connective tissue, including SSc [66]. It becomes apparent that anti-human Hsp60 should be further investigated to determine their significance.

### 1.3.2 Hsp70

Two studies have investigated the frequency of IgG and IgM anti-human Hsp70 autoabs in SSc patients (Table 3), with one showing a prevalence of 41% [28] and the other reporting much lower prevalence (IgM: 0%; IgG: 6.3%) that was comparable to healthy controls [41]. The levels of Hsp70 autoabs appeared to be elevated in SSc patients compared to healthy controls, but the significance was not explicitly stated [28]. Therefore, a potential role for anti-Hsp70 autoabs in SSc cannot be excluded.

Of interest, a recent study has proposed the involvement of autoabs against human Hsp70 in the pathogenesis of pulmonary arterial hypertension (PAH) in SSc, after detecting Hsp70 as a target of anti-fibroblast-specific autoabs [87]. Anti-fibroblast autoabs have been detected in 30% of SSc patients with PAH [85]. These autoabs have the capacity to induce the activation of fibroblasts [103], to promote a pro-inflammatory and pro-adhesive state acquisition, the production of collagen type I and the conversion of human fibroblasts to myofibroblasts [13], as well as to stimulate the generation of reactive oxygen species [4].

**Table 3** Cumulative findings of human heat shock-specific autoantibodies in systemic sclerosis

| Autoantibody | Reference(s) | IgG ab prevalence compared to HC | IgG ab prevalence in SSc | IgG ab prevalence in HC | IgG ab magnitude compared to HC | Other findings |
|---|---|---|---|---|---|---|
| Anti-Hsp60 | Jamin et al. [40], Jarjour et al. [41] | No difference | 6.3–10% | 2.8–10% | | |
| | Hirata et al. [37], Horvath et al. [38], Komiya et al. [55] | | | | Higher[a] | |
| | Dieude et al. [21], Jamin et al. [40] | | | | No difference | |
| Anti-Hsp70 | Fujimoto et al. [28] | Higher | 41% | 0% | Higher | Potential role in PAH pathogenesis |
| | Jarjour et al. [41] | No difference | 6.3% | 2.8% | | |
| Anti-Hsp90 | Conroy et al. [16], Jarjour et al. [41], Minota et al. [65] | No difference | 0% | 0–3.6 | No difference | |
| Anti-Hsp47 | Fujimoto et al. [29] | | | | Higher | No difference between diffuse cutaneous SSc and limited cutaneous SSc<br>Could potentially block the beneficial effects of Hsp47 and lead to skin fibrosis |

[a]Report of trend for statistical significance or study performed in small cohorts

### 1.3.3 Hsp90

With regard to autoabs against human Hsp90 in SSc (Table 3), three studies have reported zero or low frequency and low levels in the sera of patients [16, 41, 65]. Therefore, Hsp90 autoabs do not seem to have a role in SSc.

### 1.3.4 Hsp47

Heat shock protein 47 (Hsp47) is a molecular chaperone localized in the endoplasmic reticulum. Hsp47 is expressed by collagen-expressing cells, such as fibroblasts, and is indispensable for the synthesis, assembly and secretion of collagen [86]. IgG anti-Hsp47 autoabs were detected by immunoabsorption in 26% of SSc patients and their levels were significantly elevated in SSc patients compared to disease and healthy controls [29] (Table 3). Their frequency and levels were similar between limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) SSc patients. IgM anti-Hsp47 autoabs were not detected in SSc patients. Anti-Hsp47 positivity was not associated with clinical features of the disease nor with the presence of SSc-related autoabs. However, Hsp47 autoab positive patients had a significantly shorter disease duration compared to autoab negative patients, suggesting that Hsp47 autoabs are present only at the early phase of SSc [29]. Anti-Hsp47 abs were also detected in a murine model of SSc [29]. An important limitation of this study was that rat and not human Hsp47 was used as a substrate for detecting anti-Hsp47 in the immunoabsorption experiments, even though the homology between the two homologues surpasses 95% by basic local alignment search tool (BLAST). Considering these findings, it would be interesting to explore whether the induction of Hsp47 autoabs is associated with fibrosis, as observed in SSc by excessive collagen deposition, by interfering with the function of Hsp47 and collagen homeostasis.

## *1.4 Human Hsp Autoantibodies in Sjogren's Syndrome*

SjS is an autoimmune disease characterized by inflammation of exocrine glands, mainly salivary and lacrimal glands that leads to their destruction [88].

### 1.4.1 Hsp60

Two independent studies did not detect a significant difference in the prevalence of IgM and IgG anti-human Hsp60 autoabs in the sera of SjS patients compared to healthy controls (Table 4), ranging from 4% to 20% [40, 41]. Data on the magnitude of anti-Hsp60 autoabs did not reach a consensus, with three reporting elevated levels compared to healthy controls [37, 55, 99], two reporting comparable levels [40, 98]

and one study demonstrating significantly decreased levels in SjS patients [83]. Also, their levels did not correlate with disease activity [37].

### 1.4.2 Hsp70

Jarjour et al. reported that human IgM or IgG Hsp70 autoabs in SjS are present in 8% and that their prevalence is comparable to that noted in healthy controls [41] (Table 4).

### 1.4.3 Hsp90

Similarly, the frequency of IgM or IgG anti-human Hsp90 autoabs in SjS did not exceed 12% and was similar to healthy controls, while their titres were lower than disease controls [16, 41] (Table 4).

## *1.5 Human Hsp Autoantibodies in Behçet's Disease*

Adamantiades-Behçet's disease (BD) is a systemic, inflammatory disorder characterized by recurrent and acute inflammation manifested as oral apthous ulcers, genital ulcers, uveitis, acne-like skin lesions, and vasculitis [102].

### 1.5.1 Hsp60

IgG human Hsp60 autoabs are present in 22% of DB patients by immunoabsorption and 44% by immunoblotting (Table 5), their prevalence being higher compared to healthy controls [40]. The levels of IgG or IgA Hsp60 autoabs were increased in BD patients compared to healthy controls [23, 40, 55], although one study did not find any difference [37]. Direskeneli et al. demonstrated that both IgG and IgA autoabs targeting a peptide corresponding to aa 136–150 human Hsp60 were increased during relapses of ocular manifestations of BD compared to those in remission and that their levels were not affected by treatment [23]. Of interest, the aforementioned B cell epitope overlaps with the T cell epitope on the Hsp60 molecule [72]. However, the correlation between Hsp60 autoab levels and disease activity were not validated in another study [37]. Higher levels of these autoabs were detected in resting saliva of BD patients with moderate disease compared to patients with mild or severe disease and higher levels were associated with stomatitis for more than 2 weeks and with gingival inflammation [24].

**Table 4** Cumulative findings of human heat shock-specific autoantibodies in Sjogren's syndrome

| Autoantibody | Reference(s) | IgG ab prevalence compared to HC | IgG ab prevalence in SjS | IgG ab prevalence in HC | IgG ab magnitude compared to HC | Other findings |
|---|---|---|---|---|---|---|
| Anti-Hsp60 | Jamin et al. [40], Jarjour et al. [41] | No difference of IgG and IgM | 8–20% | 2.8–10% | | No correlation with disease activity |
| | Hirata et al. [37], Komiya et al. [55], Yokota et al. [99] | | | | Higher | |
| | Jamin et al. [40], Yokota et al. [98] | | | | No difference | |
| | Shovman et al. [83] | | | | Lower | |
| Anti-Hsp70 | Jarjour et al. [41] | No difference of IgG or IgM | 0% | 2.8% | No difference of IgG or IgM | |
| Anti-Hsp90 | Conroy et al. [16], Jarjour et al. [41] | No difference of IgG or IgM | 0–2.8% | 4–11% | IgG or IgM lower than HC | |

**Table 5** Cumulative findings of human heat shock-specific autoantibodies in Behçet's disease

| Autoantibody | Reference(s) | IgG ab prevalence compared to HC | IgG ab prevalence in BD | IgG ab prevalence in HC | IgG ab magnitude compared to HC | Other findings |
|---|---|---|---|---|---|---|
| Anti-Hsp60 | Jamin et al. [40] | Higher | 44% by Western and 22% by ELISA | <10% | | B cell epitope 136–150aa |
| | Direskeneli et al. [23], Jamin et al. [40], Komiya et al. [55] | | | | IgG or IgA higher than HC | Autoabs elevated in relapses of ocular BD |
| | Hirata et al. [37] | | | | No difference | |
| Anti-Hsp70 | Birtas-Atesoglu et al. [5], de Smet and Ramadan, (2001) | | | | Higher | |
| Anti-Hsp90 | No reports | | | | | |
| Anti-αB-crystallin | Celet et al. [10] | | | | | |
| Anti-Hsp27 | Chen et al. [11] | Higher | 57% | 0% | Higher | |

### 1.5.2 Hsp70

Data on the prevalence of anti-human Hsp70 autoabs in BD patients are still lacking (Table 5). The serum levels of these autoabs were significantly increased in BD patients compared to healthy controls [5, 20]. Hsp70 autoab levels did not correlate with Hsp70 levels in the sera of BD patients, in contrast to healthy controls, and were not linked with age, disease duration, disease activity or treatment [5].

### 1.5.3 αB-Crystallin

αB-crystallin is a small Hsp with anti-apoptotic and thermoprotecting functions, localized in the cytoplasm, but under stress conditions, the protein accumulates and can also be found in the nucleus [54]. Increased αB-crystallin has been associated with neurological diseases, including Alzheimer's disease [25, 39]. With regard to BD (Table 5), IgM and IgG anti-αB-crystallin autoabs were detected in higher titres both in the sera and in the cerebrospinal fluid of BD patients with neurological involvement compared to patients with inflammatory and non-inflammatory diseases of the central nervous system [10]. Although the authors of this study detected antibodies against αB-crystallin with an immunoabsorption method using bovine αB-crystallin, this is highly homologous with the human αB-crystallin, with four amino acid sequence difference.

### 1.5.4 Hsp27

Hsp27 is a small protein chaperone with anti-oxidant and anti-apoptotic functions, that also facilitates the refolding of denatured proteins [94]. Human Hsp27 autoabs were detected in 57% BD patients compared to 0% in healthy controls and their titres were higher compared to disease controls [11] (Table 5). By proteomic methods, Hsp27 was identified as a vascular endothelial autoantigen in patients with BD, but also in other autoimmune rheumatic diseases, including RA, SjS and SLE. Anti-Hsp27 autoabs may reduce the beneficial action of Hsp27 to endothelial cells, thereby promoting their dysfunction and, subsequent inflammatory autoimmune response.

## 2 Conclusions

Practically, all human Hsp are antigens of self-targeted immune responses. These autoimmune responses are noted in various patients with autoimmune rheumatic diseases, their prevalence ranging from one disease to another and amongst studies. The titers of anti-Hsp antibodies differ amongst patients but it is not clear whether

the magnitude of the autoabs or indeed their presence holds true clinical significance. The exact mechanisms by which these human Hsp become targets of antibody responses is less well-defined but the most studied is that assessing the role of molecular mimicry between bacterial and human Hsp. Larger, multi-centre studies investigating the diagnostic and clinical significance of these autoabs in pre-clinical or early disease states must be done. For the Hsp that substantiate a clinically meaningful role more studies on their pathogenic role in the induction of autoimmunity must be performed.

**Acknowledgements** G.E. is supported by a PhD scholarship funded by the Hellenic Scholarship Foundation (IKY, Greece). L.I.S. received lecture honoraria from Actelion, Janssen, Novartis, Sanofi Aventis, Abbvie and Roche and a grant from Bristol Meyers Squib to support the educational and research activities of the department. None of this funding relates to the scientific content of this chapter. D.P.B. received lecture honoraria/advisory board participation from Novartis, Fresenius Kabi and Roche and grants from Genesis, Aenorasis and Elpen to support the educational and research activities of the department.

**Disclosure of Interests** None of this funding relates to the scientific content of this chapter.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Alard JE, Hillion S, Guillevin L et al (2011) Autoantibodies to endothelial cell surface ATP synthase, the endogenous receptor for hsp60, might play a pathogenic role in vasculatides. PLoS One 6:e14654
2. Al-Hashimi AA, Caldwell J, Gonzalez-Gronow M et al (2010) Binding of anti-GRP78 autoantibodies to cell surface GRP78 increases tissue factor procoagulant activity via the release of calcium from endoplasmic reticulum stores. J Biol Chem 285:28912–28923
3. Baird NA, Turnbull DW, Johnson EA (2006) Induction of the heat shock pathway during hypoxia requires regulation of heat shock factor by hypoxia-inducible factor-1. J Biol Chem 281:38675–38681
4. Baroni SS, Santillo M, Bevilacqua F et al (2006) Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med 354:2667–2676
5. Birtas-Atesoglu E, Inanc N, Yavuz S, Ergun T, Direskeneli H (2008) Serum levels of free heat shock protein 70 and anti-HSP70 are elevated in Behcet's disease. Clin Exp Rheumatol 26: S96–S98
6. Blass S, Union A, Raymackers J et al (2001) The stress protein BiP is overexpressed and is a major B and T cell target in rheumatoid arthritis. Arthritis Rheum 44:761–771
7. Bodman-Smith MD, Corrigall VM, Berglin E et al (2004) Antibody response to the human stress protein BiP in rheumatoid arthritis. Rheumatology 43:1283–1287
8. Boehm J, Orth T, Van Nguyen P, Soling HD (1994) Systemic lupus erythematosus is associated with increased auto-antibody titers against calreticulin and grp94, but calreticulin is not the Ro/SS-A antigen. Eur J Clin Investig 24:248–257

9. Cao Y, Ohwatari N, Matsumoto T, Kosaka M, Ohtsuru A, Yamashita S (1999) TGF-beta1 mediates 70-kDa heat shock protein induction due to ultraviolet irradiation in human skin fibroblasts. Pflugers Archiv Eur J Physiol 438:239–244
10. Celet B, Akman-Demir G, Serdaroglu P et al (2000) Anti-alpha B-crystallin immunoreactivity in inflammatory nervous system diseases. J Neurol 247:935–939
11. Chen P, Shi L, Jiang Y et al (2015) Identification of heat shock protein 27 as a novel autoantigen of Behcet's disease. Biochem Biophys Res Commun 456:866–871
12. Cheng MY, Hartl FU, Horwich AL (1990) The mitochondrial chaperonin hsp60 is required for its own assembly. Nature 348:455–458
13. Chizzolini C, Raschi E, Rezzonico R et al (2002) Autoantibodies to fibroblasts induce a proadhesive and proinflammatory fibroblast phenotype in patients with systemic sclerosis. Arthritis Rheum 46:1602–1613
14. Choy EH, Panayi GS (2001) Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 344:907–916
15. Clarke A, Perry E, Kelly C et al (2017) Heightened autoantibody immune response to citrullinated calreticulin in bronchiectasis: implications for rheumatoid arthritis. Int J Biochem Cell Biol 89:199–206
16. Conroy SE, Faulds GB, Williams W, Latchman DS, Isenberg DA (1994) Detection of autoantibodies to the 90 kDa heat shock protein in systemic lupus erythematosus and other autoimmune diseases. Br J Rheumatol 33:923–926
17. Corrigall VM, Bodman-Smith MD, Fife MS et al (2001) The human endoplasmic reticulum molecular chaperone BiP is an autoantigen for rheumatoid arthritis and prevents the induction of experimental arthritis. J Immunol 166:1492–1498
18. Daugaard M, Rohde M, Jaattela M (2007) The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. FEBS Lett 581:3702–3710
19. de Graeff-Meeder ER, Rijkers GT, Voorhorst-Ogink MM et al (1993) Antibodies to human HSP60 in patients with juvenile chronic arthritis, diabetes mellitus, and cystic fibrosis. Pediatr Res 34:424–428
20. de Smet MD, Ramadan A (2001) Circulating antibodies to inducible heat shock protein 70 in patients with uveitis. Ocul Immunol Inflamm 9:85–92
21. Dieude M, Senecal JL, Raymond Y (2004) Induction of endothelial cell apoptosis by heat-shock protein 60-reactive antibodies from anti-endothelial cell autoantibody-positive systemic lupus erythematosus patients. Arthritis Rheum 50:3221–3231
22. Dieude M, Correa JA, Neville C et al (2011) Association of autoantibodies to heat-shock protein 60 with arterial vascular events in patients with antiphospholipid antibodies. Arthritis Rheum 63:2416–2424
23. Direskeneli H, Hasan A, Shinnick T et al (1996) Recognition of B-cell epitopes of the 65 kDa HSP in Behcet's disease. Scand J Immunol 43:464–471
24. Doino M, Yokoyama M, Sasaki Y, Kondo K, Yasuda Y, Arakawa S (2017) Evaluation of the relationship between salivary concentration of anti-heat shock protein immunoglobulin and clinical manifestations of Behcet's disease. Scand J Rheumatol 46:381–387
25. Dulle JE, Fort PE (2016) Crystallins and neuroinflammation: the glial side of the story. Biochim Biophys Acta 1860:278–286
26. Eggleton P, Ward FJ, Johnson S et al (2000) Fine specificity of autoantibodies to calreticulin: epitope mapping and characterization. Clin Exp Immunol 120:384–391
27. Faulds G, Conroy S, Madaio M, Isenberg D, Latchman D (1995) Increased levels of antibodies to heat shock proteins with increasing age in Mrl/Mp-lpr/lpr mice. Br J Rheumatol 34:610–615
28. Fujimoto M, Sato S, Ihn H, Takehara K (1995) Autoantibodies to the heat-shock protein hsp73 in localized scleroderma. Arch Dermatol Res 287:581–585
29. Fujimoto M, Hamaguchi Y, Yazawa N, Komura K, Takehara K, Sato S (2004) Autoantibodies to a collagen-specific molecular chaperone, heat-shock protein 47, in systemic sclerosis. Clin Exp Immunol 138:534–539

30. Girouard L, Laux DC, Jindal S, Nelson DR (1993) Immune recognition of human Hsp60 by Lyme disease patient sera. Microb Pathog 14:287–297
31. Goeb V, Thomas-L'Otellier M, Daveau R et al (2009) Candidate autoantigens identified by mass spectrometry in early rheumatoid arthritis are chaperones and citrullinated glycolytic enzymes. Arthritis Res Ther 11:R38
32. Gupta RS (1990) Sequence and structural homology between a mouse T-complex protein TCP-1 and the 'chaperonin' family of bacterial (GroEL, 60-65 kDa heat shock antigen) and eukaryotic proteins. Biochem Int 20:833–841
33. Handley HH, Yu J, Yu DT, Singh B, Gupta RS, Vaughan JH (1996) Autoantibodies to human heat shock protein (hsp)60 may be induced by Escherichia coli groEL. Clin Exp Immunol 103:429–435
34. Harlow L, Rosas IO, Gochuico BR et al (2013) Identification of citrullinated hsp90 isoforms as novel autoantigens in rheumatoid arthritis-associated interstitial lung disease. Arthritis Rheum 65:869–879
35. Harlow L, Gochuico BR, Rosas IO et al (2014) Anti-citrullinated heat shock protein 90 antibodies identified in bronchoalveolar lavage fluid are a marker of lung-specific immune responses. Clin Immunol 155:60–70
36. Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381:571–579
37. Hirata D, Hirai I, Iwamoto M et al (1997) Preferential binding with Escherichia coli hsp60 of antibodies prevalent in sera from patients with rheumatoid arthritis. Clin Immunol Immunopathol 82:141–148
38. Horvath L, Czirjak L, Fekete B et al (2001) Levels of antibodies against C1q and 60 kDa family of heat shock proteins in the sera of patients with various autoimmune diseases. Immunol Lett 75:103–109
39. Iwaki T, Wisniewski T, Iwaki A et al (1992) Accumulation of alpha B-crystallin in central nervous system glia and neurons in pathologic conditions. Am J Pathol 140:345–356
40. Jamin C, Dugue C, Alard JE et al (2005) Induction of endothelial cell apoptosis by the binding of anti-endothelial cell antibodies to Hsp60 in vasculitis-associated systemic autoimmune diseases. Arthritis Rheum 52:4028–4038
41. Jarjour WN, Jeffries BD, Davis JS, Welch WJ, Mimura T, Winfield JB (1991) Autoantibodies to human stress proteins. A survey of various rheumatic and other inflammatory diseases. Arthritis Rheum 34:1133–1138
42. Jones DB, Coulson AF, Duff GW (1993) Sequence homologies between hsp60 and autoantigens. Immunol Today 14:115–118
43. Jorgensen CS, Hansen KB, Jacobsen S et al (2005) Absence of high-affinity calreticulin autoantibodies in patients with systemic rheumatic diseases and coeliac disease. Scand J Clin Lab Invest 65:403–412
44. Kapsimali VD, Kanakis MA, Vaiopoulos GA, Kaklamanis PG (2010) Etiopathogenesis of Behcet's disease with emphasis on the role of immunological aberrations. Clin Rheumatol 29:1211–1216
45. Karlsson-Parra A, Soderstrom K, Ferm M, Ivanyi J, Kiessling R, Klareskog L (1990) Presence of human 65 kD heat shock protein (hsp) in inflamed joints and subcutaneous nodules of RA patients. Scand J Immunol 31:283–288
46. Kaul A, Gordon C, Crow MK et al (2016) Systemic lupus erythematosus. Nat Rev Dis Primers 2:16039
47. Kelly M, Lihua S, Zhe Z et al (2018) Transposable element dysregulation in systemic lupus erythematosus and regulation by histone conformation and Hsp90. Clin Immunol 197:6–18
48. Kenderov A, Minkova V, Mihailova D et al (2002) Lupus-specific kidney deposits of HSP90 are associated with altered IgG idiotypic interactions of anti-HSP90 autoantibodies. Clin Exp Immunol 129:169–176
49. Kilic A, Mandal K (2012) Heat shock proteins: pathogenic role in atherosclerosis and potential therapeutic implications. Autoimmune Dis 2012:502813

50. Kimura A, Sakurai T, Tanaka Y et al (2008) Proteomic analysis of autoantibodies in neuropsychiatric systemic lupus erythematosus patient with white matter hyperintensities on brain MRI. Lupus 17:16–20
51. Kimura A, Sakurai T, Yamada M et al (2012) Elevated anti-heat shock protein 60 antibody titer is related to white matter hyperintensities. J Stroke Cerebrovasc Dis 21:305–309
52. Kindas-Mugge I, Steiner G, Smolen JS (1993) Similar frequency of autoantibodies against 70-kD class heat-shock proteins in healthy subjects and systemic lupus erythematosus patients. Clin Exp Immunol 92:46–50
53. Kishore U, Sontheimer RD, Sastry KN et al (1997) The systemic lupus erythematosus (SLE) disease autoantigen-calreticulin can inhibit C1q association with immune complexes. Clin Exp Immunol 108:181–190
54. Klemenz R, Frohli E, Steiger RH, Schafer R, Aoyama A (1991) Alpha B-crystallin is a small heat shock protein. Proc Natl Acad Sci U S A 88:3652–3656
55. Komiya I, Arimura Y, Nakabayashi K et al (2011) Increased concentrations of antibody against heat shock protein in patients with myeloperoxidase anti-neutrophil cytoplasmic autoantibody positive microscopic polyangiitis. Microbiol Immunol 55:531–538
56. Li Z, Srivastava P (2004) Heat-shock proteins. Curr Protocs Immunol Appendix 1:Appendix 1T
57. Lu MC, Lai NS, Yu HC, Huang HB, Hsieh SC, Yu CL (2010) Anti-citrullinated protein antibodies bind surface-expressed citrullinated Grp78 on monocyte/macrophages and stimulate tumor necrosis factor alpha production. Arthritis Rheum 62:1213–1223
58. Lu MC, Yu CL, Yu HC, Huang HB, Koo M, Lai NS (2016) Anti-citrullinated protein antibodies promote apoptosis of mature human Saos-2 osteoblasts via cell-surface binding to citrullinated heat shock protein 60. Immunobiology 221:76–83
59. Makhnevych T, Houry WA (2012) The role of Hsp90 in protein complex assembly. Biochim Biophys Acta 1823:674–682
60. Mantej J, Polasik K, Piotrowska E, Tukaj S (2019) Autoantibodies to heat shock proteins 60, 70, and 90 in patients with rheumatoid arthritis. Cell Stress Chaperones 24:283–287
61. Mavragani CP, Sagalovskiy I, Guo Q et al (2016) Expression of long interspersed nuclear element 1 Retroelements and induction of type I interferon in patients with systemic autoimmune disease. Arthritis Rheum 68:2686–2696
62. McInnes IB, Schett G (2011) The pathogenesis of rheumatoid arthritis. N Engl J Med 365:2205–2219
63. Meroni PL, D'Cruz D, Khamashta M, Youinou P, Hughes GR (1996) Anti-endothelial cell antibodies: only for scientists or for clinicians too? Clin Exp Immunol 104:199–202
64. Minota S, Cameron B, Welch WJ, Winfield JB (1988a) Autoantibodies to the constitutive 73-kD member of the hsp70 family of heat shock proteins in systemic lupus erythematosus. J Exp Med 168:1475–1480
65. Minota S, Koyasu S, Yahara I, Winfield J (1988b) Autoantibodies to the heat-shock protein hsp90 in systemic lupus erythematosus. J Clin Invest 81:106–109
66. Mosca M, Tani C, Vagnani S, Carli L, Bombardieri S (2014) The diagnosis and classification of undifferentiated connective tissue diseases. J Autoimmun 48–49:50–52
67. Munroe ME, Lu R, Zhao YD et al (2016) Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. Ann Rheum Dis 75:2014–2021
68. Neuhaus-Steinmetz U, Rensing L (1997) Heat shock protein induction by certain chemical stressors is correlated with their cytotoxicity, lipophilicity and protein-denaturing capacity. Toxicology 123:185–195
69. Panayi GS, Corrigall VM (2014) Immunoglobulin heavy-chain-binding protein (BiP): a stress protein that has the potential to be a novel therapy for rheumatoid arthritis. Biochem Soc Trans 42:1752–1755

70. Pathak JL, Bakker AD, Verschueren P et al (2015) CXCL8 and CCL20 enhance Osteoclastogenesis via modulation of cytokine production by human primary osteoblasts. PLoS One 10:e0131041
71. Pereira I, Laurindo I, Burlingame R et al (2008) Auto-antibodies do not influence development of atherosclerotic plaques in rheumatoid arthritis. Joint Bone Spine 75:416–421
72. Pervin K, Childerstone A, Shinnick T et al (1993) T cell epitope expression of mycobacterial and homologous human 65-kilodalton heat shock protein peptides in short term cell lines from patients with Behcet's disease. J Immunol 151:2273–2282
73. Renaudineau Y, Dugue C, Dueymes M, Youinou P (2002) Antiendothelial cell antibodies in systemic lupus erythematosus. Autoimmun Rev 1:365–372
74. Rokeach LA, Haselby JA, Meilof JF et al (1991) Characterization of the autoantigen calreticulin. J Immunol 147:3031–3039
75. Routsias JG, Tzioufas AG, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM (1993) Calreticulin synthetic peptide analogues: anti-peptide antibodies in autoimmune rheumatic diseases. Clin Exp Immunol 91:437–441
76. Rudolphi U, Rzepka R, Batsford S et al (1997) The B cell repertoire of patients with rheumatoid arthritis. II. Increased frequencies of IgG+ and IgA+ B cells specific for mycobacterial heat-shock protein 60 or human type II collagen in synovial fluid and tissue. Arthritis Rheum 40:1409–1419
77. Sakkas LI, Bogdanos DP (2016) The role of B cells in the pathogenesis of systemic sclerosis. Isr Med Assoc J 18:516–519
78. Sakkas LI, Bogdanos DP, Katsiari C, Platsoucas CD (2014) Anti-citrullinated peptides as autoantigens in rheumatoid arthritis-relevance to treatment. Autoimmun Rev 13:1114–1120
79. Sanchez D, Tuckova L, Sebo P et al (2000) Occurrence of IgA and IgG autoantibodies to calreticulin in coeliac disease and various autoimmune diseases. J Autoimmun 15:441–449
80. Shaker O, Ay El-Deen MA, El Hadidi H, Grace BD, El Sherif H, Abdel Halim A (2007) The role of heat shock protein 60, vascular endothelial growth factor and antiphospholipid antibodies in Behcet disease. Br J Dermatol 156:32–37
81. Shoda H, Fujio K, Shibuya M et al (2011) Detection of autoantibodies to citrullinated BiP in rheumatoid arthritis patients and pro-inflammatory role of citrullinated BiP in collagen-induced arthritis. Arthritis Res Ther 13:R191
82. Shoda H, Hanata N, Sumitomo S, Okamura T, Fujio K, Yamamoto K (2016) Immune responses to Mycobacterial heat shock protein 70 accompany self-reactivity to human BiP in rheumatoid arthritis. Sci Rep 6:22486
83. Shovman O, Sherer Y, Gilbourd B et al (2005) Low levels of heat shock proteins-60 and -65 autoantibodies in Sjogren's syndrome. Isr Med Assoc J 7:778–780
84. Stephanou A, Conroy S, Isenberg DA et al (1998) Elevation of IL-6 in transgenic mice results in increased levels of the 90 kDa heat shock protein (hsp90) and the production of anti-hsp90 antibodies. J Autoimmun 11:249–253
85. Tamby MC, Humbert M, Guilpain P et al (2006) Antibodies to fibroblasts in idiopathic and scleroderma-associated pulmonary hypertension. Eur Respir J 28:799–807
86. Tasab M, Batten MR, Bulleid NJ (2000) Hsp47: a molecular chaperone that interacts with and stabilizes correctly-folded procollagen. EMBO J 19:2204–2211
87. Terrier B, Tamby MC, Camoin L et al (2008) Identification of target antigens of antifibroblast antibodies in pulmonary arterial hypertension. Am J Respir Crit Care Med 177:1128–1134
88. Tong L, Koh V, Thong BY (2017) Review of autoantigens in Sjogren's syndrome: an update. J Inflamm Res 10:97–105
89. Trautinger F, Trautinger I, Kindas-Mugge I, Metze D, Luger TA (1993) Human keratinocytes in vivo and in vitro constitutively express the 72-kD heat shock protein. J Invest Dermatol 101:334–338
90. Travers TS, Harlow L, Rosas IO et al (2016) Extensive citrullination promotes immunogenicity of HSP90 through protein unfolding and exposure of cryptic epitopes. J Immunol 197:1926–1936

91. van Eden W, Jansen MAA, Ludwig I, van Kooten P, van der Zee R, Broere F (2017) The enigma of heat shock proteins in immune tolerance. Front Immunol 8:1599
92. van Halm VP, Slot MC, Nurmohamed MT, Tervaert JW, Dijkmans BA, Voskuyl AE (2006) Antibodies against human 60 kDa heat shock protein are not associated with cardiovascular disease in patients with rheumatoid arthritis. Ann Rheum Dis 65:590–594
93. van Paassen P, Duijvestijn A, Debrus-Palmans L, Damoiseaux J, Vroomen M, Tervaert JW (2007) Induction of endothelial cell apoptosis by IgG antibodies from SLE patients with nephropathy: a potential role for anti-endothelial cell antibodies. Ann N Y Acad Sci 1108:147–156
94. Vidyasagar A, Wilson NA, Djamali A (2012) Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. Fibrogenesis Tissue Repair 5:7
95. Weber CK, Haslbeck M, Englbrecht M et al (2010) Antibodies to the endoplasmic reticulum-resident chaperones calnexin, BiP and Grp94 in patients with rheumatoid arthritis and systemic lupus erythematosus. Rheumatology 49:2255–2263
96. Wick G, Knoflach M, Xu Q (2004) Autoimmune and inflammatory mechanisms in atherosclerosis. Annu Rev Immunol 22:361–403
97. Yokota S, Fujii N (2010) Immunomodulatory activity of extracellular heat shock proteins and their autoantibodies. Microbiol Immunol 54:299–307
98. Yokota SI, Hirata D, Minota S et al (2000) Autoantibodies against chaperonin CCT in human sera with rheumatic autoimmune diseases: comparison with antibodies against other Hsp60 family proteins. Cell Stress Chaperones 5:337–346
99. Yokota S, Minota S, Fujii N (2006) Anti-HSP auto-antibodies enhance HSP-induced pro-inflammatory cytokine production in human monocytic cells via toll-like receptors. Int Immunol 18:573–580
100. Yu HC, Lai PH, Lai NS, Huang HB, Koo M, Lu MC (2016) Increased serum levels of anti-carbamylated 78-kDa glucose-regulated protein antibody in patients with rheumatoid arthritis. Int J Mol Sci 17:1510
101. Zampieri S, Iaccarino L, Ghirardello A et al (2005) Systemic lupus erythematosus, atherosclerosis, and autoantibodies. Ann N Y Acad Sci 1051:351–361
102. Zeidan MJ, Saadoun D, Garrido M, Klatzmann D, Six A, Cacoub P (2016) Behcet's disease physiopathology: a contemporary review. Autoimmun Highlights 7:4
103. Zhou X, Tan FK, Milewicz DM, Guo X, Bona CA, Arnett FC (2005) Autoantibodies to fibrillin-1 activate normal human fibroblasts in culture through the TGF-beta pathway to recapitulate the "scleroderma phenotype". J Immunol 175:4555–4560
104. Zhu J, Quyyumi AA, Rott D et al (2001) Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. Circulation 103:1071–1075

# DNAJA3, a Co-chaperone in Development and Tumorigenesis


**Wan-Huai Teo, Yu-Ning Fann, and Jeng-Fan Lo**

## Abstract

**Introduction**: DNAJA3, also known as Tid1 (Tumorous imaginal disc 1), is a mammalian mitochondrial DNAJ/HSP40 co-chaperone protein. DNAJA3 is predominantly localized in mitochondrial matrix which enables them to interact with mitochondrial HSP70 or other client proteins. This chapter summarizes the physiological and biochemical functions of DNAJA3 in tumorigenesis, development and mitochondrial homeostasis, and its underlying molecular mechanisms.

**Methods**: All literatures were systematically searched through EndNote and web search engines. The reported discoveries and our findings were specifically categorized into multiple roles of DNAJA3.

**Results**: DNAJA3 has been identified as tumor suppressor. Overexpression of DNAJA3 not only inhibits cancer cells proliferation but also induces cells death. In opposite, downregulation of DNAJA3 enhances malignant properties such as cell migration and invasion *in vitro*, and tumor growth *in vivo*. Acting as a tumor suppressor, DNAJA3 can interfere with the matrix metalloproteinase to repress the cancer metastasis. Additionally, DNAJA3 is involved in early embryogenesis, thymocyte development, muscular development and mitochondrial biogenesis in normal physiological condition. Mechanistically, DNAJA3 interacts with DNAK/

W.-H. Teo
Institute of Oral Biology, National Yang-Ming University, Taipei, Taiwan

Y.-N. Fann
Department & Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan

J.-F. Lo (✉)
Institute of Oral Biology, National Yang-Ming University, Taipei, Taiwan

Department & Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan

Cancer Progression Research Center, National Yang-Ming University, Taipei, Taiwan
e-mail: jflo@ym.edu.tw

Heat Shock Proteins (2021) 22: 307–323
https://doi.org/10.1007/7515_2020_33

Published Online: 16 September 2020

HSP70 to prevent aggregation of mitochondrial complex I and to contribute mtDNA maintenance.

**Conclusions**: Most of DNAJA3 client proteins are related to cancer. Therefore, it is of the importance of DNAJA3 to orchestrate its function in tumorigenesis. DNAJA3 also important in development and mitochondrial homeostasis.

**Keywords** DNAJA3 · HSP40 · HSP70 · Mitochondrial homeostasis · T cells development · Tid1 · Tumor suppressor

## Abbreviations

| | |
|---|---|
| APC | Adenomatous polyposis coli |
| CRC | Colorectal cancer |
| Cyt | Cytoplasmic |
| DCM | Dilated cardiomyopathy |
| EBV | Epstein-Barr virus |
| EGFR | Epidermal growth factor receptor |
| EOPD | Early onset Parkinson's |
| ER | Endoplasmic reticulum |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| hERG | Human ether-a-go-go-related gene |
| HGF | Hepatocyte growth factor |
| HNSCC | Head and neck squamous cell carcinoma |
| HSP | Heat shock protein |
| HSV | Herpes simplex virus type 1 |
| HTLV-1 | Human T cell leukemia virus type 1 |
| MEF | Mouse embryonic fibroblast |
| Mem | Membrane |
| MetR | c-Met receptor tyrosine kinase |
| Mito | Mitochondria |
| NSCLC | Non-small cell lung carcinoma |
| Opa1 | Optic atrophy 1 |
| RCC-CICs | Renal cell carcinoma cancer- initiating cells |
| Tid1 | Tumorous imaginal disc 1 |
| TNFα | Tumor necrosis factor-alpha |
| VHL | Von Hippel-Lindau protein |
| YY1 | Yin Yang 1 |

# 1 Introduction

Heat shock proteins (HSP) are also known as molecular chaperones. They play an important role as safeguard in cells. Most of them protect cells from pathological, environmental and pharmacological stress factors. The major role of HSP is to ensure the correct conformation of nascent peptides during proteins synthesis or upon cellular stress. Further, they help to prevent aggregation of misfolded proteins, and to eliminate them if unrepairable. HSP are classified according to their molecular weight such as HSP70, HSP90, HSP60 HSP40, HSP27 and HSP110. Of the HSP, HSP40, as known as DNAJ protein, contains the largest family members of HSP. It has approximately 49 members which are decoded from DNAJ genes (Kampinga et al. 2009). The DNAJ family is classified into three subclasses: DNAJA, DNAJB and DNAJC based on their similarity to *E. coli* DNAJ proteins. The J domains of HSP40 are highly conserved and through which the HSP40 functions as a co-chaperone to interact with the DNAK/HSP70. HSP40s modulate proteins synthesis, folding and membrane translocation through stimulating the ATPase activity of HSP70, then stabilize their interaction with other substrate proteins (Bascos et al. 2017). In addition to regulate proteins synthesis, HSP40 proteins also have been found to regulate other cellular signaling, and to participate in cancer development and progression. In this chapter, we discuss the physiological and pathological roles of HSP40 members especially focusing on DNAJA3, a mitochondrial DNAJ protein.

## *1.1 HSP40/DNAJs: An Overview*

HSP40/DNAJs are the co-chaperones of HSP70. They are a huge family and could be classified into three subclasses, DNAJA (Type I), DNAJB (Type II) and DNAJC (Type III). The classification of three subclasses are based on the presence or absence of glycine/phenylalanine-rich region (G/F), cysteine-repeat domain, and C-terminal domain (Cheetham and Caplan 1998; Kampinga and Craig 2010). DNAJA proteins are able to work without the interaction with HSP70 for preventing proteins aggregation and proteins binding. DNAJB members depend on the interaction with HSP70 to prevent protein degradation but not the polypeptide binding (Fan et al. 2004). DNAJC members are completely dependent on interacting with HSP70 to exert their function as co-chaperones (Fan et al. 2003; Fan et al. 2004). HSP40/DNAJs members interact with HSP70 through the binding of J domains and stimulation of ATPase activity (Mayer and Bukau 2005; Qiu et al. 2006). HSP40/DNAJs proteins are dispersed within different compartments such as nuclei (N), endoplasmic reticulum (ER), mitochondria (Mito), cytoplasmic (Cyt) and cell membrane (Mem). Although the members of HSP40 are diverse, most of their roles have not been thoroughly understood. Table 1 summarized the subcellular locations and the major roles of HSP40 members.

**Table 1** Summary of human DNAJ family members

| Family | Gene | Chromosome Location | Cellular Location | Function | References |
|---|---|---|---|---|---|
| **HSP40/ DnaJA** | **DNAJA1 (HDJ-2)** | 9p13-p12 | N/ Cyt | Inhibiting tumor growth of C6 glioblastoma cells | Meshalkina et al. (2016) |
| | **DNAJA3 (Tid1)** | 16p13.3 | Cyt/ Mito | Required for early embryonic development and T cells early development; Suppression on HBV replication in human hepatoma cells | Lo et al. 2004; Lo et al. (2005), Sohn et al. (2006) |
| | **DNAJA4** | 15q25.1 | Cyt | Interacting with SERB and enhancing cholesterol synthesis | Robichon et al. (2006) |
| | **DNAJB1** | 19p13.12 | N/Cyt | Interacting with PDCD5 and inhibiting p53 mediated apoptosis in cancer cells | Cui et al. (2015) |
| **HSP40/ DnaJB** | **DNAJB4 (HLJ1)** | 1p31.1 | Mem/ Cyt | YY1 regulating DNAJB4 expression and inhibiting cancer cells invasion. | Wang et al. (2005) |
| | **DNAJB6** | 11q24.3 | N/Cyt | Suppressing tumorigenicity and metastasis of breast cancer cells. | Mitra et al. (2008) |
| | **DNAJB8** | 3q21.3 | N/ Cyt | Maintenance of RCC CICs | Nishizawa et al. (2012) |
| | **DNAJB9 (MDG1)** | 7q31 | ER | Inhibiting pro-apoptotic function of p53 | Lee et al. (2015a, b) |
| **HSP40/ DnaJC** | **DNAJC6** | 1p31.3 | Cyt | Association with juvenile parkinsonism | Köroğlu et al. (2013) |
| | **DNAJC15 (MCJ)** | 13q14.1 | Cyt | Interacting with mitochondrial complex I of the ETC and functioning as a negative regulator of respiratory chain | Hatle et al. (2013) |
| | **DNAJC25** | 9q31.3 | Mem | Suppression on HCC | Liu et al. (2012) |

### 1.1.1 DNAJA1

HSP40 members are found to involve in cancer development. However, they might play dual roles, pro-oncogenic or anti-cancerous. DNAJA1 (HDJ-2) is found to promote colorectal cancer (CRC) cell proliferation *in vitro*, and tumor growth and metastasis *in vivo*. The HSP inhibitor, KNK437, represses the E2F transcription factor 1 (E2F1) activity activated by DNAJA1 to arrest the cell cycle. KNK437 significantly suppresses the tumor growth of DNAJA1 expressing cancer cells (Yang et al. 2019). Meshalkina, et al. show that up-regulation of DNAJA1 in C6 cells leads to aggressive tumorigenesis whereas knockdown of DNAJA1 inhibits the *in vivo*

tumor growth. In opposite, the invasiveness of C6 spheroid is enhanced along with the knockdown of DNAJA1 co-chaperone (Meshalkina et al. 2016). Strikingly, DNAJA1 could function as a double-edged sword in cancer development.

### 1.1.2 DNAJA3

DNAJA3, also known as Tid1, has been found to interact with various proteins, including the several key regulators of cellular signaling such as adenomatous polyposis coli (APC), Von Hippel-Lindau protein (VHL), p53, epidermal growth factor receptor (EGFR), Galectin 7 and etc. (Bae et al. 2005; Kurzik-Dumke and Czaja 2007; Chen et al. 2009, 2018; Qian et al. 2010; Trinh et al. 2010), in addition to the other heat shock proteins (Cheng et al. 2001; Sarkar et al. 2001; Lu et al. 2006; Kurzik-Dumke and Czaja 2007). DNAJA3 also interacts with viral proteins such as Hepatitis B virus core protein (HBV) /HBX, Epstein-Barr virus (EBV)-encoded BARF1 protein and Herpes simplex virus type 1 (HSV) /UL9 protein, Human T cell leukemia virus type 1 (HTLV-1) Tax protein and Human papilloma virus-16 (HPV-16) E7 oncoprotein (Schilling et al. 1998; Cheng et al. 2001; Eom and Lehman 2002; Sohn et al. 2006; Wang et al. 2006) (Fig. 1). DNAJA3 can act as a tumor suppressor since overexpression of Tid1 inhibits the cell proliferation, migration and invasion *in vitro*, and also suppresses the tumor growth and recurrences in head and neck squamous cell carcinoma (HNSCC) *in vivo* (Chen et al. 2009; Chen et al. 2018). Additonally, suppress of DNAJA3 protein enhances cells growth and decreases apoptosis in osteosarcoma cancer cells (Edwards and Munger 2004). Taken togather, these data support the tumor-suppressive role of DNAJA3. Apart of these, DNAJA3 also plays an important role in early embryogenesis and T cells development (Lo et al. 2004; Lo et al. 2005). The $Tid^{-/-}$ mouse embryos fail to develop and die between E4.5 and E7.5 (Lo et al. 2005). The $lck\text{-}Tid1^{f/f}$ mice, with Tid1 gene deletion specifically in early T cells, are found to develop thymic atrophy with a significant reduction in thymocytes (Lo et al. 2004).

### 1.1.3 DNAJA4

DNAJA4 is one of the members of the DNAJA family with a molecular weight around 40 kDa. In addition to its conventional functions through interacting with HSP70 (Hafizur et al. 2004), DNAJA4 is found to be involved in cholesterol biosynthesis (Robichon et al. 2006) and human ether-a-go-go-related gene (hERG) potassium channel quality control (Ahrendt and Braun 2010). There is a study reveals that DNAJA4 can interact with NS2 protein of the classical swine fever virus, and regulates the viral replication and pathogenesis (Kang et al. 2012). This implies a possible mechanistic interaction between DNAJA4 and viral proteins.

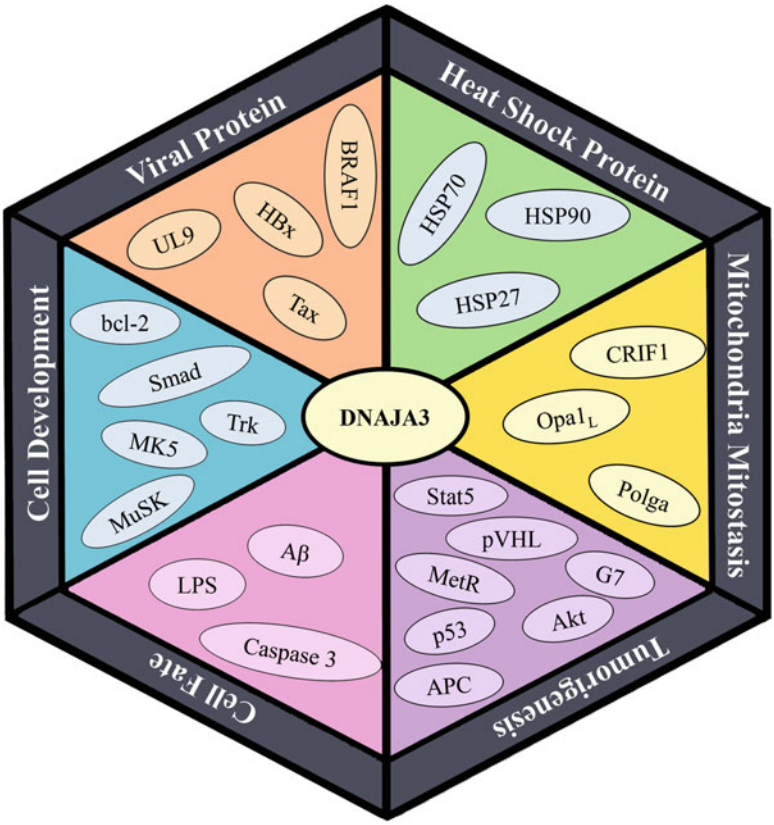



**Fig. 1** Functional roles of DNAJA3 and its interacting proteins
DNAJA3 is found to interact with diverse partners, including viral proteins, heat shock proteins, and key regulators involved in tumorigenesis, mitostasis, cell fate and cell development

### 1.1.4 DNAJB1

HSP70-DNAJB1 chaperones interaction is found to prevent the nitric oxide-induced apoptosis in COS-7 cells and macrophages (Gotoh et al. 2001, 2004). In addition, the interaction between DNAJB1and PDCD5 inhibits p53-mediated apoptosis, and knockdown of DNAJB1 suppresses proliferation of cancer cells *in vitro* (Cui et al. 2015).

### 1.1.5 DNAJB4

DNAJB4 (HLJ1) can function as a tumor suppressor by inhibiting cell proliferation, invasion and tumorigenesis, and the expression of DNAJB4 is negatively correlated with clinical prognosis in non-small cell lung carcinoma (NSCLC) patients.

Additionally, elevation of DNAJB4 in NSCLC cells causes cell cycle arrest through activating the STAT1/p21 axis (Tsai et al. 2006). Further, through the activation of transcription factor Yin Yang 1 (YY1), DNAJB4 is up-regulated to inhibit tumor invasion (Wang et al. 2005). DNAJB4 is also found to be a substrate for caspase-3, via upregulation of HLJ1, it enhances UV-induced apoptosis in NSCLC cells (Lin et al. 2010).

### 1.1.6 DNAJB6

DNAJB6 proteins consist of two isoforms, isoform DNAJB6a has 326 amino acids, whereas DNAJB6b isoform is shorter and has 242 amino acids. The isoform b lacks the carboxyl terminal but contains an additional 10 amino acids (KEQLLRLDNK) (Edo De Bock et al. 2010). DNAJB6a suppresses tumorigenesis and metastasis of breast cancer cells and its protein level is significantly lower in the aggressive breast cancer cells. It has been demonstrated that overexpression of DNAJB6a in breast cancer cells inhibits the cell migration and invasion *in vitro*. Additionally, DNAJB6a overexpression reduces orthotopic tumor growth in xenograft mice *in vivo*. DNAJB6a overexpression, again, down regulates the malignant activity and reverse the mesenchymal phenotype in breast cancer cells (Mitra et al. 2008; Mitra et al. 2010). Clinically, nuclear localization of DNAJB6 is associated with prognosis of esophageal cancer patients. Patients with low expression of DNAJB6 in nuclear have high risk of death (Yu et al. 2015).

### 1.1.7 DNAJB8

DNAJB8 seems to maintain the renal cell carcinoma cancer-initiating cells (RCC-CICs) properties. Overexpression of DNAJB8 increases the RCC-CICs sub-population cells and promotes the tumorigenesis (Nishizawa et al. 2012).

### 1.1.8 DNAJB9

DNAJB9, also known as MDG1 is located in the ER. The external perturbation such as treatment with stress inducers (tunicamycin, thapsigargin, heat, methanol, ethanol and sodium chloride) can induce DNAJB9 expression (Pröls et al. 2001; Shen et al. 2002; Berger et al. 2003). It has been reported that DNAJB9 can inhibit tunicamycin-induced cell death in SK-N-SH cells (Kurisu et al. 2003). Further, J-domain of DNAJB9 interacts with p53 to inhibit the pro-apoptotic function of p53 (Lee et al. 2015a, b).

### 1.1.9 DNAJC6

Juvenile parkinsonism, known as early onset Parkinson's (EOPD) disease, is a rare disease with syndrome onset prior to age 21 years. DNAJC6 mutations are found in EOPD patients from several countries including Arab, Turkey, Dutch and Brazil (Edvardson et al. 2012; Köroğlu et al. 2013; Elsayed et al. 2016; Olgiati et al. 2016). *DNAJC6* encodes the HSP40 Auxilin, a protein selectively expressed in neurons. DNAJC6 mutations reduce the expression of Auxilin (Eisenberg and Greene 2007; Park et al. 2015), and might impair the synaptic vesicle recycling, subsequently, to lead to the EOPD (Edvardson et al. 2012).

### 1.1.10 DNAJC15

DNAJC15 (MCJ,) is a 16 kDa HSP40/Type III family protein, and its' CpG island at the first exon is found to be methylated. Upon methylation, low protein level of DNAJC15 is expressed and would lead to chemo-resistan phenotype by modulating the permeability of mitochondria and controling the influx of anti-cancer drugs (Shridhar et al. 2001; Strathdee et al. 2004; Sinha and D'silva 2014). Additionally, DNAJC15 negatively modulates the respiratory chain activity by interacting with complex I (Hatle et al. 2013).

### 1.1.11 DNAJC25

DNAJC25 is identified as tumor suppressor, and it is localized in the cytoplasm. In hepatocellular carcinoma (HCC), the expression of DNAJC25 is significantly downregulated in cancer tissues compared to adjacent normal tissues. Overexpression of DNAJC25 not only inhibits the growth of cancer cells colony but also increases the cell apoptosis (Liu et al. 2012).

HSP40 members have distinct roles in regulating the cellular signaling and physiological functions. They are associated with various diseases including cancer initiation, cancer progress and physiological processes. In the followings, we would like to discuss more about DNAJA3 on its functional roles in different clinical pathophysiology.

### 1.1.12 DNAJA3 (Tid1)

DNAJA3 is first identified by a yeast-two hybrid screening where DNAJA3 is found to form a complex with the human papillomavirus E7 oncoprotein. DNAJA3/Tid1 is the mammalian homolog to the *Drosophila* tumor suppressor protein Tid56. DNAJA3 contains a conserve J-domain which is the characteristic of the DNAJ family proteins. It interacts with HSP70 family numbers via stimulation of the

ATPase activity (Schilling et al. 1998). Human *DNAJA3* gene is located at chromosome 16p13.3 with a size about 34 kb. *DNAJA3* gene is composed of 12 exons and separated by 11 introns (Yin and Rozakis-Adcock 2001). *DNAJA3* gene encodes two alternative splice forms, Tid1-L and Tid1-S, which respectively responsible in encoding two cytosolic (hTid50 and hTid48) and the other two mitochondrial (hTid43 and hTid40) proteins. Tid1-L fully incorporates all exons and Tid1-S decoded from an in-frame deletion of 50 amino acids which correspond precisely to exon 5 (Yin and Rozakis-Adcock 2001; Kurzik-Dumke and Czaja 2007). Tid1-L is found to have a greater cytosolic stability whereas Tid1-S is predominantly expressed in mitochondrial (Lu et al. 2006). Tid1 has various interact partners. Previous studies have shown that Tid1 interacts with HSP client partners including heat shock proteins, viral proteins and various regulators of cell signaling and growth factors. Here, we further describe the functional roles of Tid1 in different pathologies and physiology aspect, mainly in cells development, the impact of tumorigenesis and mitochondrial homeostasis. The identified functions of Tid1 and its interacting proteins are summarized in Table 2.

**Table 2** Summary of DNAJA3 / Tid1 functions

| Function | Interacting protein | Roles | References |
|---|---|---|---|
| Development | HSP70 | Development of early embryonic cells and sustains of cells survival. | Lo et al. (2004) |
| | bcl-2 | Development early thymocytes | Lo et al. (2005) |
| | Mortalin | Neuronal development | Patra et al. (2019) |
| Tumorigenesis | Jak2 | Modulates IFN-gamma-mediated transcriptional activity | Sarkar et al. (2001) |
| | ErbB-2 | Suppresses and attenuates the expression level of ErbB-2 | Kim et al. (2004), Jan et al. (2011) |
| | MetR | Expression of Tid1-S isoform enhanced cell migration | Copeland et al. (2011) |
| | STAT5 | Prevents the expression and transcriptional activation of the oncogenic STAT5b | Dhennin-Duthille et al. (2011) |
| | AKT | Attenuates EGFR-dependent signaling; regulate the localization of EGFR in lung cancer | Chen et al. (2009) |
| | APC | Suppresses the pro-apoptotic function of APC | Qian et al. (2010) |
| | Galectin 7 | Interacts with Galectin 7 and suppresses Galectin-7-induced tumorigenesis and metastasis HNSCC | Chen et al. (2018) |
| Mitochondrial biogenesis | HSP70 | Prevent complex I aggregation | Ng et al. (2014) |
| | $Opa1_L$ | Leading cristae opening, decreased OXPHOS, and triggering of mitochondrial fragmentation | Lee et al. (2015a, b) |

## Development

HSP can be transcriptionally regulated by various physiological processes which are not typically associated with cell stress but include the cell cycle, cell proliferation and cell differentiation (Milarski and Morimoto 1986; Hang et al. 1995; Truman et al. 2012). These observations suggest that HSP may also have critical functions during cell growth or development. DNAJA3/Tid1 is found to participate in the early embryonic development. Initially, Tid1 conventional mutant mice have been generated and by mating *Tid1*$^{flox/+}$ mice with transgenic FVB/N-TgN (ACTB-Cre) mice (Jackson Laboratories) to generate *Tid1*$^{+/-}$ heterogenous mice. Subsequently, the interbreeding of the *Tid1*$^{+/-}$ mice would generate the *Tid*$^{-/-}$ embryos without Tid1 expression. The Tid$^{-/-}$ embryos cannot be detected as early as embryonic day 7.5 (E7.5). This suggests that Tid1 is essential for early embryonic development. In addition, the interaction of J-domain with HSP70 is important to maintain the survival of mouse embryonic fibroblast (MEF) cells (Lo et al. 2004).

Interestingly, Tid1 also plays a crucial role in T cells development. Tid1 is required for the T-cell transition from double-negative (DN: CD4$^-$/CD8$^-$) 3 to double-positive (DP: CD4$^+$/CD8$^+$) stages. Thymocyte-specific Tid1-deficient mice (*Lck-Tid1*$^{f/f}$) is generated and indicates a dramatic reduction of double-positive and single-positive thymocytes in the *Lck-Tid1*$^{f/f}$ thymus. Tid1 deletion in early T cell development not only inhibits cell proliferation but also induces DN4 cells death. Furthermore, overexpression of bcl-2 transgene is able to restore the T lymphocyte proliferation and differentiation in Tid1 knockout mice. Together, Tid1 coupled up with bcl-2 regulation is important in early thymocyte development and sustains the cell survival (Lo et al. 2005).

Mortalin is found to modulate its biochemical function through the interaction with the J-domain of Tid1. Indeed, both isoforms, Tid1-L and Tid1-S, can interact with mortalin to mediate protein refolding (Goswami et al. 2010). Patra et al. have reported that patients suffering of ataxia and developmental delay are detected with homozygous variant c.452G > C (p.(Arg151Thr)) in Tid1 genome. This variant obstructs its translocation into mitochondrial and impairs the chaperone activity, subsequently, affects the neuron development (Patra et al. 2019).

## Tumorigenesis

The pathological functions of Tid1 have been characterized in several cancer types, including CRC, breast cancer, lung cancer, ovarian cancer and HNSCC (Kurzik-Dumke and Czaja 2007; Traicoff et al. 2007; Chen et al. 2009, 2018; Jan et al. 2011; Wang et al. 2017). Traicoff et al. have reported that the expression of Tid1 and the Patched proteins is positively correlated in CRC tissues. Further, the Tid1 expression profile is also relatively correlated with CRC progression (Traicoff et al. 2007). Additionally, this Tid1 expression pattern is also associated with the loss of APC and elevation of HSP70. Moreover, the APC deficiency is independent with β-catenin degradation (Kurzik-Dumke and Czaja 2007). Interestingly, the two isoforms of

Tid1, Tid1-L and Tid1-S, display opposite biological activities. Tid1-L seems to promote apoptosis but Tid1-S suppresses it. Tid1-L enhances the cell apoptosis along with the treatment of mitomycin c (DNA-damaging agent) or tumor necrosis factor-alpha (TNFα). In addition, overexpression of Tid1-S mutant also increases the apoptotic effect. Together, Tid1-L suppresses the cell apoptosis (Syken et al. 1999). Furthermore, Tid1-S inhibits cell apoptosis through antagonizing the apoptotic function of the N-terminal region of APC protein (Qian et al. 2010).

Overexpression of Tid1-L is shown to inhibit cell growth and to cause cell death in U2OS cells in a J-domain dependent manner. Additionally, Tid1-L suppresses the NF-κB signaling via stabilizing the IκB to repress the IκK activity (Cheng et al. 2005). Empirically, the interaction between Tid1-L and ErbB-2 is identified by yeast two-hybrid assay. Further, overexpression of Tid1 inhibits cell proliferation and induces cell death only in ErbB-2 high expression breast carcinoma cell lines. Additionally, Tid1 promotes ErbB-2 ubiqutination and consequent proteasome mediated degradation to trigger programed cell death by inhibiting the MAPK pathway (Kim et al. 2004). Kim et al. further demonstrate that ectopically expressing of both mutant Tid1 isoforms enhances *in vitro* cell migration and *in vivo* metastasis of breast cancer cell line MB-MDA-231, along with the elevation of IL-8 expression. Conversely, overexpression of wild type Tid1 inhibits the IL-8 induced tumor cell growth, cell invasion and metastasis (Kim et al. 2005).

In RCC, it has been shown that Tid1 can interact with c-Met receptor tyrosine kinase (MetR), with a preferential binding between MetR and Tid1-S isoform. Overexpression of Tid1-S not only induces hepatocyte growth factor (HGF)-mediated MetR auto-phosphorylation but also enhances cell migration. In opposite, Tid1-L isoform inhibits the MetR activation (Copeland et al. 2011). Tid1 has also been shown specifically bind to STAT5b but not STAT5a in hematopoietic cells. Tid1 negatively regulates the expression of the oncogenic STAT5b and STAT5b downstream transcriptional activation. The inhibition of STAT5b mediated by Tid1 results in suppression of hematopoietic cells transformation (Dhennin-Duthille et al. 2011).

In HNSCC, we have reported that the prognosis of HNSCC patients is correlated with the expression of Tid1. Clinically, the patients with higher expression of Tid1 have better overall survival. Additionally, we demonstrate that overexpression of Tid1 in HNSCC cells enables the inhibition of *in vitro* cell proliferation, migration, invasion, anchorage-independent growth, and *in vivo* xenotransplantation tumorigenicity. Molecularly, Tid1 interacts with EGFR. Further, overexpression of Tid1 in HNSCC cells not only promotes EGFR degradation but also attenuates the EGFR activity by blocking the AKT activation. Nevertheless, the ectopic expression of downstream constitutive activated AKT rescues cell apoptosis with Tid1 overexpressing in HNSCC cells (Chen et al. 2009).

Most recently, we report that Tid1 represses the oncogenic effect of Galectin-7 and consequently inhibits the metastasis of HNSCC. We first identify the interaction between Tid1 and Galectin-7 by affinity chromatography plus mass spectrometry analysis with overexpressing of HA-tagged-Tid1-L-wt or Tid1-L-mut, respectively, in 293 T cells. Clinically, we observe that elevated expression of Galectin-7 predicts

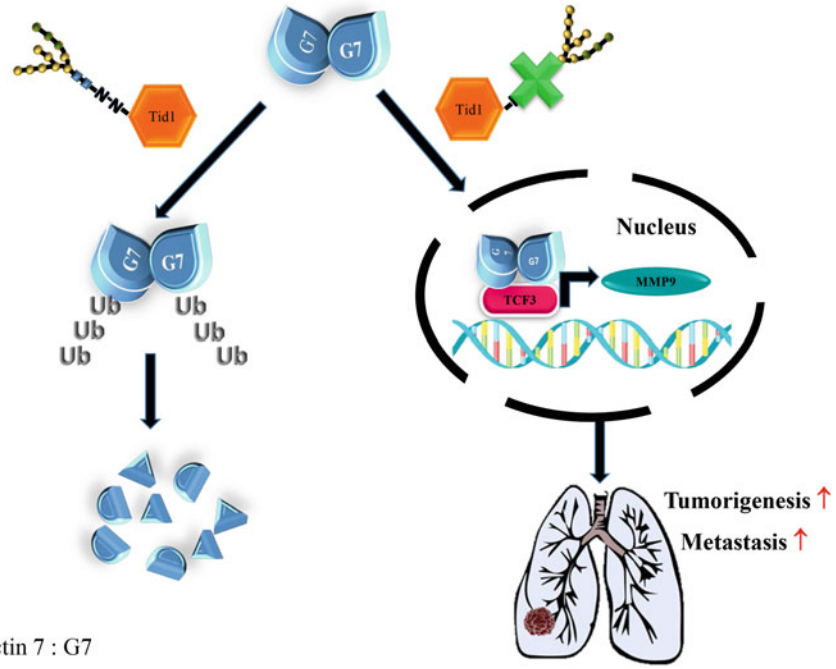



**Fig. 2** Tid1 suppressing G7-TCF3-MMP9 axis and inhibiting tumorigenesis and metastasis The schematic demonstrating the negative regulation of Galectin-7 mediated by glycosylated Tid1 in HNSCC

a worse survival of HNSCC patients. We also discover the negative association between the expression of Tid1 and Galectin-7 protein in HNSCC patients. In HNSCC cell lines, the expression of Galectin-7 protein is upregulated in a metastatic HNSCC cells, whereas the expression of Tid1 protein is down regulated. Interestingly, we also discover that Tid1 interacts with Galectin-7 through its N-linked glycosylation. The interaction between Tid1 and Galectin-7 enhances degradation of ubiquitinated Galectin-7. Our *in vitro* and *in vivo* findings consistently indicate that Tid1 diminishes Galectin-7-mediated tumorigenesis and metastasis. And this metastatic activity is through the Galectin-7/TCF3/MMP9 axis (Chen et al. 2018). Together, we provide a potential treatment in HNSCC progression by overexpressing of Tid1-L and/or targeting the oncogenic Galectin-7 (Fig. 2).

## Mitochondrial Homeostasis; Mitostasis

Chaperones are crucial to assure mitochondrial homeostasis (mitostasis). Mitostasis is a specialized form of homeostasis in mitochondrial, which is to maintain the number and quality of mitochondrial over time in cells (Castro et al. 2018). In fact, co-chaperones also play a key role in maintaining mitostasis. It is reported that the

interaction between HSP70 and Tid1 helps to prevent complex I aggregation. It also participates in the mtDNA maintenance (Ng et al. 2014). Lee et al. have showed that mitochondrial fragmentation is due to the deregulation of Tid1 and it is Drp1 dependent. Therefore, Tid1 may play important role together with HSP70 in modulating mitochondrial morphology. Lee et al. demonstrate that expressing the mutant Tid1 with the loss-of-function DNAJ domain abrogates the molecular balance between Tid1 and HSP70 which is crucial for their chaperones activity and maintaining mitochondrial morphology. Moreover, overexpression or knockdown of Tid1 causes accumulation of optic atrophy 1 (Opa1) in fragmented mitochondria, subsequently, to impair the cristae development, OXPHOS, and ATP production (Lee et al. 2015a, b).

Tid1 can play a critical role in mitochondrial mitostasis through the chaperone activity on DNA polymerase γ (Polga). In mice with Tid1 knockout specifically in cardiomyocytes, they develop dilated cardiomyopathy (DCM) and die before 10 weeks of age. Furthermore, the decreasing copy number of mtDNA and the respiratory chain defect are also observed in cardiomyocytes with Tid1 deletion (Hayashi et al. 2006).

Recently, we report that up-regulation of Tid1 protein is observed in differentiated myoblast cells (C2C12). Tid1 knockdown impairs the differentiation ability of C2C12 myoblasts by reducing mitochondrial activity and intracellular ATP amounts, consequently, it results in energy imbalance and promotion of cells apoptosis. We have also established transgenic mice with skeletal muscle specific (*HSA-Cre*) Tid1 deletion to characterize the physiological function of Tid1 during skeletal muscle development. The *HSA-Tid1*$^{f/f}$ mice are severe muscular dystrophy and with reduced motor activity. Additionally, activity of ATP sensor (p-AMPK) and mitochondrial biogenesis protein (PGC-1α) is compromised in *HSA-Tid1*$^{f/f}$ mice. Taken together, the deficiency of Tid1 in skeletal muscle not only reduces the ATP production but also diminishes the mitochondrial activity (Cheng et al. 2016). However, the detailed molecular mechanism regulated by co-chaperone, Tid1, through which to mediate the muscular dystrophy remaining to be investigated.

## 2 Conclusions

Overall, this review has covered the important physiological functions of most the DNAJ family proteins. Of note, co-chaperon DNAJA3 (Tid1) certainly has participated in various cell regulation including cell development, tumorigenesis, mitostasis, viral replication and immune modulation. However, we mainly discuss about the physiological function of Tid1 in cell development, tumorigenesis and mitostasis. There is no doubt that J domain is critical for DNAJA3 function, it enables DNAJA3 to interact with HSP70. We have also found that most of DNAJA3 substrates are related to carcinogenesis. Hence, the participation of DNAJA3 in tumorigenesis and metastasis inhibition is intuitive. Nevertheless,

DNAJA3 is also important in maintaining the mitochondrial integrity. Drugs development on targeting DNAJA3 should benefit future cancer treatment.

**Acknowledgements** This study was supported by grants from National Yang-Ming University (108CRC-T402) and the Ministry of Science and Technology, Taiwan (MOST 107-2314-B-010-024-MY3).

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

Ahrendt E, Braun JE (2010) Channel triage: emerging insights into the processing and quality control of hERG potassium channels by DnaJA proteins 1, 2 and 4. Channels 4:335–336

Bae MK, Jeong JW, Kim SH et al (2005) Tid-1 interacts with the von Hippel-Lindau protein and modulates angiogenesis by destabilization of HIF-1alpha. Cancer Res 65:2520–2525

Bascos NAD, Mayer MP, Bukau B, Landry SJ (2017) The Hsp40 J-domain modulates Hsp70 conformation and ATPase activity with a semi-elliptical spring. Protein Sci 26:1838–1851

Berger BJ, Müller TS, Buschmann IR et al (2003) High levels of the molecular chaperone Mdg1/ERdj4 reflect the activation state of endothelial cells. Exp Cell Res 290:82–92

Castro JP, Wardelmann K, Grune T, Kleinridders A (2018) Mitochondrial chaperones in the brain: safeguarding brain health and metabolism? Front Endocrinol 9:196

Cheetham ME, Caplan AJ (1998) Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. Cell Stress Chaperones 3:28

Chen CY, Chiou SH, Huang CY et al (2009) Tid1 functions as a tumour suppressor in head and neck squamous cell carcinoma. J Pathol 219:347–355

Chen YS, Chang CW, Tsay YG et al (2018) HSP40 co-chaperone protein Tid1 suppresses metastasis of head and neck cancer by inhibiting Galectin-7-TCF3-MMP9 axis signaling. Theranostics 8:3841–3855

Cheng H, Cenciarelli C, Shao Z et al (2001) Human T cell leukemia virus type 1 tax associates with a molecular chaperone complex containing hTid-1 and Hsp70. Curr Biol 11:1771–1775

Cheng H, Cenciarelli C, Nelkin G et al (2005) Molecular mechanism of hTid-1, the human homolog of Drosophila tumor suppressor l (2) Tid, in the regulation of NF-κB activity and suppression of tumor growth. Mol Cell Biol 25:44–59

Cheng LH, Hung KF, Lee TC et al (2016) Mitochondrial co-chaperone protein Tid1 is required for energy homeostasis during skeletal myogenesis. Stem Cell Res Ther 7:185

Copeland E, Balgobin S, Lee C, Rozakis-Adcock M (2011) hTID-1 defines a novel regulator of c-met receptor signaling in renal cell carcinomas. Oncogene 30:2252

Cui X, Choi H-K, Choi Y-S et al (2015) DNAJB1 destabilizes PDCD5 to suppress p53-mediated apoptosis. Cancer Lett 357:307–315

Dhennin-Duthille I, Nyga R, Yahiaoui S et al (2011) The tumor suppressor hTid1 inhibits STAT5b activity via functional interaction. J Biol Chem 286:5034–5042

Edo De Bock C, Lin Z, Mekkawy AH, Byrne JA, Wang Y (2010) Interaction between urokinase receptor and heat shock protein MRJ enhances cell adhesion. Int J Oncol 36:1155–1163

Edvardson S, Cinnamon Y, Ta-Shma A et al (2012) A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One 7:e36458

Edwards KM, Munger K (2004) Depletion of physiological levels of the human TID1 protein renders cancer cell lines resistant to apoptosis mediated by multiple exogenous stimuli. Oncogene 23:8419–8431

Eisenberg E, Greene LE (2007) Multiple roles of auxilin and hsc70 in clathrin-mediated endocytosis. Traffic 8:640–646

Elsayed L, Drouet V, Usenko T et al (2016) A novel nonsense mutation inDNAJC6Expands the phenotype of autosomal-recessive juvenile-onset Parkinson's disease. Ann Neurol 79:335–337

Eom CY, Lehman IR (2002) The human DnaJ protein, hTid-1, enhances binding of a multimer of the herpes simplex virus type 1 UL9 protein to oris, an origin of viral DNA replication. Proc Natl Acad Sci U S A 99:1894–1898

Fan C-Y, Lee S, Cyr DM (2003) Mechanisms for regulation of Hsp70 function by Hsp40. Cell Stress Chaperones 8:309

Fan C-Y, Lee S, Ren H-Y, Cyr DM (2004) Exchangeable chaperone modules contribute to specification of type I and type II Hsp40 cellular function. Mol Biol Cell 15:761–773

Goswami AV, Chittoor B, D'Silva P (2010) Understanding the functional interplay between mammalian mitochondrial Hsp70 chaperone machine components. J Biol Chem 285:19472–19482

Gotoh T, Terada K, Mori M (2001) hsp70-DnaJ chaperone pairs prevent nitric oxide-mediated apoptosis in RAW 264.7 macrophages. Cell Death Differ 8:357

Gotoh T, Terada K, Oyadomari S, Mori M (2004) hsp70-DnaJ chaperone pair prevents nitric oxide- and CHOP-induced apoptosis by inhibiting translocation of Bax to mitochondria. Cell Death Differ 11:390

Hafizur RM, Yano M, Gotoh T, Mori M, Terada K (2004) Modulation of chaperone activities of Hsp70 and Hsp70-2 by a mammalian DnaJ/Hsp40 homolog, DjA4. J Biochem 135:193–200

Hang H, He L, Fox MH (1995) Cell cycle variation of Hsp70 levels in HeLa cells at 37 C and after a heat shock. J Cell Physiol 165:367–375

Hatle KM, Gummadidala P, Navasa N et al (2013) MCJ/DnaJC15, an endogenous mitochondrial repressor of the respiratory chain that controls metabolic alterations. Mol Cell Biol 33:2302–2314

Hayashi M, Imanaka-Yoshida K, Yoshida T et al (2006) A crucial role of mitochondrial Hsp40 in preventing dilated cardiomyopathy. Nat Med 12:128

Jan CI, Yu CC, Hung MC et al (2011) Tid1, CHIP and ErbB2 interactions and their prognostic implications for breast cancer patients. J Pathol 225:424–437

Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11:579

Kampinga HH, Hageman J, Vos MJ et al (2009) Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 14:105–111

Kang K, Guo K, Tang Q et al (2012) Interactive cellular proteins related to classical swine fever virus non-structure protein 2 by yeast two-hybrid analysis. Mol Biol Rep 39:10515–10524

Kim S-W, Chao T-H, Xiang R et al (2004) Tid1, the human homologue of a Drosophila tumor suppressor, reduces the malignant activity of ErbB-2 in carcinoma cells. Cancer Res 64:7732–7739

Kim S-W, Hayashi M, Lo J-F et al (2005) Tid1 negatively regulates the migratory potential of cancer cells by inhibiting the production of interleukin-8. Cancer Res 65:8784–8791

Köroğlu Ç, Baysal L, Cetinkaya M, Karasoy H, Tolun A (2013) DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. Parkinsonism Relat Disord 19:320–324

Kurisu J, Honma A, Miyajima H, Kondo S, Okumura M, Imaizumi K (2003) MDG1/ERdj4, an ER-resident DnaJ family member, suppresses cell death induced by ER stress. Genes Cells 8:189–202

Kurzik-Dumke U, Czaja J (2007) Htid-1, the human homolog of the Drosophila melanogaster l(2) tid tumor suppressor, defines a novel physiological role of APC. Cell Signal 19:1973–1985

Lee B, Ahn Y, Kang SM et al (2015a) Stoichiometric expression of mtHsp40 and mtHsp70 modulates mitochondrial morphology and cristae structure via Opa1L cleavage. Mol Biol Cell 26:2156–2167

Lee H, Kim J, Kim K, Heo J, Kwak S, Han J (2015b) Genotoxic stress/p53-induced DNAJB9 inhibits the pro-apoptotic function of p53. Cell Death Differ 22:86

Lin S-Y, Hsueh C-M, Yu S-L et al (2010) HLJ1 is a novel caspase-3 substrate and its expression enhances UV-induced apoptosis in non-small cell lung carcinoma. Nucleic Acids Res 38:6148–6158

Liu T, Jiang W, Han D, Yu L (2012) DNAJC25 is downregulated in hepatocellular carcinoma and is a novel tumor suppressor gene. Oncol Lett 4:1274–1280

Lo JF, Hayashi M, Woo-Kim S et al (2004) Tid1, a cochaperone of the heat shock 70 protein and the mammalian counterpart of the Drosophila tumor suppressor l(2)tid, is critical for early embryonic development and cell survival. Mol Cell Biol 24:2226–2236

Lo JF, Zhou H, Fearns C, Reisfeld RA, Yang Y, Lee JD (2005) Tid1 is required for T cell transition from double-negative 3 to double-positive stages. J Immunol 174:6105–6112

Lu B, Garrido N, Spelbrink JN, Suzuki CK (2006) Tid1 isoforms are mitochondrial DnaJ-like chaperones with unique carboxyl termini that determine cytosolic fate. J Biol Chem 281:13150–13158

Mayer M, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670

Meshalkina DA, Shevtsov MA, Dobrodumov AV et al (2016) Knock-down of Hdj2/DNAJA1 co-chaperone results in an unexpected burst of tumorigenicity of C6 glioblastoma cells. Oncotarget 7:22050

Milarski KL, Morimoto RI (1986) Expression of human HSP70 during the synthetic phase of the cell cycle. Proc Natl Acad Sci 83:9517–9521

Mitra A, Fillmore RA, Metge BJ et al (2008) Large isoform of MRJ (DNAJB6) reduces malignant activity of breast cancer. Breast Cancer Res 10:R22

Mitra A, Menezes ME, Shevde LA, Samant RS (2010) DNAJB6 induces degradation of β-catenin and causes partial reversal of mesenchymal phenotype. J Biol Chem 285:24686–24694

Ng AC-H, Baird SD, Screaton RA (2014) Essential role of TID1 in maintaining mitochondrial membrane potential homogeneity and mitochondrial DNA integrity. Mol Cell Biol 34:1427–1437

Nishizawa S, Hirohashi Y, Torigoe T et al (2012) HSP DNAJB8 controls tumor-initiating ability in renal cancer stem–like cells. Cancer Res 72:2844–2854

Olgiati S, Quadri M, Fang M et al (2016) D NAJC 6 mutations associated with early-Onset P Arkinson's disease. Ann Neurol 79:244–256

Park B-C, Yim Y-I, Zhao X, Olszewski MB, Eisenberg E, Greene LE (2015) The clathrin-binding and J-domains of GAK support the uncoating and chaperoning of clathrin by Hsc70 in the brain. J Cell Sci 128:3811–3821

Patra M, Weiss C, Abu-Libdeh B et al (2019) A novel variant of the human mitochondrial DnaJ protein, Tid1, associates with a human disease exhibiting developmental delay and polyneuropathy. Eur J Hum Genet 27:1072–1080

Pröls F, Mayer MP, Renner O et al (2001) Upregulation of the cochaperone Mdg1 in endothelial cells is induced by stress and during in vitro angiogenesis. Exp Cell Res 269:42–53

Qian J, Perchiniak EM, Sun K, Groden J (2010) The mitochondrial protein hTID-1 partners with the caspase-cleaved adenomatous polyposis cell tumor suppressor to facilitate apoptosis. Gastroenterology 138:1418–1428

Qiu X-B, Shao Y-M, Miao S, Wang L (2006) The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. Cell Mol Life Sci CMLS 63:2560–2570

Robichon C, Varret M, Le Liepvre X et al (2006) DnaJA4 is a SREBP-regulated chaperone involved in the cholesterol biosynthesis pathway. Biochim Biophys Acta (BBA)-Mol Cell Biol Lipids 1761:1107–1113

Sarkar S, Pollack BP, Lin KT et al (2001) hTid-1, a human DnaJ protein, modulates the interferon signaling pathway. J Biol Chem 276:49034–49042

Schilling B, De-Medina T, Syken J, Vidal M, Münger K (1998) A novel human DnaJ protein, hTid-1, a homolog of the Drosophila tumor suppressor protein Tid56, can interact with the human papillomavirus type 16 E7 oncoprotein. Virology 247:74–85

Shen Y, Meunier L, Hendershot LM (2002) Identification and characterization of a novel endoplasmic reticulum (ER) DnaJ homologue, which stimulates ATPase activity of BiP in vitro and is induced by ER stress. J Biol Chem 277:15947–15956

Shridhar V, Bible KC, Staub J et al (2001) Loss of expression of a new member of the DNAJ protein family confers resistance to chemotherapeutic agents used in the treatment of ovarian cancer. Cancer Res 61:4258–4265

Sinha D, D'silva P (2014) Chaperoning mitochondrial permeability transition: regulation of transition pore complex by a J-protein, DnaJC15. Cell Death Dis 5:e1101

Sohn SY, Kim SB, Kim J, Ahn BY (2006) Negative regulation of hepatitis B virus replication by cellular Hsp40/DnaJ proteins through destabilization of viral core and X proteins. J Gen Virol 87:1883–1891

Strathdee G, Davies B, Vass J, Siddiqui N, Brown R (2004) Cell type-specific methylation of an intronic CpG island controls expression of the MCJ gene. Carcinogenesis 25:693–701

Syken J, De-Medina T, Münger K (1999) TID1, a human homolog of the Drosophila tumor suppressor l (2) TID, encodes two mitochondrial modulators of apoptosis with opposing functions. Proc Natl Acad Sci 96:8499–8504

Traicoff JL, Chung J-Y, Braunschweig T et al (2007) Expression of EIF3-p48/INT6, TID1 and patched in cancer, a profiling of multiple tumor types and correlation of expression. J Biomed Sci 14:395

Trinh DL, Elwi AN, Kim SW (2010) Direct interaction between p53 and Tid1 proteins affects p53 mitochondrial localization and apoptosis. Oncotarget 1:396–404

Truman AW, Kristjansdottir K, Wolfgeher D et al (2012) CDK-dependent Hsp70 phosphorylation controls G1 cyclin abundance and cell-cycle progression. Cell 151:1308–1318

Tsai M-F, Wang C-C, Chang G-C et al (2006) A new tumor suppressor DnaJ-like heat shock protein, HLJ1, and survival of patients with non–small-cell lung carcinoma. J Natl Cancer Inst 98:825–838

Wang C-C, Tsai M-F, Hong T-M et al (2005) The transcriptional factor YY1 upregulates the novel invasion suppressor HLJ1 expression and inhibits cancer cell invasion. Oncogene 24:4081

Wang L, Tam JP, Liu DX (2006) Biochemical and functional characterization of Epstein-Barr virus-encoded BARF1 protein: interaction with human hTid1 protein facilitates its maturation and secretion. Oncogene 25:4320–4331

Wang TH, Lin YH, Yang SC, Chang PC, Wang TC, Chen CY (2017) Tid1-S regulates the mitochondrial localization of EGFR in non-small cell lung carcinoma. Oncogenesis 6:e361

Yang S, Ren X, Liang Y et al (2019) KNK437 restricts the growth and metastasis of colorectal cancer via targeting DNAJA1/CDC45 axis. Oncogene:1–13

Yin X, Rozakis-Adcock M (2001) Genomic organization and expression of the human tumorous imaginal disc (TID1) gene. Gene 278:201–210

Yu VZ, Wong VC-L, Dai W et al (2015) Nuclear localization of DNAJB6 is associated with survival of patients with esophageal cancer and reduces AKT signaling and proliferation of cancer cells. Gastroenterology 149:1825–1836. e1825

# Therapeutic Potential of Heat Shock Proteins in Human Inflammation/ Autoimmune Skin Diseases: Future Directions


**Paul Chukwudi Ikwegbue, Neerish Revaprasadu, and Abidemi Paul Kappo**

## Abstract

**Introduction** Despite being a major physical protective barrier, the human skin is also a major source of several immune cells that participate in innate and adaptive immune responses therefore, it is described as "first line of immune defence". The involvement of heat shock proteins (HSP) in skin immune responses has been demonstrated by their expression in response to skin stressors such as UV irradiation, heat, environmental, microbial invasion and in several inflammatory-autoimmune skin disorders, which ultimately present them as potential therapeutic targets. The loss of immunological tolerance to the critical self-antigens which leads to dysregulation of immune responses and amplified inflammatory reactions are the major characteristic features underlying most inflammatory-autoimmune diseases. In spite of the recent successes recorded with novel immunosuppressive biological therapies and the use of atopic medications in the treatment of inflammatory/autoimmune skin diseases, their use remains a burden because they neither provide permanent solution to the interaction between pathogenic and protective immune responses nor offer permanent state of medicine-free disease remission. Our aim is to better understand the key players in the pathophysiology of various inflammatory-autoimmune skin disorders, which would have significant impact towards improved therapy.

**Methods** We reviewed all the relevant literatures on the therapeutic potential of heat shock proteins in human inflammation/autoimmune skin diseases.

P. C. Ikwegbue
Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa, Zululand, South Africa

N. Revaprasadu
Department of Chemistry, University of Zululand, KwaDlangezwa, Zululand, South Africa

A. P. Kappo (✉)
Molecular Biophysics and Structural Biology Group, Department of Biochemistry, University of Johannesburg, Kingsway Campus, Auckland Park, South Africa
e-mail: akappo@uj.ac.za

Heat Shock Proteins (2021) 22: 325–340
https://doi.org/10.1007/7515_2020_36

Published Online: 4 October 2020

**Result** Several studies have reported the therapeutic potential of HSP in inflammatory/autoimmune skin diseases owing to their ability to induce the regulation of regulatory T-cells, which play critical role in induction and dysregulation of immune response leading to the progression of several inflammatory/autoimmune diseases.

**Conclusions** Despite the progress made in determining the key players in pathophysiology of various inflammatory/autoimmune skin disorders, therapeutic treatment remain burdensome as most of these treatments are restricted to conventional immunosuppressive methods. These therapies however, do not provide permanent solution to the interaction between pathogenic and protective immune responses. With HSP being one of the key regulators of immune response to autoimmune inflammatory diseases, targeting HSP or HSP inhibitors in these diseases could provide a novel therapeutic approach in the treatment of autoimmune/inflammatory diseases.

**Keywords** Atopic dermatitis · Autoimmune diseases · Bullous pemphigoid · Heat shock proteins (HSP) · Inflammation · Psoriasis · Skin

## Abbreviations

| | |
|---|---|
| AD | Atopic dermatitis |
| BP | Bullous Pemphigoid |
| DCs | dendritic cells |
| HSP | heat shock proteins |
| IFN-γ | interferon-γ |
| IgE | immunoglobulin E |
| IL | interleukins |
| LPS | lipopolysaccharides |
| MMPs | matrix metalloproteinases |
| PBMC | peripheral blood mononuclear cell |
| ROS | reactive oxygen species |
| SALT | skin-associated lymphoid tissue |
| Th1 | T helper 1 |
| TLRs | toll-like receptors |
| TNF-α | tumor necrosis factor-α |
| Treg | regulatory T cells |

## 1 Introduction

The skin is the largest external organ that provides both physical and microbial protection to the human body. It is composed of two main layers: epidermis and dermis [1]. The epidermis, being the outermost layer of the skin creates a waterproof

barrier and plays an important role in the skin tone. This layer is composed of keratinized, stratified-squamous epithelium, closely packed with 95% of cells called keratinocytes, which produces keratin: a fibrous structural protein with an important role in hardness and water-resistance properties of the skin [2]. The dermis on the other hand, is considered the core integumentary system of the skin, and a layer lying beneath the epidermis, consisting of blood vessels, nerves, sweat glands, hair follicles, as well as tough connective tissues such as collagen and elastin fibres which provide structural support to the human skin [3]. Apart from protecting against physical barriers and external injuries (such as UV radiation, environmental stresses and microbial invasion), the skin like other parts of body is actively involved in immune responses and dynamic cellular interactions in order to maintain and regulate several key processes such as wound healing, angiogenesis, disease prevention and inflammation [4]. In addition, it has been hypotheiszed that the skin is the first line of immune defence and functions in immune surveillance. This is supported by the skin immunity and skin-associated lymphoid tissue (SALT) concept, which reveals the skin houses several types of immune cells (such as T and B cells, dendritic cells, mast cells, langerhan's cells) that participate in both innate and adaptive immune responses [5].

Inflammation of the skin is described as an irritation of the skin from internal or external causes characterized by pain, itching, redness and dryness. Inflammation works to detect and neutralize invading pathogens including cancer cells and infectious agents that stimulate or triggers an immune response, hence playing a prominent role in the maintenance of the overall body health, as well as regulation of skin homeostasis. Additionally, when short-lived, inflammatory responses are said to be beneficial to the human body because it helps clear invading pathogens and initiates the process of healing and repair. However, long term inflammation has been associated with several inflammatory diseases of the skin including psoriasis, dermatitis and bullous pemphigoid [6].

As a result of constant exposure of the skin to microbial pathogens and other stressful stimuli (such as UV irradiation, cold, allergens, heat and reactive oxygen species), the skin frequently produces larger number of heat shock proteins (HSP), which enables its response to these stressors. These proteins are broadly classified into various groups according to their molecular weight, function, cellular location and sequence homology. They include ATP-dependent HSP (Hsp110, Hsp100, Hsp90, Hsp70, Hsp60/65 and Hsp40) and ATP-independent HSP (small heat shock proteins-Hsp27 to Hsp10) [7]. The expression and synthesis of heat shock proteins or otherwise known as stress proteins is a universal phenomena taking place in all living cells studied including humans [8]. The study and identification of heat-inducible proteins date back to the early days of Ritossa in 1962, when he observed the dramatic increase in the expression of HSP in the salivary gland of the fruit fly *Drosophila melanogaster* in response to elevated temperature [9, 10]. Following the discovery of HSP, it was thought their functions were restricted to inducible signals such as heat, viral and bacterial infections, exercise, heavy metals, ischemia, gravity, oxidative stress, nutrient deficiency, inflammatory diseases and cancer [11]. Subsequent studies have however demonstrated that some of these proteins are

constitutively expressed in different cellular compartments to perform various cellular functions ranging from protein folding, degradation of misfolded peptides, transportation of organelles across cellular membrane to signal transduction, hence giving them the less complimentary name of "molecular chaperones" [12, 13]. Although their expression pattern varies, it is noteworthy to state that these proteins do not work in isolation but are dependent on co-chaperoning activities or oligomerization to execute their biological functions [14].

Since discovery till date, it is not surprising that HSP have been the subject of huge strides in biological research, owing to their ever-present, abundant expression and diverse effects in the pathology of many diseases, especially human inflammatory diseases and cancer [15]. The roles of HSP in human skin inflammatory diseases, most especially psoriasis, atopic dermatitis, bullous pemphigoid and in other skin diseases, has been well documented. In fact, HSP has been reported to form part of the immune response capable of modulating inflammatory cascades through the inhibition of pro-inflammation mediators, hence encouraging its crucial role in the pathophysiology of several skin inflammatory disorders. Primarily, HSP are intracellular proteins involved in protein folding, refolding, degradation and translocation of organelles, however, their ubiquitous nature in the pathogenesis of several human diseases have escalated their therapeutic applications, Thus, herein the therapeutic potential of heat shock proteins in the following skin inflammatory diseases: psoriasis, atopic dermatitis and bullous pemphigoid is discussed.

## *1.1 Roles of HSP in Skin Inflammatory Diseases*

Unresolved inflammatory reactions have been linked to various clinical manifestations, immunopathology and pathogenesis of several human inflammatory diseases. As a result, many studies have been oriented towards understanding and resolving the mechanisms of inflammatory reactions. These mechanisms include: apoptosis of inflammatory leukocytes, production of lipid mediators, macrophage repolarization and production of specific cytokines. In addition, proper regulation of these mechanisms are very important in controlling and managing inflammatory diseases, hence any powerful immune-modulator that regulates propagation of these mechanisms can be seriously considered as potential therapy in human inflammatory diseases. The roles of HSP in modulating immune cascades leading to inflammatory reactions via inhibition of pro-inflammatory cytokines or mediators has been elucidated [11, 16]. For instance, most chronic inflammatory diseases are autoimmune diseases caused by the dysregulation and inappropriate stimulation of the immune system leading to a loss of tolerance to self-antigens, which degenerates into the immune system attacking its own cells.

### *1.2 Atopic Dermatitis*

Atopic dermatitis (AD) is a common, chronic inflammatory skin disease characterized by intense itching or highly pruritic and inflammatory eczematous lesions that often leads to substantial sleep disturbance, as well as skin trauma, with significant impact on the quality of life of the affected persons and care givers [17]. Recently, AD has become one of the most common chronic skin disorders that affects nearly 5% of the population in developed countries and an increase in the life-time prevalence of AD has been reported in the last 30 years. In fact, it is estimated that about 10–20% of children and 1–2% of adults suffers from this skin illness in the Western countries [18]. Furthermore, it is noteworthy to mention that children with AD are very susceptible to developing asthma, food allergies (eggs and peanut) and allergic rhinitis, the latter is more likely to cause severe form of the disease [19, 20].

Although the pathogenesis of AD is still unclear, it has been reported that the complex interactions between subvert skin barrier function, infectious agents, environmental factors and immune dysregulations contribute immensely to the pathology of AD [21]. A genetic defect in the gene that codes for filaggrin, a filament aggregating protein known to bind keratin fibres in epithelial cells has been associated with the disruption of the epidermal layer, which is a hallmark of AD pathogenesis and its clinical manifestation [22]. Filaggrin protein is a protective barrier that plays an important role in structural organization, as well as in the maintenance of skin homeostatic balance in general [23]. A disrupted filaggrin layer allows for the entering of allergens and microbes into the dermis, thus provoking systemic allergic responses such as immunoglobulin E (IgE) reaction, leading to a direct interaction between antigens (specifically *Staphylococcus aureus*, which is predominantly present in nearly 90% of AD patients) from the external environment and the dermis' immune cells (such as Th2 & Th1 cells, chemokines and cytokines), which intensifies scratching, itching and inflammation: the characteristic features of AD [21, 24, 25]. In addition, AD can emanate as a result of imbalance or interaction disturbance between Th2 and regulatory T cells leading to disruption of epidermal cells and complex interaction of skin immune cells, perpetuating the itchy-scratch cycle as proposed by Hägermark and Wahlgren [26], which result in the loss of skin barrier integrity causing dryness of the skin [27] as shown in Fig. 1.

Interestingly, AD skin diseased are prone to similar stressful signals such as infection, UV irradiation, mechanical injuries, miscellaneous topic medications and environmental stimuli, which also enhances the induction of HSP expression, of which their cytoprotective functions in inflammatory diseases are well documented ([28]). More so, the heat shock protein family HSP60 in particular has been reported to act as bystander antigen due to its wide expression in the inflammation sites and recognition by immune-competent T cells. Evidence from skin lesions of patients suffering from Behcet's disease, atherosclerosis, juvenile idiopathic arthritis and diabetes mellitus, present Hsp60 as a potential candidate in the management of various inflammatory diseases ([29, 30]). In almost all the AD skin lesions studied,

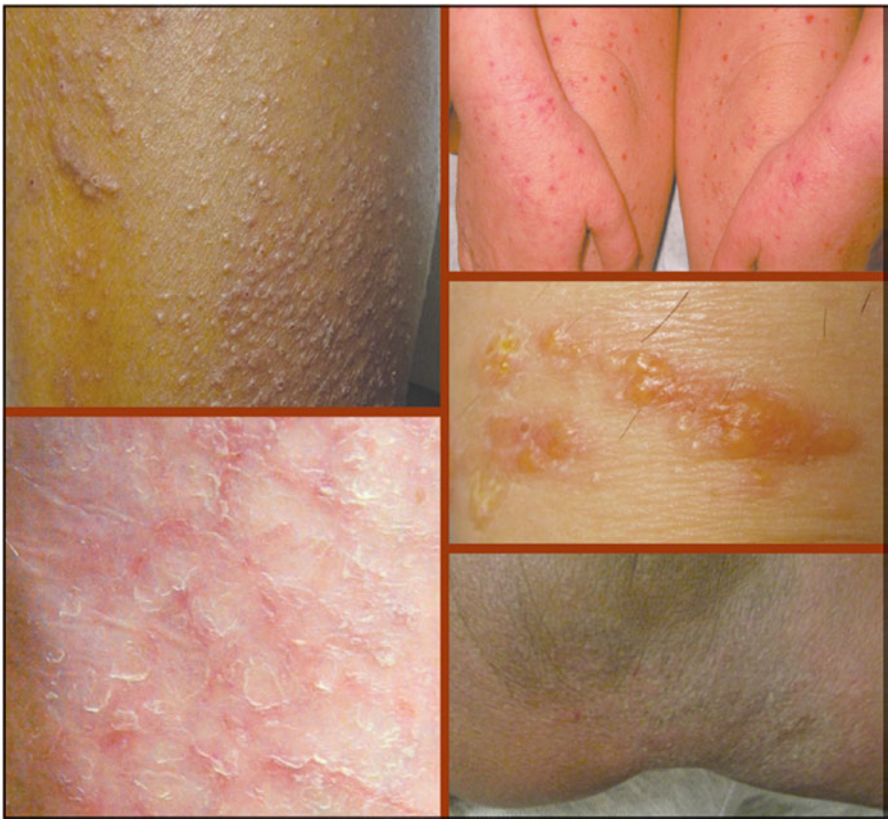


**Fig. 1** The skin of atopic dermatitis patients (ranging from acute atopic dermatitis in its weeping, blistering form; eczema herpeticum in a young girl; close-up photograph of the skin demonstrating dramatic xerosis to subacute atopic dermatitis in its dry, scaly, papular form; leg of an infant with atopic dermatitis demonstrating xerosis). Diagram taken from Berke et al. [24]

infiltrating cells and keratinocytes expresses various heat shock proteins (Hsp65 and Hsp72/73) and the intensity of their expression seems to correlate with the severity of the disease lesions [28]. Taken together, these findings suggest roles for HSP in the pathogenesis of AD, although the exact mechanisms that mediate this relationship in AD skin lesions are poorly understood. However, based on the existing evidence of enhanced expression of HSP27 in response to environmental stress factors in AD patients [31] and considering the roles of HSP in the healing and repairing processes in general, it is safe to speculate that over-expression of these proteins in the skin of AD patients promotes the repairing processes through inflammatory and innate immune responses.

### *1.3 Psoriasis*

Psoriasis is a common, multifactorial, chronic, autoimmune inflammatory skin disorder that can be triggered by a complex interaction between environmental factors (e.g. stress, physical trauma, cigarette smoking and infection), immunology and genetic factors, which sometimes lead to disfiguring features in affected individuals [32]. Generally, psoriasis affects approximately 1–3% of the world's population, and is caused by different contributing factors, although these factors vary from individual to individual [33, 34]. While the actual cause of psoriasis is not fully understood, it believed that dyregulation of auto-immune T cells network, which include various cytokines (IL-17, IL-12, IL-22) resulting in abnormal proliferation and differentiation of keratinocytes leads to the "cellular immune confusion" with the immune system attacking its own cells. Consequently, the process of production and regulation of immune cells in the keratinocytes switches to overdrive, speeding up the abnormal production and regulation of the immune cells in that area of the skin [35]. This process, together with colonizing cells (in-flowing leucocytes-neutrophils) and several inflammatory cytokines secreted by the activated keratinocytes leads to the formation of psoriatic/scaly plaques characterised by pustular lesions [36, 37] as shown in Fig. 2; warmth and redness, which are the hallmarks of inflammatory response. There are several types of psoriasis, which include: inverse, guttate, erythrodermic and plague, with latter being the most severe and common form of psoriasis. The re-occurring, blistering red/pink spots, stigma and disfiguring nature of psoriatic infections often poses serious social, psychological and financial challenges on the infected individual [38, 39].

Recently, growing knowledge of the role of innate and adaptive immunity in several inflammatory diseases including psoriasis exist. The roles of HSP in modulating immune response such as Th-1-type cytokine and Toll-like receptors (TLRs) as well as in psoriatic plaque have been well documented. These TLRs play crucial roles in mediating innate immune responses through the induction of proinflammatory cytokines production via the NF-$_k$B mediated transduction pathways, as well as in recognizing numerous microbial-derived molecules [40, 41]. Furthermore, studies have shown that HSP are greatly expressed in psoriatic skin lesions and could have a huge role in the pathogenesis of psoriatic plaque ([42, 43, 44]). Although their exact function in psoriatic skin is not well known, it is believed that HSP could engage with innate immunity through the activation of IL-12, thereby contributing to Th1 cell that mediates the transition from symptomless to psoriatic skin lesion [45]. Similarly, in keratinocytes, binding of Hsp90 to CD91 receptor and activation of dendritic cells (DCs) results in antigen presentation, migration and production of proinflammatory cytokines. Considering the crucial role of activated DCs in the initiation of the psoriatic skin lesions, the secretion of Hsp90 by the stressed keratinocytes could play a prominent role in the mediation and pathogenesis of psoriasis [43]. Additionally, the chaperoning activities of Hsp90 promotes IL-17 signal, which induces proinflammatory cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$), contributing to the amplified loop of psoriatic lesion. A recent finding

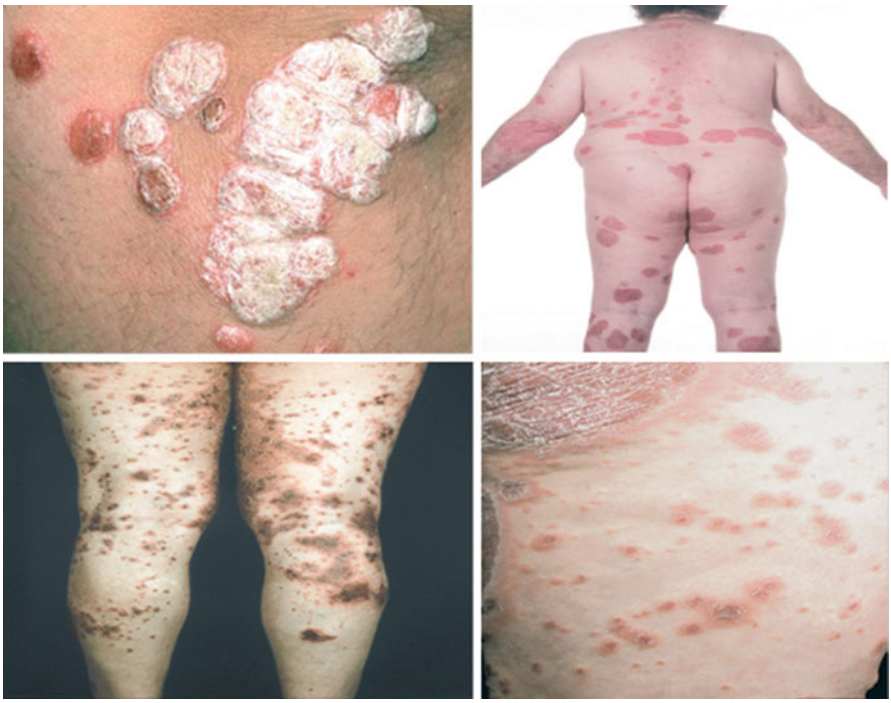


**Fig. 2** The psoriatic skin plaque (ranging from single plaque of psoriasis, well demarcated and heavily scaled, chronic plaque psoriasis to inverse psoriasis). Diagram taken from Griffiths and Barker [38]

suggest Hsp90 involvement in the inflammation of the psoriatic lesion could provide a link between innate immune activation, keratinocytes stress and perpetuation of psoriatic inflammation therefore, inhibiting Hsp90 activities could be a promising therapeutic target towards improved psoriasis therapy [43]. Nevertheless, a result from topical treatment of alfalfa-derived Hsp70 (aHsp70) on mice psoriatic skin lesion suggests that its expression might decrease psoriasis pathogenesis through modulation of T cell activation, since psoriasis is an immune-related disease characterized by proliferation of keratinocytes, although exact mechanism needs to be elucidated in future studies [37].

## *1.4 Bullous Pemphigoid*

Bullous Pemphigoid (BP) is the most common autoimmune and inflammatory skin disorder characterized by subepidermal blistering due to the disruption of dermal-epidermal junction by the binding of autoantibodies against the hemidesmosomal

component of BP180 and BP230. Bullous Pemphigoid is an acquired autoimmune condition that mostly affects elderly people over the age of 60 years, even though the rare incidence at infancy and early childhood development has been reported. Additionally, BP occur evenly in men and in women, though the clinical manifestation and severity of the disease differ from person to person and individuals over the age of 80 years are at high risk of developing the severe of form of the disease.

Although what triggers the immune malfunction that leads to the development and pathogenesis of BP is not clearly understood. However, it is believed to arise from binding of the autoantibodies to hemidesmosomal component of BP180 and BP230 proteins, resulting in the activation of component complements at basement membrane zone (BMZ) or dermal-epidermal junction [46]. This binding results in the degranulation of mast cells and releasing of proinflammatory cytokines from keratinocytes together with immigrating eosinophils, macrophages and neutrophils into the skin. Additionally, the subsequent release of reactive oxygen species (ROS) and proteinases from the surrounding infiltrating inflammatory cells, enhances the separation of epidermis from dermis and the formation of blisters-filled fluid characterised by tense itchy-blisters and erosion, urticarial and pruritic plaques [47, 48] as seen in Fig. 3. Other factors such as drugs (some medications), bacterial or viral infections, physical and mechanical traumas, as well as other autoimmune/inflammatory diseases such as rheumatoid arthritis, thyroid disorders, malignancies and diabetes mellitus have been reported to associate with triggering BP in some patients [49].

BP are predominantly malfunctions of autoantibodies or immune cells, dysregulation of inflammatory responses and stress, as such, any immunodulating and form of stress inducible molecules capable of eliciting these responses could play a role in the pathogenesis of BP. The roles of heat shock proteins in many

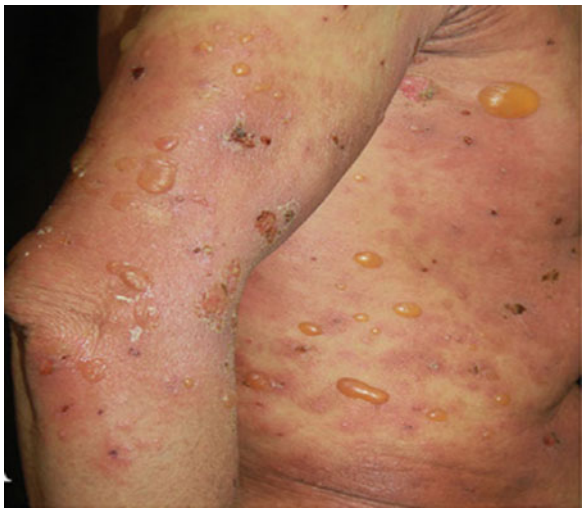


**Fig. 3** The skin of Bullous Pemphigoid patient (showing large, tense bullae, and erythematous patches at dermal-epidermal junction filled with infiltrating inflammatory cells). Diagram taken from Fang et al. [46]

chronic inflammation as well as in autoimmune diseases such rheumatoid arthritis, systematic lupus, psoriasis and BP, have been widely reported [28]. For instance, the high expression of Hsp90 in blistering lesions of BP patients has been documented, although its serum level expression did not correlate with autoantibodies against the BP180 NC16A enzyme complex [50]. It was hypothesised that anti-BP180 NC16A autoantibodies could induce intracellular expression of Hsp90 via the generation of ROS and cytokines proinflammatory mediators, which induces inflammatory response [49]. More so, studies have shown that the overexpression of Hsp90 in BP patients has been observed in both bullous skin lesion and serum treated with human keratinocytes (HaCaT), suggesting that Hsp90 could be an effective treatment target in an experimental epidermolysis bullosa acquisita-mouse model [51, 52]. Taken together, these findings are indications of the crucial role that heat shock proteins could play in the pathogenesis of BP, even though the exact mechanism of action is unclear at the moment and thus requires future studies.

## *1.5 Therapeutic Potential of Heat Shock Proteins in Human Inflammatory/Autoimmune Skin Diseases*

Unresolved inflammation response and loss of tolerance to self-antigens caused by dysregulation and inappropriate stimulation of the immune system leading to an attack by its own cells are the hallmark of inflammatory and autoimmune diseases initiation. Although the actual causes of autoimmune dysregulation and its progression to chronic inflammation are still not fully understood. However, it has been reported that genetic mutation, lifestyle, environmental factors, persistence viral or bacterial infections, are the major contributing factors that cause these diseases [53]. In addition, these stress factors that mediate chronic inflammation or autoimmune diseases, also activate the synthesis of heat shock proteins. The roles of HSP in proinflammatory cytokines and signalling pathways that promote inflammatory or autoimmune diseases have been exploited and are still an ongoing area of research. In fact, overexpression of Hsp60 has been reported to induce the secretion of several cytokines (IL-6, IL-12 and IL-15), as well as increased expression of nitric oxide and TNF-α via CD14 and p38 MAPK signalling pathway in human monocytes [54]. Furthermore, an enhanced expression of Hsp60 in mammalian cell lines proved it could be a key for T cell and antibody responses in both chronic inflammatory diseases and atherosclerosis [55, 56]. In addition, considering that Hsp70 can mediate the induction of TNF-α, IL-1 and IL-6 via CD14-dependent pathway ultimately suggest a direct role of HSP in inflammatory propagation [57].

The concept of heat shock proteins in therapeutics initially arose from a study on cross-reactive immunity to human Hsp60, which revealed that T cells cross-reactivity with HSP60 could induce diabetes in mice. Subsequent study then found that the administration of *Mycobacterium tuberculosis* Hsp65 in mice could either induce or repress diabetes [58]. In addition, the findings that recombinant Hsp10 can

inhibit the inflammatory changes induced by lipopolysaccharides (LPS) in mice and in macrophages prompted the attempt to commercialize Hsp10 as a therapeutic target and small-scale clinical trials for XToll, a modified form of Hsp10 [59, 60].

For instance, in patients with psoriasis plaque, short-term treatment with Hsp10 [recombinant chaperonin 10 (Cpn10)] led to a rapid reduction of the disease parameters. Probably, this was achieved through the modification of activity in chronic inflammatory and decrease in the release of inflammatory cytokines such as IL-1 and TNF-α, which play key roles in mediating inflammatory response and immune response in general [60, 61]. This finding suggests that Cpn10 may regulate a wide array of inflammatory responses in skin inflammatory diseases and may provide a range of therapeutic approaches in these diseases.

Furthermore, studies have shown overexpression of Hsp60 in peripheral blood mononuclear cell (PBMC) of children with AD skin lesion compare to that of non-lesional skin. The expression of Hsp60-reactive T cells in these children correlates with up-regulation of pro-inflammatory cytokine IFNγ and dowregulation of anti-inflammatory cytokine IL-10 [30]. This study together with the finding that demonstrates the expression of Hsp60 at site of inflammation and that Hsp60 can modulate the regulation of cytokines and Th1 phenotype in the skin lesion of dogs with atopic dermatitis [62], suggests the immunomodulating activities of Hsp60, as well as its contribution to the local inflammatory response in AD patients. Based on these findings, it is safe to speculate that Hsp60-reactive T cells plays a role in the pathogenesis of AD and future studies oriented towards investigating the immunomodulating properties and inflammatory response of Hsp60 in AD, could provide a novel therapeutic target in the management of the disease. Additionally, the expression of Hsp27 in the skin of AD and its correlation with environmental stress in human keratinocytes and in exercised skin, could be indicative of its cytoprotective role and biological defence responses to the disruption of skin barriers (filaggrin) in AD [31].

More so, the implication of Hsp90 in inflammation-associated immunological processes has been demonstrated by its essential role in various transcription factors and signalling molecules, which participate in cellular inflammation cascades such as induction of several pro-inflammatory cytokines, participation in auto-antigen presentation, as well as interaction with auto-antigenic proteins by acting as a potent promoter of the immune network outside of the cells [63]. The report that basement membrane-degrading matrix metalloproteinases (MMPs) released from surrounding cells also participates in blister formation and tissue injury in autoimmune bullous diseases rather than their initial role in tumour cellular invasion [64, 65], makes it an interesting aspect that inhibitors of Hsp90 could present therapeutic target in autoimmune diseases like BP. In fact, the growing pharmacological evidence of Hsp90 inhibitors in bullous autoimmune-inflammatory disorders has gained momentum as demonstrated by ameliorating effects of Hsp90 blockers in preclinical rodent experiments in other autoimmune inflammatory disorders such as systemic lupus erythematosus, encephalomyelitis and rheumatoid arthritis [66–68]. Although, it is largely unknown whether extracellular and intracellular dyregulation of Hsp90 in BP patients is primarily due to the pathogenesis or secondary changes induced by

inflammation. However, for the fact that this chaperone is involved in many intracellular signalling which directly associated with production of inflammatory cytokines such as IL-6 and IL-8 (these cytokines play a major transcriptional factor role in the pathogenesis of BP) and this role in keratinocytes is believed to be of one the earliest stage of BP pathophysiology leading to the formation of blisters, thus suggests it may play a crucial role in the pathophysiology of BP [68–70]. Based on the aforementioned studies that highlight the important roles of HSP in modulating regulatory T cells (Tregs), which is critical in mediating inflammatory/autoimmune diseases, it can be hypothesized that studies targeting HSP or/and their inhibitors in inflammatory/autoimmune skin disorders could provide a good therapeutic approach in the treatment and management of these diseases as proposed in the model shown in Fig. 4.

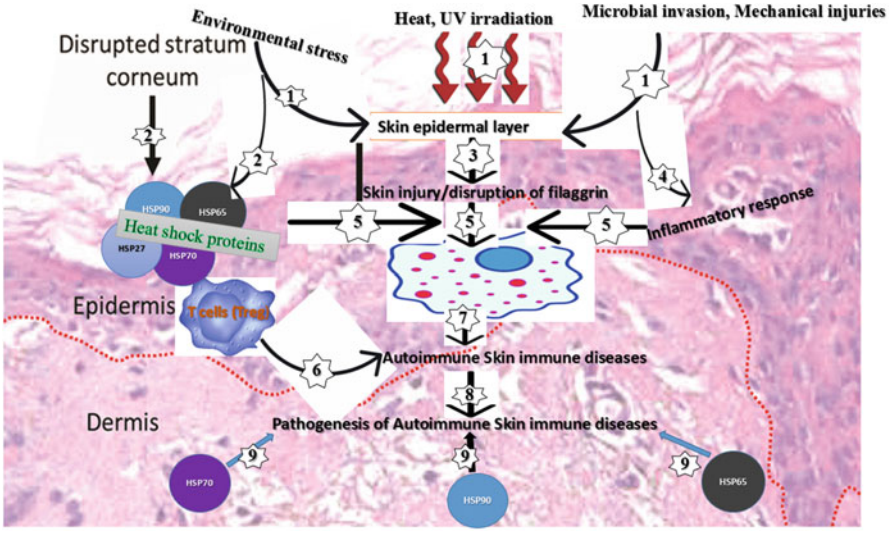



**Fig. 4** Model proposing the disruption of skin epidermal layer, initiation, progression and pathogenesis of inflammatory/autoimmune diseases. (1) The stressors such UV irradiation, environmental factors, microbial invasion and other mechanical injuries that damages skin epidermal cells leading to cellular skin stress; (2) disruption of stratum corneum and environmental stressors such heat, UV radiation and microbial invasion induces expression of heat shock protein (Hsp90, Hsp70, Hsp60 and Hsp27); (3) these stressors causes skin injury/disruption of filaggrin protein; (4) microbial invasion and mechanical injuries to the skin provokes inflammatory response which aimed at clearing the invading pathogen and initiating cellular repair; (5) disruption of skin layers also induce the synthesis of cytoprotective heat shock proteins as well as inflammatory reaction which form part of immune response in general; (6) up-regulation of T cells (Tregs) which play critical roles in the initiation and progression of autoimmune diseases; (7) the interaction between immune response together with regulatory T cells can sometimes lead to "immune confusion" of immune system attacking its own cells which degenerates to autoimmune diseases; (8) pathogenesis of autoimmune diseases; (9) up-regulation and involvement of heat shock proteins in the pathogenesis and clinical manifestation of autoimmune disorders

## 2 Conclusions

Despite the progress made in determining the key players in pathophysiology of various inflammatory/autoimmune skin disorders, their therapeutic treatment remains a burden as most of these treatments are restricted to conventional immunosuppressive methods such as corticosteroid, atopic medications and more advanced biological methods that focus on supressing chronic inflammation. These therapies however, do not provide permanent solution to the interaction between pathogenic and protective immune responses and in most cases, patients are subjected to severe side effects. Therefore, there is a need for researchers to develop safe therapies with high efficacy to carefully balance this interaction in the complex environment. HSP are one of the key regulators of immune response to autoimmune inflammatory diseases and have been considered as potential therapeutic targets in the treatment of these diseases due to their ability to induce the production of immune regulatory T (Treg) cells, which plays critical role in the clinical manifestation of autoimmune-inflammatory disorders. Thus, targeting HSP or HSP inhibitors in these diseases could provide a novel therapeutic approach in the treatment of autoimmune-inflammatory skin disorders.

**Acknowledgements** Authors will like to acknowledge Dr. Sesan John Owonubi for proofreading this manuscript. None of the authors received any kind of funding towards the work presented in this article.

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Human** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Oomens CW, van Vijven M, Peters GW (2017) Skin mechanics. In: Biomechanics of living organs. Academic, London, pp 347–357
2. Hendriks FM, Brokken D, Oomens CWJ, Bader DL, Baaijens FPT (2006) The relative contributions of different skin layers to the mechanical behavior of human skin in vivo using suction experiments. Med Eng Phys 28:259–266
3. Benítez JM, Montáns FJ (2017) The mechanical behavior of skin: structures and models for the finite element analysis. Comput Struct 190:75–107
4. Matejuk A (2018) Skin immunity. Arch Immunol Ther Exp 66:45–54
5. Quaresma JAS (2019) Organization of the skin immune system and compartmentalized immune responses in infectious diseases. Clin Microbiol Rev 32:00034–00018
6. Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444(7121):860–867
7. Batulan Z, Pulakazhi Venu VK, Li Y, Koumbadinga G, Alvarez-Olmedo DG, Shi C, O'Brien ER (2016) Extracellular release and signaling by heat shock protein 27: role in modifying vascular inflammation. Front Immunol 7:285

8. Ikwegbue P (2019) Investigation of the interaction between heat shock protein 70.14 (Hsp 70.14) and RBBP6 RING finger domain. Master of Science Dissertation, University of Zululand, South Africa
9. Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in Drosophila. Experientia 18:571–573
10. Candido EPM (2001) Heat shock proteins A2-Brenner, Sydney. In: Miller JH (ed) Encyclopedia of genetic. Academic, New York, pp 914–915
11. Ikwegbue PC, Masamba P, Oyinloye BE, Kappo AP (2018) Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. Pharmaceuticals 11:1–12
12. Alqarni AS, Ali H, Iqbal J, Owayss AA, Smith BH (2019) Expression of heat shock proteins in adult honey bee (Apis mellifera L.) workers under hot-arid subtropical ecosystems. Saudi J Biol Sci 26(7):1372–1376
13. Li Z, Srivastava P (2004) Heat-shock proteins. In: Coligan JE et al (eds) Current protocols in immunology, p Appendix 1: Appendix 1T. https://doi.org/10.1002/0471142735.ima01ts58
14. Zininga T, Ramatsui L, Shonhai A (2018) Heat shock proteins as immunomodulants. Molecules 23(11):E2846
15. Pockley AG (2002) Heat shock proteins, inflammation, and cardiovascular disease. Circulation 105(8):1012–1017
16. Edkins AL, Price JT, Pockley AG, Blatch GL (2017) Heat shock proteins as modulators and therapeutic targets of chronic disease: an integrated perspective. Philos Trans R Soc Lond Ser B Biol Sci 373(1738):20160521
17. Weidinger S, Klopp N, Rummler L, Wagenpfeil S, Novak N, Baurecht HJ, Groer W, Darsow U, Heinrich J, Gauger A, Schafer T (2005) Association of NOD1 polymorphisms with atopic eczema and related phenotypes. J Allergy Clin Immunol 116:177–184
18. Larsen FS, Hanifin J (2002) Epidemiology of atopic dermatitis. Immunol Allergy Clin N Am 22:1–24
19. Akdis CA, Akdis M, Bieber T, Bindslev-Jensen C, Boguniewicz M, Eigenmann P, Hamid Q, Kapp A, Leung DY, Lipozencic J, Luger TA (2006) Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. Allergy 61:969–987
20. Tsakok T, Marrs T, Mohsin M, Baron S, du Toit G, Till S, Flohr C (2016) Does atopic dermatitis cause food allergy? A systematic review. J Allergy Clin Immunol 137:1071–1078
21. Kapur S, Watson W, Carr S (2018) Atopic dermatitis. Allergy Asthma Clin Immunol 14:2–52
22. Avena-Woods C (2017) Overview of atopic dermatitis. Am J Manag Care 23(8 Suppl):S115–S123
23. Bandier J, Ross-Hansen K, Carlsen BC, Tanassi JT, Johansen JD, Heegaard NH (2016) Quantification of epidermal Filaggrin in human skin and its response to skin irritation. J Invest Dermatol 136:1296–1299
24. Berke R, Singh A, Guralnick M (2012) Atopic dermatitis: an overview. Am Fam Physician 86:35–42
25. Buske-Kirschbaum A, Geiben A, Hellhammer D (2001) Psychobiological aspects of atopic dermatitis: an overview. Psychother Psychosom 70:6–16
26. Hägermark Ö, Wahlgren CF (1995) Treatment of itch. Semin Dermatol 14(4):320–325
27. Yosipovitch G, Hundley JL (2004) Practical guidelines for relief of itch. Dermatol Nurs 16:325–332
28. Ghoreishi M, Yokozeki H, Hua WM, Nishioka K (2000) Expression of 27 KD, 65 KD and 72/73 KD heat shock protein in atopic dermatitis: comparison with those in normal skin and contact dermatitis. J Dermatol 27:370–379
29. Ergun T, İnce Ü, Ekşioğlu-Demiralp E, Direskeneli H, Gürbüz O, Gürses L, Aker F, Akoğlu T (2001) HSP 60 expression in mucocutaneous lesions of Behcet's disease. J Am Acad Dermatol 45:904–909

30. Kapitein B, Aalberse JA, Klein MR, de Jager W, Hoekstra MO, Knol EF, Prakken BJ (2013) Recognition of self-heat shock protein 60 by T cells from patients with atopic dermatitis. Cell Stress Chaperones 18:87–95
31. Niiyama S, Yoshino T, Matsukuma S, Mukai H (2016) Heat shock protein 27 kDa content in stratum Corneum correlates with severity of atopic dermatitis. Acta Derm Venereol 96:976–977
32. Albanesi C, Madonna S, Gisondi P, Girolomoni G (2018) The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis. Front Immunol 9:1549
33. Christophers E (2001) Psoriasis – epidemiology and clinical spectrum. Clin Exp Dermatol 26:314–320
34. Wu W, Debbaneh M, Moslehi H, Koo J, Liao W (2014) Tonsillectomy as a treatment for psoriasis: a review. J Dermatolog Treat 25:482–486
35. Buquicchio R, Foti C, Ventura MT (2018) The psoriasis pathogenesis and the metabolic risk. Open Dermatol J 12:70–79
36. Lowes MA, Suárez-Fariñas M, Krueger JG (2014) Immunology of psoriasis. Annu Rev Immunol 32:227–255
37. Seifarth FG, Lax JEM, Harvey J, DiCorleto PE, Husni ME, Chandrasekharan UM, Tytell M (2018) Topical heat shock protein 70 prevents imiquimod-induced psoriasis-like inflammation in mice. Cell Stress Chaperones 23(5):1129–1135
38. Griffiths CE, Barker JN (2007) Pathogenesis and clinical features of psoriasis. Lancet 370:63–271
39. Raho G, Koleva DM, Garattini L, Naldi L (2012) The burden of moderate to severe psoriasis. PharmacoEconomics 30:1005–1013
40. Heldwein KA, Fenton MJ (2002) The role of toll-like receptors in immunity against mycobacterial infection. Microbes Infect 4:937–944
41. Kaisho T, Akira S (2000) Critical roles of toll-like receptors in host defense. Crit Rev Immunol 20(5):393–405
42. Boehncke WH, Dahlke A, Zollner TM, Sterry W (1994) Differential expression of heat shock protein 70 (HSP70) and heat shock cognate protein 70 (HSC70) in human epidermis. Arch Dermatol Res 287:68–71
43. Kakeda M, Arock M, Schlapbach C, Yawalkar N (2014) Increased expression of heat shock protein 90 in keratinocytes and mast cells in patients with psoriasis. J Am Acad Dermatol 70:683–690
44. Wang WM, Jin HZ (2019) Heat shock proteins and psoriasis. Eur J Dermatol 29:121–125
45. Curry JL, Qin JZ, Bonish B, Carrick R, Bacon P, Panella J, Robinson J, Nickoloff BJ (2003) Innate immune-related receptors in normal and psoriatic skin. Arch Pathol Lab Med 127:178–186
46. Fang H, Zhang Y, Li N, Wang G, Liu Z (2018) The autoimmune skin disease bullous pemphigoid: the role of mast cells in autoantibody-induced tissue injury. Front Immunol 9:407
47. Schulze F, Kasperkiewicz M, Zillikens D, Schmidt E (2013) Bullöses Pemphigoid. Hautarzt 64:931–945
48. Okon L, Werth V (2014) Bullous Pemphigoid, mucous membrane Pemphigoid and pemphigus vulgaris: an update on pathobiology. Curr Oral Health Rep 1:180–189
49. Raveendran MN, Srinivasan SM (2017) Postpartum bullous pemphigoid-A case report. Our Dermatol Online/Nasza Dermatologia Online 8:2
50. Tukaj S, Kleszczyński K, Vafia K, Groth S, Meyersburg D, Trzonkowski P, Ludwig RJ, Zillikens D, Schmidt E, Fischer TW, Kasperkiewicz M (2013) Aberrant expression and secretion of heat shock protein 90 in patients with bullous pemphigoid. PLoS One 8:7–0496
51. Kasperkiewicz M, Tukaj S, Gembicki AJ, Silló P, Görög A, Zillikens D, Kárpáti S (2014) Evidence for a role of autoantibodies to heat shock protein 60, 70, and 90 in patients with dermatitis herpetiformis. Cell Stress Chaperones 19:837–843
52. Kasperkiewicz M, Müller R, Manz R, Magens M, Hammers CM, Somlai C, Westermann J, Schmidt E, Zillikens D, Ludwig RJ, Orosz A (2011) Heat-shock protein 90 inhibition in

autoimmunity to type VII collagen: evidence that nonmalignant plasma cells are not therapeutic targets. Blood 117:6135–6142
53. Ikwegbue PC, Masamba P, Mbatha LS, Oyinloye BE, Kappo AP (2019) Interplay between heat shock proteins, inflammation and cancer: a potential cancer therapeutic target. Am J Cancer Res 9:2–242
54. Dubey A, Prajapati KS, Swamy M, Pachauri V (2015) Heat shock proteins: a therapeutic target worth to consider. Vet World 8(1):46–51
55. Grundtman C, Kreutmayer SB, Almanzar G, Wick MC, Wick G (2011) Heat shock protein 60 and immune inflammatory responses in atherosclerosis. Arterioscler Thromb Vasc Biol 31:960–968
56. Tukaj S, Kaminski M (2019) Heat shock proteins in the therapy of autoimmune diseases: too simple to be true? Cell Stress Chaperones 24:475–479
57. Lovett MC, Coates JR, Shu Y, Oglesbee MJ, Fenner W, Moore SA (2014) Quantitative assessment of hsp70, IL-1β and TNF-α in the spinal cord of dogs with E40K SOD1-associated degenerative myelopathy. Vet J 200:312–317
58. Elias D, Markovits D, Reshef T, van der Zee R, Cohen IR (1990) Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein. Proc Natl Acad Sci 87:1576–1580
59. Johnson BJ, Le TT, Dobbin CA, Banovic T, Howard CB, Flores FDML, Vanags D, Naylor DJ, Hill GR, Suhrbier A (2005) Heat shock protein 10 inhibits lipopolysaccharide-induced inflammatory mediator production. J Biol Chem 280:4037–4047
60. Pockley AG, Henderson B (2017) Extracellular cell stress (heat shock) proteins—immune responses and disease: an overview. Philos Trans R Soc B Biol Sci 373:20160–20522
61. Williams B, Vanags D, Hall S, McCormack C, Foley P, Weiss J, Johnson B, Latz E, Feeney D (2008) Efficacy and safety of chaperonin 10 in patients with moderate to severe plaque psoriasis: evidence of utility beyond a single indication. Arch Dermatol 144:683–685
62. Jassies-van der Lee A, Rutten V, Van Kooten P, Van der Zee R, Willemse T (2008) Intradermal injection of Hsp60 induces cytokine responses in canine atopic and healthy skin. Cell Stress Chaperones 13:387–391
63. Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev Immunol 2(3):185–194
64. Schulz R, Dobbelstein M, Moll UM (2012) HSP90 inhibitor antagonizing MIF: the specifics of pleiotropic cancer drug candidates. Onco Targets Ther 1:1425–1426
65. Kasperkiewicz M, Zillikens D, Schmidt E (2012) Pemphigoid diseases: pathogenesis, diagnosis, and treatment. Autoimmunity 45:55–70
66. Rice JW, Veal JM, Fadden RP, Barabasz AF, Partridge JM, Barta TE, Dubois LG, Huang KH, Mabbett SR, Silinski MA, Steed PM (2008) Small molecule inhibitors of Hsp90 potently affect inflammatory disease pathways and exhibit activity in models of rheumatoid arthritis. Arthritis Rheum 58:3765–3775
67. Yun TJ, Harning EK, Giza K, Rabah D, Li P, Arndt JW, Luchetti D, Biamonte MA, Shi J, Lundgren K, Manning A (2011) EC144, a synthetic inhibitor of heat shock protein 90, blocks innate and adaptive immune responses in models of inflammation and autoimmunity. J Immunol 186:563–575
68. Tukaj S, Zillikens D, Kasperkiewicz M (2015) Heat shock protein 90: a pathophysiological factor and novel treatment target in autoimmune bullous skin diseases. Exp Dermatol 24:567–571
69. Van den Bergh F, Eliason SL, Burmeister BT, Giudice GJ (2012) Collagen XVII (BP 180) modulates keratinocyte expression of the proinflammatory chemokine, IL-8. Exp Dermatol 21:605–611
70. Tukaj S, Węgrzyn G (2016) Anti-Hsp90 therapy in autoimmune and inflammatory diseases: a review of preclinical studies. Cell Stress Chaperones 21:213–218

# Role of HSP in the Pathogenesis of Age-Related Inflammatory Diseases


**Asmaa F. Khafaga, Ahmed E. Noreldin, and Islam M. Saadeldin**

## Abstract

**Introduction** Heat shock proteins (HSP) are pivotal players in the normal cellular physiological processes and possess regulatory functions in pathogenesis of age-related disorders. HSP as chaperons are participating in protein folding, proper protein conformation, and prevention of undesired protein aggregation. In here, we provide the essential roles of HSP in inflammation with special focus on the ageing-related inflammatory diseases such as Alzheimer's disease, Parkinson's disease, diabetes, rheumatoid arthritis, and atherosclerosis.

**Methods** A literature based collection of articles in the available search engines (PubMed and Google Scholar).

**Results** We show the interrelation of HSP and inflammation-related ageing disorders such as Alzheimer's disease, Parkinson's disease, diabetes, rheumatoid arthritis, and atherosclerosis.

**Conclusions** Understanding the critical roles of HPS would help in designing and manufacturing therapeutics for ameliorating the symptoms associated with age-related diseases.

A. F. Khafaga
Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Edfina, Egypt

A. E. Noreldin
Histology and Cytology Department, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt

I. M. Saadeldin (✉)
Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia
e-mail: isaadeldin@ksu.edu.sa; islamms@zu.edu.eg

Heat Shock Proteins (2021) 22: 341–371
https://doi.org/10.1007/7515_2020_29

Published Online: 9 September 2020

**Keywords** Ageing · Alzheimer's disease · Atherosclerosis · Cancer · Diabetes · HSP · Parkinson disease · Rheumatoid arthritis

## Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| ALS | amyotrophic lateral sclerosis |
| Aβ | amyloid β peptides |
| CRP | C-reactive protein |
| HSF | heat shock factor |
| HSP | heat shock proteins |
| IL | interleukins |
| PD | Parkinson's disease |
| RA | Rheumatoid arthritis |
| ROS | reactive oxygen species |
| SOD | superoxide dismutase |
| TGFβ | transforming growth factor-β |
| TNF | tumor necrosis factor |

## 1 Introduction

In 1962, Ritossa has been discovered Heat chock proteins (HSP). They are a family of highly conserved ubiquitous proteins. This family composed of group of different molecular weight proteins including HSP10, Hsp27, HSP40, Hsp60, Hsp70, Hsp90 and Hsp110 [25]. However, Hsp70 and 90 are the two major types that have the potential to bind to the unfolded protein helping them to folded and synthesized properly [25, 120]. Some types of this protein could express substantially, while the most of them expressed under stress conditions [166]. A variety of environmental or physiologic stress could lead to production and activation of HSP such as inflammation, hypoxia, chemotherapy, infections, as well as thermal injury [1, 86, 157–161]. In living systems, HSP have essential activities including polypeptides folding, proteins transportation, and formation of multiprotein complexes [62]. Moreover, they can prevent apoptosis, cleared aggregated proteins, and ameliorates the cytotoxic impact of toxic proteins.

During aging, declined HSP expression was reported in several tissues particularly muscle, liver, neurons, and vascular system [100, 133], with resultant protein aggregation, a commonly noted feature in neurodegenerative disorders [57, 64]. On the other hand, it is now well documented that, aging is related to presence of high concentration of pro-inflammatory cytokines such as interleukins (IL), IFNα, IFNβ, C-reactive protein (CRP), tumor necrosis factor (TNF), and transforming growth factor-β (TGFβ) [49, 55]. This inflammatory response becomes evolutionarily benefit during adulthood. However, during aging, it becomes detrimental due to inactive

natural selection [53]. Inflamm-ageing is a chronic inflammatory response associated to the physiologic aging. It is the primary risk factor for the common age-related pathologies including malignancy, dementia, and cardiovascular disorders. Moreover, it may be considered as generalized health indicator for mobility disability, impairment of daily activities, and premature death [105, 156, 162].

Recently, HSP proteins were reported for their anti-inflammatory and antiapoptotic effect. Therefore, they have the potential to modulate and reduce the responses against various inflammatory cytokines. Consequently, understanding for the essential role of HSP in pathogenesis of age-related chronic inflammatory diseases may be a promising target to block the establishment of those diseases [44]. The aim of the present work is to update knowledge concerning the key role of HSP in pathogenesis of age-related inflammatory diseases including neurodegenerative disorders (such as Alzheimer's disease and Parkinson's disease), vascular disorders (such as Atherosclerosis), diabetes, rheumatoid arthritis, and neoplastic changes.

## *1.1 History and Types of HSP*

HSP are a collection of common and highly preserved proteins. According to their size, HSP have been categorized into two groups: small molecular weight HSP and high molecular weight HSP. The first group contains four families: Hsp60, Hsp70, Hsp90, and Hsp110. Some of these proteins have continuous expression whereas stressful conditions induce the expression of the others [166]. High molecular weight HSP are ATP-dependent chaperones and need cochaperones to modify their conformation and ATP binding. On the other hand, small molecular weight HSP are ATP independent chaperones. HSP are stimulated by many environmental and physiological factors, such as inflammation, hypoxia, temperature stress, anticancer chemotherapy, or infections [86].

## *1.2 Role of HSP in the Inflammatory Mechanism*

The best biological stimulant to induce the innate immunity response is the invasion of a foreign molecule. Innate immune recognition receptors decide to respond or ignore that stimulus by activation of PAMP (pathogen-associated molecular patterns) molecules, i.e. factors linked with groups of pathogens (for example bacterial CpG DNA, lipopolysaccharides, etc.). Other pathway for the induction of innate immunity is 'danger theory' [119]. By this hypothesis, the innate immunity can be induced by endogenous substances produced by the stressed or damaged tissue. Based on this theory, stressed cells can transfer stress to other cells. The stress signals produced by cells can be the HSP stimulated in response to the damage, so they are possible candidates for signaling cellular stress or tissue damage. Some of

HSP such as Hsp70 and Hsp60 have been detected to be capable of signaling by TLR-4, TLR-2, and CD14 [8, 187]. Now, it is well established that Hsp70 is in charge of the stimulation of monocytes, macrophages, natural killer cells, dendritic cells, hepatocytes, etc. [26, 42, 58, 174]. Furthermore, extracellular HSP have been detected to work as powerful immunosuppressive or immunostimulatory molecules according to the various circumstances [124].

In addition to Hsp70, other HSP have been detected in the extracellular matrix, such as Hsp27 [108], Grp78/BIP [45, 99], Hsp90 [184], and Hsp60 [124]. The biological relation of these factors has been enhanced by the presence of Hsp70 in the serum of patients having myocardial infarction [47], chronic inflammation [135], coronary artery disease [212, 216], lung injury [59], infections [135], ischemia/reperfusion events [75], cancer [12], diabetes [137], hypertension during pregnancy [128], etc. HSP can also be detected at lower levels in the serum of healthy individuals [145]. Interestingly, the existence of Hsp70 in plasma is linked to enhanced survival of sever ill patients [217]. Other extracellular HSP such as Hsp90 [170], Hsp60 [211], and Hsp27 [108] have also been correlated with many diseases like coronary heart disease, pancreatic carcinoma, systemic lupus erythematosus or cancer metastasis.

Cytoprotection is the main role of HSP. Some investigations have revealed the number of cells surviving elevated when the temperature increased till 43 °C. This thermotolerance was attributed to the increased formation of HSP [104]. Some reports suggested the role of HSP in the suppression of stress-activated kinases. [56] revealed that preheating the human leukemic cells resulted in decreased cell death after heat shock, which was correlated with p38 activation and suppression of JNK. This influence might be made by HSP. This overexpression suppressed the stress kinase-activating influences of ultraviolet irradiation, $H_2O_2$ and heat. Park et al. indicated that Hsp72 inhibits the JNK signaling pathway by prohibition of JNK phosphorylation by its upstream kinase SEK1 [140]. Moreover, Hsp70 has been involved in the suppression of IKKγ and following synthesis of IKK complexes [163]. As for the impact of NfkB in inflammation, the suppression of its kinase, IKK, has specific therapeutic importance for inflammation. Furthermore, overexpression of Hsp70 prevents sepsis-induced lung injury in rats through suppression of the IKK complex [193].

## *1.3 Role of HSP in Aging-Related Diseases*

The aggregation of oxidized proteins is an essential feature of the major neurological diseases, and many investigations have revealed that elevating HSP levels can have useful influences [101]. HSP have been detected to decrease the symptoms of Alzheimer's disease, a disease caused by the aggregation of β-amyloid in neurons [148]. The aggregation of β-amyloid was decreased after the over-expression of HSP

in primary neurons and neural blastoma cells [189]. In an in vitro study, Hsp70 was revealed to help in the digestion of amyloid plaques by enhancing microglia [90].

In Parkinson's disease, cytoplasmic protein aggregates (Lewy bodies) containing numerous proteins such as HSP, α-synuclein, ubiquitin and parkin are present in neurons [51]. Cells have increased levels of HSPB5 (αB crystalline) were detected to have a significantly decreased number of Lewy bodies [23]. Although, Amyotrophic lateral sclerosis (ALS) has no known cause, there is a powerful proof connecting this neurodegenerative disease with dysfunctional superoxide dismutase (SOD). It has been detected that Hsp70 interacts with ubiquitin to aim the degradation of dysfunctional SOD by proteasome [186]. Treatment of mice with HSP inducer, arimoclomol, lowered the progression of ALS [93]. ROS participate in the development of multiple sclerosis lesions, and an elevation in HSP expression with the beginning of multiple sclerosis has been detected [132]. Antioxidant therapies have a beneficial effect for multiple sclerosis [167], and an elevation in HSP is accompanied by the decrease of plaques in multiple sclerosis [152].

In addition to having a pivotal role in the treatment of age-related diseases, modification of HSP levels reveals a healthy lifespan and elevating longevity of humans. The function of HSR and HSP across species means that several influences observed in lower organisms could be utilized in humans. Many investigations on C. elegans have revealed marked elevations in life-span with the increased expression of HSP [191]. Moreover, enhanced longevity because of caloric restriction demanded a functional HSR pathway in C. elegans [175]. In Drosophila, HSP revealed the ability to elevate the lifespan of Drosophila, where overexpression of HSP22 was detected to enhance protection against stress and expand longevity [129]. Moreover, Murine models have assisted to reveal the mechanisms behind elevated life-span. Mouse embryonic fibroblasts were detected to reach replicative senescence far sooner at elevated levels of oxidative stress [142], and mice lacking the ubiquitin ligase/co-chaperone CHIP reveal a lowered lifespan with a quickly ageing phenotype [126]. A comparison between short-lived Mus musculus and long-lived Peromyscus leucopus, two closely related species of mice, revealed that the difference in life-span correlated with a variance in oxidative stress tolerance [37]. Moreover, caloric restriction, which is known to increase maximal life-span in murine models, was detected to keep levels of Hsp70 and Hsp60, which normally both decrease during ageing [35].

Exposure of human fibroblasts to frequent heat stress was detected to elevate the levels of Hsp70, Hsp27, HspA8 and Hsp90 and elevate tolerance to oxidative stress; however, no elevation in their Hayflick limit was detected [52]. Extracellular (secreted) HSP, in contrast to intracellular HSP, could have detrimental influences and be pro-inflammatory. Importantly, plasma levels of Hsp70, when compared to controls, were inversely correlated with longevity and significantly decreased in centenarian offspring [178].

### 1.4 Role of HSP in Alzheimer's Disease

Recently, Alzheimer's disease (AD) has a major effect on the international public health. AD characterized by the abnormal synthesis of Tau and amyloid-peptides (Aβ), resulting in the pathological creation of intracellular neurofibrillary tangles (NFTs) and extracellular senile plaques. Insoluble Aβ with a sequence between 38 and 42 amino acids created senile plaques in brains of AD patients [178]. According to the amyloid hypothesis, β-amyloid precursor protein (APP) which is a trans-membrane protein created Aβ peptides. Aβ42 is the main motif in amyloid plaques and creates the most toxic oligomers. Therefore, the increased synthesis of Aβ stimulates cell death, finally resulting in dementia [141]. Moreover, the pathological hyperphosphorylation of protein Tau and its misfolding and accumulation within the cytoplasm resulted in the intra-cellular NFT lesion [63].

The major cause of neuron's injury in AD is because of stress stimulated by the misfolding of Tau and Aβ peptides, inducing the synthesis of toxic oligomers and finally NFTs, the significant of the chaperones in AD has been proofed in the last two decades [113]. Among molecular chaperones, Heat Shock Proteins (HSP) are major constituent of the chaperone and Hsp90, Hsp70 and Hsp60 are deemed target of special superiority in AD [116] and as cancer [32].

Molecular chaperones modify protein activity, organize protein folding and target misfolded or accumulated proteins for degradation or for refolding. HSP are pivotal to ease the protein folding process [39]. They share in various mechanisms to guard the cells against stress-related mechanisms hurtful to the cell [39]. So, as detected in different neurodegenerative diseases, failure of these cellular mechanisms can lead to pathogenic lesions. Several data revealed that HSP organize protein misfolding in many neurodegenerative diseases, such as AD, showing preventative roles and/or working as pathogenic factors. Stress-induced proteins like chaperones have been reported to work as preventive molecules for cells of the nervous system [116]. Several proofs revealed that oxidative stress is a characteristic of PD and AD [208]. Abnormal aggregation of Tau and Aβ proteins and mitochondrial dysfunction can share in making the imbalance between antioxidant and oxidant mechanisms defining oxidative damage in AD patients [208]. In the brain, oxidative stress can make destruction that share in neuronal loss [2]. Reactive oxygen species (ROS) can aggregate inside cells and have negative influences on all biological molecules, determining, for instance, enzyme inactivation, nucleic acid breakage, lipid peroxidation and polysaccharide depolymerization. Under these stress circumstances, the expression of the genes encoding HSP was stimulated [2]. Furthermore, increased levels of ROS and mitochondrial dysfunction might synthesis a vicious circle sharing in AD progression and instauration [116].

Recently, several results were acquired from research on anti-cancer agents. Some compounds of therapeutic impacts were detected but clinical trials are not granted till now. So, acquisition further information is essential and several questions should be lighted, such as: (i) mode of action of HSP inhibitors; (ii) AD biochemical pathways related to HSP; (iii) sensing of client/HSP protein-protein

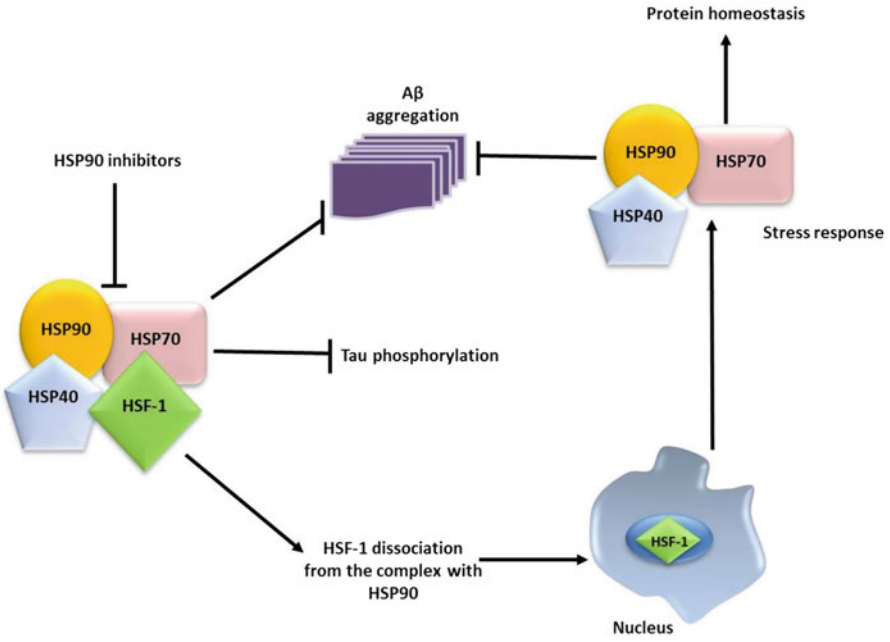



**Fig. 1 Down-regulation of Hsp90 in Alzheimer's disease induce decline in aggregation and hyperphosphorylation of Tau protein**. In cellular stress and Hsp90 inhibitors, Heat Shock Factor 1 (HSF-1) dissociates from the chaperone and induces the activation of heat shock genes within nucleus and stress response through production of Hsp90, Hsp70 and Hsp40, restoring protein homeostasis

interactions at the molecular level; (iv) selection of stress-induced versus constitutive HSP [138, 151]. In conclusion, HSP targeting might be the fundamental for potential drugs in the polypharmacological approach and multitargeted drug discovery and toward a complex disease such as AD. The role of HSP in the pathogenesis of Alzheimer's disease is illustrated in Fig. 1.

## *1.5 Role of HSP in Parkinson's Disease*

Parkinson's disease (PD) is the second most popular neurodegenerative disorder influencing 1% of the population over 60 [41]. People having PD suffer from cardinal motor symptoms comprise muscular rigidity, bradykinesia, gait decay or rest tremor but often cause nonmotor symptoms, like psychiatric symptoms and cognitive sickness. Loss of the dopaminergic neurons of the *substantia nigra* (SN) *pars compacta* lead to most of symptoms accompanied by PD result from [91]. Recently, PD is handled surgically, by deep brain stimulation (DBS) and, pharmacologically, by supporting dopamine tone (e.g., dopamine replacement with

L-dopa) [91]. As the disease advances L-dopa remediation is accompanied by complications comprising dyskinesia and motor fluctuation. DBS is limited to a group of patients suffering from L-dopa-induced complications and L-dopa responsive motor symptoms, but without marked psychiatric disturbance or cognitive sickness. Interestingly, both interventions result in symptomatic cure and do not slow the progression of PD.

Thus, there is a requirement for a remedy targeting the main sources of the disease. By the pathological view, PD cause the existence of proteinaceous intracellular aggregates composed primarily of $\alpha$-synuclein, called Lewy pathology (Lewy neurites and Lewy bodies). Multiplications and missense mutations of the SNCA gene, which encodes for $\alpha$-synuclein, induce the tendency of $\alpha$-synuclein to self-accumulate and cause heritable forms of PD and therefore involving $\alpha$-synuclein accumulation in the pathogenesis of the disease [147, 172]. While there is suspicion concerning the specific form of accumulates ("species") that are neurotoxic, novel proof supposes that $\alpha$- synuclein toxicity is granted by soluble oligomeric species [36, 92, 179]. Due to the pivotal role of $\alpha$-synuclein accumulation in PD, researchers study about the nature and modification of the molecular pathways in charge of directing protein misfolding and folding, decreasing abnormal protein aggregation and keeping proper protein confirmation, gives a potential path for distinguishing a disease altering strategy.

Early proof involving molecular chaperones in the pathobiology of PD concluded from the detection by Auluck et al. [11] that Hsp70 overexpression alleviate $\alpha$-synuclein-mediated dopaminergic neurodegeneration in a Drosophila model. This indicates that Hsp70 can have a neuroprotective role in PD. Posteriorly, McLean et al. [122] reported that the overexpression of Hsp70 and Hsp40 family members decreases the synthesis of $\alpha$-synuclein accumulates in vitro and that Lewy bodies colocalize with multiple chaperone proteins. Molecular chaperones were involved in the pathobiology of PD by the detection of mutations within the promoter region upstream of both inducible and expressed Hsp70 family members elevate the danger of PD [201]. Moreover, mutations in the HspA9 (mortalin), Hsp70, were indicated to enhance the progress of PD [43]; on the other hand, other groups indicate mutations in HspA9 are not a common reason of early-onset PD as they are also detected in patient controls [54].

The ability of Hsp70 overexpression to improve $\alpha$-synuclein toxicity has been well studied in yeast by autonomous groups which have revealed that Hsp70 overexpression can reduce $\alpha$-synuclein in mediated cell death [50] and decrease high molecular weight aggregates in rodent models of PD [102, 127]. Hsp70 overexpression was detected to be preventive against cell death caused by the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), mitochondrial complex I inhibitor, both in vivo [46] and in vitro [149]. $\alpha$-synuclein aggregation is not characteristic to the toxin model, but $\alpha$-synuclein is necessary for MPTP-induced cell death as revealed by the opposition of $\alpha$-synuclein null mice to MPTP [40]. On the other hand, mitochondrial HspA9 may play a pivotal role in the mitochondrial flaws inspired by the pathological A53T mutant $\alpha$-synuclein as HspA9 knockdown

prevents against the mitochondrial fragmentation and elevated tendency to the complex I inhibitor, rotenone, stimulated by A53T overexpression [111].

The mechanism, Hsp70 decreases α-synuclein toxicity, appears to be dependent mechanism on both its function in protein degradation and its refolding activity by the ALP and UPS. Mutations which change the ATPase function of Hsp70 (K71S) cancel its preventive influence on α-synuclein toxicity, suggesting that Hsp70 folding activity is essential for its preventive function [102]. Importantly, this mutation has no influence on the ability of Hsp70 to inhibit α-synuclein accumulation [102], indicating that Hsp70 utilizes clear mechanisms to decrease the aggregation and toxicity and of α-synuclein. Moreover, Hsp70 can ease disassemble of preformed α-synuclein aggregates [134]. Gao et al. [60] revealed that an Hsp70 machine composed of HspH2, DNAJB1, and HspA8 could efficiently disaggregate created α-synuclein fibrils in vitro.

Many studies have indicated that CMA may play a pivotal role in alleviating α-synuclein toxicity [204]. Promoted α-synuclein expression in both paraquat and transgenic models of PD leads to the enhancement of HspA8 and LAMP2A expression and a larger movement of α-synuclein into the lysosomes [115]. Furthermore, both HspA8 and LAMP2A have decreased expression in the SN of PD patients [3], and a novel investigation revealed a link between the α-synuclein aggregation and loss of LAMP2A in postmortem PD brains [131]. Importantly, the detected reduction in HspA8 and LAMP2A expression anatomically overlaps with an elevation in miRNAs able to translationally suppress both HspA8 and LAMP2A [4], and implicate miRNAs in PD-associated chaperone dysregulation. In conclusion, the ability of Hsp70 and its cochaperones to disaggregate, refold, and aim for destruction of toxic α-synuclein species indicates that molecular chaperones can have a pivotal role in the pathobiology of PD. The role of HSP in the pathogenesis of Parkinson disease is illustrated in Fig. 2.

## *1.6 Role of HSP in Diabetes*

Diabetes is a condition implicated a chronic elevation of blood glucose levels (hyperglycemia). This disease is classified into 2 types: type 1, which is accompanied by the demolition of pancreatic beta cells leading to scanty insulin production; and type 2, which embraces a range of disorders that finally result in hyperglycemia [196]. Both types of diabetes elevate the potential for the development of microvascular disorders, such as neuropathy, retinopathy and nephropathy, and for macrovascular disorders [10].

It has been mentioned that these HSP chaperones are accompanied by several clinical disorders, containing diabetes. HSP have been involved in the sources of type 1 diabetes and in the cure of the obesity and insulin resistance implicated in type 2 diabetes [34]. Reduced expression of Hsp70 and suppression of heat shock factor-1 (HSF-1) have been reported in different tissues of rats with type 1 diabetes. Inhibition of HSP 70 levels by diabetes is accompanied by elevation in tissue

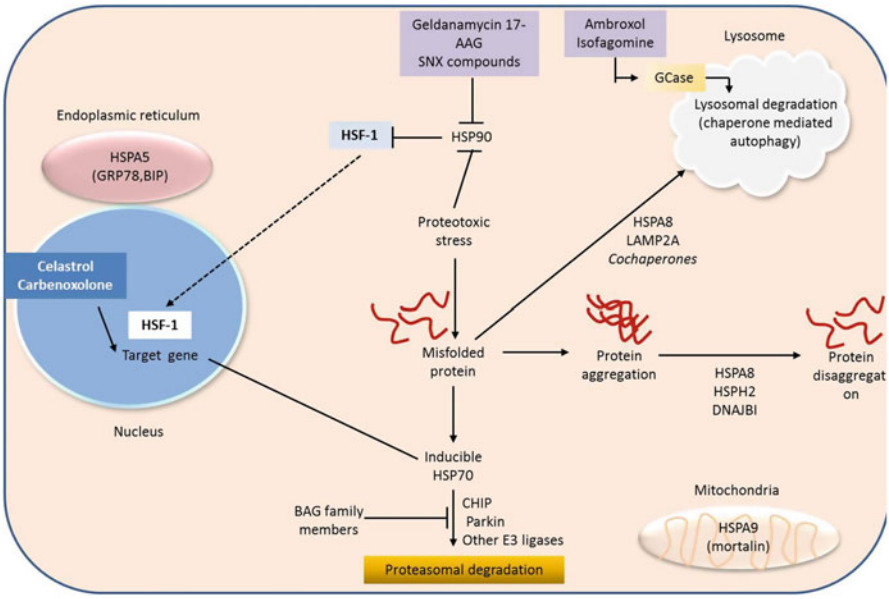



**Fig. 2 Proposed role of molecular and small molecule chaperones in proteostasis in Parkinson disease**. At normal state, Hsp90 bind to HSF-1 and block its activity. However, in presence of Hsp90 inhibitors (such as SNX compounds, geldanamycin, 17-AAG) or proteotoxic stress, active HSF-1 separated from Hsp90 and translocates to the nucleus where it stimulates the expression of Hsp70. Members of inducible Hsp70 family induce proteasomal degradation via a pathway activated by E3 ligases, CHIP, and Parkin. This degradation is prevented by members of BAG family and enhanced by celastrol and carbenoxolone (small molecule HSF-1 activators). In Proteotoxic stress, the misfolded proteins directed for degradation via the interactions of autophagy-lysosome system with various chaperone-mediated autophagy. Chaperone/cochaperone complexes can play a role in disaggregation of already formed protein aggregates. Also, the pharmacologic chaperones such as isofagomine and ambroxol can activate glucocerebrosidase (GCase) in the lysosome to further stimulation for chaperone-mediated autophagy. In the endoplasmic reticulum and mitochondria, chaperone are regulated by specific members of the Hsp70 family, HspA5 and HspA9, respectively

inflammation. Moreover, the same authors mentioned that normal Hsp70 and HSF-1 stimulation by endurance exercise has been prevented by diabetes [185].

Many investigations have revealed reduced expression of HSP in patients with type 1 and 2 diabetes. It appears that the decrease in chaperon activity in diabetic patients is one of the major causes for beginnings of diabetic problems. So, researchers are seeking to use various techniques, inclusive pharmaceutical and chemical compounds, thermotherapy and exercise, to stimulate the expression of HSP [81]. Former investigations suggest that an elevation in protein stability and a decrease in protein glycation can markedly reduce the complications of diabetes [85].

Diabetes is a disorder including elevated glycation, oxidation and inflammation; therefore, it would have been foretold that levels of HSP could be highly preventive in persons suffering from diabetes. However, results of investigations in humans and animals with diabetes detected reduced HSP expression. Therefore, the paradoxically reduced levels of HSP confirm the destruction caused by diabetes lesions. Intracellular HSP, through blocking nuclear factor-*k*B (NF-*k*B) activation, have anti-inflammatory influences on cells. Protein kinase C activation by NF-*k*B is a primary pathway resulting in diabetes-induced cytokine gene expression. Therefore, reduced levels of HSP in cases of diabetes will elevate the activity of NF-*k*B and confirm inflammation [80].

Type 2 diabetes mellitus is age related; it can lower longevity and fast several traits accompanied by aging. HSP are factors which have a pivotal role in aging and longevity [81]. Patients with type 2 diabetes have elevated incidence of neurodegenerative diseases, such as Parkinson and Alzheimer diseases. In Parkinson and Alzheimer diseases, amyloid precursor aggregation may result in a common loss of insulin signaling in the pancreatic beta cells and in the brain. Moreover, loss of insulin signaling results in reduced HSP in beta cells or neurons, which leads to abnormal protein aggregation and function. It has been mentioned that administration of Hsp70 and insulin can lower amyloid aggregation in the brain [81].

Bimoclomol is a drug that can elevate the fluidity of membrane and expands the activity of HSF-1, therefore can elevate the levels of Hsp70. It has been mentioned that bimoclomol lowers tissue damage, enhances wound healing, ameliorates insulin sensitivity in animal models of diabetes and decreases diabetes complications [74]. Lipoic acid administration in patients with neuropathy and type-1 diabetes was accompanied by normalization of the low level of Hsp72. This Influence was attributed to clinical amelioration in the neuropathy in these patients. It has been mentioned that thiazolidinediones, carvedilol and exercise elevate HSP. The anti-inflammatory action of on the pancreatic beta cells could be linked to the elevation of Hsp70 levels by this drug. Nitric oxide is a powerful inducer of HSP expression. Drugs that retrieve the secretion of nitric oxide from blood vessels, such as angiotensin-converting enzyme inhibitors, beta-adrenergic blockers, thiazolidinediones and HMG-CoA reductase inhibitors, are correlated to outstanding results in clinical trials of diabetes. Near-infrared light therapy releases nitric oxide from endothelium and, thus, treat diabetic neuropathy.

Finally, the oral or intravenous administration of HSP is impractical due to the intracellular position of HSP. On the other hand, it has been mentioned that liposomal delivery of Hsp72 into renal tubular cells blocks induction of NF-*k*B tumor necrosis factor and, so, blocks ischemia-induced apoptosis. It is an important finding that several drugs or conditions that may elevate HSP levels and also block NF-kB (i.e. statins [76], exercise [150], pentoxifyllin and carvedilol [79].

## *1.7 Role of HSP in Atherosclerosis*

Atherosclerosis is an old disease slowly progressing disease that becomes manifested in the middle age or later, even if it begins in childhood [155]. In past, atherothrombosis is considered as the first killer of the aging people in the developed countries; however, dramatic increase in its incidence in the developing countries was recently reported. Currently, around 39% of death cases reported in the U.K. is concerning to atherosclerosis, however about 12 million of American citizens suffered atherosclerosis-related diseases [13]. This disease is characterized by lipids deposition, especially of low-density lipoproteins (LDLs), on the endothelial layer of medium- sized and large arteries, together with remodeling of arterial walls and severe infiltration of immune cells, forming the characteristic plaques called atheroma. Although the signs of disease have been discovered in Egyptian mummies more than 4000 years old [180], the lipid composition of atheroma and the combined mononuclear infiltration were first described about two centuries ago [121]. However, the scientists have reached to the inflammatory hallmarks in progress and pathogenesis of atherosclerosis over the past 30–40 years [73]. Recently, several researches proved that inflammation is the first steps of atherosclerosis [109]. They concluded that the expression of adhesion molecule on the endothelial cells, such as vascular adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin, beside the activation of macrophages, T lymphocytes, mast cells, and several cytokines suggesting involvement of inflammatory and immune processes in the pathogenic progress of atherosclerosis [67, 72].

From another sight of view, the key role played by the immune reactivity in the pathogenesis of atherosclerosis confirmed the essential contribution of inflammatory process. Many investigators induced experimental atherosclerosis in rabbits by high fat diet, and examined the therapeutic activities of immunosuppressive drugs, authors concluded marked prevention for plaque formation and inflammatory infiltration in the aorta; they outlined the relationship between the declined production of local inflammatory and immune cells and the reduction in cholesterol content in the arterial walls [68, 190].

Normally, cells subjected to stress stimuli, such as oxidized LDL, heat shock, infectious, surgical, mechanical stress, or cytokine activation, will respond by production of increased levels of HSP to protect themselves from stress stimuli [16]. Accordingly, it was described that HSP could expressed in high levels in cardiovascular tissues to initiate the inflammatory process, and that they may be expressed during the progress of atherosclerosis as an autoantigen [143].

In 1990, Berberian et al. reported for the first time the increased expression of Hsp70 in arteries of human and rabbits. Authors concluded that the distribution of HSP in arteries was correlated to necrosis, lipid accumulation, and macrophages infiltration in human atheroma. Interestingly, Hsp70 was found to be concentrated mainly in the central thickened portions of atheroma around the accumulated lipid and sites of tissue necrosis [88]. On contrary, some of the most complex plaques contained foci of smooth muscle cells without obvious relation to necrosis or

increased expression of HSP [87]; where Hsp70 was produced in arterial wall cells even in dendritic cells [20]. Authors concluded that HSP production was increased within the depth of plate, particularly in macrophages and associated to necrotic tissue.

Consistently, Xu et al. [207] indicated that Hsp70 is overexpressed in the advanced atherosclerotic lesions. Authors found that Hsp70 ameliorates the NFκB activation, suggesting its anti-inflammatory potential. In another studies, authors declared that the levels of Hsp70 in plasma have a direct [203] and inverse [119] relation to atherosclerosis severity. Additional investigations concluded that administration of Hsp70 promoted the production of pro-inflammatory (such as IL-6) [9] and anti-inflammatory (such as Treg) cytokines [194]. Interestingly, Hsp70 could be considered as a favor factor for progression of atherosclerosis as well as mononuclear inflammatory infiltration. This theory confirmed in study designed by Xie et al. [203]; authors concluded that feeding diet with high-cholesterol level led to increased levels of Hsp70 in plasma. Additionally, the exogenous supplementation of Hsp70 promotes production of adhesion molecules within mononuclear cells in peripheral blood. In contrast, Madrigal-Matute et al. [114] observed that overexpression of Hsp70 was associated with declined oxidative stress and inflammatory response in the walls of arteries; suggesting its protective potential. Therefore, the promoting and inhibition effect Hsp70 against atherosclerosis are still a debate matter [18].

On the other hand, several investigations focused on the role of Hsp90 in atherogenesis. It was observed that the overexpression of Hsp90 is related to instability of atheroma. As well, the inhibition of Hsp90 led to declined production of inflammatory cells and oxidative stress due to reduced activation of transcription factors (such as the activators of transcription and NFκB signal transducers). Interestingly, the suppression of Hsp90 activity could be benefit in promoting the overexpression of Hsp70, with subsequent inhibition of the proinflammatory response and atherogenesis [114].

Recently, a growing body of evidence suggested the direct atherogenic potential for Hsp60; where increased expression of Hsp60 usually precedes the growth of atherosclerotic plaque [95]. In humans, the increased level of circulating Hsp60 and anti-Hsp60 are correlated to thickness of carotid artery wall [202], atherosclerotic lesions [146], and atherosclerosis-associated morbidity and mortality [205]. Furthermore, early atherosclerotic lesion was induced by transfer of Hsp60 reactive T cells [197]; where specific immunity of T-cell to Hsp60 is induced (Knoflach et al. 2007). In addition, administration of Hsp60 might induce or suppress atherogenesis, based on administration route, and the involved co-stimulatory molecules. Administration of Hsp60 parenterally activate infiltration of Hsp60-specific T cells, with subsequent secretion for anti-Hsp60 antibodies, pro-inflammatory cytokines, accumulations of macrophages and lipid, and atheroma formation. However, administration of Hsp60 via oral or nasal route reduced the atherosclerotic lesions, due to induction of Tregs and anti-inflammatory mediators including interleukin-10 (IL-10) and transforming growth factor beta (TGF-β) [197]. In human atherosclerosis, Kleindienst and colleagues indicated that Hsp60 was identified on smooth muscle cells, mononuclear

inflammatory cells, and endothelial cells of aorta and carotid artery compared to the small blood vessels that had no sclerotic lesions. The positive correlation between the severity of atherosclerosis and the produced Hsp60 was also confirmed by Hammerer-Lercher et al. [70]. In another investigation, the expressions of Hsp60 and Hsp70 in the aortic tree showed positive correlation with the progress of atherosclerosis in apoE-deficient mice [95]. The main expression sites for both HSP were within macrophages, smooth muscle cells, endothelium, and CD3 T lymphocytes [95].

Interestingly, results from another study revealed that Hsp47 might be also involved in atherogenesis [198]. Strong expression of Hsp47 was proved locally in atherosclerotic arteries (particularly in the collagenous areas) but not in normal artery. HSP47 was expressed mainly in cells produce type I procollagen [154]. Results from this study suggested the role of Hsp47 in atheroma formation in human coronary. In addition, authors concluded the upregulation of Hsp47 as a response to stress; this conclusion might indicate the possible role of Hsp47 in plaque stability.

Hsp27 is an intracellular chaperone that possesses an important role in stabilization of RNA, beside its role in the antioxidant and antiapoptotic responses [14]. In atherosclerosis, extracellular production of Hsp27 from atheroma was evident; may be due to cellular damage or as a co-secretion with exosomes or lysosomes. After secretion, in the extracellular space, Hsp27 able to binds with several receptors on cell membrane of inflammatory immune cells and endothelial cells, such as CD14, CD36, CD40, CD91, scavenger receptor A (SR-A), and toll like receptors (TLRs) as TLR2, TLR3, and TLR4 [19]. Interestingly, data from available research concluded the ameliorative role played by Hsp27 during atherogenesis. On the same line, the definition of Hsp27 as an estrogen receptor-associated protein could explain the ameliorative role played by estrogens during atherogenesis [153]. Consistent with that, several studies demonstrated that atheroma has low content of Hsp27 [117], and therefore, low circulating levels of Hsp27 indicates more severe atherosclerotic lesions [169]. On contrary, overexpression of Hsp27 may protect against atherogenesis [38]. The protective potential of Hsp27 against atherosclerotic disease may attribute to its suppressive activity for NFκB activation [14], involvement in lipid homeostasis via competing with LDL in binding to SR-A, with subsequent formation of foam cell [14], and declined the cholesterol content in atheroma the serum [38]. The role of HSP in the pathogenesis of atherosclerosis is illustrated in Fig. 3.

## *1.8 Role of HSP in Rheumatoid Arthritis*

Rheumatoid arthritis (RA) is a widely known chronic inflammatory disease that particularly affects the aging population. It occurs due to damage of the synovial membranes of joints via infiltration of mononuclear and/or polynuclear inflammatory cells including macrophages, lymphocytes, and neutrophils [69, 123]. Usually, during the course of RA, patient developed severe pain due to progressive injury or

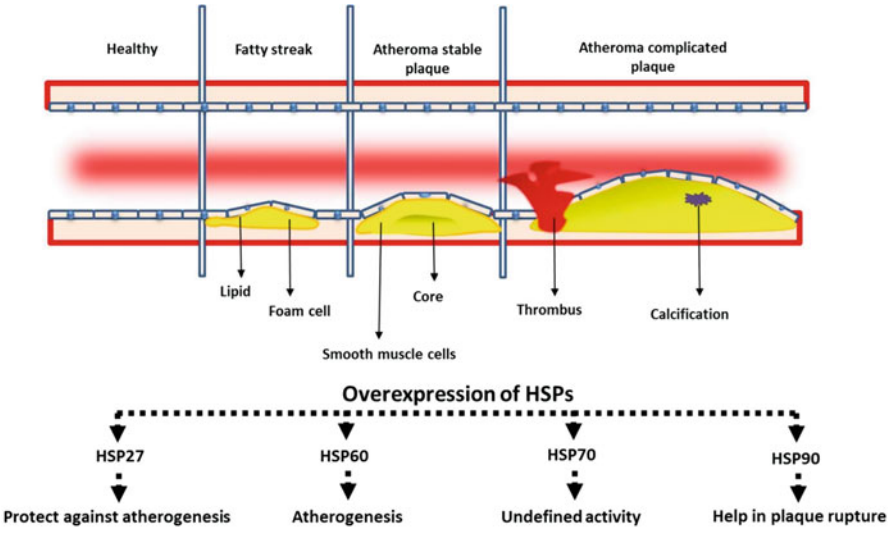



**Fig. 3 The role of HSP in the pathogenesis of atherosclerosis**. Overexpression of Hsp27 is protects against atherogenesis; however, overexpression of Hsp60 help in atherogenesis. In addition, Hsp90 aggravates atheroma by help plaque rupture

even complete loss of bone and cartilage around the inflamed joint. Generally, the pathogenesis of this disease is complicated; however, the pathologic events associated to RA suggesting an autoimmune cause in form of T-cell-mediated chronic inflammatory response [209].

On the other hand, it has been observed that overexpression of HSP family might be involved in RA pathogenesis; where increased expression can regulate the progress of disease [83]. The inflammatory events and other stress factors occur in synovial membrane are able to increase HSP expression. In RA, the hypoxia and reperfusion injury of the rheumatoid joint, lead to production of high levels of reactive oxygen species (ROS) [77]. Subsequently, this increased production of ROS and the high synovial content of inflammatory mediators (such as tumor necrosis factor (TNF)- a and interleukin (IL-l) will act as stress factors [199]. Consistently, Schett et al. [165] concluded that, in RA but not osteoarthritic, heat shock transcription factor 1 (HSFl) was activated and undergo hyper-phosphorylation and nuclear translocation that could lead to regulation of Hsp70 transcription. In another investigation, authors used cultured RA SM synovial fibroblasts to study the expression of Hsp70 in AR, they observed that the proinflammatory mediators including IL-l0 and TNF-$\alpha$ were able to upregulate expression of Hsp70 in cultured fibroblasts (Luo et al. 2008). In another research groups, authors declared that several types of HSP and chaperones were overexpressed in RA such as human Hsp27, Hsp90α, Hsp60 [168], and Hsp65 [83]. On contrary, Worthington et al. [200] concluded that human Hsp65 was expressed equally in RA and control non-inflamed synovia.

Moreover, Hsp60 was expressed equally in mitochondria of RA and osteoarthritis, as assessed by immunohistology [22].

Karlsson-Parra et al. [96] observed that human Hsp65 (huHsp65) was overexpressed at the cartilage-pannus junction; they concluded that the eroding front possessed the maximum expression compared to SM itself. Interestingly, the same authors identified the expression of huHSP65 in rheumatoid nodules; where rheumatoid nodules are the pathognomonic histologic and clinical feature of RA. This expression could be attributed to the presence of non-caseated hypoxic center, where hypoxia act as stress factor lead to increased expression of HSP.

Lewthwaite et al. [106] observed the correlation between huHsp60 circulating in plasma and the psychosocial and physiologic stress. Consistent with this finding, the increased levels of circulating huHsp60 was correlated to carotid atherosclerotic plaque [202]. Generally, in osteoarthritis, HSP are produced mainly from the chondrocytes [171]. However, in RA patient, they synthesized mainly in synovial intimal cells [112]. Therefore, it could be concluded that the inflammatory signaling in this tissue are able to initiate the production of HSP; with subsequent protection for the host cells.

In another theory, RA may define as autoimmune-inflammatory disease; where the immune system attacks the synovial fluid-membranes exist in different joints. Neglect treatment of RA could result in severe inflammatory response [173]. This inflammation has the potential to attract several immune components such as immune chemokine, cytokines, and lymphocytes to the infection area [84]. As a response to RA infection, the synthesis of HSP particularly Hsp70 is increased. It is now well documented that Hsp70 possess an anti-apoptotic property through inhibition of proinflammatory and proapoptotic factors such as Caspases and JNK (Jun N-terminal) signaling, cytochrome c release, and apoptosome formation [66]. Therefore, the overexpression of Hsp70 in synovial membrane during the infection with rheumatoid arthritis fibroblast-like synoviocyte (RA-FLSs) is not surprisingly. Herein, Hsp70 acts to control the inflammatory process through blocking of pro-inflammatory signaling, and to regulate the effect of T-cells [165].

In another study, Kang et al. [94] observed repression of Hsp70 produced in RA fibroblast-like synoviocytes (FLSs) after treatment with sodium nitroprusside (SNP) in an in vitro experiment. Authors reported that Hsp70 downregulated cells showed better survive compared to control cells. It was concluded that downregulation of Hsp70 protects RA FLSs against apoptosis induced via nitric oxide production through activation of the Akt signaling pathway. However, the real in vivo function of Hsp70 in the RA is still not fully clear. By considering these findings, we can conclude that inhibition of Hsp70 in RA may be used as a therapeutic approach to control the severe inflammatory response occurs in RA. Additionally, van Roon et al. [188] noted that T-cells collected from patients suffered RA have the potential to react with huHsp60 to suppress the activation of the pro-inflammatory mediator (TNF-$\alpha$) via induction of Th2 cytokine regulator. However, this regulation is not reported for Hsp65 isolated from Mycobacterium tuberculosis [144]. Consistently, several investigators attributed this response of T-cell to self-Hsp70 and Hsp60 to production of the regulatory mediators (interleukin-4 and interleukin-10), with

subsequent prevention of arthritic diseases [6, 97, 144]. Taken together, it is may conclude that huHsp60 and mycobacterial Hsp60 might be considered as promising potential vaccines against autoimmune inflammatory diseases.

## *1.9 Role of HSP in Cancer*

The progressive loss of physiologic and immunologic potency is a characteristic feature for the elderly [33]. Growing body of evidences has proved cancer augmentation by aging, which may be due to age- associated immune dysregulation [182], with subsequent poor prognosis [98]. Interestingly, around 50% of malignancies are diagnosed in aging patient over than 65 years old [78]. Several investigations concluded the anti-apoptotic activities of HSP. Therefore, it is not surprising that the high levels of HSP may have the potential to protect malignant cells against therapy-induced apoptosis [89]. The apoptotic process may occur in either intrinsic or extrinsic pathways. Whatever pathway, the final event is induction of caspases proteases, which is cleaved enzymatically leading to activation of the apoptotic stimulus [28]. In the intrinsic pathway, apoptosome is formed by mitochondria; where cytochrome c released from mitochondria to the cytosol, and then interact with pro-caspase-9 and cytosolic apoptosis protease activating factor-1 (APAF-1) forming apoptosome. Apoptosome is responsible for initiation of apoptotic cascade via activation of pro-caspase-3 [177].

Currently, it has been well known that Hsp27 and Hsp70 have the potential to inhibit the formation of apoptosome, with in turn inhibition of apoptosis. Another theory for inhibition of apoptosis by Hsp27 is direct binding to APAF-1, which subsequently led to inhibition of apoptosome formation [61]. Additionally, Hsp90 suppress pro-caspase-9 activation by cytochrome c [139]. On the other hand, the extrinsic apoptotic pathway work via binding to the respective ligands of death receptors (TNF receptor 1, TNF receptor superfamily, apoptosis antigen-1), leading to their activation and formation of death inducing complex at the plasma membrane. This complex activates the pro-caspase-8, which in turn induce direct or indirect activation of caspases [21].

In another research group, authors cleared that phosphorylated dimers of Hsp27 can bind to Daxx protein competitively with FAS; lead to subsequent interference with FAS-mediated apoptotic pathway [31]. Additionally, Bruey et al. [24] investigate the interaction between Hsp27 and cytochrome *c*. Authors concluded that Hsp27 can block Caspase activation via its binding to cytochrome c and inhibition for interaction with procaspase-9 and apoptotic protease activating factor-1 (APAF-1). In CD133 + colorectal cancer stem cells, activation of Hsp27 inhibits the cleavage of caspase-3 and -9 in the apoptosis pathway. However, its inhibition promotes apoptotic cascade in CD133+ cells [110]. Additionally, inhibition of Hsp27 activation up-regulated the activity of caspase-3 in glioblastoma cells [107].

Moreover, the anti-apoptotic activity of Hsp90 could be discussed by its ability to bind to the anti-apoptotic protein (such as AKT1) and suppress its activation, which

in turn enhanced cell surviving [62]. In contrast, several investigators concluded the pro- apoptotic activities of Hsp60 in in vitro experiment [164]. In addition to the antiapoptotic and proapoptotic activities of HSP members, some members such as Hsp27 are essential also in regulation, progression, and metastasis of tumor cells. Interestingly, blocking of Hsp27 led to decline in matrix metalloproteinase (MMP), epithelial-to-mesenchymal transition, migration, and metastasis of neoplastic cells [65]. In addition, in human prostatic malignancy, Hsp27 has the potential to up-regulates MMP2 activity stimulated by transforming growth factor b (TGF-b), lead to promoting cell invasion [206]. Additionally, Hsp27 reported to enhance the neoplastic migration in bladder malignancy [210], and promote metastasis of epithelial ovarian cancer to peritoneum [215].

Thuringer et al. [181] studied effect of Hsp27 on progression and metastasis of breast cancer. They concluded that Hsp27 has a direct role in enhancement of angiogenic activity and neoplastic migration via upregulating gene transcription of vascular endothelial growth factor (VEGF) and activated VEGF receptor type 2. In another study, [136] found that Hsp27 inhibit p53-induced activation of p21 in neoplastic cells, with in turn regulation of p53 signaling. Moreover, proliferation of lung cancer cells could be enhanced by Hsp27-induced activation of activator protein-1 [214]. However, in gastric adenocarcinoma, cancer progression could be enhanced by the C-X-C chemokine receptor type 1 (CXCR1); CXCR1 has the potential to decrease Hsp27 expression, indicating the relationship between cancer progress, Hsp27, and CXCR1 [82].

It has been reported that Hsp90AA1 is involved in enhancement of invasiveness and mobility of cancer cells [195], where it is required for the invasion of fibrosarcoma cells [48]. On these bases, Hsp90AA1 found to enhance the in vitro invasion of breast cancer and melanoma, with in turn increment of the metastatic activities. Also, serum Hsp90AA1 increased in breast, liver, pancreas, and lung cancer in correlation to degree of malignancy [192]. However, inhibition of Hsp90AA1 suppresses the metastatic invasion in mouse melanoma [176]. This enhancement of HSP against tumor invasion potential may attribute to their binding to the extracellular receptors activating ERK1/2 and PI3K-Akt pathways [71]. Additionally, Tsuneki et al. [183] reported that HspA9 is released from oral squamous carcinoma cells, and then interact with podoplanin; that is an adhesion molecule responsible for the invasion potential of tumor. Moreover, HspB6 has a role in angiogenesis, progression, and migration. For example, overexpression of HspB6 led to increase density of heart capillaries in mice [213].

In recent years, many researchers studied the extracellular and intracellular localization of HSP in tumor cells. In normal cells, it is uncommon to localized Hsp60 on the cell membrane. However, this localization is frequent in malignant cells [27, 29]. In addition, it was reported that Hsp60 is exist in exosomes released from malignant cells in human [125]. The extracellular HSP have several functions; one of them is immune modulation. For instance, TNFα and IL-6 were produced in mast cells under stimulation of HspA1A via activation of toll-like receptors 2 and 4 (TLR4, TLR2) [130] and interleukin 12 (IL-12) [15]. Additionally, treatment with HspA1A led to activation of macrophage and production of TNFα [5]. Recently, in vitro studies were performed on murine leukemia monocytes and hepatocellular

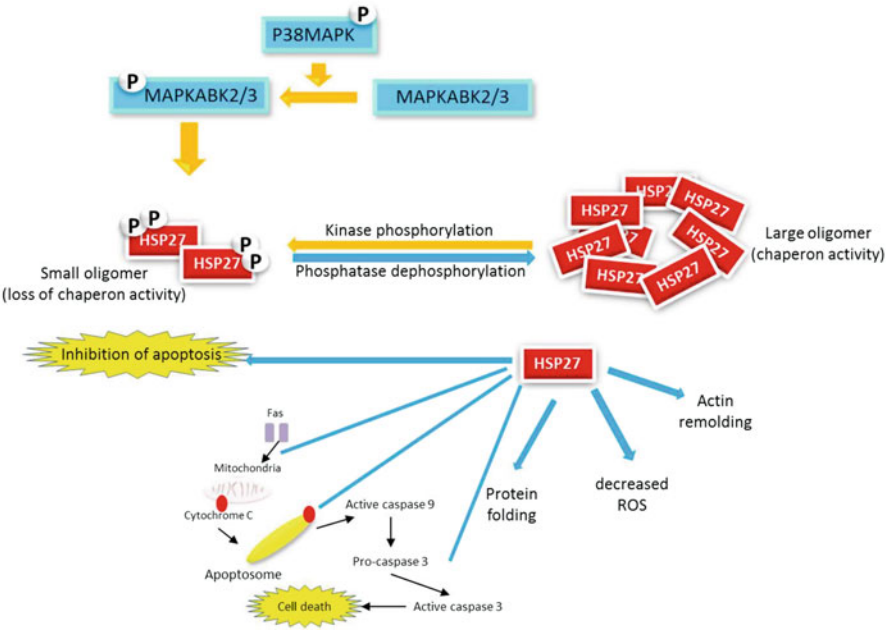



**Fig. 4 Schematic representation for the role of Hsp27 in carcinogenesis**. Hsp27 suppressed apoptosis with subsequent enhancement for tumorigenesis. Unphosphorylated Hsp27 present as large oligomers and possess chaperonage activities. Phosphorylated Hsp27 switches to smaller oligomers and lose their chaperonage activities and initiate their pro-oncogenic activities. Hyperactivation of Hsp27 induces inhibition of apoptosis

carcinoma cell line, author concluded release of HspA1A, Hsp90AA1, and HSPD1 from exosomes, which enhancing the activity of macrophages, natural killer, and mononuclear cells [103]. In contrast, Chalmin et al. [30] described the immunosuppressive role of HspA1A released from exosome; HspA1A is reported to suppress tumor immune surveillance via activation of myeloid-derived suppressor cells. In addition, in colorectal carcinoma, secretion of HspH1 led to differentiation of macrophage, with in turn anti-inflammatory profile and pro-tumor effect [17]. However, in primary breast tumor cells, released HspB1 led to monocytes differentiate into proangiogenic macrophages [7]. The role of HSP in the pathogenesis of cancer is summarized in Fig. 4.

## 2 Conclusions

HSP are the cornerstone for repairing damaged proteins resulted exposure of cells to different stresses including the age-related disorders. Hence, enhancing and modulation of HSP functions would help the human welfare through promoting healthy lifespan and elevating the longevity of humans.

**Acknowledgements** We would like to thank the Deanship of Scientific Research and RSSU at King Saud University for their technical support.

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Abdelnour SA, Abd El-Hack ME, Khafaga AF, Arif M, Taha AE, Noreldin AE (2019) Stress biomarkers and proteomics alteration to thermal stress in ruminants: a review. J Therm Biol 79:120–134
2. Abdul HM, Butterfield DA (2007) Involvement of PI3K/PKG/ERK1/2 signaling pathways in cortical neurons to trigger protection by cotreatment of acetyl-L-carnitine and alpha-lipoic acid against HNE-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. Free Radic Biol Med 42:371–384
3. Alvarez-Erviti L, Rodriguez-Oroz MC, Cooper JM, Caballero C, Ferrer I, Obeso JA, Schapira AH (2010) Chaperone-mediated autophagy markers in Parkinson disease brains. Arch Neurol 67:1464–1472
4. Alvarez-Erviti L, Seow Y, Schapira AH, Rodriguez-Oroz MC, Obeso JA, Cooper JM (2013) Influence of microRNA deregulation on chaperone-mediated autophagy and α-synuclein pathology in Parkinson's disease. Cell Death Dis 4:e545
5. Anand PK, Anand E, Bleck CK, Anes E, Griffiths G (2010) Exosomal Hsp70 induces a pro-inflammatory response to foreign particles including mycobacteria. PLoS One 5:e10136
6. Anderton SM, Van Der Zee R, Prakken B, Noordzij A, Van Eden W (1995) Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. J Exp Med 181:943–952
7. Arispe N, Doh M, De Maio A (2002) Lipid interaction differentiates the constitutive and stress-induced heat shock proteins Hsc70 and Hsp70. Cell Stress Chaperones 7:330–338
8. Asea A (2003) Chaperokine-induced signal transduction pathways. Exerc Immunol Rev 9:25–33
9. Asea A, Kraeft S-K, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK (2000) Hsp70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6:435
10. Atalay M, Oksala N, Lappalainen J, Laaksonen DE, Sen CK, Roy S (2009) Heat shock proteins in diabetes and wound healing. Curr Protein Pept Sci 10:85–95
11. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM (2002) Chaperone suppression of α-synuclein toxicity in a Drosophila model for Parkinson's disease. Science 295:865–868
12. Azuma K, Shichijo S, Takedatsu H, Komatsu N, Sawamizu H, Itoh K (2003) Heat shock cognate protein 70 encodes antigenic epitopes recognised by HLA-B4601-restricted cytotoxic T lymphocytes from cancer patients. Br J Cancer 89:1079–1085
13. Barylski M, Małyszko J, Rysz J, Myśliwiec M, Banach M (2011) Lipids, blood pressure, kidney–what was new in 2011? Arch Med Sci 7:1055
14. Batulan Z, Pulakazhi Venu VK, Li Y, Koumbadinga G, Alvarez-Olmedo DG, Shi C, O'Brien ER (2016) Extracellular release and signaling by heat shock protein 27: role in modifying vascular inflammation. Front Immunol 7:285

15. Bausero MA, Gastpar R, Multhoff G, Asea A (2005) Alternative mechanism by which IFN-γ enhances tumor recognition: active release of heat shock protein 72. J Immunol 175:2900–2912
16. Benjamin IJ, McMillan DR (1998) Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. Circ Res 83:117–132
17. Berthenet K, Boudesco C, Collura A, Svrcek M, Richaud S, Hammann A, Causse S, Yousfi N, Wanherdrick K, Duplomb L, Duval A, Garrido C, Jego G (2016) Extracellular Hsp110 skews macrophage polarization in colorectal cancer. Onco Targets Ther 5:e1170264
18. Bielecka-Dabrowa A, Barylski M, Mikhailidis DP, Rysz J, Banach M (2009) HSP 70 and atherosclerosis–protector or activator? Expert Opin Ther Targets 13:307–317
19. Binder CJ, Chang M-K, Shaw PX, Miller YI, Hartvigsen K, Dewan A, Witztum JL (2002) Innate and acquired immunity in atherogenesis. Nat Med 8:1218
20. Bobryshev YV, Lord RS (2002) Expression of heat shock protein–70 by dendritic cells in the arterial intima and its potential significance in atherogenesis. J Vasc Surg 35:368–375
21. Boldin MP, Goncharov TM, Goltseve YV, Wallach D (1996) Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1-and TNF receptor–induced cell death. Cell 85:803–815
22. Boog CJ, de Graeff-Meeder ER, Lucassen MA, van der Zee R, Voorhorst-Ogink MM, van Kooten PJ, Geuze HJ, van Eden W (1992) Two monoclonal antibodies generated against human hsp60 show reactivity with synovial membranes of patients with juvenile chronic arthritis. J Exp Med 175:1805–1810
23. Braak H, Del Tredici K, Sandmann-Kiel D, Rub U, Schultz C (2001) Nerve cells expressing heat-shock proteins in Parkinson's disease. Acta Neuropathol 102:449–454
24. Bruey J-M, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo A-P, Kroemer G, Solary E (2000) Hsp27 negatively regulates cell death by interacting with cytochrome c. Nat Cell Biol 2:645
25. Calderwood S (2007) Molecular chaperones and the ubiquitin proteasome system in aging. In: The ubiquitin proteasome system in the central nervous system. Nova, New York, pp 537–552
26. Calderwood SK, Gong J (2016) Heat shock proteins promote cancer: it's a protection racket. Trends Biochem Sci 41:311–323
27. Campanella C, Bucchieri F, Merendino AM, Fucarino A, Burgio G, Corona DF, Barbieri G, David S, Farina F, Zummo G (2012) The odyssey of Hsp60 from tumor cells to other destinations includes plasma membrane-associated stages and Golgi and exosomal protein-trafficking modalities. PLoS One 7:e42008
28. Cappello F, Bellafiore M, Palma A, Bucchieri F (2002) Defective apoptosis and tumorigenesis: role of p53 mutation and Fas/FasL system dysregulation. Eur J Histochem 46:199–208
29. Cappello F, de Macario EC, Di Felice V, Zummo G, Macario AJ (2009) Chlamydia trachomatis infection and anti-Hsp60 immunity: the two sides of the coin. PLoS Pathog 5: e1000552
30. Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, Boireau W, Rouleau A, Simon B, Lanneau D, De Thonel A, Multhoff G, Hamman A, Martin F, Chauffert B, Solary E, Zitvogel L, Garrido C, Ryffel B, Borg C, Apetoh L, Rebe C, Ghiringhelli F (2010) Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. J Clin Invest 120:457–471
31. Charette SJ, Landry J (2000) The interaction of Hsp27 with Daxx identifies a potential regulatory role of Hsp27 in Fas-induced apoptosis. Ann N Y Acad Sci 926:126–131
32. Chatterjee S, Burns TF (2017) Targeting heat shock proteins in cancer: a promising therapeutic approach. Int J Mol Sci 18:1978
33. Chen W, Zheng R, Zhang S, Zeng H, Xia C, Zuo T, Yang Z, Zou X, He J (2017) Cancer incidence and mortality in China, 2013. Cancer Lett 401:63–71
34. Chung J, Nguyen A-K, Henstridge DC, Holmes AG, Chan MHS, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA,

Vigh L, Hevener A, Febbraio MA (2008) HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci 105:1739–1744
35. Colotti C, Cavallini G, Vitale RL, Donati A, Maltinti M, Del Ry S, Bergamini E, Giannessi D (2005) Effects of aging and anti-aging caloric restrictions on carbonyl and heat shock protein levels and expression. Biogerontology 6:397–406
36. Conway KA, Harper JD, Lansbury PT (1998) Accelerated in vitro fibril formation by a mutant α-synuclein linked to early-onset Parkinson disease. Nat Med 4:1318–1320
37. Csiszar A, Labinskyy N, Zhao X, Hu F, Serpillon S, Huang Z, Ballabh P, Levy RJ, Hintze TH, Wolin MS, Austad SN, Podlutsky A, Ungvari Z (2007) Vascular superoxide and hydrogen peroxide production and oxidative stress resistance in two closely related rodent species with disparate longevity. Aging Cell 6:783–797
38. Cuerrier CM, Chen Y-X, Tremblay D, Rayner K, McNulty M, Zhao X, Kennedy CR, de BelleRoche J, Pelling AE, O'Brien ER (2013) Chronic over-expression of heat shock protein 27 attenuates atherogenesis and enhances plaque remodeling: a combined histological and mechanical assessment of aortic lesions. PLoS One 8:e55867
39. Czarnecka AM, Campanella C, Zummo G, Cappello F (2006) Mitochondrial chaperones in cancer: from molecular biology to clinical diagnostics. Cancer Biol Ther 5:714–720
40. Dauer W, Kholodilov N, Vila M, Trillat AC, Goodchild R, Larsen KE, Staal R, Tieu K, Schmitz Y, Yuan CA, Rocha M, Jackson-Lewis V, Hersch S, Sulzer D, Przedborski S, Burke R, Hen R (2002) Resistance of α-synuclein null mice to the parkinsonian neurotoxin MPTP. Proc Natl Acad Sci U S A 99:14524–14529
41. de Lau LM, Breteler MM (2006) Epidemiology of Parkinson's disease. Lancet Neurol 5:525–535
42. De Maio A (2011) Extracellular heat shock proteins, cellular export vesicles, and the Stress Observation System: a form of communication during injury, infection, and cell damage. It is never known how far a controversial finding will go! Dedicated to Ferruccio Ritossa. Cell Stress Chaperones 16:235–249
43. De Mena L, Coto E, Sanchez-Ferrero E, Ribacoba R, Guisasola LM, Salvador C, Blazquez M, Alvarez V (2009) Mutational screening of the mortalin gene (HSPA9) in Parkinson's disease. J Neural Transm (Vienna) 116:1289–1293
44. de Toda IM, De la Fuente M (2015) The role of Hsp70 in oxi-inflamm-aging and its use as a potential biomarker of lifespan. Biogerontology 16:709–721
45. Delpino A, Castelli M (2002) The 78 kDa glucose-regulated protein (GRP78/BIP) is expressed on the cell membrane, is released into cell culture medium and is also present in human peripheral circulation. Biosci Rep 22:407–420
46. Dong Z, Wolfer DP, Lipp HP, Bueler H (2005) Hsp70 gene transfer by adeno-associated virus inhibits MPTP-induced nigrostriatal degeneration in the mouse model of Parkinson disease. Mol Ther 11:80–88
47. Dybdahl B, Slordahl SA, Waage A, Kierulf P, Espevik T, Sundan A (2005) Myocardial ischaemia and the inflammatory response: release of heat shock protein 70 after myocardial infarction. Heart 91:299–304
48. Eustace BK, Sakurai T, Stewart JK, Yimlamai D, Unger C, Zehetmeier C, Lain B, Torella C, Henning SW, Beste G (2004) Functional proteomic screens reveal an essential extracellular role for hsp90α in cancer cell invasiveness. Nat Cell Biol 6:507
49. Ferrucci L, Semba RD, Guralnik JM, Ershler WB, Bandinelli S, Patel KV, Sun K, Woodman RC, Andrews NC, Cotter RJ (2010) Proinflammatory state, hepcidin, and anemia in older persons. Blood 115:3810–3816
50. Flower TR, Chesnokova LS, Froelich CA, Dixon C, Witt SN (2005) Heat shock prevents α-synuclein-induced apoptosis in a yeast model of Parkinson's disease. J Mol Biol 351:1081–1100
51. Foley P, Riederer P (2000) Influence of neurotoxins and oxidative stress on the onset and progression of Parkinson's disease. J Neurol 247(Suppl 2):Ii82–Ii94

52. Fonager J, Beedholm R, Clark BF, Rattan SI (2002) Mild stress-induced stimulation of heat-shock protein synthesis and improved functional ability of human fibroblasts undergoing aging in vitro. Exp Gerontol 37:1223–1228
53. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S (2017) Inflammaging and 'Garb-aging'. Trends Endocrinol Metab 28:199–212
54. Freimann K, Zschiedrich K, Bruggemann N, Grunewald A, Pawlack H, Hagenah J, Lohmann K, Klein C, Westenberger A (2013) Mortalin mutations are not a frequent cause of early-onset Parkinson disease. Neurobiol Aging 34:2694.e2619-e2620
55. Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA, Witkowski JM, Franceschi C (2018) Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? Front Immunol 8:1960
56. Gabai VL, Meriin AB, Mosser DD, Caron AW, Rits S, Shifrin VI, Sherman MY (1997) Hsp70 prevents activation of stress kinases. A novel pathway of cellular thermotolerance. J Biol Chem 272:18033–18037
57. Gagliano N, Grizzi F, Annoni G (2007) Mechanisms of aging and liver functions. Dig Dis 25:118–123
58. Gaipl US, Multhoff G, Scheithauer H, Lauber K, Hehlgans S, Frey B, Rodel F (2014) Kill and spread the word: stimulation of antitumor immune responses in the context of radiotherapy. Immunotherapy 6:597–610
59. Ganter MT, Ware LB, Howard M, Roux J, Gartland B, Matthay MA, Fleshner M, Pittet JF (2006) Extracellular heat shock protein 72 is a marker of the stress protein response in acute lung injury. Am J Physiol Lung Cell Mol Physiol 291:L354–L361
60. Gao X, Carroni M, Nussbaum-Krammer C, Mogk A, Nillegoda NB, Szlachcic A, Guilbride DL, Saibil HR, Mayer MP, Bukau B (2015) Human Hsp70 disaggregase reverses Parkinson's-linked α-synuclein amyloid fibrils. Mol Cell 59:781–793
61. Garrido C, Bruey J-M, Fromentin A, Hammann A, Arrigo AP, Solary E (1999) Hsp27 inhibits cytochrome c-dependent activation of procaspase-9. FASEB J 13:2061–2070
62. Garrido C, Gurbuxani S, Ravagnan L, Kroemer G (2001) Heat shock proteins: endogenous modulators of apoptotic cell death. Biochem Biophys Res Commun 286:433–442
63. Gerson JE, Castillo-Carranza DL, Kayed R (2014) Advances in therapeutics for neurodegenerative tauopathies: moving toward the specific targeting of the most toxic tau species. ACS Chem Neurosci 5:752–769
64. Gestwicki JE, Garza D (2012) Protein quality control in neurodegenerative disease. In: Progress in molecular biology and translational science. Prog Mol Biol Transl Sci 107:327–353. https://doi.org/10.1016/B978-0-12-385883-2.00003-5
65. Gibert B, Simon S, Dimitrova V, Diaz-Latoud C, Arrigo A-P (2013) Peptide aptamers: tools to negatively or positively modulate HSPB1 (27) function. Philos Trans R Soc B Biol Sci 368:20120075
66. Gill C, Meyer M, FitzGerald U, Samali A (2006) The role of heat shock proteins in the regulation of apoptosis. In: Stress response: a molecular biology approach. Research Signpost, Trivandrum, pp 1–25
67. Gimbrone MA Jr, Topper JN, Nagel T, Anderson KR, Garcia-Cardeña G (2000) Endothelial dysfunction, hemodynamic forces, and Atherogenesis[a]. Ann N Y Acad Sci 902:230–240
68. Greenstein SM, Sun S, Calderon TM, Kim DY, Schreiber TC, Schechner RS, Tellis VA, Berman JW (2000) Mycophenolate mofetil treatment reduces atherosclerosis in the cholesterol-fed rabbit. J Surg Res 91:123–129
69. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J (2018) Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res 6:1–14
70. Hammerer-Lercher A, Mair J, Bonatti J, Watzka SB, Puschendorf B, Dirnhofer S (2001) Hypoxia induces heat shock protein expression in human coronary artery bypass grafts. Cardiovasc Res 50:115–124
71. Hance MW, Dole K, Gopal U, Bohonowych JE, Jezierska-Drutel A, Neumann CA, Liu H, Garraway IP, Isaacs JS (2012) Secreted Hsp90 is a novel regulator of the epithelial to mesenchymal transition (EMT) in prostate cancer. J Biol Chem 287:37732–37744

72. Hansson GK (2001) Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol 21:1876–1890
73. Hansson GK, Libby P (2006) The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 6:508
74. Hargitai J, Lewis H, Boros I, Rácz TM, Fiser A, Kurucz I, Benjamin I, Vígh L, Pénzes Z, Csermely P, Latchman DS (2003) Bimoclomol, a heat shock protein co-inducer, acts by the prolonged activation of heat shock factor-1. Biochem Biophys Res Commun 307:689–695
75. Hecker JG, McGarvey M (2011) Heat shock proteins as biomarkers for the rapid detection of brain and spinal cord ischemia: a review and comparison to other methods of detection in thoracic aneurysm repair. Cell Stress Chaperones 16:119–131
76. Heneka MT, Gavrilyuk V, Landreth GE, O'Banion MK, Weinberg G, Feinstein DL (2003) Noradrenergic depletion increases inflammatory responses in brain: effects on IκB and Hsp70 expression. J Neurochem 85:387–398
77. Hitchon CA, El-Gabalawy HS (2004) Oxidation in rheumatoid arthritis. Arthritis Res Ther 6:265
78. Hong H, Wang Q, Li J, Liu H, Meng X, Zhang H (2019) Aging, cancer and immunity. J Cancer 10:3021
79. Hooper PL, Hooper JJ (2005) Loss of defense against stress: diabetes and heat shock proteins. Diabetes Technol Ther 7:204–208
80. Hooper PL, Hooper PL (2009) Inflammation, heat shock proteins, and type 2 diabetes. Cell Stress Chaperones 14:113–115
81. Hooper PL, Balogh G, Rivas E, Kavanagh K, Vigh L (2014) The importance of the cellular stress response in the pathogenesis and treatment of type 2 diabetes. Cell Stress Chaperones 19:447–464
82. Hu W, Wang J, Luo G, Luo B, Wu C, Wang W, Xiao Y, Li J (2013) Proteomics-based analysis of differentially expressed proteins in the CXCR1-knockdown gastric carcinoma MKN45 cell line and its parental cell. Acta Biochim Biophys Sin 45:857–866
83. Huang M-N, Yu H, Moudgil KD (2010) The involvement of heat-shock proteins in the pathogenesis of autoimmune arthritis: a critical appraisal. Semin Arthritis Rheum 40:164–175
84. Ikwegbue PC, Masamba P, Oyinloye BE, Kappo AP (2018) Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. Pharmaceuticals 11:2
85. Jafarnejad A, Bathaie SZ, Nakhjavani M, Hassan MZ, Banasadegh S (2008) The improvement effect of L-Lys as a chemical chaperone on STZ-induced diabetic rats, protein structure and function. Diabetes Metab Res Rev 24:64–73
86. Jindal S (1996a) Heat shock proteins: applications in health and disease. Trends Biotechnol 14:17–20
87. Johnson AD, Berberian PA, Tytell M, Bond MG (1993) Atherosclerosis alters the localization of Hsp70 in human and macaque aortas. Exp Mol Pathol 58:155–168
88. Johnson AD, Berberian PA, Tytell M, Bond MG (1995) Differential distribution of 70-kD heat shock protein in atherosclerosis: its potential role in arterial SMC survival. Arterioscler Thromb Vasc Biol 15:27–36
89. Joly A-L, Wettstein G, Mignot G, Ghiringhelli F, Garrido C (2010) Dual role of heat shock proteins as regulators of apoptosis and innate immunity. J Innate Immun 2:238–247
90. Kakimura J, Kitamura Y, Takata K, Umeki M, Suzuki S, Shibagaki K, Taniguchi T, Nomura Y, Gebicke-Haerter PJ, Smith MA, Perry G, Shimohama S (2002) Microglial activation and amyloid-beta clearance induced by exogenous heat-shock proteins. FASEB J 16:601–603
91. Kalia LV, Lang AE (2015) Parkinson's disease. Lancet 386:896–912
92. Kalia LV, Kalia SK, McLean PJ, Lozano AM, Lang AE (2013) α-Synuclein oligomers and clinical implications for Parkinson disease. Ann Neurol 73:155–169
93. Kalmar B, Novoselov S, Gray A, Cheetham ME, Margulis B, Greensmith L (2008) Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS. J Neurochem 107:339–350

94. Kang EH, Kim DJ, Lee EY, Lee YJ, Lee EB, Song YW (2009) Downregulation of heat shock protein 70 protects rheumatoid arthritis fibroblast-like synoviocytes from nitric oxide-induced apoptosis. Arthritis Res Ther 11:R130
95. Kanwar RK, Kanwar JR, Wang D, Ormrod DJ, Krissansen GW (2001) Temporal expression of heat shock proteins 60 and 70 at lesion-prone sites during atherogenesis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 21:1991–1997
96. Karlsson-Parra A, Soderstrom K, Ferm M, Ivanyi J, Kiessling R, Klareskog L (1990) Presence of human 65 kD heat shock protein (hsp) in inflamed joints and subcutaneous nodules of RA patients. Scand J Immunol 31:283–288
97. Kaul G, Thippeswamy H (2011) Role of heat shock proteins in diseases and their therapeutic potential. Indian J Microbiol 51:124–131
98. Kendal WS (2008) Dying with cancer: the influence of age, comorbidity, and cancer site. Cancer 112:1354–1362
99. Kern J, Untergasser G, Zenzmaier C, Sarg B, Gastl G, Gunsilius E, Steurer M (2009) GRP-78 secreted by tumor cells blocks the antiangiogenic activity of bortezomib. Blood 114:3960–3967
100. Khafaga AF, Noreldin AE, Taha AE (2019) The adaptogenic anti-ageing potential of resveratrol against heat stress-mediated liver injury in aged rats: role of Hsp70 and NF-kB signalling. J Therm Biol 83:8–21
101. Kim HJ, Hwang NR, Lee KJ (2007) Heat shock responses for understanding diseases of protein denaturation. Mol Cells 23:123–131
102. Klucken J, Shin Y, Masliah E, Hyman BT, McLean PJ (2004) Hsp70 reduces α-synuclein aggregation and toxicity. J Biol Chem 279:25497–25502
103. Kovalchin JT, Wang R, Wagh MS, Azoulay J, Sanders M, Chandawarkar RY (2006) In vivo delivery of heat shock protein 70 accelerates wound healing by up-regulating macrophage-mediated phagocytosis. Wound Repair Regen 14:129–137
104. Landry J, Bernier D, Chretien P, Nicole LM, Tanguay RM, Marceau N (1982) Synthesis and degradation of heat shock proteins during development and decay of thermotolerance. Cancer Res 42:2457–2461
105. Leonardi GC, Accardi G, Monastero R, Nicoletti F, Libra M (2018) Ageing: from inflammation to cancer. Immun Ageing 15:1
106. Lewthwaite J, Owen N, Coates A, Henderson B, Steptoe A (2002) Circulating human heat shock protein 60 in the plasma of British civil servants: relationship to physiological and psychosocial stress. Circulation 106:196–201
107. Li J, Hu W, Lan Q (2012) The apoptosis-resistance in t-AUCB-treated glioblastoma cells depends on activation of Hsp27. J Neuro-Oncol 110:187–194
108. Liao WC, Wu MS, Wang HP, Tien YW, Lin JT (2009) Serum heat shock protein 27 is increased in chronic pancreatitis and pancreatic carcinoma. Pancreas 38:422–426
109. Libby P (2012) Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol 32:2045–2051
110. Lin S-P, Lee Y-T, Wang J-Y, Miller SA, Chiou S-H, Hung M-C, Hung S-C (2012) Survival of cancer stem cells under hypoxia and serum depletion via decrease in PP2A activity and activation of p38-MAPKAPK2-Hsp27. PLoS One 7:e49605
111. Liu FT, Chen Y, Yang YJ, Yang L, Yu M, Zhao J, Wu JJ, Huang F, Liu W, Ding ZT, Wang J (2015) Involvement of mortalin/GRP75/mthsp70 in the mitochondrial impairments induced by A53T mutant α-synuclein. Brain Res 1604:52–61
112. Lundy SK, Sarkar S, Tesmer LA, Fox DA (2007) Cells of the synovium in rheumatoid arthritis. T lymphocytes. Arthritis Res Ther 9:202
113. Macario AJL, De Macario EC (2001) Molecular chaperones and age-related degenerative disorders. In: Advances in cell aging and gerontology. Adv Cell Aging Gerontol 7:131–162. https://doi.org/10.1016/S1566-3124(01)07018-3
114. Madrigal-Matute J, López-Franco O, Blanco-Colio LM, Muñoz-García B, Ramos-Mozo P, Ortega L, Egido J, Martín-Ventura JL (2010) Heat shock protein 90 inhibitors attenuate inflammatory responses in atherosclerosis. Cardiovasc Res 86:330–337

115. Mak SK, McCormack AL, Manning-Bog AB, Cuervo AM, Di Monte DA (2010) Lysosomal degradation of α-synuclein in vivo. J Biol Chem 285:13621–13629
116. Marino Gammazza A, Bavisotto CC, Barone R, de Macario EC, Macario AJ (2016) Alzheimer's disease and molecular chaperones: current knowledge and the future of chaperonotherapy. Curr Pharm Des 22:4040–4049
117. Martin-Ventura JL, Duran MC, Blanco-Colio LM, Meilhac O, Leclercq A, Michel J-B, Jensen ON, Hernandez-Merida S, Tuñón J, Vivanco F (2004) Identification by a differential proteomic approach of heat shock protein 27 as a potential marker of atherosclerosis. Circulation 110:2216–2219
118. Martin-Ventura JL, Leclercq A, Blanco-Colio LM, Egido J, Rossignol P, Meilhac O, Michel J-B (2007) Low plasma levels of Hsp70 in patients with carotid atherosclerosis are associated with increased levels of proteolytic markers of neutrophil activation. Atherosclerosis 194:334–341
119. Matzinger P (2002) An innate sense of danger. Ann N Y Acad Sci 961:341–342
120. Mayer M, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670
121. Mayerl C, Lukasser M, Sedivy R, Niederegger H, Seiler R, Wick G (2006) Atherosclerosis research from past to present—on the track of two pathologists with opposing views, Carl von Rokitansky and Rudolf Virchow. Virchows Arch 449:96–103
122. McLean PJ, Kawamata H, Shariff S, Hewett J, Sharma N, Ueda K, Breakefield XO, Hyman BT (2002) TorsinA and heat shock proteins act as molecular chaperones: suppression of α-synuclein aggregation. J Neurochem 83:846–854
123. Mehana E-SE, Khafaga AF, El-Blehi SS (2019) The role of matrix metalloproteinases in osteoarthritis pathogenesis: an updated review. Life Sci 234:116786
124. Merendino AM, Bucchieri F, Campanella C, Marciano V, Ribbene A, David S, Zummo G, Burgio G, Corona DF, Conway de Macario E, Macario AJ, Cappello F (2010a) Hsp60 is actively secreted by human tumor cells. PLoS One 5:e9247
125. Merendino AM, Bucchieri F, Campanella C, Marcianò V, Ribbene A, David S, Zummo G, Burgio G, Corona DF, de Macario EC (2010b) Hsp60 is actively secreted by human tumor cells. PLoS One 5:e9247
126. Min JN, Whaley RA, Sharpless NE, Lockyer P, Portbury AL, Patterson C (2008) CHIP deficiency decreases longevity, with accelerated aging phenotypes accompanied by altered protein quality control. Mol Cell Biol 28:4018–4025
127. Moloney TC, Hyland R, O'Toole D, Paucard A, Kirik D, O'Doherty A, Gorman AM, Dowd E (2014) Heat shock protein 70 reduces α-synuclein-induced predegenerative neuronal dystrophy in the α-synuclein viral gene transfer rat model of Parkinson's disease. CNS Neurosci Ther 20:50–58
128. Molvarec A, Prohaszka Z, Nagy B, Szalay J, Fust G, Karadi I, Rigo J Jr (2006) Association of elevated serum heat-shock protein 70 concentration with transient hypertension of pregnancy, preeclampsia and superimposed preeclampsia: a case-control study. J Hum Hypertens 20:780–786
129. Morrow G, Samson M, Michaud S, Tanguay RM (2004) Overexpression of the small mitochondrial Hsp22 extends Drosophila life span and increases resistance to oxidative stress. FASEB J 18:598–599
130. Mortaz E, Redegeld FA, Nijkamp FP, Wong HR, Engels F (2006) Acetylsalicylic acid-induced release of Hsp70 from mast cells results in cell activation through TLR pathway. Exp Hematol 34:8–18
131. Murphy KE, Gysbers AM, Abbott SK, Spiro AS, Furuta A, Cooper A, Garner B, Kabuta T, Halliday GM (2015) Lysosomal-associated membrane protein 2 isoforms are differentially affected in early Parkinson's disease. Mov Disord 30:1639–1647
132. Mycko MP, Papoian R, Boschert U, Raine CS, Selmaj KW (2004) Microarray gene expression profiling of chronic active and inactive lesions in multiple sclerosis. Clin Neurol Neurosurg 106:223–229

133. Nagai Y, Fujikake N, Popiel HA, Wada K (2010) Induction of molecular chaperones as a therapeutic strategy for the polyglutamine diseases. Curr Pharm Biotechnol 11:188–197
134. Nillegoda NB, Bukau B (2015) Metazoan Hsp70-based protein disaggregases: emergence and mechanisms. Front Mol Biosci 2:57
135. Njemini R, Lambert M, Demanet C, Mets T (2003) Elevated serum heat-shock protein 70 levels in patients with acute infection: use of an optimized enzyme-linked immunosorbent assay. Scand J Immunol 58:664–669
136. O'Callaghan-Sunol C, Gabai VL, Sherman MY (2007) Hsp27 modulates p53 signaling and suppresses cellular senescence. Cancer Res 67:11779–11788
137. Oglesbee MJ, Herdman AV, Passmore GG, Hoffman WH (2005) Diabetic ketoacidosis increases extracellular levels of the major inducible 70-kDa heat shock protein. Clin Biochem 38:900–904
138. Oroz J, Kim JH, Chang BJ, Zweckstetter M (2017) Mechanistic basis for the recognition of a misfolded protein by the molecular chaperone Hsp90. Nat Struct Mol Biol 24:407–413
139. Pandey P, Saleh A, Nakazawa A, Kumar S, Srinivasula SM, Kumar V, Weichselbaum R, Nalin C, Alnemri ES, Kufe D (2000) Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. EMBO J 19:4310–4322
140. Park HS, Lee JS, Huh SH, Seo JS, Choi EJ (2001) Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. EMBO J 20:446–456
141. Parodi J, Sepulveda FJ, Roa J, Opazo C, Inestrosa NC, Aguayo LG (2010) Beta-amyloid causes depletion of synaptic vesicles leading to neurotransmission failure. J Biol Chem 285:2506–2514
142. Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S, Campisi J (2003) Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. Nat Cell Biol 5:741–747
143. Pockley AG (2002) Heat shock proteins, inflammation, and cardiovascular disease. Circulation 105:1012–1017
144. Pockley AG (2003) Heat shock proteins as regulators of the immune response. Lancet 362:469–476
145. Pockley AG, Multhoff G (2008) Cell stress proteins in extracellular fluids: friend or foe? Novartis Found Symp 291:86–95; discussion 96–100, 137–140
146. Pockley AG, Wu R, Lemne C, Kiessling R, de Faire U, Frostegård J (2000) Circulating heat shock protein 60 is associated with early cardiovascular disease. Hypertension 36:303–307
147. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047
148. Pratico D (2008) Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. Trends Pharmacol Sci 29:609–615
149. Quigney DJ, Gorman AM, Samali A (2003) Heat shock protects PC12 cells against MPP+ toxicity. Brain Res 993:133–139
150. Radak Z, Chung HY, Naito H, Takahashi R, Jung KJ, Kim HJ, Goto S (2004) Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise. FASEB J 18:749–750
151. Radli M, Rudiger SGD (2018) Dancing with the Diva: Hsp90-Client interactions. J Mol Biol 430:3029–3040
152. Raska M, Weigl E (2005) Heat shock proteins in autoimmune diseases. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 149:243–249
153. Rayner K, Chen Y-X, McNulty M, Simard T, Zhao X, Wells DJ, de Belleroche J, O'Brien ER (2008) Extracellular release of the atheroprotective heat shock protein 27 is mediated by estrogen and competitively inhibits acLDL binding to scavenger receptor-A. Circ Res 103:133–141

154. Rocnik E, Chow LH, Pickering JG (2000) Heat shock protein 47 is expressed in fibrous regions of human atheroma and is regulated by growth factors and oxidized low-density lipoprotein. Circulation 101:1229–1233
155. Rodríguez-Iturbe B, Johnson R (2018) Heat shock proteins and cardiovascular disease. Physiol Int 105:19–37
156. Ruparelia N, Chai JT, Fisher EA, Choudhury RP (2017) Inflammatory processes in cardiovascular disease: a route to targeted therapies. Nat Rev Cardiol 14:133
157. Saadeldin IM, Swelum AA, Elsafadi M, Mahmood A, Alfayez M, Alowaimer AN (2018) Differences between the tolerance of camel oocytes and cumulus cells to acute and chronic hyperthermia. J Therm Biol 74:47–54
158. Saadeldin IM, Swelum AA, Noreldin AE, Tukur HA, Abdelazim AM, Abomughaid MM, Alowaimer AN (2019a) Isolation and culture of skin-derived differentiated and stem-like cells obtained from the Arabian camel (Camelus dromedarius). Animals 9:378
159. Saadeldin IM, Swelum AA, Tukur HA, Alowaimer AN (2019b) Thermotolerance of camel (Camelus dromedarius) somatic cells affected by the cell type and the dissociation method. Environ Sci Pollut Res Int 26:29490–29496
160. Saadeldin IM, Abdel-Aziz Swelum A, Elsafadi M, Mahmood A, Osama A, Shikshaky H, Alfayez M, Alowaimer AN, Magdeldin S (2020a) Thermotolerance and plasticity of camel somatic cells exposed to acute and chronic heat stress. J Adv Res 22:105–118
161. Saadeldin IM, Swelum AA, Zakri AM, Tukur HA, Alowaimer AN (2020b) Effects of acute hyperthermia on the Thermotolerance of cow and sheep skin-derived fibroblasts. Animals 10:545
162. Salimi S, Shardell MD, Seliger SL, Bandinelli S, Guralnik JM, Ferrucci L (2018) Inflammation and trajectory of renal function in community-dwelling older adults. J Am Geriatr Soc 66:804–811
163. Salminen A, Paimela T, Suuronen T, Kaarniranta K (2008) Innate immunity meets with cellular stress at the IKK complex: regulation of the IKK complex by Hsp70 and Hsp90. Immunol Lett 117:9–15
164. Samali A, Cai J, Zhivotovsky B, Jones DP, Orrenius S (1999) Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of Jurkat cells. EMBO J 18:2040–2048
165. Schett G, Redlich K, Xu Q, Bizan P, Groger M, Tohidast-Akrad M, Kiener H, Smolen J, Steiner G (1998) Enhanced expression of heat shock protein 70 (hsp70) and heat shock factor 1 (HSF1) activation in rheumatoid arthritis synovial tissue. Differential regulation of hsp70 expression and hsf1 activation in synovial fibroblasts by proinflammatory cytokines, shear stress, and antiinflammatory drugs. J Clin Invest 102:302–311
166. Schlesinger MJ (1990) Heat shock proteins. J Biol Chem 265:12111–12114
167. Schreibelt G, van Horssen J, van Rossum S, Dijkstra CD, Drukarch B, de Vries HE (2007) Therapeutic potential and biological role of endogenous antioxidant enzymes in multiple sclerosis pathology. Brain Res Rev 56:322–330
168. Sedlackova L, Sosna A, Vavrincova P, Frydl J, Guerriero V, Raynes DA, Hromadnikova I (2011) Heat shock protein gene expression profile may differentiate between rheumatoid arthritis, osteoarthritis, and healthy controls. Scand J Rheumatol 40:354–357
169. Seibert TA, Hibbert B, Chen Y-X, Rayner K, Simard T, Hu T, Cuerrier CM, Zhao X, de Belleroche J, Chow BJ (2013) Serum heat shock protein 27 levels represent a potential therapeutic target for atherosclerosis: observations from a human cohort and treatment of female mice. J Am Coll Cardiol 62:1446–1454
170. Shimp SK 3rd, Chafin CB, Regna NL, Hammond SE, Read MA, Caudell DL, Rylander M, Reilly CM (2012) Heat shock protein 90 inhibition by 17-DMAG lessens disease in the MRL/lpr mouse model of systemic lupus erythematosus. Cell Mol Immunol 9:255–266
171. Siebelt M, Jahr H, Groen HC, Sandker M, Waarsing JH, Kops N, Muller C, van Eden W, de Jong M, Weinans H (2013) Hsp90 inhibition protects against biomechanically induced osteoarthritis in rats. Arthritis Rheum 65:2102–2112

172. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K (2003) α-Synuclein locus triplication causes Parkinson's disease. Science 302:841
173. Smolen JS, Breedveld FC, Burmester GR, Bykerk V, Dougados M, Emery P, Kvien TK, Navarro-Compan MV, Oliver S, Schoels M, Scholte-Voshaar M, Stamm T, Stoffer M, Takeuchi T, Aletaha D, Andreu JL, Aringer M, Bergman M, Betteridge N, Bijlsma H, Burkhardt H, Cardiel M, Combe B, Durez P, Fonseca JE, Gibofsky A, Gomez-Reino JJ, Graninger W, Hannonen P, Haraoui B, Kouloumas M, Landewe R, Martin-Mola E, Nash P, Ostergaard M, Ostor A, Richards P, Sokka-Isler T, Thorne C, Tzioufas AG, van Vollenhoven R, de Wit M, van der Heijde D (2016) Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. Ann Rheum Dis 75:3–15
174. Specht HM, Ahrens N, Blankenstein C, Duell T, Fietkau R, Gaipl US, Gunther C, Gunther S, Habl G, Hautmann H, Hautmann M, Huber RM, Molls M, Offner R, Rodel C, Rodel F, Schutz M, Combs SE, Multhoff G (2015) Heat shock protein 70 (Hsp70) peptide activated natural killer (NK) cells for the treatment of patients with non-small cell lung Cancer (NSCLC) after Radiochemotherapy (RCTx) – from preclinical studies to a clinical phase II trial. Front Immunol 6:162
175. Steinkraus KA, Smith ED, Davis C, Carr D, Pendergrass WR, Sutphin GL, Kennedy BK, Kaeberlein M (2008) Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in Caenorhabditis elegans. Aging Cell 7:394–404
176. Stellas D, Karameris A, Patsavoudi E (2007) Monoclonal antibody 4C5 immunostains human melanomas and inhibits melanoma cell invasion and metastasis. Clin Cancer Res 13:1831–1838
177. Takayama S, Reed JC, Homma S (2003) Heat-shock proteins as regulators of apoptosis. Oncogene 22:9041
178. Terry DF, Wyszynski DF, Nolan VG, Atzmon G, Schoenhofen EA, Pennington JY, Andersen SL, Wilcox MA, Farrer LA, Barzilai N, Baldwin CT, Asea A (2006) Serum heat shock protein 70 level as a biomarker of exceptional longevity. Mech Ageing Dev 127:862–868
179. Tetzlaff JE, Putcha P, Outeiro TF, Ivanov A, Berezovska O, Hyman BT, McLean PJ (2008) CHIP targets toxic α-synuclein oligomers for degradation. J Biol Chem 283:17962–17968
180. Thompson RC, Allam AH, Lombardi GP, Wann LS, Sutherland ML, Sutherland JD, Soliman MA-T, Frohlich B, Mininberg DT, Monge JM (2013) Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. Lancet 381:1211–1222
181. Thuringer D, Jego G, Wettstein G, Terrier O, Cronier L, Yousfi N, Hébrard S, Bouchot A, Hazoumé A, Joly A-L (2013) Extracellular Hsp27 mediates angiogenesis through toll-like receptor 3. FASEB J 27:4169–4183
182. Tomihara K, Curiel TJ, Zhang B (2013) Optimization of immunotherapy in elderly cancer patients. Crit Rev Oncog 18:573–583
183. Tsuneki M, Maruyama S, Yamazaki M, Xu B, Essa A, Abé T, Babkair H, Cheng J, Yamamoto T, Saku T (2013) Extracellular heat shock protein A9 is a novel interaction partner of podoplanin in oral squamous cell carcinoma cells. Biochem Biophys Res Commun 434:124–130
184. Tsutsumi S, Neckers L (2007) Extracellular heat shock protein 90: a role for a molecular chaperone in cell motility and cancer metastasis. Cancer Sci 98:1536–1539
185. Tytell M, Hooper PL (2001) Heat shock proteins: new keys to the development of cytoprotective therapies. Expert Opin Ther Targets 5:267–287
186. Urushitani M, Kurisu J, Tateno M, Hatakeyama S, Nakayama K, Kato S, Takahashi R (2004) CHIP promotes proteasomal degradation of familial ALS-linked mutant SOD1 by ubiquitinating Hsp/Hsc70. J Neurochem 90:231–244
187. Vabulas RM, Ahmad-Nejad P, da Costa C, Miethke T, Kirschning CJ, Hacker H, Wagner H (2001) Endocytosed Hsp60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/

interleukin-1 receptor signaling pathway in innate immune cells. J Biol Chem 276:31332–31339
188. van Roon JA, van Eden W, van Roy JL, Lafeber FJ, Bijlsma JW (1997) Stimulation of suppressive T cell responses by human but not bacterial 60-kD heat-shock protein in synovial fluid of patients with rheumatoid arthritis. J Clin Invest 100:459–463
189. Veereshwarayya V, Kumar P, Rosen KM, Mestril R, Querfurth HW (2006) Differential effects of mitochondrial heat shock protein 60 and related molecular chaperones to prevent intracellular beta-amyloid-induced inhibition of complex IV and limit apoptosis. J Biol Chem 281:29468–29478
190. von Vietinghoff S, Koltsova EK, Mestas J, Diehl CJ, Witztum JL, Ley K (2011) Mycophenolate mofetil decreases atherosclerotic lesion size by depression of aortic T-lymphocyte and interleukin-17–mediated macrophage accumulation. J Am Coll Cardiol 57:2194–2204
191. Walker GA, Lithgow GJ (2003) Lifespan extension in C. elegans by a molecular chaperone dependent upon insulin-like signals. Aging Cell 2:131–139
192. Wang X, Song X, Zhuo W, Fu Y, Shi H, Liang Y, Tong M, Chang G, Luo Y (2009) The regulatory mechanism of Hsp90α secretion and its function in tumor malignancy. Proc Natl Acad Sci U S A 106:21288–21293
193. Weiss YG, Bromberg Z, Raj N, Raphael J, Goloubinoff P, Ben-Neriah Y, Deutschman CS (2007) Enhanced heat shock protein 70 expression alters proteasomal degradation of IkappaB kinase in experimental acute respiratory distress syndrome. Crit Care Med 35:2128–2138
194. Wendling U, Paul L, van der Zee R, Prakken B, Singh M, van Eden W (2000) A conserved mycobacterial heat shock protein (hsp) 70 sequence prevents adjuvant arthritis upon nasal administration and induces IL-10-producing T cells that cross-react with the mammalian self-hsp70 homologue. J Immunol 164:2711–2717
195. Whitesell L, Lindquist SL (2005) Hsp90 and the chaperoning of cancer. Nat Rev Cancer 5:761
196. Whitham M, Febbraio MA (2010) HSP and diabetes. In: Asea AAA, Pedersen BK (eds) Heat shock proteins and whole body physiology. Springer Netherlands, Dordrecht, pp 3–18
197. Wick C (2016) Tolerization against atherosclerosis using heat shock protein 60. Cell Stress Chaperones 21:201–211
198. Williams RS (2000) Heat shock protein 47: a chaperone for the fibrous cap? Circulation 101:1227–1228. https://doi.org/10.1161/01.cir.101.11.1227
199. Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D (2014) The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediat Inflamm 2014:19
200. Worthington J, Rigby AS, MacGregor AJ, Silman AJ, Carthy D, Ollier WE (1993) Lack of association of increased antibody levels to mycobacterial hsp65 with rheumatoid arthritis: results from a study of disease discordant twin pairs. Ann Rheum Dis 52:542–544
201. Wu YR, Wang CK, Chen CM, Hsu Y, Lin SJ, Lin YY, Fung HC, Chang KH, Lee-Chen GJ (2004) Analysis of heat-shock protein 70 gene polymorphisms and the risk of Parkinson's disease. Hum Genet 114:236–241
202. Xiao Q, Mandal K, Schett G, Mayr M, Wick G, Oberhollenzer F, Willeit J, Kiechl S, Xu Q (2005) Association of serum-soluble heat shock protein 60 with carotid atherosclerosis: clinical significance determined in a follow-up study. Stroke 36:2571–2576
203. Xie F, Zhan R, Yan L-C, Gong J-B, Zhao Y, Ma J, Qian L-J (2016) Diet-induced elevation of circulating Hsp70 may trigger cell adhesion and promote the development of atherosclerosis in rats. Cell Stress Chaperones 21:907–914
204. Xilouri M, Brekk OR, Landeck N, Pitychoutis PM, Papasilekas T, Papadopoulou-Daifoti Z, Kirik D, Stefanis L (2013) Boosting chaperone-mediated autophagy in vivo mitigates α-synuclein-induced neurodegeneration. Brain 136:2130–2146
205. Xu Q, Wick G (1996) The role of heat shock proteins in protection and pathophysiology of the arterial wall. Mol Med Today 2:372–379
206. Xu L, Chen S, Bergan R (2006) MAPKAPK2 and Hsp27 are downstream effectors of p38 MAP kinase-mediated matrix metalloproteinase type 2 activation and cell invasion in human prostate cancer. Oncogene 25:2987

207. Xu Q, Metzler B, Jahangiri M, Mandal K (2011) Molecular chaperones and heat shock proteins in atherosclerosis. Am J Phys Heart Circ Phys 302:H506–H514
208. Yan MH, Wang X, Zhu X (2013) Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. Free Radic Biol Med 62:90–101
209. Yap H-Y, Tee SZ-Y, Wong MM-T, Chow S-K, Peh S-C, Teow S-Y (2018) Pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development. Cell 7:161
210. Yu L, Yuan X, Wang D, Barakat B, Williams E, Hannigan G (2014) Selective regulation of p38β protein and signaling by integrin-linked kinase mediates bladder cancer cell migration. Oncogene 33:690–701
211. Zhang X, He M, Cheng L, Chen Y, Zhou L, Zeng H, Pockley AG, Hu FB, Wu T (2008) Elevated heat shock protein 60 levels are associated with higher risk of coronary heart disease in Chinese. Circulation 118:2687–2693
212. Zhang X, Xu Z, Zhou L, Chen Y, He M, Cheng L, Hu FB, Tanguay RM, Wu T (2010) Plasma levels of Hsp70 and anti-Hsp70 antibody predict risk of acute coronary syndrome. Cell Stress Chaperones 15:675–686
213. Zhang X, Wang X, Zhu H, Kranias EG, Tang Y, Peng T, Chang J, Fan G-C (2012) Hsp20 functions as a novel cardiokine in promoting angiogenesis via activation of VEGFR2. PLoS One 7:e32765
214. Zhang S, Hu Y, Huang Y, Xu H, Wu G, Dai H (2015) Heat shock protein 27 promotes cell proliferation through activator protein-1 in lung cancer. Oncol Lett 9:2572–2576
215. Zhao M, Shen F, Yin Y, Yang Y, Xiang D, Chen Q (2012) Increased expression of heat shock protein 27 correlates with peritoneal metastasis in epithelial ovarian cancer. Reprod Sci 19:748–753
216. Zhu J, Quyyumi AA, Wu H, Csako G, Rott D, Zalles-Ganley A, Ogunmakinwa J, Halcox J, Epstein SE (2003) Increased serum levels of heat shock protein 70 are associated with low risk of coronary artery disease. Arterioscler Thromb Vasc Biol 23:1055–1059
217. Ziegler TR, Ogden LG, Singleton KD, Luo M, Fernandez-Estivariz C, Griffith DP, Galloway JR, Wischmeyer PE (2005) Parenteral glutamine increases serum heat shock protein 70 in critically ill patients. Intensive Care Med 31:1079–1086

# Heat Shock Factor Network in Kidney Diseases


**Kinga Musiał and Danuta Zwolińska**

## Abstract

**Introduction** Heat shock response (HSR) pathway is a highly conserved cellular process. HSF1 is a master transcriptional regulator responsible for expression of several important heat shock proteins (HSP), which can effectively protect critical client proteins from misfolding and degradation, thus maintaining intracellular integrity under stressed conditions. Recent studies have demonstrated the direct connections between HSR players and tumor cell survival, validating HSR players as novel molecular targets in anticancer treatment. Small molecule screening has produced some promising HSR inhibitors for anticancer treatment. In this article, we will be summarizing the main findings from HSR inhibitors on recent clinical and preclinical studies.

**Methods** The authors reviewed all the relevant papers of HSR inhibitors with an emphasis on human and animal studies.

**Results** More than 18 unique chemical identities have been discovered with confirmed inhibition of HSR pathway. Among them, two natural products and their derivatives are currently in various phases of clinical studies. Detailed works are required to define the exact mechanisms of actions (MOA) for these compounds.

**Conclusions** Many hurdles in clinical application still need to be effectively addressed, such as undesirable drug toxicity and off target effects; narrow therapeutic window; poor PK/PD profiles, etc. Recent reports on synergistic drug combination, advanced prodrug design, smart nanoparticle packaging, and RNA aptamer selection offer promising solutions to overcome these challenges. Future advancements in this fast-growing area can potentially lead to the next-generation cancer therapeutics.

K. Musiał (✉) · D. Zwolińska
Department of Pediatric Nephrology, Wrocław Medical University, Wrocław, Poland
e-mail: kinga.musial@umed.wroc.pl

Heat Shock Proteins (2021) 22: 373–385
https://doi.org/10.1007/7515_2020_30

Published Online: 9 September 2020

**Keywords** Acute kidney injury · Chronic kidney disease · Heat shock factor 1 · Heat shock protein · Ischemia reperfusion injury · Tubular function

## Abbreviations

| | |
|---|---|
| AMPK | 5′ adenosine monophosphate-activated protein kinase |
| ATP | 5′ adenosine triphosphate |
| ccRCC | clear cell renal cell carcinoma |
| CKD | chronic kidney disease |
| CR+/CR- | presence/absence of glomerular amyloid deposition by the Congo Red staining |
| CryAB | crystallin-αB |
| FE | fractional excretion |
| GBM | glomerular basement membrane |
| HBA | Hsp90-binding agent |
| HD | hemodialysis |
| HIF1 | hypoxia-inducible factor 1 |
| HO | heme-oxygenase |
| HSE | heat shock element |
| HSF | heat shock factors |
| Hsf1 | heat shock factor 1 |
| Hsf1-M30 | Hsf1 hemizygous mice |
| HSF-KO | Hsf1 functional knockout mice |
| Hsf1 KD cells | Hsf1 knockdown rat proximal tubular cells |
| Hsf1 NC cells | rat proximal tubular negative control cells |
| HSP | heat shock proteins |
| Hsp25 | heat shock protein 25 |
| KIM-1 | kidney injury molecule-1 |
| LDH | lactate dehydrogenase |
| M30 | Hsf-1 homozygous mice |
| PGC1α | peroxisome proliferator-activated receptor-γ coactivator 1 α |
| PFP | podocyte foot processes |
| PTM | post-translational modifications |
| RBP-4 | retinol binding protein-4 |
| RPTC | renal proximal tubular cells |
| S1R | Sigma-1 receptor |
| TGF-β1 | transforming growth factor- β1 |
| TTR+/TTR- | presence/absence of TTRV30M glomerular deposition |
| TTRV30M | mice with the presence of mutated transthyretin |
| VDBP | vitamin D binding protein |
| WT | wild-type mice |

# 1 Introduction

Heat shock transcription factors (HSF) are DNA-binding proteins, regulating gene expression at the transcriptional level. Their structure remained evolutionary conserved throughout species. HSF family in humans contains six members: Hsf1, Hsf2, Hsf4, Hsf5, HsfX and HsfY [3]. When first discovered, HSF were meant to activate, under thermal stress, genes encoding protein chaperones – heat shock proteins (HSP). The recognition of heat shock element (HSE) DNA binding site was triggered by harmful conditions, like high temperature, heavy metals or oxidants, causing protein misfolding. Proteotoxicity resulted in gene activation and chaperone production, protecting cells from death. Hsf1 is the main player in the group of heat shock factors and the major regulator of system controlling the quality of proteins (called proteostasis), thus promoting cell survival. Hsf2 is mainly observed in testes and seems to regulate tumor activation or suppression. Hsf4 localization concerns eye lenses, brain, heart, pancreas and muscles. HsfX and HsfY are located on the X and Y chromosomes, although their role is not fully understood. Hsf1 is the most abundant representative of the family and the multifunctional player in both health and disease. It is also the only member of the HSF family, able to regulate HSP expression. Another intriguing aspect of its function is the multistep activation, requiring the presence of heat shock proteins, that in turn may inactivate Hsf1.

## *1.1 HSF Link to Heat Shock Response*

The following steps of Hsf1 activity form the sequence of heat shock response: de-repression and trimerization, translocation to the nucleus, DNA binding and transcriptional activation and protein stability [6]. Inactive Hsf1 is most probably a monomer, which turns into an activated form of a homotrimer. This oligomerization is necessary, but not sufficient for transcriptional activity. The essential trigger is a chaperone complex, consisting of 3 heat shock proteins – Hsp40, Hsp70 and Hsp90, bound to Hsf1. This binding prevents Hsf1 from transcriptional activity. However, when the amount of unfolded/misfolded proteins in the area increases, they competitively bind HSP and release Hsf1. This action makes the activation of HSP and release of their free forms possible. The feedback reaction to increased concentration of HSP is the Hsf1 inactivation. Activated Hsf1 migrates from the cytosol into the nucleus, where transcription may take place.

## *1.2 HSF Beyond Heat Shock Response*

Hsf1 influences multiple events beyond heat shock response and controls the transcription of genes encoding proteins other than HSP (the so-called non-HSP

target genes). They are engaged in various processes, including cell cycle, apoptosis, autophagy, aging or immune function [1]. The multifaceted nature of HSF activity is also pictured by various ways of its regulation and its dependence on metabolic conditions. Multi-chaperone complex is not the only tool for Hsf1 regulation. Post-translational modifications (PTM), like acetylation, phosphorylation and sumoylation, seem other powerful mechanisms modifying Hsf1 activity. Metabolic stress is another Hsf1–related process. Nutrient deprivation is a classic example of metabolic stress, coordinated by AMP kinase. Its activity is focused on ATP overproduction and reduction of its expenditure, maintaining optimal milieu for cell survival. Meanwhile, AMPK suppresses Hsf1, making cells more prone to proteotoxic conditions. Mitochondrial activity is also an energy-consuming and Hsf1-dependent process. Hsf1 activates the transcription of peroxisome proliferator-activated receptor-γ coactivator 1 α (PGC1α). In consequence, PGC1α increases invasiveness of cancer cells, triggering distant metastases, whereas PGC1α dysfunction is responsible for neurodegenerative diseases or increased deposition of lipids in white adipose tissue. In reverse, Hsf1 activity seems to be strongly dependent on the metabolic conditions, like e.g. in the case of acute kidney injury.

## *1.3 HSF in Kidney Diseases*

The role of heat shock factors in kidney diseases is hardly known and available data come mainly from animal and *in vitro* studies. However, even such scarcity makes a promising perspective for future investigation in this area. Among multiple nephrological subjects, ischemia-reperfusion injury in the kidney seems the best example of stressful condition, when Hsf1 can show the multifaceted nature of its activity.

### 1.3.1 Hsf1 in Renal Ischemia-Reperfusion Injury

Hsf1 dependence on ATP has found its reflection in the phenomenon of ischemic renal injury, where all players are interdependent in a finely tuned way. Experimental renal ischemia in rats, caused by 45-min aortic occlusion, reduced cortical ATP, activated Hsf1 and then induced Hsp70 mRNA [15]. Both Hsf1 activation and Hsp70 induction turned out “dose-dependent”. Cortical ATP concentration, monitored through NMR in real time, did not influence the heat shock response until kept above 60% of its control level. The ATP reduction to 35–50% of preocclusion values, gained after the 90% constriction of the vessel, triggered HSF overactivity, yet kept Hsp70 mRNA expression at low levels. Only after 15-min total vessel occlusion, when cellular ATP crossed the 20–25% border of its control concentration, further activation of Hsf1 and, subsequently, Hsp70 mRNA transcription, took place. The interesting findings concerned also the following reperfusion phase, showing sequential connections between HSF and HSP. Hsf1 binding to HSE was

commenced after 15 min of occlusion, increased after 30 min of reflow, then started declining by 2 h of reflow. Hsp70 mRNA expression acted similarly, but with a slight delay: it was first detected at 15 min of reflow, aggravated until 2-h period, and began to decline by 6 h. These preliminary results have established the borderline safety value of ATP concentration at the 50% level, suggesting that ATP depletion below this threshold is a sufficient stimulus to trigger stress response during the ischemia phase through HSF1 activation. It also became evident that reflow, with reperfusion injury, accounts for further enhancement of stress response, and is a major trigger of Hsp70 mRNA accumulation.

When Hsf1 activation, subsequent to ATP depletion, became evident, the question arose about the nature of the critical signal, activating stress response directly. Van Why et al. studied that matter *in vitro* [16]. They focused on early events accompanying ATP depletion in renal epithelial cells, like changes in free calcium and cytoskeletal proteins, leading to disintegration of cell membrane and loss of cell polarity. It turned out that the degree of Hsf1 activation is adjusted to the severity of injury and it seems an adaptive reaction, leading first to sublethal and potentially reversible changes. Most probably, changes in calcium are responsible for Hsf1 translocation from cytoplasm to the nucleus, as well as for the further enhancement of stress response. Thus, the final list of events leading to HSF activation would start with the depletion of cellular ATP and subsequent accumulation of abnormal (denatured and aggregated) proteins, triggering chaperones like Hsp70, that in turn activate Hsf1. Increase in calcium may play an additional role in cell disruption and loss of polarity. The whole process seems precisely tuned and the degree of Hsf1 activation strongly depends on the level of energy deprivation.

The following project governed by Eickelberg et al. [2] aimed at distinguishing between *in vivo* and *in vitro* conditions of HSF activation, in confrontation to another stress-responsive transcription factor – HIF (hypoxia-inducible factor)1. The *in vivo* experiment of renal ischemia in rats resulted in rapid activation of both transcription factors and expression of their target genes – Hsp72 and HO (heme-oxygenase) 1. To differentiate between two factors and the ways of their activation, the *in vitro* experiment was performed on porcine proximal tubule cells. Both Hsf1 and HIF1 were stimulated separately by the oxygen and ATP deprivation. The ATP depletion was able to activate Hsf1 *in vitro,* whereas hypoxia had no impact on its function. On the opposite, HIF1 was activated by oxygen deprivation, yet ATP depletion could not influence its activity. These results have shown the discrepancy between *in vivo* and *in vitro* conditions of renal ischemia phenomenon. Meanwhile, it became evident that ischemia-reperfusion injury influences in the same time many regulatory pathways that may trigger gene transcription through independent mechanisms. Whether such multiplicity is an insurance for infallibility, is to be elucidated. The intriguing question is, whether such divergence of activation pathways serves a certain goal, that is, whether it provides various independent tools for protection against lethal cell damage.

The logical way to verify whether Hsf1 plays a protective role, was to inhibit it specifically and examine the functional consequences of Hsf1 decoy. To empower the strength of the proof, Sreedharan et al. [13] performed the investigation on

immature cells, which by definition present with increased tolerance to stress. The study concerned the immature renal tubules of rats at 1, 3 and 10 postnatal days of life and renal tubules of mature animals. The first observation concerned baseline levels of Hsf1 expression: these in immature uninjured proximal tubule cells increased gradually from day 1 through day 3 until day 10 of postnatal life. On the 10th day, they were similar to those in mature animals subjected to 45-min ischemia. Therefore, immature cells presented with amplified baseline heat shock response, when compared to mature counterparts. This was, most probably, the mechanism responsible for increased protection against anoxia.

Another step of the experiment was the Hsf1 inhibition by the means of its incubation with cyclic oligonucleotide decoy, homologous in sequence to heat shock element (HSE). The effect of decoy on cellular integrity was verified by the percentage of lactate dehydrogenase (LDH) released from damaged cells, versus non-released LDH in the cells. Before injury, baseline release of LDH was comparable in immature and mature tubules (9% vs. 13%). After injury (45 min of anoxia followed by 1 h of reoxygenation), LDH release increased in both groups, but with evident discrepancies. Immature tubules tolerated anoxia to a greater extent (23% LDH release) than mature cells (40%). When decoy treatment was introduced, immature tubules have undergone damage to greater extent, yet comparable to that of mature cells (33% vs. 40%). Moreover, the degree of damage was proportional to the dose of decoy. Thus, in the *in vitro* conditions, Hsf1 undoubtedly plays a major role in the resistance of immature tubules to anoxia, although other components are yet to be established.

HSP expression in the course of Hsf1 induction is one of the main mechanisms by which the cells are protected against ischemic injury. Thus, the logical consequence was the experiment verifying the role of Hsf1 as a master regulator of stress response *in vivo*. Therefore, the Hsf1 functional knockout mice (HSF-KO) and wild-type (WT) mice have undergone bilateral ischemic renal injury [14]. Once again, the results obtained from the *in vivo* experiments differed from the observations in the above mentioned *in vitro* studies.

Baseline expression of Hsp70 and Hsp25, as well as serum creatinine, were comparable in both strains. Predictably, 45 min of injury and 24 h of reflow have triggered Hsp70 and Hsp25 overexpression in WT mice, whereas in HSF-KO mice such response was absent. Thus, Hsf1 regulated the induction of HSP following ischemia, but was not responsible for the control of constitutive expression of inducible HSP. However, although ischemia and reflow elevated serum creatinine concentration in all animals, the values in HSF-KO animals were significantly lower than in the WT littermates. Moreover, histopathological assessment revealed no difference in the degree of tubular injury between WT and HSF-KO mice, whereas the medullary vascular congestion, typical for ischemic renal injury, was seen only in WT mice. It became evident that, paradoxically, HSF-KO mice were better protected against ischemia-reperfusion injury than the WT littermates.

In order to reveal the source of this anti-ischemic protection in HSF-KO mice, the mononuclear cells isolated from the kidneys were analyzed by flow cytometry. Baseline number of CD4+ and CD8+ T cells did not differ between HSF-KO and

WT mice. After 45 min of ischemia and 1 h of reflow, the number of cells in WT mice has doubled, whereas in HSF-KO mice has not changed. However, the latter strain has presented with the higher amount of Foxp3+ T-regulatory cells, when compared to WT mice, both before and after ischemia. The suppression of CD25+ Foxp3+ T cells with anti-CD25 in HSF-KO mice worsened the kidney function. Therefore, immunomodulatory Foxp3+ cells turned out the major systemic protectors against ischemic injury, blocking the infiltration of pro-inflammatory CD4+ and CD8+ cells. The results of this study helped to understand the duality of the Hsf1 impact on cells through HSP. Inducible HSP, localized in the renal tubules, protected them against ischemia. In the same time, they facilitated systemic pro-inflammatory mechanisms in microvasculature and outside the tubules, acting detrimentally.

Another example of tight HSF – HSP relations was the *in vitro* experiment with geldanamycin, Hsp90-binding agent (HBA) [4]. The theory behind the experiment was that HBA releases Hsf1 from multichaperone complex, thus enabling its activation and stimulation of HSP. Indeed, in human renal adenocarcinoma cells, pretreatment with HBA triggered upregulation of Hsp90 and Hsp70, protected the cells from oxidative stress and reduced renal damage during ischemia-reperfusion injury. However, for the final proof of direct Hsf1 engagement into HSP upregulation and nephroprotection, cell transfection was needed. Transfection of the adenocarcinoma cells with Hsf1 (short interfering) siRNA resulted in decreased cell viability and normalization of formerly increased Hsp70. Thus, the HBA-mediated expression of HSP turned out Hsf1-dependent.

Hsf1 activation and heat shock response also revealed the discrepancies in the susceptibility to ischemia/reperfusion injury, related to sex differences. Hosszu et al. [5] have tested the impact of estrogen receptors on Hsf1 activation and stress response in human cells, as well as post-ischemic kidney function and damage in female, male and ovariectomized female Wistar rats. First, human proximal tubular epithelial cells were treated with 17β-estradiol, in order to verify its potential impact on Hsf1 activity. Indeed, 17β-estradiol triggered Hsf1 production and translocation to the nuclei of these cells. There, transcription of heat shock response genes was activated and Hsp72 produced. Thus, the direct activation of stress response through Hsf1 was shown. The results of this *in vitro* study on protective influence of estrogens on stress response were confirmed by the *in vivo* results in rats. Post-ischemic evaluation of serum creatinine showed the lowest concentrations in female rats, when compared to male and ovariectomized animals. On the opposite, renal damage was earlier and more pronounced in male rats, which presented with tubular necrosis, hyalinization and interstitial lesions.

Hsf1 showed similar discrepancy regarding gender – baseline expression levels in female rats were higher than in males, then increased by 2 and 24 h of reperfusion in both groups, still being superior in females. Hsp72 showed similar discrepancies concerning gender and time. The dynamics of Hsp27 changes differed slightly. Baseline levels in females were the highest, then equalized to those in males by 2 h of reperfusion and became higher again by 24 h reperfusion. Interestingly, Hosszu et al. [5] have also described another sex-dependent protective mechanism against ischemia reperfusion injury. This renoprotection was maintained by Sigma-1

receptor (S1R) – a highly conserved chaperone expressed in central nervous system and peripheral tissues. The authors have identified S1R in human proximal tubular cells and have shown that treatment with 17β-estradiol activates it by alterations in localization. Moreover, although baseline S1R expression was comparable in females and males, 2 h of reperfusion triggered its increase, but only in females. However, this activation was transient and S1R returned to baseline level by the 24 h reperfusion. Again, it was shown that ischemic conditions trigger concomitantly various chaperones, as if the dysfunction of one protective mechanism (Hsf1) could be compensated by the activity of an additional signaling pathway (S1R).

### 1.3.2 HSF in Chronic Kidney Disease

Hsf1 proved its utility as a protective factor, triggering stress response and heat shock protein overactivity, in acute stress conditions. Thus, the logical consequence was the question whether it can serve as a marker of chronic changes in renal tubules. However, there are no data on such *in vitro* experiments or animal studies, testing the behavior of Hsf1 in chronic kidney disease, and the data from clinical studies are scarce. Our investigation [9] has shown the early rise of Hsf1 both in serum and urine of children with chronic kidney disease. The added value of these results came from the combination of serum and urine concentrations, giving the overall picture of tubular function through the tool of fractional excretion (FE), defined as a proportion of serum and urine concentrations of a molecule, with reference to serum and urine creatinine values. FE revealed the compensatory abilities of renal tubules to adapt to increased protein load in urine, but also defined the moment when these adaptive possibilities use out and irreversible damage commences. It became evident that Hsf1 was a more sensitive marker of chronic stress conditions in renal tubules than other known indices of kidney injury or dysfunction, like kidney injury molecule (KIM)-1, retinol binding protein (RBP)-4 or vitamin D binding protein (VDBP). Not surprisingly, the results have also confirmed the sequence of events concerning the activation of stress response. In detail, Hsf1 rise preceded the increase of Hsp27 concentration, irrespective of the analyzed notion (serum or urine). Consequently, FE Hsf1 values have risen above those from the control group already in patients with CKD stage 2, whereas FE Hsp27 reached that border no sooner than in patients with CKD stage 4.

### 1.3.3 HSF on Chronic Hemodialysis

Propensity towards infections, immune dysfunction or impaired wound healing are characteristic features of chronic kidney disease, aggravating in patients on chronic hemodialysis (HD). Monocyte/macrophage population plays the main role in this process. Reuter et al. [11] tested the function of macrophages in rats with renal insufficiency and in patients on chronic hemodialysis. In rats, the baseline expression of Hsp72 in sick and healthy animals was comparable. However, when stress factor

appeared, the induction turned out insufficient. When monocytes from patients on chronic hemodialysis were triggered by heat stress, the expression of Hsf1 mRNA and Hsp72 mRNA/protein increased, although the response was weaker than in healthy controls. These results were the proof for the impaired macrophage stress response and reduced resistance of cells to apoptotic stress. Surprisingly, this impairment was rather due to chronic kidney disease, because no difference was observed after a single HD session.

### 1.3.4 HSF in Renal Cell Carcinoma

Hsf1 triggers HSP transcription in order to prevent all proteins from misfolding and improve their survival. Thus, such protective activity covers also proteins essential for tumorigenesis, like p53. Among HSP stimulated by Hsf1, Hsp90 seems of a paramount importance because of its impact on the activation of hypoxia inducible factor-1 (HIF-1). The latter is one of the main players in the oncogenesis related to clear cell renal cell carcinoma (ccRCC). When the role of Hsf1 and HSP in various types of cancer was analyzed, the most important question was about their prognostic ability towards tumor progression and cancer-specific mortality. In the case of ccRCC, the combination of HSP (Hsp27, Hsp60, Hsp70, Hsp90) and Hsf1 was analyzed in patients with diagnosis of ccRCC, after radical or partial nephrectomies [17]. Under normal conditions, proximal renal tubules expressed all tested HSP, whereas Hsf1 prevailed in distal tubules and collecting ducts. Tumor tissues showed expression of Hsp60, Hsp70 and Hsf1, increasing with the advancement of grades and stages on histopathological assessment. In detail, Hsp60 and Hsp70 high expression correlated with grade and stage, whereas increased expression of Hsf1 showed positive correlation with stage. However, only the simultaneous overexpression of at least 3 parameters from those mentioned above, has proved its prognostic value for long –term cancer – specific mortality. Hsp60, Hsp70, Hsp90 and Hsf1 proved the prognostic significance in univariate analysis, whereas Hsp60 and Hsp90 remained significant in multivariate analysis. Thus, Hsf1 in combination with HSP could distinguish the group of patients with high risk of cumulative cancer-specific mortality.

## *1.4 HSF Targeted Action on Specific Renal Structures*

### 1.4.1 HSF-Podocyte Interaction

*In vitro* data have confirmed relations between stress response and HSF/HSP activity. However, probably due to its ubiquitous expression in tissues, less investigation has focused on impact of HSF on particular cells/structures/proteins. One of the examples of such direct interaction was the testing of mouse model of amyloidosis. Petrakis et al. [10] studied the connections between expression of various slit

diaphragm proteins, width of podocyte foot processes (PFP), glomerular basement membrane (GBM) thickness, the presence of mutated transthyretin (TTRV30M) and/or amyloid deposition in the kidney, and the degree of renal damage. All above mentioned parameters were evaluated in conditions of either hemizygocity or homozygocity for Hsf1, showing significant differences between these two conditions. The severity of changes was graded according to the absence (TTR -) or presence (TTR +) of TTRV30M glomerular deposition by the use of anti-human TTR immunostaining, as well as the absence (CR -) or presence (CR +) of glomerular amyloid deposition by the Congo Red staining. The podocyte number was assessed with WT1 as a specific marker. Additionally, the mean fluorescence intensity for nephrin and podocin in the glomeruli, as well as their mRNA levels, were assessed.

In Hsf1 hemizygous (Hsf1-M30) mice, the number of podocytes was higher than in the homozygous littermates (M30 mice). Besides, all Hsf1-M30 mice were TTR+, whereas M30 mice were either TTR+ or TTR -. Thus, the combination of Hsf1 hemizygocity and TTR deposition additionally increased the podocyte count. Regarding nephrin and podocin protein content in the glomeruli, Hsf1-M30 mice presented with lower fluorescence intensity than M30 mice. Moreover, among Hsf1-M30 animals, TTR deposition additionally decreased nephrin and podocin levels, whereas in M30 mice no such effect was observed. Likewise, both GBM thickness and PFP width, aggravated along with TTR deposition and stayed independent of amyloid deposition. Interestingly, mRNA levels of both proteins showed no difference between Hsf1 hemizygous and homozygous mice. Therefore, Hsf1 hemizygocity alone could not modulate nephrin or podocin content at the post-translational level. However, in the presence of TTRVM30 deposition, it turned out a sufficient trigger of podocyte dysfunction.

### 1.4.2 HSF – Renal Tubule Interaction

The direct impact of Hsf1 on renal tubules was analyzed in mice during cisplatin-induced kidney injury and in cultured renal proximal tubular cells (RPTC) undergoing apoptosis due to cisplatin treatment [7]. In mice, cisplatin induced Hsf1 and a small heat shock protein crystallin-αB (CryAB) at the 2nd, and at the 3rd day of drug intake, respectively. Meanwhile, there was no change in the expression of Hsp27 or Hsp90, whereas Hsp70 presented with marginal induction at the 2nd day, which decreased the following day. In the *in vitro* experiment, cisplatin in RPTC was used for 0, 4, 8, and 24 h. Hsf1 induction was the earliest among all analyzed proteins – it started after 4 h and continued until 24 h. CryAB rose significantly after 8 h. Hsp70 activation was minimal at that time, whereas Hsp90 did not change. Meanwhile, cisplatin-induced apoptosis was limited during first 8 h, but concerned 45% of cells at 24 h.

Cisplatin stimulated also the translocation of Hsf1 from the cytosol into the nucleus, giving the final proof of its activation. The essential question was whether the activation of Hsf1 could be of a protective manner. Thus, the Hsf1 knockdown

cells (with the Hsf1 expression level decreased by 85%) were tested in the cisplatin milieu. Apoptosis concerned 58% of Hsf1 knockdown cells and 33% of negative control cells, confirming the protective and anti-apoptotic role of Hsf1. In detail, Hsf1 knockdown cells sensitized renal tubular cells to apoptosis by the activation of intrinsic pathways – accumulation of Bax in mitochondria, as well as release of cytochrome c from mitochondria to the cytosol.

To further delineate the real nature of connection between Hsf1 and CryAB, Hsf1 knockdown cells were tested for CryAB expression. Not surprisingly, the latter was decreased. However, in the conditions of CryAB overexpression, gained by hemagglutinin-CryAB transfection, apoptosis was significantly reduced. Therefore, Hsf1 anti-apoptotic activity worked, at least in part, through CryAB induction.

In their most recent investigation, Lou et al. have continued the subject of influence that Hsf1 may have on renal tubular cells, taking into account the role of transforming growth factor (TGF)-β1-Smad 2/3 signaling pathway [8]. In detail, Hsf1 knockdown (KD) rat kidney proximal tubular cells (RPTC) and Hsf1 negative control (NC) cells have undergone a series of assays. First, the impact of Hsf1 on RPTC was analyzed by testing the migration of cells 6, 12 and 24 h after the creation of wounds on the dishes, where cells were sieved. The migration rate in Hsf1 KD cells was higher than in Hsf1 NC cells, at any time point tested. The wound closure rates, defined as a percentage of healing distance in relation to the width of initial wound, were: 26% vs. 12% after 6 h, 46% vs. 32% after 12 h, and 97% vs. 87% after 24 h, in KD and NC cells, respectively. When TGF-β1 expression was analyzed in the selected time points, it turned out weak just after wound formation, but increased with time, especially at the edge of the wound. Moreover, the TGF-β1 fluorescence intensity was higher in Hsf1 KD than in Hsf1 NC cells, and was accompanied by the Smad2/3 phosphorylation – the following step of TGF-β1 signaling pathway. These results suggested for the first time that Hsf1 inhibits migration of proximal tubular cells, whereas TGF-β1 promotes it. Moreover, they showed that, most probably, RPTC suppression by Hsf1 is not direct, but acts through TGF-β1-Smad 2/3 signaling pathway activation.

To confirm the nature of Hsf1 – TGF-β1 relation, the cells were treated with TGF-β1 inhibitor (SB431542) before the wound formation. Indeed, the migration rates diminished in both KD and NC cells, and the decrement range was more evident in the case of Hsf1 KD counterparts (after 0-6 h: by 32% in KD cells and by 9% in NC cells; after 6-12 h: by 30% in KD cells and by 22% in NC cells).

Furthermore, the transwell assay tested the invasive ability of RPTC to penetrate the Matrigel Invasion Chamber. As expected, Hsf1 KD cells were more invasive than Hsf1 NC ones, whereas TGF-β1 inhibitor diminished the invasive ability of Hsf1 KD cells to greater extent than that of Hsf1 NC cells (by 70% and 52%, respectively). No doubt, Hsf1 revealed its nature of tubular cell migration inhibitor, cooperating in this field with TGF-β1. This observation is concordant with the most recent suggestions of Hsf1 inhibition as a potential mechanism for use in next-generation anti-cancer therapeutics [12].

## 2 Future Prospects

Animal studies and *in vitro* experiments still predominate in the HSF-related nephrological area. The clinical context of Hsf1 activity in humans was only analyzed in patients with chronic kidney disease and renal cell carcinoma. Therefore, it is high time for studies in the area of clinical nephrology, showing the vast possibilities of HSF use in everyday practice, for example as an early marker of ischemic injury or a protective agent against tubular damage.

## 3 Conclusions

HSF always play in concert with other factors, being the master regulators and *spiritus movens* of their further activity. Their cooperation with other transcription factors, HSP, other chaperones or growth factors, seems finely tuned in time and adjusted to severity of injury. It is known that HSF act mainly through heat shock response, but they may well cooperate with other signaling pathways and influence selected processes through actions of other regulators.

Therefore, Hsf1 is not an individualist, but rather the element of a complex network acting differently in various conditions, not a "single shot hero", but a player waiting for a "second hit" to fully visualize its power in action.

**Acknowledgements** No funding was required for this review.

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** All procedures performed in the studies cited in this review, involving human participants, were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approvals were granted by local ethics committees (see references for details).

**Ethical Approval for Studies Involving Animals** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed in the studies cited in this review. Approvals were granted by local ethics committees (see references for details).

## References

1. Barna J, Csermely P, Vellai T (2018) Roles of heat shock factor 1 beyond the heat shock response. Cell Mol Life Sci 75:2897–2916
2. Eickelberg O, Seebach F, Riordan M et al (2002) Functional activation of heat shock factor and hypoxia-inducible factor in the kidney. J Am Soc Nephrol 13:2094–2101

3. Gomez-Pastor R, Burchfiel E, Thiele DJ (2018) Regulation of heat shock transcription factors and their roles in physiology and disease. Nat Rev Mol Cell Biol 19:4–19
4. Harrison EM, Sharpe E, Bellamy CO et al (2008) Heat shock protein 90-binding agents protect renal cells from oxidative stress and reduce kidney ischemia-reperfusion injury. Am J Physiol Renal Physiol 295:F397–F405
5. Hosszu A, Antal Z, Veres-Szekely A et al (2018) The role of Sigma-1 receptor in sex-specific heat shock response in an experimental rat model of renal ischaemia/reperfusion injury. Transpl Int 31:1268–1278
6. Li J, Labbadia J, Morimoto RI (2017) Rethinking HSF1 in stress, development and organismal health. Trends Cell Biol 27:895–905
7. Lou Q, Hu Y, Ma Y, Dong Z (2016) Heat shock factor 1 induces crystallin-αB to protect against cisplatin nephrotoxicity. Am J Physiol Renal Physiol 311:F94–F102
8. Lou Q, Li Y, Hou B et al (2019) Heat shock transcription factor 1 affects kidney tubular cell migration by regulating the TGF-β1-Smad2/3 signaling pathway. Mol Med Rep 20:4323–4330
9. Musiał K, Zwolińska D (2018) Fractional excretion as a new marker of tubular damage in children with chronic kidney disease. Clin Chim Acta 480:99–106
10. Petrakis I, Mavroeidi V, Stylianou K et al (2013) Hsf-1 affects podocyte markers NPHS1, NPHS2 and WT1 in a transgenic mouse model of TTRVal30Met-related amyloidosis. Amyloid 20:164–172
11. Reuter S, Bangen P, Edemir B et al (2009) The HSP72 stress response of monocytes from patients on haemodialysis is impaired. Nephrol Dial Transplant 24:2838–2846
12. Sharma C, Seo YH (2018) Small molecule inhibitors of HSF1-activated pathways as potential next-generation anti-cancer therapeutics. Molecules 23:2757. https://doi.org/10.3390/molecules23112757
13. Sreedharan R, Riordan M, Wang S, Thulin G, Kashgarian M, Siegel NJ (2005) Reduced tolerance of immature renal tubules to anoxia by HSF-1 decoy. Am J Physiol Renal Physiol 288:F322–F326
14. Sreedharan R, Chen S, Miller M, Haribhai D, Williams CB, Van Why SK (2014) Mice with an absent stress response are protected against ischemic renal injury. Kidney Int 86:515–524
15. Van Why SK, Mann AS, Thulin G, Zhu X-H, Kashgarian M, Siegel NJ (1994) Activation of heat-shock transcription factor by graded reductions in renal ATP, in vivo, in the rat. J Clin Invest 94:1518–1523
16. Van Why SK, Kim S, Geibel J, Seebach FA, Kashgarian M, Siegel NJ (1999) Thresholds for cellular disruption and activation of the stress response in renal epithelia. Am J Phys 277:F227–F234
17. Wu P-S, Chang Y-H, Pan C-C (2017) High expression of heat shock proteins and heat shock factor-1 distinguishes an aggressive subset of clear cell renal cell carcinoma. Histopathology 71:711–718

# Binary Role of Heat Shock Proteins in Cancer Immunotherapy: A Detailed Perspective


**B. V. Sunil Kumar, Priya K. Gopal, Ankita Gurao, and Ramneek Verma**

## Abstract

**Introduction** It is a well-accepted fact that Heat Shock Proteins (HSP) have immuno-stimulatory roles. HSP can act as self-antigens or as either adjuvants or molecular chaperones in stimulating both the innate and adaptive immune systems. The role of HSP as cancer vaccines is being tested with successful immunization comprising gp96, HSP 90, and HSP 70 either bound to synthetic or natural peptides. These chaperones stimulate the immune system involving antigen-presenting cells leading to activation of cytotoxic T-lymphocytes and also lead to stimulation of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-12, and GM-CSF as well as C-C chemokines such as MCP-1, MIP-1, and RANTES. In this article, the authors have attempted to encapsulate in brief the alternative role played by HSP in progression and mitigation of cancer, emphasizing the later one as an attribute of their immuno-stimulatory role achieved by activation of various immune cells.

**Methods** The articles emphasizing the HSP' immuno-stimulatory or immunogenic roles in cancer were searched in PubMed by filtering last 20 year's collection and reviewed systematically.

**Results** HSP stimulate the immune system when directly used as antigens in cancer therapy; however, it reports some adverse effects as well. The most successful design of cancer vaccine has been the use of HSP as efficient immuno-adjuvants in complex form with antigens specific to the cancer type. They mediate both innate as well as adaptive immune responses, therefore are comprehensively studied for cancer immune signaling.

B. V. S. Kumar (✉) · A. Gurao · R. Verma
Department of Microbial and Environmental Biotechnology, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India
e-mail: drbvskumar@gmail.com; sunilkumar@gadvasu.in

P. K. Gopal
Department of Microbiology, Muralidhar Girls' College, Kolkata, West Bengal, India

Heat Shock Proteins (2021) 22: 387–405
https://doi.org/10.1007/7515_2020_34

Published Online: 9 September 2020

**Conclusions** This chapter briefly reviews the recent advancements in our understanding of the dual role played by HSP in cancer immunotherapy, as an immunogen, and as an immuno-modulator.

**Keywords** Adjuvant · Diagnosis · HSP · Immune-modulation · Immunogen · Vaccine

## Abbreviations

| | |
|---|---|
| APC | antigen presenting cells |
| ATP/ADP | adenosine triphosphate/adenosine diphosphate |
| CD | cluster of differentiation |
| CMT | canine mammary tumor |
| DAMP | damage-associated molecular patterns |
| GM-CSF | granulocyte-macrophage colony stimulating factor |
| GP96/GRP94 | endoplasmin/glucose-regulated protein 94 |
| HIF | hypoxia inducible factor |
| HLA | human leukocyte antigen |
| HSP | heat shock protein |
| IFN | interferon |
| IL-12 | interleukin-12 |
| kDa | kilo Dalton |
| LOX | lysyl oxidase |
| MCP-1 | monocyte chemoattractant protein 1 |
| MHC | major histocompatibilty complex |
| MIP-1 | macrophage inflammatory protein-1 |
| PAMP | pathogen-associated molecular patterns |
| PRR | pattern recognition receptor |
| RA | rheumatoid arthritis |
| RANTES | regulated on activation, normal T-cell expressed and secreted |
| SLE | systemic lupus erythematosus |
| TLR | toll like receptors |
| TME | tumor microenvironment |
| TNF | tumor necrosis factor |
| TSA/TAA | tumor specific antigen/tumor associated antigen |

## 1 Introduction

Stress is a detrimental factor in all life forms. Invariably in either of its type- biotic and abiotic, it disturbs the minuscule balance in a living system leading to disruption in the homeostasis. Proteins play a whole gamut of localized and global functions in cell/tissue. Similarly, certain classes of proteins function to restore or repair other

proteins; this includes the Heat Shock Proteins (HSP), which represent a multigene protein family.

The HSP are molecular chaperones that are reported to be highly conserved among living forms for a long span of evolutionary timescale [60, 104]. Apparently, this class of protein protects cellular proteins from heat stress, and also against some other abiotic stresses like $Ca^{+2}$ excess, cold shock, UV radiation, too little or excess of physical exertion and the defective versions of HSP associated with several proteinopathies [27, 45, 60, 108]. The HSP, which are generally regarded as highly conserved in all life forms, are not linked evolutionarily, making them pliable to diverge into homologs amid various factors such as tissue-specific requirements and accessing target [104]. In the last few decades, increased exposure to a diverse range of abiotic stressors such as pollutants, radiations, heavy metals, etc. have undoubtedly ventured organisms to accumulate more stress. Therefore, leading to increased functional load on HSP for chaperoning the proteome; further, the HSP have been linked for their steady transformations in the course of changing spatiotemporal environmental conditions [19, 43, 68]. This humungous demand has led to more and more diversification of heat shock proteins.

The above discussion manifests the primary definition of HSP as any protein displaying chaperonage activity, and their nomenclature has been done mainly based on the molecular weights (from 8 to 150 KDa) [46]. The human genome assembly reveals more than a hundred HSP, belonging to 17 well-described classes [46, 57] (Table 1, Fig. 1). This vast family includes α-A crystallin, α-B crystallin, Calnexin, Calreticulin, Gp96, Grp170, Grp78, HSC 70, HSC 74, HSP 110, HSP 27, HSP 40, HSP 47, HSP 60, HSP 70, HSP 90α, and HSP 90β. The fashion these HSP interact with the target proteins varies; either they interact directly with the surface of target protein, e.g., HSP70, HSP60, HSP90, and HSP110 or aggregate as complex to evolve into a tailor-made interaction site for the target, e.g., small HSP. The cascade of interactions of such chaperones makes the target proteins resistant to further damages, and contrarily, the former's elevated levels could also inhibit apoptosis [14].

Conventionally the heat shock proteins are considered to be prime tools devised by the living system to restore homeostasis; nevertheless, of late, researchers have started considering their dual-sided aspects such as immunity and apoptosis, as well [38, 58]. As for immunity, the flip side of HSP could be to promote autoimmunity, whereas for apoptosis, they could be associated with indefinite cell division leading to malignancy. Further, metastasis, followed by malignancy, causes an imbalance in proteostasis (homeostasis of proteins), where chaperonage activity of HSP is again called upon [4, 14, 101]. More critically, the HSP' liabilities have also been demonstrated for developing resistance to the different cancer therapies and treatments [2].

**Table 1** Different classes of HSP [46]

| S. No | Protein name | Gene name | UniProtKB | Length | Domain/ Family |
|---|---|---|---|---|---|
| 1 | **Alpha-crystallin A**<br>Heat shock protein beta-4<br>Short name: HspB4 | CRYAA<br>Synonyms: CRYA1, HSPB4 | P02489 | 173 | Alpha crystallin/ Hsp20 domain (52–164) |
| 2 | **Alpha-crystallin B**<br>Alternative name(s):<br>Alpha(B)-crystallin, Heat shock protein beta-5, Short name: HspB5, Renal carcinoma antigen, NY-REN-27, Rosenthal fiber component | CRYAB<br>Synonyms: CRYA2, HSPB5 | P02511 | 175 | Alpha crystallin/ Hsp20 domain (56–164) |
| 3 | **Calnexin**<br>Alternative name(s):<br>IP90, Major histocompatibility complex class I antigen-binding protein p88, p90 | Name: CANX | P27824 | 592 | Calreticulin family (71–440) |
| 4. | **Calreticulin**<br>Alternative name(s): CRP55, Calregulin, Endoplasmic reticulum resident protein 60, Short name: ERp60, HACBP, grp60 | Name: CALR<br>Synonyms: CRTC | P27797 | 417 | Calreticulin family (23–257, 259–332) |
| 5 | **Gp96**<br>Alternative name(s):<br>Endoplasmin, 94 kDa glucose-regulated protein, Short name: GRP-94, Heat shock protein 90 kDa beta member 1, Tumor rejection antigen 1, gp96 homolog | HSP 90B1<br>Synonyms: GRP94, TRA1 | P14625 | 803 | HSP90 family (257–773) |
| 6 | Grp170<br>Alternative name(s):<br>Hypoxia up-regulated protein 1, 150 kDa oxygen-regulated protein (ORP-150), 170 kDa glucose-regulated protein (GRP-170) | Name: HYOU1<br>Synonyms: GRP170, ORP150 | Q9Y4L1 | 999 | HSP70 family (36–632) |
| 7 | Grp78<br>Alternative name(s):<br>Endoplasmic reticulum chaperone BiP, 78 kDa | Name: HSP A5<br>Synonyms: GRP781 | P11021 | 654 | HSP70 family (30–635) |

(continued)

**Table 1** (continued)

| S. No | Protein name | Gene name | UniProtKB | Length | Domain/ Family |
|---|---|---|---|---|---|
| | glucose-regulated protein (GRP-78), Binding-immunoglobulin protein (BiP1), Heat shock protein 70 family protein (HSP70 family protein), Heat shock protein family A member, Immunoglobulin heavy chain-binding protein | | | | |
| 8 | **Hsc70** | HSP A8 | P11142 | 646 | HSP70 family (6–612) |
| | Alternative name(s): | Synonyms: HSC 70, HSP 73, HSP A10 | | | |
| | Heat shock cognate 71 kDa protein Heat shock 70 kDa protein 8 | | | | |
| | Lipopolysaccharide-associated protein 1 (LAP-1, LPS-associated protein 1) | | | | |
| 9 | **Hsc74** | HSP A9 | P38646 | 679 | HSP70 family (55–652) |
| | Stress-70 protein, mitochondrial | Synonyms: GRP75, HSP A9B, mt-HSP 70 | | | |
| | Alternative name(s): | | | | |
| | 75 kDa glucose-regulated protein (GRP-75) | | | | |
| | Heat shock 70 kDa protein 9 | | | | |
| | Mortalin (MOT) | | | | |
| | Peptide-binding protein 74 | | | | |
| | (PBP74) | | | | |
| 10. | **HSP1** | HSP E1 | P61604 | 102 | Cpn 10 family (9–100) |
| | 10 kDa heat shock protein, mitochondrial | | | | |
| | Short name: | | | | |
| | Hsp10 | | | | |
| | Alternative name(s): | | | | |
| | 10 kDa chaperonin | | | | |
| | Chaperonin 10 | | | | |
| | Short name: | | | | |
| | CPN10 | | | | |
| | Early-pregnancy factor | | | | |
| | Short name: | | | | |
| | EPF | | | | |

(continued)

**Table 1** (continued)

| S. No | Protein name | Gene name | UniProtKB | Length | Domain/ Family |
|---|---|---|---|---|---|
| 11 | **Hsp110** | HSP H1 | Q92598 | 858 | HSP70 family (3–704) |
| | Alternative name(s): | Synonyms: HSP 105, HSP 110, KIAA0201 | | | |
| | Heat shock protein 105 kDa | | | | |
| | Antigen NY-CO-25 | | | | |
| | Heat shock 110 kDa protein | | | | |
| 12 | **Hsp27** | HSPB1 | P04792 | 205 | HSP20 domain (88–183) |
| | Heat shock protein beta-1 | Synonyms: HSP 27, HSP 28 | | | |
| | (HspB1), 28 kDa heat shock protein, Estrogen-regulated 24 kDa protein, Heat shock 27 kDa protein | | | | |
| | (HSP 27), Stress-responsive protein 27 (SRP27) | | | | |
| 13 | **Hsp40** | DNAJB1 | P25685 | 340 | DnaJ_C domain (4–65; 164–323) |
| | Alternative name(s): | Synonyms: DNA J1, HDJ1, HSP F1 | | | |
| | DnaJ homolog subfamily B member 1, DnaJ protein homolog 1, Heat shock 40 kDa protein 1 (HSP40), Heat shock protein 40, Human DnaJ protein 1 (hDj-1) | | | | |
| 14 | **Hsp47** | SERPINH1 | P50454 | 418 | Serpin domain (48–409) |
| | Alternative name(s): | Synonyms: CBP1, CBP2, HSP 47, SERPINH2 | | | |
| | Serpin H1 | ORF names: PIG14 | | | |
| | 47 kDa heat shock protein | | | | |
| | Arsenic-transactivated protein 3 (AsTP3) | | | | |
| | Cell proliferation-inducing gene 14 protein | | | | |
| | Collagen-binding protein (Colligin) | | | | |
| | Rheumatoid arthritis-related antigen RA-A47 | | | | |
| 15 | Hsp60 | HSP D1 | P10809 | 573 | Cpn60_TCP1 domain (47–546) |
| | Alternative name(s): | Synonyms: HSP 60 | | | |
| | 60 kDa chaperonin | | | | |
| | Chaperonin 60 (CPN60), | | | | |

(continued)

**Table 1** (continued)

| S. No | Protein name | Gene name | UniProtKB | Length | Domain/ Family |
|---|---|---|---|---|---|
| | Heat shock protein 60 (HSP-60) | | | | |
| | HuCHA60 | | | | |
| | Mitochondrial matrix protein P1 | | | | |
| | P60 lymphocyte protein | | | | |

## *1.1 HSP Expression in Different Types of Cancers*

Till date, more than 100 types of cancers have been characterized in human beings. The correlation of different HSP' expression with various types of cancer involves a complex set of events (Fig. 1) (https://www.cancer.gov/types; [46]). Moreover, the progression of cancer accumulates at every stage, de-novo molecular milieu, which further multiplexes the interactome [20]. A cascade of molecular and physiological events occurs during tumorigenesis, and at every cascade, there are some showstoppers or leading receptors/ enzymes/ growth factors with their clientship to one or more class of HSP (Fig. 1). These HSP prevent oppression of growth signals during tumorigenesis by their tendency to bring out proteostasis [14]. Similarly, some HSP viz. the small HSP, HSP 70, and HSP 90 have also been reported to chaperone mutated P53 [78], channelizing them towards maintaining the lifespan of cancer cells. It could also be contemplated that HSP have a regulatory affair with telomerase. Reports on yeast chaperones have concluded that the *hsp*82p (the gene encoding HSP 90) promotes the binding of telomerase with DNA complex [98] and thereby preventing apoptosis.

Narrowing down the discussion of tumor biology to the next hierarchy, the TME (tumor microenvironment) has been argued for its essential role in tumor progression [103]. HSP have also been demonstrated for their essential role in the maintenance of the tumor microenvironment; otherwise, the tumor growth would be suppressed [33]. Metastasis involves angiogenesis; HSP90 has been indirectly shown to promote angiogenesis by chaperoning the factors involved in sensing the hypoxic tumor niche (HIFs) [44]. Another prospect of HSP involvement in TME is very obvious that any deviation from the normal physiological state in the cellular environment demands HSP to come into action and respond to the stress [51].

At the mechanistic level, participation of HSP at different stages is, therefore, a part and parcel of their chaperonage activity. But when considering all the different types of cancers characterized in human beings, the entire set of HSP responds individually in various fashion. In the last two decades (1999 onwards), there has been 353% increase in the research publications linking HSP with cancer (PubMed repository) than the earlier two decades (1979–1998), leading to an enormous data demonstrating HSP expression in various cancer types [86]. Table 2 summarizes HSP' expression in various types of cancer.

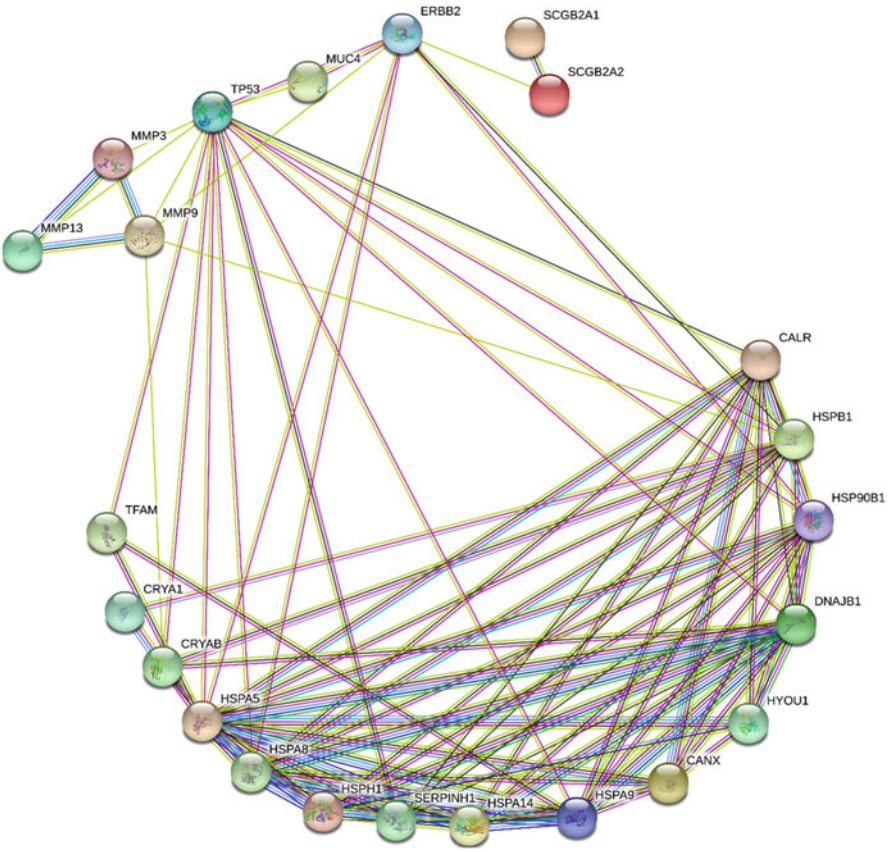



**Fig. 1 The interactome complex elucidating interaction of various tumor specific/associated proteins with 15 representative members from heat shock proteins' family constructed using online tool- STRING 11.0** (https://string-db.org/). Sky blue lines (●) indicate the interactions derived from curated databases, magenta lines (●) indicate the interactions that are experimentally determined, green lines (●) indicate interactions predicted using gene neighbor-hood method, navy blue lines (●) indicate interactions predicted using gene co-occurance, apple green lines (●) indicate predicted interactions by text mining, and violet lines (●) indicate interactions predicted using protein homology. The proteins studied in STRING interactome are as follows, **SCGB2A2**- Mammaglobin-A; **SCGB2A1**- Mammaglobin-B; **CANX**- Calnexin; **HSPB1**- Heat shock protein beta-1; **DNAJB1**- DnaJ homolog subfamily B member 1; **MMP13**- Collagenase 3; **TP53**- Cellular tumor antigen p53; **ERBB2**- Receptor tyrosine-protein kinase erbB-2; **HSPA9**- Stress-70 protein; **HSP90B1**- Endoplasmin; **MMP3**- Stromelysin-1; **HSPH1**- Heat shock protein 105; **CALR**- Calreticulin; **HSPA5**–78 kDa glucose-regulated protein; **MMP9**- Matrix metalloproteinase-9; **HSPA14**- Heat shock 70 kDa protein 14; **MUC4**- Mucin-4; **TFAM**- Transcription factor A; **HSPA8**- Heat shock cognate 71 kDa protein; **CRYAB**- Alpha-crystallin B chain; **SERPINH1**- Serpin H1; **HYOU1**- Hypoxia up-regulated protein 1; **CRYA1**- Alpha-crystallin A chain

**Table 2** HSP expression in various types of cancer

| Chaperone/ HSP | Upregulation | Downregulation |
|---|---|---|
| HSP27 | Human breast cancer [3], Rectal cancer [99], Hepatocellular carcinoma [59], ovarian cancer [75] | -NA- |
| HSP60 | Cervial cancer [40], Prostate cancer ([34, 25]), Breast cancer [42, 25, 37], Ductal carcinoma [25], Ovarian cancer [25] | Bladder cancer [55], Hepatocellular carcinoma [59] |
| HSP70 | T-cell lymphoma [87], Breast Cancer [82], Colorectal cancer [39], Lung Cancer [61], Oral cancer [48] | Cholangiocarcinoma [13] |
| HSP90 | Breast cancer ([22, 77]), Pancreatic cancer [54], Colon carcinoma (Drecoll) | -NA- |

## *1.2 HSP as Immunogens*

The interactome of extracellular HSP and the immune system is too complicated. The HSP have quintessential roles in the regulation of the immune system. This makes them dominant antigens and promising candidates/components of antitumor vaccines [16]. HSP carry the fingerprint of each cancer cell type expressing them during disease state [76]. Therefore, the use of HSP-based vaccines helps to circumnavigate the need to identify specific antigens for an individual type of tumor. This type of vaccine protocol has been evaluated in various types of cancers like gastrointestinal cancers, melanoma, pancreatic cancers, lymphoma, renal cell carcinoma, and so on [74].

Several patterns of HSP' role in generating immune response has been addressed to. One pattern explains HSP as classical antigens [23, 49, 74]. Hence being foreign, HSP can elicit cellular and antibody-mediated immune effects [52]. HSP are believed to be highly conserved; however, species-specific differences occur in the corresponding HSP, which are read as foreign epitopes by the body's immune system [63, 70]. In another pattern, it is exemplified that the immune response could be directed to epitopes of self HSP [53]. HSP have been highlighted more profoundly as critical antigens in both autoimmune diseases as well as in autoimmunity experimental models [28]. HSP 70 and HSP 90 have been implicated in around 50% of patients with systemic lupus erythematosus (SLE) [66, 67]. Some early studies carried out in rheumatoid arthritis (RA) suggest that a T-cell response to HSP 65 was recognized when presented by HLA-DP [29, 47]. In a separate study set, HSP 70 has been reported explicitly over-expressed in tumor tissues such as various squamous cell carcinomas of the head and neck, prostate carcinoma, sarcomas, haematological malignancies and so on [31, 69]. HSP 70 alone is reported to bring about the maturation of dendritic cells, activate the all immunological functions of Natural killer cells, IFN-γ secretion, and also lead to both pro and anti-inflammatory cytokines [88]. Pre-activated natural killer cells recognize membrane-bound HSP 70, which serves as tumor-specific antigen [35].

In several cancer types like prostate carcinoma, osteosarcoma, gastric, liver, lung, and breast cancer, HSP, especially HSP 27, are related to poor prognosis and

treatment outcomes. Particularly in the breast cancer microenvironment, a high concentration of HSP 27 has been reported, which correlates with the expression of IL-10, IL-6, and TNF-α [24]. Elevated levels of HSP 27 have been reported to promote carcinogenesis by inducing cytoprotection, multidrug resistance, suppression of apoptotic protein, and up-regulation of MAPK pathway proteins [36, 102]. HSP 27 has also been reported to be upregulated in canine mammary tumors (CMT) and has been implicated in CMT diagnosis and prognosis [12] and thus could be targeted for CMT immunotherapy.

The role of HSP as antigen alone has been limited, as in some of the studies, it was shown that crude HSP antigens led to autoimmune disorders in those cases [106]. Also, the most successful and elaborately studied vaccine design for cancer therapy is the use of HSP as adjuvant or mini-chaperons. This use of HSP based vaccines has proven to reduce the cytotoxic effects in cancer patients compared to radiotherapy or chemotherapy as it is highly targeted and specific.

### *1.3 Immuno-Modulatory Role of HSP*

HSP have been tested as cancer vaccines, apart from acting as antigens eliciting an immune response. These molecular chaperones can also act as immuno-modulators/adjuvants to stimulate the immunogenicity of the client proteins, which they bind covalently or non-covalently [21, 92]. Usually, these client proteins bind with the HSP with low affinity and low specificity [11]. Various peptides/fragments of antigens are associated with different types of HSP. These client proteins include those derived from virus-infected cells or bacterial proteins, tumor antigens, and alloantigens from MHC-mismatched cells [11, 17, 72, 79, 80]. HSP not only stimulate the innate immune response but can also present antigens to antigen-presenting cells (APCs), thereby inducing a specific acquired immune response [93]. Native HSP isolated from any organism indeed carries client polypeptides of the source organism and thus, can elicit immune response against that particular polypeptide. The development of autologous cancer vaccines relies on this principle of HSP binding. [21].

Initially, HSP were not considered as PAMPs but were thought to be danger-associated molecular patterns (DAMPs) [6, 21], that serve as alternative ligands for PRRs and signal non-infectious cellular damage, and aid in activating the innate immune response [9, 21]. However, now, it is evident that HSP can be actively secreted into the extracellular environment by tumor cells or those affected by viral infections [95]. These HSP have been reported to elicit non-specific secretion of cytokines from cells of the mammalian innate immune system, thereby up-regulating co-stimulatory molecules to activate APC like the dendritic cells [7, 15].

### 1.3.1 Microbial HSP as Immuno-Modulators

Microbial HSP like Mycobaterial HSP 70 [26] has also been reported to possess immuno-modulatory properties. Cytokine production in monocytes is enthused by Mycobacterial HSP 70, which interacts with TLR2 and TLR4 in a CD14-dependent manner [56]. The bacterial chaperone HSP 70 is found to stimulate the production of IL-12p40 and TNF-$\alpha$ in human beings by binding to CCR5 and CD40 on human dendritic cells [105]. In the past, a complex of Mycobacterial HSP 70 and influenza-A virus nucleoprotein has been reported to elicit viral peptide-specific T-cell response in mice without any added adjuvant [83]. Mycobacterial recombinant HSP 70, when complexed with p24 of HIV-1, has been found to elicit humoral and cellular immune responses against the virus [96]. Pneumococcal rDnaJ (rHSP 40) has also been shown to activate TLR4 pathway leading to induction of dendritic cell maturation and possess adjuvant like property [107]. Recently, we have found that recombinant *Brucella* HSP40, when co-immunized with *Brucella* rOmp22, elicits CMI to a greater extent and may act as an immune-modulator [65]. Co-immunization of rOmp22 with *Brucella* rDnaJ (HSP 40) resulted in the up-regulation of IL-12 mRNA expression and an increase in serum IgG2a levels.

### 1.3.2 Eukaryotic HSP as Immuno-Modulators

Eukaryotic HSP have been found to modulate the adaptive immune response against tumor specific/associated antigens (TSA/TAA). HSP often interact with their client antigens, when TSA/TAA is a client protein for an HSP, the adaptive immune response is elicited [93]. HSP 70 has a binding pocket for the client proteins, ATP/ADP, upon interaction with the chaperone, brings about a change in the conformation of HSP 70 which ultimately exposes its binding pocket for interaction with the associated peptides [21, 84]. HSP 90, on the other hand, exists as a homodimer, and the peptide binding cleft is located between the two sub-units, which also functions in conjugation with smaller co-chaperones in an ATP/ADP dependent manner [21, 81]. The HSP complexed exogenous peptides may enter the MHC I pathway and prime CD8+ T cells because of their ability to cross-present before the APCs [41, 71]. HSP 90 is involved in the cytosolic pathway of antigen cross-presentation wherein the peptide first gets internalized in the cell through receptors like Scavenger receptor-A, Scavenger receptor-F1, stabilin-1, LOX-1, and then gets trafficked to the cytosol and enters the classical MHC I pathway [41, 71]. It seems that different involvement of various receptors for selective internalization may account for better immunogenicity of various HSP, and the nature of the HSP-complexed peptides and APC type determines the pathway of antigen cross-presentation [10, 21, 97].

Although several prophylactic strategies have been adapted to prevent various infectious diseases successfully, immunological approaches for cancer therapy have been reticent. The tumor cells are altered self cells, which can evade the host immune

response, and they elicit a feeble immune response. Attaching 'danger signals' to the tumor antigens may enhance their antigen presentation capability. HSP, when complexed with such antigens, may boost up both the innate and adaptive immune response against the antigen [85]. These complexes enter the MHC class I or II pathway through specific TLR or scavenger receptors [93].

There has been a keen interest in the last few decades toward harnessing the immuno-modulatory and adjuvant properties of HSP for formulating prophylactic and therapeutic vaccines against virus-induced human cancer. The efficacy of human HSP 70 as an effective immune adjuvant has been assessed in the past for the development of a therapeutic protein vaccine against human papilloma virus-induced cervical cancer in women. An optimized E7 protein of HPV16 was linked to human HSP 70 and used as a therapeutic vaccine. The construct proved to induce a robost E7-specific CD8+ T cell immune response and exhibited a significant therapeutic effect against E7-expressing tumor cells [109].

### 1.3.3 HSP Activate Dendritic Cells

The binary (antigen and adjuvant) roles of HSP are exploited in the development of anti-tumor vaccines. Apart from activation of dendritic cells, some HSP are also involved in secretion of the pro-inflammatory cytokines viz. interleukin (IL)-12 and tumor necrosis factor (TNF)-α, and chemokines [5, 62, 85, 100]. Maturation of APCs has also been reported to be induced by CD14, TLR-2, or TLR-4 mediated internalization of HSP 70 [1]. The endoplasmic reticulum paralog of HSP 90 (Grp96), on the other hand, up-regulates MHC-II and CD86 surface expression and induces IL-6, IL-1, TNF-a, and IL-12 secretion thereby leading to activation of dendritic cells [1, 90]. It is understood that dendritic cells orchestrate both innate and adaptive immunity. Thus, HSP may induce antigen-specific T-cell responses by activating dendritic cells.

### 1.3.4 HSP Activate Natural Killer Cells

Another sub-set of cells that mediate anti-tumor response is the natural killer cells (NK cells), which have a vital role in innate immunity as well as cytokine production. They tend to kill infected and tumor cells, as well. Some HSP boost up NK cell activity also, thereby enhancing the latter's tumor regressing/rejection activity [50]. CD94/NKG2A is an inhibitory receptor expressed on NK cells that diminish their activity. Human histocompatibility leukocyte antigen (HLA) can interact with the CD94/NKG2A receptors of NK cells. During oxidative stress, HSP 60 signal peptide interacts with HLA-E and hinder CD94/NKG2A recognition, thereby enhancing NK cell's ability to detect and act upon stressed cells, including tumor cells [64]. NK cells also mediate their role of killing cells through secretion of perforin, which disintegrates the target cell membrane inducing apoptosis. It has been suggested that gp96 peptide complexes are required for perforin dependent

sustained NK cell activation and clonal expansion of CTLs [94]. HSP 70, when expressed on the tumor cell membrane, has also been reported to stimulate the migratory and cytolytic activity of NK cells and induces apoptosis of tumor cells [30].

### 1.3.5 HSP to Act Well during Oxidative Stress

Interestingly, it has been observed that the immunogenicity of HSP-based vaccines is increased following oxidative stress [8, 88]. The reason behind those mentioned above may be that, the expression and quantity of immunogenic peptides differ in stressed and non-stressed cells. Moreover, immunogenic peptides are better chaperoned by stress-inducible HSP than their constitutively expressed counterparts. It is also reported that immunity against HSP-chaperoned peptides is tumor specific and the protective immunity is mediated only against autologous tumors, not the allogeneic ones [88, 91]. Moreover, HSP–peptide complexes from healthy sources are incompetent in stimulating T cell-mediated immunity [88].

It is worth mentioning here that the anti-tumor specific immunity of HSP-peptide complexes is highly dose-dependent. While low doses were found to be effective, a higher dose of these complexes turned out to be ineffective or even immunosuppressive. It induced immune tolerance through TLR2 and TLR4 mediated signaling [18]. With the knowledge of immuno-modulatory properties of HSP, these chaperones have widely been used in the treatment of cancers. In preclinical studies, in-situ induction of HSP70 expression (at the tumor site) has been reported to have a significant therapeutic potential [32, 73, 89] as well.

## 2 Conclusions

In conclusion, it could be assumed that various HSP have antigenic as well as immunomodulating effects. They are able to bind to a variety of client proteins and elicit immune response against the interacting proteins in a specific manner. In the future, multimodality anti-tumor therapy based on HSP in combination with radio, chemo, and/ or hyperthermia therapy could be a treatment option for further clinical trials.

**Acknowledgements** The authors acknowledge Department of Biotechnology, Ministry of Science and Technology (Grant ID: BT/PR6434/GBD/27/414/2012) for carrying out research on diagnostic implication of HSP 90 and HSP 27 on mammary tumors and University Grant Commission, India (Grant number: F.No. 42-54/2013SR), for carrying out research on evaluating immuno-modulating effect of *Brucella* rHSP 40.

**Disclosure of Interests** All the authors declare they have no conflict of interest.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed for the above mentioned projects. Approval was granted by Institutional animal ethics committee (IAEC), GADVASU, vide memo no. IAEC/2015/30-63 and VMC/12/3901-35 for UGC and DBT sponsored projects respectively.

## References

1. Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK (2000) HSP70 stimulates cytokine production through a CD14- dependent pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6:435–442
2. Azad AA, Zoubeidi A, Gleave ME, Chi KN (2015) Targeting heat shock proteins in metastatic castration-resistant prostate cancer. Nat Rev Urol 12(1):26
3. Banerjee S, Lin CF, Skinner KA, Schiffhauer LM, Peacock J, Hicks DG, Redmond EM, Morrow D, Huston A, Shayne M, Langstein HN, Miller-Graziano CL, Strickland J, O'Donoghue L, De AK (2011) Heat shock protein 27 differentiates tolerogenic macrophages that may support human breast cancer progression. Cancer Res 71:318–327
4. Bastola P, Oien DB, Cooley M, Chien J (2018) Emerging cancer therapeutic targets in protein homeostasis. AAPS J 20(6):94
5. Basu S, Srivastava PK (1999) Calreticulin, a peptide-binding chaperone of the endoplasmic reticulum, elicits tumor- and peptide-specific immunity. J Exp Med 189:797–802
6. Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK (2000) Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-κB pathway. Int Immunol 12(11):1539–1546
7. Basu S, Binder RJ, Ramalingam T, Srivastava PK (2001) CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity 14(3):303–313
8. Belli F, Testori A, Rivoltini L, Maio M, Andreola G, Sertoli MR, Gallino G, Piris A, Cattelan A, Lazzari I, Carrabba M, Scita G, Santantonio C, Pilla L, Tragni G, Lombardo C, Arienti F, Marchiano A, Queirolo P, Bertolini F, Cova A, Lamaj E, Ascani L, Camerini R, Corsi M, Cascinelli N, Lewis JJ, Srivastava P, Parmiani G (2002) Vaccination of metastatic melanoma patients with autologous tumor-derived heat shock protein gp96-peptide complexes: clinical and immunologic findings. J Clin Oncol 20(20):4169–4180
9. Bianchi ME (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. J Leuk Biol 81(1):1–5
10. Binder RJ (2009) Hsp receptors: the cases of identity and mistaken identity. Curr Opin Mol Ther 11(1):62–71
11. Binder RJ (2014) Functions of heat shock proteins in pathways of the innate and adaptive immune system. J Immunol 193:5765–5771
12. Birdi R, Sunil Kumar BV, Gupta K, Kashyap N, Kumar A (2019) Circulating level of heat shock protein 27 is elevated in dogs with mammary tumors. 3 Biotech 9:229. https://doi.org/10.1007/s13205-019-1765-x
13. Boonjaraspinyo S, Boonmars T, Kaewkes S, Laummaunwai P, Pinlaor S, Loilome W, Yongvanit P, Wu Z, Puapairoj A, Bhudhisawasdi V (2012) Down-regulated expression of HSP70 in correlation with clinicopathology of cholangiocarcinoma. Pathol Oncol Res 18 (2):227–237

14. Calderwood SK, Gong J (2016) Heat shock proteins promote cancer: it's a protection racket. Trends Biochem Sci 41(4):311–323
15. Calderwood SK, Theriault J, Gray PJ, Gong J (2007) Cell surface receptors for molecular chaperones. Methods 43(3):199–206
16. Calderwood SK, Gong J, Murshid A (2016) Extracellular HSP: the complicated roles of extracellular HSP in immunity. Front Immunol 7:159
17. Castelli C, Ciupitu AM, Rini F, Rivoltini L, Mazzocchi A, Kiessling R, Parmiani G (2001) Human heat shock protein 70 peptide complexes specifically activate antimelanoma T cells. Cancer Res 61:222–227
18. Chandawarkar RY, Wagh MS, Srivastava PK (1999) The dual nature of specific immunological activity of tumor-derived gp96 preparations. J Exp Med 189:1437–1442
19. Chen B, Feder ME, Kang L (2018) Evolution of heat shock protein expression underlying adaptive responses to environmental stress. Mol Ecol 27(15):3040–3054
20. Ciocca DR, Calderwood SK (2005) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 10(2):86
21. Colaco CA, Bailey CR, Walker KB, Keeble J (2013) Heat shock proteins: stimulators of innate and acquired immunity. Biomed Res Int 2013:461230. https://doi.org/10.1155/2013/461230
22. Crouch B, Murphy H, Belonwu S, Martinez A, Gallagher J, Hall A, Soo MS, Lee M, Hughes P, Haystead T, Ramanujam N (2017) Leveraging ectopic Hsp90 expression to assay the presence of tumor cells and aggressive tumor phenotypes in breast specimens. Sci Rep 7 (1):17487
23. Davenport MP, McKenzie KR, Basten A, Britton WJ (1992) The variable C-terminal region of the Mycobacterium leprae. 70-kilodalton heat shock protein is the target for humoral immune responses. Infect Immun 60:1170–1177
24. De AK, Kodys KM, Yeh BS, Miller-Graziano C (2000) Exaggerated human monocyte IL-10 concomitant to minimal TNF-alpha induction by heatshock protein 27 (Hsp27) suggests Hsp27 is primarily an antiinflammatory stimulus. J Immunol 165:3951–3958
25. Desmetz C, Bibeau F, Boissiere F, Bellet V, Rouanet P, Maudelonde T, Mange A, Solassol J (2008) Proteomics-based identification of HSP60 as a tumor-associated antigen in early stage breast cancer and ductal carcinoma in situ. *J*. Proteome Res 7:3830–3837
26. Dhakal J, Brah GS, Agrawal RK, Pawar HN, Kaur D, Verma R (2013) Over-expression of gene encoding heat shock protein 70 from Mycobacterium tuberculosis and its evaluation as vaccine adjuvant. Indian J Med Microbiol 31(2):123–129
27. Feige U, Morimoto RI, Yahara I, Polla B (eds) (1996) Stress-inducible cellular responses, vol 77, Birkhäuser
28. Gaston JSH (1991) Heat shock proteins and autoimmunity. Semin Immunol 3:35
29. Gaston JSH, Life PF, van der Zee R, Jenner PJ, Colston MJ, Tonks S, Bacon PA (1991) Epitope specificity and MHC restriction of rheumatoid arthritis synovial T cell clones which recognize a mycobacterial 65 kD heat shock protein. Int Immun 3:965–972
30. Gastpar R, Gehrmann M, Bausero MA, Asea A, Gross C, Schroeder JA, Multhoff G (2005) Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. Cancer Res 65:5238–5247
31. Gehrmann M, Schmetzer H, Eissner G, Haferlach T, Hiddemann W, Multhoff G (2003) Membrane-bound heat shock protein 70 (Hsp70) in acute myeloid leukemia: a tumor specific recognition structure for the cytolytic activity of autologous NK cells. Haematologica 88:474–476
32. Geng H, Zhang GM, Xiao H, Yuan Y, Li D, Zhang H, Qiu H, He YF, Feng ZH (2006) HSP70 vaccine in combination with gene therapy with plasmid DNA encoding sPD-1 overcomes immune resistance and suppresses the progression of pulmonary metastatic melanoma. Int J Cancer 118:2657–2664
33. Giri B, Garg B, Sethi V, Modi S, Banerjee S, Ramakrishnan S, Saluja A, Dudeja V (2017) Pancreatic cancer requires HSP70 in the tumor microenvironment for cancer growth. J Am Coll Surg 225(4):e124–e125

34. Glaessgen A, Jonmarker S, Lindberg A, Nilsson B, Lewensohn R, Ekman P, Valdman A, Egevad L (2008) Heat shock proteins 27, 60 and 70 as prognostic markers of prostate cancer. APMIS 116:888–895
35. Gross C, Koelch W, DeMaio A, Arispe N, Multhoff G (2003) Cell surface-bound heat shock protein 70 (Hsp70) mediates perforin-independent apoptosis by specific binding and uptake of granzyme B. J Biol Chem 278:41173–41181
36. Guay J, Lambert H, Gingras-Breton G, Lavoie JN, Huot J, Landry J (1997) Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of heat shock protein 27. J Cell Sci 110:357–368
37. Hamrita B, Chahed K, Kabbage M, Guillier CL, Trimeche M, Chaieb A, Chouchane L (2008) Identification of tumor antigens that elicit a humoral immune response in breast cancer patients' sera by serological proteome analysis (SERPA). Clin Chim Acta 393:95–102
38. Harboe M, Quayle AJ (1991) Heat shock proteins: friend and foe? Clin Exp Immunol 86(1):2
39. Hwang TS, Han HS, Choi HK, Lee YJ, Kim YJ, Han MY, Park YM (2003) Differential, stage-dependent expression of Hsp70, Hsp110 and Bcl-2 in colorectal cancer. J Gastroenterol Hepatol 18(6):690–700
40. Hwang YJ, Lee SP, Kim SY, Choi YH, Kim MJ, Lee CH, Lee JY, Kim DY (2009) Expression of heat shock protein 60 kDa is upregulated in cervical cancer. Yonsei Med J 50:399–406
41. Ichiyanagi T, Imai T, Kajiwara C, Mizukami S, Nakai A, Nakayama T, Udono H (2010) Essential role of endogenous heat shock protein 90 of dendritic cells in antigen cross-presentation. J Immunol 185(5):2693–2700
42. Isidoro A, Casado E, Redondo A, Acebo P, Espinosa E, Alonso AM, Cejas P, Hardisson D, Fresno Vara JA, Belda-Iniesta C, Gonzalez-Baron M, Cuezva JM (2005) Breast carcinomas fulfill the Warburg hypothesis and provide metabolic markers of cancer prognosis. Carcinogenesis 26:2095–2104
43. Jones LM, Eves-van den Akker S, van-Oosten Hawle, P., Atkinson, H.J. and Urwin, P. E. (2018) Duplication of hsp-110 is implicated in differential success of Globodera species under climate change. Mol Biol Evol 35(10):2401–2413
44. Joseph JV, Conroy S, Pavlov K, Sontakke P, Tomar T, Eggens-Meijer E, Balasubramaniyan V, Wagemakers M, den Dunnen WF, Kruyt FA (2015) Hypoxia enhances migration and invasion in glioblastoma by promoting a mesenchymal shift mediated by the HIF1α–ZEB1 axis. Cancer Lett 359(1):107–116
45. Kakkar V, Meister-Broekema M, Minoia M, Carra S, Kampinga HH (2014) Barcoding heat shock proteins to human diseases: looking beyond the heat shock response. Dis Model Mech 7:421–434
46. Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower LE (2009) Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chap 14(1):105–111
47. Kaufmann SH, Schoel B, Embden JDAV, Koga T, Wand-Württenberger A, Munk ME, Steinhoff U (1991) Heat shock protein 60: implications for pathogenesis of an protection against bacterial infections. Immunol Rev 121:67–90
48. Kaur J, Srivastava A, Ralhan R (1998) Expression of 70-kDa heat shock protein in oral lesions: marker of biological stress or pathogenicity. Oral Oncol 34(6):496–501
49. Kellner H, Wen J, Wang J, Raybourne RB, Williams KM, Yu DT (1994) Serum antibodies from patient with ankylosing spondylitis and Reiter's syndrome are reactive with HLA-B27 cells transfected with the Mycobacterium tuberculosis hsp60 gene. Infect Immun 62:484–491
50. Kelly JM, Darcy PK, Markby JL, Godfrey DI, Takeda K, Yagita H, Smyth MJ (2002) Induction of tumor-specific T cell memory by NK cell mediated tumor rejection. Nat Immunol 3:83–90
51. Khajah MA, Almohri I, Mathew PM, Luqmani YA (2013) Extracellular alkaline pH leads to increased metastatic potential of estrogen receptor silenced endocrine resistant breast cancer cells. PLoS One 8(10):e76327

52. Lamb JR, Ivanyi J, Rees ADM, Rothbard J, Howland K, Young RA, Young DB (1987) Mapping of T cells epitopes using recombinant antigens and synthetic peptides. EMBO J 6:1245–1249
53. Lamb JR, Bal V, Mendez-Samperio P, Mehlert ASA, Rothbard J, Jindal S, Young RA, Young DB (1989) Stress proteins may provide a link between the immune response to infection and autoimmunity. Int Immun 1:191–196
54. Lang SA, Moser C, Gaumann A, Klein D, Glockzin G, Popp FC, Dahlke MH, Piso P, Schlitt HJ, Geissler EK, Stoeltzing O (2007) Targeting heat shock protein 90 in pancreatic cancer impairs insulin-like growth factor-I receptor signaling, disrupts an interleukin-6/signal-transducer and activator of transcription 3/hypoxia-inducible factor-1alpha autocrine loop, and reduces orthotopic tumor growth. Clin Cancer Res 13:6459–6468
55. Lebret T, Watson RW, Molinie V, O'Neill A, Gabriel C, Fitzpatrick JM, Botto H (2003) Heat shock proteins HSP27, HSP60, HSP70, and HSP90: expression in bladder carcinoma. Cancer 98:970–977
56. Lehner T, Bergmeier LA, Wang Y, Tao L, Sing M, Spallek R, van der Zee R (2000) Heat shock proteins generate beta-chemokines which function as innate adjuvants enhancing adaptive immunity. Eur J Immunol 30(2):594–603
57. Li Z, Srivastava P (2004) Heat-shock proteins current protocols in immunology, Coligan JE, et al.
58. Lianos GD, Alexiou GA, Mangano A, Mangano A, Rausei S, Boni L, Dionigi G, Roukos DH (2015) The role of heat shock proteins in cancer. Cancer Lett 360(2):114–118
59. Lim SO, Park SG, Yoo JH, Park YM, Kim HJ, Jang KT, Cho JW, Yoo BC, Jung GH, Park CK (2005) Expression of heat shock proteins (HSP27, HSP60, HSP70, HSP90, GRP78, GRP94) in hepatitis B virus-related hepatocellular carcinomas and dysplastic nodules. World J Gastroenterol 11:2072–2079
60. Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191
61. Małusecka E, Zborek A, Krzyzowska-Gruca S, Krawczyk Z (2001) Expression of heat shock proteins HSP70 and HSP27 in primary non-small cell lung carcinomas. An immunohistochemical study. Anticancer Res 21(2A):1015–1021
62. Manjili MH, Wang XY, MacDonald IJ, Arnouk H, Yang GY, Pritchard MT, Subjeck JR (2004) Cancer immunotherapy and heat-shock proteins: promises and challenges. Expert Opin Biol Ther 4:363–373
63. Matthews RC (1992) The 14th C. L. Oakley Lecture. Candida albicans HSP 90: link between protective and auto immunity. J Med Microbio 36:367–370
64. Michaelsson J, Teixeira de Matos C, Achour A, Lanier LL, Karre K, Soderstrom K (2002) A signal peptide derived from hsp60 binds HLA-E and interferes with CD94/NKG2A recognition. J Exp Med 196:1403–1414
65. Minhas P, Sunil Kumar BV, Verma R (2019) Evaluation of immuno-modulating effect of recombinant heat shock protein 40 of Brucella abortus in mice. 3 Biotech. https://doi.org/10.1007/s13205-019-1905-3
66. Minota S, Cameron B, Welch W, Winfield JB (1988a) Autoantibodies to the constitutive 73-kD member of the hsp70 family of heat shock proteins in systemic lupus erythematosus. J Exp Med 168:1475
67. Minota S, Koyasu S, Yahara I, Winfield JB (1988b) Autoantibodies to the heat shock protein hsp90 in systemic lupus erythematosus. J Clin Invest 81:106
68. Moya A, Huisman L, Foret S, Gattuso JP, Hayward DC, Ball EE, Miller DJ (2015) Rapid acclimation of juvenile corals to CO2-mediated acidification by upregulation of heat shock protein and Bcl-2 genes. Mol Ecol 24(2):438–452
69. Multhoff G, Botzler C, Jennen L, Schmidt J, Ellwart J, Issels R (1997) Heat shock protein 72 on tumor cells: a recognition structure for natural killer cells. J Immunol 158:4341–4350
70. Munk ME, Schoel B, Modrow S, Karr RW, Young RA, Kaufmann SHE (1989) T lymphocytes from healthy individuals with specificity to self-epitopes shared by the mycobacterial and human 65 kDa heat shock protein. J Immun 143:2844–2849

71. Murshid A, Gong J, Calderwood SK (2012) The role of heat shock proteins in antigen cross-presentation. Front Immunol 3:1–10
72. Navaratnam M, Deshpande MS, Hariharan MJ, Zatechka DS Jr, Srikumaran S (2001) Heat shock protein-peptide complexes elicit cytotoxic T-lymphocyte and antibody responses specific for bovine herpesvirus 1. Vaccine 19:1425–1434
73. Nishikawa M, Otsuki T, Ota A, Guan X, Takemoto S, Takahashi Y, Takakura Y (2010) Induction of tumor-specific immune response by gene transfer of Hsp70- cell-penetrating peptide fusion protein to tumors in mice. Mol Ther 18:421–428
74. Oki Y, Younes A (2004) Heat shock protein-based cancer vaccines. Expert Rev Vaccines 3 (4):403–411
75. Olejek A, Damasiewicz-Bodzek A, Bodzek P, Wielkoszynski T, Zamlynski J, Stoltny P, Skutil M (2009) Concentrations of antibodies against heat shock protein 27 in the sera of women with ovarian carcinoma. Int J Gynecol Cancer 19:1516–1520
76. Pardoll DM (1994) The new look of tumor antigen in the 90's. Nature 369:357–358
77. Pick E, Kluger Y, Giltnane JM, Moeder C, Camp RL, Rimm DL, Kluger HM (2007) High HSP90 expression is associated with decreased survival in breast cancer. Cancer Res 67 (7):2932–2937
78. Pinhasi-Kimhi O, Michalovitz D, Ben-Zeev A, Oren M (1986) Specific interaction between the p53 cellular tumor antigen and major heat shock proteins. Nature 320(6058):182–185
79. Qian J, Wang S, Yang J, Xie J, Lin P, Freeman ME III, Yi Q (2005) Targeting heat shock proteins for immunotherapy in multiple myeloma: generation of myeloma-specific CTLs using dendritic cells pulsed with tumor-derived gp96. Clin Cancer Res 11:8808–8815
80. Rapp UK, Kaufmann SH (2004) DNA vaccination with gp96-peptide fusion proteins induces protection against an intracellular bacterial pathogen. Int Immunol 16:597–605
81. Richter K, Reinstein J, Buchner J (2007) A Grp on the Hsp90 mechanism. Mol Cell 28 (2):177–179
82. Rohde M, Daugaard M, Jensen MH, Helin K, Nylandsted J, Jäättelä M (2005) Members of the heat-shock protein 70 family promote cancer cell growth by distinct mechanisms. Genes Dev 19(5):570–582
83. Roman E, Moreno C (1996) Synthetic peptides non-covalently bound to bacterial hsp70 elicit peptide-specific T-cell responses in vivo. Immunology 88:487–492
84. Saibil HR, Ranson NA (2002) The chaperonin folding machine. Tr Biochem Sci 27 (12):627–632
85. Segal BH, Wang XY, Dennis CG, Youn R, Repasky EA, Manjili MH, Subjeck JR (2006) Heat shock proteins as vaccine adjuvants in infections and cancer. Drug Discov Today 11 (11–12):534–540
86. Seigneuric R, Mjahed H, Gobbo J, Joly AL, Berthenet K, Shirley S, Garrido C (2011) Heat shock proteins as danger signals for cancer detection. Front Oncol 1:37
87. Seo JS, Park YM, Kim JI, Shim EH, Kim CW, Jang JJ, Kim SH, Lee WH (1996) T cell lymphoma in transgenic mice expressing the human Hsp70gene. Biochem Biophys Res Commun 218(2):582–587
88. Shevtsov M, Multhoff G (2016) Heat shock protein–peptide and hsp-based immunotherapies for the treatment of cancer. Front Immunol 7:171. https://doi.org/10.3389/fimmu.2016.00171
89. Shevtsov MA, Kim AV, Samochernych KA, Romanova IV, Margulis BA, Guzhova IV, Yakovenko IV, Ischenko AM, Khachatryan WA (2014) Pilot study of intra-tumoral injection of recombinant heat shock protein 70 in the treatment of malignant brain tumors in children. Onco Targets Ther 7:1071–1081
90. Singh-Jasuja H, Scherer HU, Hilf N, Arnold-Schild D, Rammensee HG, Toes RE, Schild H (2000) The heat shock protein gp96 induces maturation of dendritic cells and down-regulation of its receptor. Eur J Immunol 30:2211–2215
91. Srivastava PK (1994) Heat shock proteins in immune response to cancer: the fourth paradigm. Experientia 50:1054–1060

92. Srivastava P (2002a) Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Ann Rev Immunol 20:395–425
93. Srivastava P (2002b) Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev Immunol 2(3):185–194
94. Strbo N, Oizumi S, Sotosek-Tokmadzic V, Podack ER (2003) Perforin is required for innate and adaptive immunity induced by heat shock protein gp96. Immunity 18:381–390
95. Sunil Kumar BV, Bhardwaj R, Mahajan K, Kashyap N, Kumar A, Verma R (2018) The over expression of HSP90B1 is associated with tumorigenesis of canine mammary glands. Mol Cell Biochem 440:23–31
96. Suzue K, Young RA (1996) Adjuvant-free hsp70 fusion protein system elicits humoral and cellular immune responses to HIV-1 p24. J Immunol 156(2):873–879
97. Tobian AAR, Canaday DH, Boom WH, Harding CV (2004) Bacterial heat shock proteins promote CD91-dependent class I MHC cross-presentation of chaperoned peptide to CD8+ T cells by cytosolic mechanisms in dendritic cells versus vacuolar mechanisms in macrophages. J Immunol 172(9):5277–5286
98. Toogun OA, DeZwaan DC, Freeman BC (2008) The hsp90 molecular chaperone modulates multiple telomerase activities. Mol Cell Biol 28(1):457–467
99. Tweedle EM, Khattak I, Ang CW, Nedjadi T, Jenkins R, Park BK, Kalirai H, Dodson A, Azadeh B, Terlizzo M, Grabsch H (2010) Low molecular weight heat shock protein HSP27 is a prognostic indicator in rectal cancer but not colon cancer. Gut 59(11):1501–1510
100. Udono H, Srivastava PK (1994) Comparison of tumor-specific immunogenicities of stress-induced proteins gp96, hsp90 and hsp70. J Immunol 152:5398–5403
101. Vahid S, Thaper D, Zoubeidi A (2017) Chaperoning the cancer: the proteostatic functions of the heat shock proteins in cancer. Recent Pat Anti Cancer 12(1):35–47
102. Vidyasagar A, Wilson NA, Djamali A (2012) Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. Fibrogenes Tissue Repair 5:7
103. Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, Gong Z, Zhang S, Zhou J, Cao K, Li X (2017) Role of tumor microenvironment in tumorigenesis. J Cancer 8(5):761
104. Waters ER (2014) Conservative innovation. The mixed-up evolutionary history of the heat-shock proteins. Biochemist 36:9
105. Whittall T, Wang Y, Younson J, Kelly C, Bergmeier L, Peters B, Singh M, Lehner T (2006) Interaction between the CCR5 chemokine receptors and microbial HSP70. Eur J Immunol 36 (9):2304–2314
106. Wood C, Srivastava P, Bukowski R, Lacombe L, Gorelov AI, Gorelov S, Mulders P, Zielinski H, Hoos A, Teofilovici F, Isakov L (2008) An adjuvant autologous therapeutic vaccine (HSPPC-96; vitespen) versus observation alone for patients at high risk of recurrence after nephrectomy for renal cell carcinoma: a multicentre, open label, randomised phase III trial. Lancet 372:145–154
107. Wu Y, Cui J, Zhang X, Gao S, Ma F, Yao H, Sun X, He Y, Yin Y, Xu W (2017) Pneumococcal DnaJ modulates dendritic cell-mediated Th1 and Th17 immune responses through Toll-like receptor 4 signaling pathway. Immunobiology 222:384–393
108. Xu Q (2002) Role of heat shock proteins in atherosclerosis. Arterioscler Thromb Vasc Biol 22:1547–1559
109. Zong J, Wang C, Liu B, Liu M, Cao Y, Sun X, Yao Y, Sun G (2013) Human hsp70 and HPV16 oE7 fusion protein vaccine induces an effective antitumor efficacy. Oncol Rep 30 (1):407–412

# The Role of Heat Shock Proteins in Reproductive Functions

**Ayman Abdel-Aziz Swelum, Nesrein M. Hashem, Ahmed I. Abo-Ahmed, Mohamed E. Abd El-Hack, and Mohamed Abdo**

## Abstract

**Introduction** Heat shock response (HSR) pathway is a highly conserved cellular process. Heat shock factor 1 (HSF1) is a master transcriptional regulator responsible for expression of several important heat shock proteins (HSPs), which can effectively protect critical client proteins from misfolding and degradation, thus maintaining intracellular integrity under stressed conditions. Recent studies have demonstrated the direct connections between HSR players and tumor cell survival, validating HSR players as novel molecular targets in anticancer treatment. Small molecule screening has produced some promising HSR inhibitors for anticancer treatment. In this article, we will be summarizing the main findings from HSR inhibitors on recent clinical and preclinical studies.

**Methods** The authors reviewed all the relevant papers of HSR inhibitors with an emphasis on human and animal studies.

A. A.-A. Swelum (✉)
Department of Animal production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Sharkia, Egypt
e-mail: aymanswelum@zu.edu.eg; aymanswelum@yahoo.com; aswelum@ksu.edu.sa

N. M. Hashem
Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

A. I. Abo-Ahmed
Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt

M. E. Abd El-Hack
Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

M. Abdo
Department of Anatomy and Embryology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt

Heat Shock Proteins (2021) 22: 407–427
https://doi.org/10.1007/7515_2020_32

Published Online: 9 September 2020

**Results** More than 18 unique chemical identities have been discovered with confirmed inhibition of HSR pathway. Among them, two natural products and their derivatives are currently in various phases of clinical studies. Detailed works are required to define the exact mechanisms of actions (MOA) for these compounds.

**Conclusions** Many hurdles in clinical application still need to be effectively addressed, such as undesirable drug toxicity and off target effects; narrow therapeutic window; poor pharmacokinetic/pharmacodynamic (PK/PD) profiles, etc. Recent reports on synergistic drug combination, advanced prodrug design, smart nanoparticle packaging, and ribonucleic acid (RNA) aptamer selection offer promising solutions to overcome these challenges. Future advancements in this fast-growing area can potentially lead to the next-generation cancer therapeutics.

**Keywords** Fertility · Heat shock proteins · Oocyte · Reproduction · Sperm

## Abbreviations

| | |
|---|---|
| ART | assisted reproductive technology |
| CD | Cluster of differentiation |
| CDC | Cell division control |
| DCs | Dendritic cells |
| DNA | Deoxyribonucleic acid |
| HbA1c | glycated hemoglobin |
| HELLP | H (hemolysis), EL (elevated liver enzymes), LP (low platelet count). |
| HSF | Heat shock factor |
| HSFY | Heat shock transcription factor Y linked |
| HSPs | Heat shock proteins |
| HSR | Heat shock response |
| ICSI | intracytoplasmic sperm injection |
| IVF | in vitro fertilization |
| LOX | Lectin-like oxidized low-density lipoprotein receptor |
| MOA | Mechanisms of actions |
| NK | natural killer |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| PK/PD | Pharmacokinetic/pharmacodynamic |
| RNA | Ribonucleic acid |
| ROS | Reactive oxygen species |
| Th | T helper |
| TNF | Tumor necrosis factor |
| VDAC2 | Voltage-dependent anion-selective channel protein 2 |

# 1 Introduction

Heat shock proteins (HSPs) having molecular mass range from 15 to 110 kDa are known as one of the major molecular chaperone proteins in eukaryotic cells. In mammals, the HSPs are commonly divided according to their molecular weight into the HSP100 (HSPH), HSP90 (HSPC), HSP70 (HSPA), HSP60 (HSPD), HSP40 and HSP27 (HSPB) families. They are present in the cytosol and different cellular components including mitochondria, endoplasmic reticulum, and nucleus, although their expression levels exhibit cell-type-specific patterns. The well-investigated HSPs in mammals are those with molecular masses of 60, 70, 90, and 110 kDa [20]. However, HSPs have been known for first time as stress-related proteins, strong evidence now refers to its protective role even under non-pathological/stressful conditions. The expression level of HSPs in cells is changed when the cell exposure to different stresses such as hyperthermia, hypothermia, hypoxia, hyperoxia, oxidative stress, viral infection and energy depletion [20, 42]. Generally, HSPs have a protective function, in that they allow maintenance of cellular homeostasis under stressful/lethal cellular conditions, mainly by conferring maintenance of cellular protein homeostasis (prevention of proteins aggregation, contribution to protein folding and refolding, recognition of non-native protein conformation, transmeins, fixing damaged and/or defective proteins and targeting misfolded proteins) [35]. Heat shock response (HSR) pathway is a highly conserved cellular process. Heat shock factor 1 (HSF1) is a master transcriptional regulator responsible for expression of several important HSPs, which can effectively protect critical client proteins from misfolding and degradation, thus maintaining intracellular integrity under stressed conditions. Recent studies have demonstrated the direct connections between HSR players and tumor cell survival, validating HSR players as novel molecular targets in anticancer treatment. Small molecule screening has produced some promising HSR inhibitors for anticancer treatment. Therefore, this article aimed to summarize the main findings from HSR inhibitors on recent clinical and preclinical studies.

# 2 The Role of Heat Shock Proteins (HSPs) in Male Reproductive Functions

## *2.1 HSPs Overview and Expression in Male Reproductive System*

In males, HSPs are expressed throughout the testicular germ cells, Sertoli cells and along of the male reproductive tract, indicating their pivotal role in maintaining male reproductive functions. Several studies have shown the pivotal role of HSPs as functional and structural candidates required to fulfill the reproductive function in male (Table 1). Both large and small molecular weights HSPs have been isolated

**Table 1** Expression of HSP70 throughout the human reproductive organs/cells and their putative roles

| | Reproductive organ/cell | | | |
|---|---|---|---|---|
| Organ/cell | Testis | Epididymis | Vas deferens | Sperm |
| Site of expression | Spermatogenic cells<br>Sertoli cells<br>Leydig cell | Principal and ciliated<br>Cells<br>Macrophage localized cells | Basal cells | Cytoplasm<br>Nucleus<br>Mitochondria<br>Sperm surface |
| Putative function/role | Spermatogenesis<br>Steroidogenesis<br>Protection against thermal and oxidative stresses | Sperm maturation<br>Protection against sperm autoantigens | No information | Capacitation<br>Sperm-ova recognition<br>Sperm-zona pellucida interaction |
| Reference | Ji et al. [16] | Xun et al. [45]<br>Légaré et al. [21] | Légaré et al. [21] | Hiyama et al. [13]<br>Dun et al. [7]<br>Neuer et al. [30] |

from different male reproductive organs. HSPs have been identified in the surface of sperm cell membrane of bull, boar, mouse, rat, and human. Of HSPs, HSP70 family members seem to be one of the major molecules of the sperm cell membrane surface related to different sperm functions as *capacitation*, fertilization and motility [13]. In testis, testis specific HSP70 isomers (HSP70-2 and HSC70T) were described for first time in mice spermatogenic cells, later their homologues HSPA2 was discovered in human testis). Besides HSPA2, two others human HSPs known as HSPA1L and HSPH3 are also expressed in the human testis. In mice, HSP70-2 has been shown to be essential for the completion of male germ cells meiotic division. Also, small molecular weights HSPs are expressed in the testis. For example, HSP9 is expressed in spermatogonia, spermatocytes, and round spermatids. Other small molecular, cysteine-rich HSPS, known as HSPB10 or outer dense fiber protein 1 with a 27kD protein is expressed in the pseudostratified columnar epithelium of epididymis [45], and serves as structural component in the sperm tail [46]. Overall, expression of different HSPs throughout the male reproductive system indicates their critical roles in maintaining male reproductive functions under unstressed and stressed conditions.

## 2.2 *Heat Shock Proteins and Spermatogenesis*

Spermatogenesis is one of the essential functions of the testis. The completion of this process depends on occurrence of a series chain of *mitotic* and *meiotic* divisions of germ cells, spermatocytogenesis, which is followed by cell metamorphose

transformation of spermatid into mature sperm cell, *spermiogenesis*. The spermatogenesis process is a very complex biological process that needs precision control; thus, it is expected that HSPs and their regulators (heat shock factors, HSF) are crucial client proteins for the progression of this process. Disruption of HSPs has been linked to incompetence in spermatogenesis, sperm maturation and fertilization [7]. For example, *heat shock* transcription factor Y linked (HSFY) deletion causes a severe alteration in spermatogenesis. Also, HSPC1 or HSPA2 knock-out results in lack of post-meiotic germ cell, arrest T vacuolization and lack of post-meiotic, leading to complete infertility [3, 16]. In human, lack of HSPH3 expression has been related to defects in spermatogenesis, suggesting that this chaperone has a unique role during germ cell differentiation.

Many studies have shown that HSPA, belonging to HSP70, HSP60 and HSP90 families, are of the most important HSPs for spermatogenesis process in many mammalian species [7, 12]. In human testis, HSPA2 (HSP70) is expressed in meiotic spermatocytes, and being prominent during elongating spermatids and mature sperm cells [16]. During spermatogenesis process, HSPA2 is required for cell division control (CDC2) to form a heterodimer with cyclin B1 which is necessary for the progression of meiosis in the germ cells of male mice [8]. In spermatid cells, HSPA2 possess a new function as it can act as a chaperone of spermatid-specific deoxyribonucleic acid (DNA) packaging transition proteins (transition proteins 1, 2, and 4) [12]. Also, HSPA8, a HSP70 member, has been identified during late stages of spermiogenesis and is responsible for the phosphorylation of the histones H1, H2A, H2AX and H3. Phosphorylation of such proteins may be important for DNA packaging and chromatin compaction, as indicated by reduced sperm numbers, impaired DNA condensation, abnormal sperm morphology and motility in Tssk6 gene-disrupted male mice (reviewed by [7]). Similarly, HSP90 is required for completion of spermatogenesis, particularly the differentiation of spermatid into mature sperm cell, Jha et al. [15] confirmed the critical role of HSP90 in stabilization and activation of the testis-specific serine/threonine family of protein kinases that are related to male fertility and highly expressed in spermatid cells.

Expression of some HSPs seems to be more specific during spermatogenesis process, for example, in human, HSPD1 (HSP60) contribute to spermatocytogenesis but not to spermiogenesis, also it is important chaperone for sperm capacitation and sperm-zona pellucid interaction [7].

Small HSPs such as HSPB1 (HSP27) is also expressed in human testes, especially in cytoplasm of Sertoli cells and germ cells possessing mitotic activity (spermatogonia and spermatocytes) but not in sperm cells. HSPB1 has the ability to serve as a cytoplasmic protein assembly machinery that generates further proteins needed for divisions which explains its highly expression levels in germ cell during mitotic division. In human, testes with maturation arrest have reduced expression levels of HSPB1, indicating its association with development of male germ cell Also, expression of the HSPB1 in spermatids has been associated with histologic onset of maturation to the stage of sperm, since HSP27 can regulate microfilament organization in sperm tail [3, 16].

In addition to the role of HSPs, different HSFs also responsible for maintaining proper spermatogenesis by controlling expression levels of HSPs [43]. Under non-stress circumstances, HSFs (HSF1 and HSF2) are normally expressed in spermatocytes and spermatids which are characterized by extensive chromatin remodeling in order to regulate expression of different HSPs [16]. Under stress circumstances, activation of HSF1, HSFs, can adversely affect spermatogenesis by acting as a proapoptotic factor in spermatogenic cells [43]. Overall, HSPs family/isomer, HSPs expression level, time of HSPs expression and HSPs location are highly varied during spermatogenesis process and are specie-dependent (Table 2). Further studies are required in this field to increase our knowledge regarding the role of these proteins in spermatogenesis.

## *2.3 Heat Shock Proteins and Sperm Functions*

Sperm cell has unique structure and possesses different structural and functional changes in order to deliver the male genetic content to ova in right time, which require the action of chaperone proteins such as HSPs. Several studies have shown the role of HSPs in the progression and maintenance of sperm physical traits and sperm function during normal fertilization [13, 31] or after exposure of sperm cell to different environmental challenges (thermal stress or freezing) [6, 19, 47]. In both bovine [18] and boar [37] spermatozoa, the re-localization and redistribution of HSP70 from the acrosomal area to the equatorial segment and post-acrosomal regions during induced capacitation and acrosome reaction suggests a potential role of these molecules during fertilization. Among different mammalian species, HSPA8 facilitates the ability of sperm cells to bind to oviductal epithelial cells, and decreases sperm mitochondrial activity that are linked to enhanced *in vitro* fertilization (IVF) outcomes. These effects have been mainly related to the ability of HSPA8 to repair membrane damage of sperm cells by modulating cholesterol/phospholipid ratio in sperm cell membrane, conferring adequate stability and fluidity for sperm cell membrane [26]. Also, HSP90 has been found localized in the sperm tail of many mammalian species and seems to play a role in mediating sperm fertility [32].

Interestingly, sperm cells can express HSPs pre- or post-ejaculation, indicating their role in regulating all sperm functions in both male and female reproductive tract. For example, Hiyama et al. [13] reported that HSP70 can bind to the sperm surface by interacting with voltage-dependent anion-selective channel protein 2 (VDAC2), which leads to activation of sperm motility. This binding appears to play an important role in sperm migration within the oviduct as vaginal infusion of anti-HSP70 antibody can dramatically inhibit fertilization in vivo. Recently, HSPA8 has been suggested to play role in human sperm–egg recognition [31]. Both HSPD1 and HSPE1 chaperones appear in the sperm cell membrane surface during capacitation process and sperm-zona pellucid interaction [41].

Under stress conditions, low levels of HSP70 expression by sperm cells after freeze-thawing process has been related to low sperm cell membrane integrity and

**Table 2** Expression of different heat shock proteins (HSPs) and heat shock factors (HSFs) in different spermatogenic cells throughout the spermatogenesis process in human and mice

| HSPs | HSP family | Spermatogenesis[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Spermatocytogenesis | | | *Spermiogenesis* | | |
| | | Spermatogonia | Primary spermatocyte | Secondary spermatocyte | Round spermatid | Elongated spermatid | Sperm |
| **Human germ cells** | | | | | | | |
| HSPB1 | HSP27 | + | + | + | + | + | − |
| HSPD1 | HSP60 | + | + | − | − | − | − |
| HSPA5 | HSP70 | + | + | + | + | + | + |
| HSPA2 | HSP70 | − | + | + | + | + | + |
| HSPC1 | HSP90 | + | + | + | ni | ni | ni |
| **Mice germ cells** | | | | | | | |
| HSPE1 | HSP10 | + | − | − | + | + | − |
| HSPD1 | HSP60 | + | + | + | + | − | − |
| HSPA5 | HSP70 | − | + | + | + | − | − |
| HSPA2 | HSP70 | − | + | + | + | − | ni |
| HSPC4 | HSP90 | + | + | + | + | + | − |
| HSPH3 | HSP110 | − | + | + | + | + | ni |
| HSPH2 | HSP110 | + | + | + | + | + | ni |
| HSF1 | HSP factor | − | + | + | + | − | ni |
| HSF2 | HSP factor | − | + | + | + | − | − |

+ expressed, − not-expressed and *ni* no available information

[a]The information presented in the table is adopted from Ji et al. [16]

retarded sperm motility [47]. On the other hand, sperm cells resistance to cryopreservation might be strengthened by the increased expression levels of HSP70 [6, 19] or HSP90 [42]. HSP90 could protect sperm from oxidative damage and improve sperm quality after freezing–thawing through direct resistance to reactive oxygen species (ROS) or activation of nitric oxide synthase (NOS) to produce nitric oxide (NO) that can eliminate free radical molecules. In addition, HSP90 might contribute to changes during repair that occur in protamine–DNA complexes and in structural damage to chromatin as observed during boar sperm cryopreservation [11].

## *2.4 Male Sub-fertility*

Studies in the field of male infertility have shown a relationship between expression patterns of HSPs/HSFs throughout the reproductive system and emergence of physiological, pathological and microbial infertility factors. Taking varicocele case as an example, over expression of HSPA4, HSF1 and HSF2 were observed in the sperm of men with varicocele (dilatation of veins along the spermatic cord) and oligozoospermia, while high expression levels of HSP90 and HSFY were observed in oligozoospermia cases, independent of varicocele, and in cases with varicocele and normozoospermia, respectively [10], thus varicocele is correlated with different degrees of infertility. In chryptochid men, expression levels of HSP70 was higher in epididymis and vas deferens than normal men, supposing the epididymal protective role of HSP70 [21]. Thus, among different studies, HSPs/HSFs expressions in subfertile/infertile cases have been related either to their protective role or apoptotic-inducing role [9, 16]. These contradictory assumptions could be explained by the multi-biological function of HSPs; Apoptosis occurred during spermatogenesis was controlled by HSPs to ensure an enough quantity of germ cells, matching the helpful power of Sertoli cells and eliminating the diseased sperm cells. In some patients suffering from varicocele or other detrimental situations, the protective effects made by HSPs/HSFs thrive to counteract the adverse factors and consequently maintain fertility. On the other hand, some members of HSps family can activate the apoptosis pathway in order to control the quality of germ cells. Overexpression of HSPs/HSFs, such as HSPA and HSF1, excessively activated the apoptosis pathway in germ cells. Consequently, male germ cells underwent massive apoptosis and therefore impaired male fertility [40]. Taken together, HSPs seem to have a vital impact on sperm quality. HSPs/HSFs are essential for spermatogenesis, which can promote the normal germ cells survival and inhibit apoptosis under physiological or pathological conditions. On the other hand, HSPs/HSFs may activate apoptosis pathway to eliminate the abnormal germ cells [16].

Interestingly, HSPs can contribute immunologically to male infertility; Naaby-Hansen and Herr [29] showed an association between genital tract infection, immunity to HSP70 and reproductive failure. This researcher and his co-worker reported that some HSP chaperones on the surface of human sperm share epitopes with Chlamydia trachomatis HSP70, which allows molecular cross-reactivity between

HSP70-like antigens from Chlamydia trachomatis and human sperm, leading to antibody-mediated blockade of gamete interaction.

## 3 The Role of Heat Shock Proteins (HSPs) in Female Reproductive Functions

### *3.1 Oogenesis and Female Fertility*

Heat shock proteins support cells to remain alive with adverse environmental conditions by inhibition denaturation of protein. So, the physiological and pathological abilities for heat shock proteins are enormous. HSPS affect the all point of reproduction [30]. They are considered among the first proteins produced during development of mammalian embryo. Powerful oogenesis represents the main fundamental step preceding embryo development, important expression patterns and functions for HSPS have considered during these processes. Several types of HSPS; HSP90 is an abundant, constitutively expressed chaperone consisting around 1–2% of total cellular protein under non-stress conditions. Two main cytoplasmic isoforms of HSP90 present: HSP90α and HSP90β, which may arose by gene duplication roughly 500 million years ago [1]. The isoform specificity is limited not only to the biochemical level, but holding the functional role of HSP90 in cell differentiation and development. On the other hand, HSP90α has been confirmed to perform a regulatory role in muscle cell differentiation of zebrafish, while it is shown to prevent cellular differentiation of embryonic carcinoma cells to trophectoderm. HSP90β plays a major service in trophoblast differentiation, and HSP90β-deficient homozygous mice with normal expression of HSP90α be unsuccessful to form placental labyrinths [1]. Over and above, expression of HSP90β is discovered throughout the germ cell lineage from too early stages of development until adult oocytes. Studies have reported that HSP90β may be required for early embryonic development [2]. Experiments from the lab confirmed that HSP90β is expressed in the ovary and amply in the oocytes and the early embryo. Heat shock proteins represent the main products which are created following gene transcription initiation inside the fertilized zygote. As soon as blastocyst phase begin, induction of HSP70 made by the stress induction. The ovarian follicles developments are finished before birth. These follicles still fertile while the female accomplish maturation, and thus, the ordinary menstrual cycle elicit only one follicle. Autophagy shows to be involved both in protect the viability of oocytes during embryogenesis and in the interval between birth and puberty [30]. In all probability, HSP70 induction inside the ovary as a result of infection, inflammation, oxidative stress, exposure to an environmental toxin down-regulates autophagy and that way will increases susceptibility to premature ovarian failure. In a mouse example, it is shown that expression of anti-hsp60 and anti-hsp70 antibodies growth inhibiting effect at unique developmental stages of embryos. A continuous infection with unknown bacteria could induce the production

of antibodies to HSPs. Hyperthermia and other environmental stress considered a critical factor for female germ line. HSP expression appears to play an integral role during oogenesis in mammals; the preservation of HSP expression in evolutionary organisms shores the hypothesis of the fundamental role of HSP during development of germ cell. HSPs are found, for example, in ovarian nurse cells of Drosophila where they are subsequently moved to the oocyte, in mammalian oocytes HSP occurs for heat induction that is adjusted by the specific stage of oocyte development. In mouse oocytes, the heat shock reaction is utmosted during the growth phase of the oocyte and turn down with completed oocyte and follicle differentiation. Thus, the ability of mouse oocytes to persist a hard heat shock response is highest during early follicular growth and disappears prior to ovulation. Furthermore, growing oocytes spontaneously clear high levels of HSP70 and HSP90. HSP70 is present at huge levels in the preovulatory oocyte, later, its synthesis holds shortly after germinal vesicle breakdown, which is undetectable inside released ovulated oocyte at the time of fertilization. After meiosis, HSP70 synthesis masked completely. This is interesting to remind, because it is known that mammalian oocytes are very heat sensitive. Since fully developed oocytes are unable to express the heat inducible HSP70, this could clarify why mammalian oocytes offer an atypical and degenerate characteristic after exposure to hyperthermic stress. The anomalies covered multinuclear eggs, and first polar body oversize. In vitro, high temperature reduced the number of oocytes following second metaphase as well as it minimized the ratio of conception; therefore, HSPs play indispensable role throughout the ovulation stage. The ovulation is fundamental characters of an inflammatory response; HSPS have an essential function in the concept of ovulation and the conservation of the metabolic action following ovulation and existence of the ovum. Successful completion of the fertilization procedures and the beginning of the first mitotic division signal the starting of embryonic development. Characteristic aspects of expression of HSPS are exactly correlated to main procedures during the pre-implantation stage; HSP70 starts to be expressed alongside the initiation of embryonic genome effectiveness up to the blastocyst phase. Common feature of early stages of mammalian embryonic development is a shortage of stimulated HSP production which caused by the embryonic gene transcription deprivation. When the transcription recommences, most HSPS are induced by stress. At the 8-cell stage, the mouse embryo does not synthesize inducible HSP, although after heat shock, it synthesizes high level of cognate HSP70, when an eight-cell stage nucleus is conveyed into a one-cell embryo cytoplast. One-cell stage embryo does not create any HSP70 in the first hours following the manipulation. However, the reconstructed embryos produce both the cognate HSP70 and the inducible HSP70 at the exact time in relation to the development of the recipient cytoplast.

HSPs are immune-dominant antigens of many bacterial pathogens, such as Chlamydia trachomatis, which are considered as the chief reasons of tubal infertility. Mammals with fertility complications had a former infection in the genital tract, have developed sensitivity to microbial HSP, and a continuous with asymptomatic infection may excite the immune system to microbial HSP epitopes. Antibodies to either human or bacterial HSPs are found in great concentrations in sera and fluid of hydro-

salpinx of various mammals with IVF. Stress-inducible HSP70 has been recognized promptly earlier during the blastocyst stage. Interestingly, the stresses accompanying early embryonic development and differentiation stages leading to stimulation of HSP70 to enable optimal protein activity. Autophagy has been discovered earlier at 4 h after fertilization of oocyte by sperm in order to eliminate any spermatogenic mitochondria which could be present in the cytoplasm of the fertilized ovum and to prevent any ovum macro-molecules which may interfere with development [44]. Autophagy induction discovered in pregnancy was found to encourage the propagation and action of regulatory T lymphocytes which might decrease the extent of anti-fetal immune reaction [30]. While embryonic development progresses, activity of autophagy restrained throughout the morula and blastocyst phases and the boosted creation of HSP70 during this time proposes decreasing the level of autophagy. Autophagy and HSP70 induction have contrasting reactions with HSP70 being prevailing. HSP70 induction was revealed to inhibit the autophagy both in-vitro and in-vivo. HSPs were found to be considerably expressed by embryos, during gametogenesis, and in differentiation and stem cell models, and were found to be tissue and stage-dependent. That is why HSPs were needed for their chaperone activity in developmental pathways, which are thought to be more demanding for protein homeostasis. Throughout the female embryonic development, oogonia start meiotic division at embryonic day 13.5 and the ova stopped at prophase I till the accomplishment of their growth. HSF1 ova display numerous deviances from this process. Firstly, the germinal vesicle is disrupted followed by resumption of meiosis upon physiological hormonal stimulation during delay of the estrus cycle. HSF1 ova also undergo a partial stopover in Metaphase I. HSP90a is the main HSP expressed by fully grown ova and strikingly down-regulated by lack of HSF1 [1].

HSP70 and HSP60, play a great role throughout oogenesis. Development of pre-implantation stage embryo calls HSP support. Monoclonal antibodies formed against heat shock proteins inhibit *in vitro* development of bovine and mouse embryos. Presence of antibodies for heat shock proteins is directly linked with women infertility undergoing IVF. Affections of the cilia of fallopian tube by bacteria gains immune reactions to preserved sites of the microbial HSP60 which are also expressed on the homologous human HSP60. All of the above impedes with fertilization rate either natural or IVF. The HSP70 also exists in amniotic fluid for the modulation of immune responses [44]. Amniotic fluid supports the fetus defenses against microorganisms invading the amniotic cavity as well as inhibits stimulation of a forceful pro-inflammatory immune reaction that might trigger the premature parturition and ejection of the embryo. Latest studies have involved the inducible HSP70 as supporting phase in intra-amniotic immune responses. Extraembryonic membranes of the fetus were accelerated to show obvious HSP70 subsequent to insinuation to lipopolysaccharide, a constituent of the gram-negative bacterial cell wall [25]. The density of HSP70 in the amniotic fluid is directly interrelated with the level of the pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α) in the amniotic fluid proposing that HSP70 stimulates TNF-α or HSP70 is stimulated as a result of an intra-amniotic pro-inflammatory immune reaction [44].

### *3.2 Pregnancy and Parturition*

HSPs are pervasive, highly conserved molecules, and intracellular proteins with molecular chaperone plus cytoprotective importance. The principal complexes of the HSPS70 family are HSPS72 activated during cell stress, and HSPS73 which is fundamentally expressed in all cells. Both of them have identical amino acid chain and implicated in protein translocation from the cytosol to the mitochondria and endoplasmic reticulum; and in folding of protein throughout and next to synthesis. During non-stressful circumstances, expressed elements of each HSP family are present in most organelles such as the cytoplasm, nucleus, mitochondria and endoplasmic reticulum. Interaction of HSPS70 family with peptides and proteins displays a critical function in cell and organ existence. HSPS are activated in reply to cellular stresses involving chemicals, physical damage, nutritional deprivation, ischemia, viral infections, oxidative stress, heat shock and ultraviolet radiation. HSPS70 (HSPA1A) is found in the peripheral blood circulation of healthy pregnant and non-pregnant mammals. During normal pregnancy, deficiency of circulating HSPS70 level is obvious, and shows a direct relationship with age of pregnancy while an inverse relationship with age of the mother. Ability of extracellular HSPS70 to induce inherent and adaptive pro-inflammatory T helper type 1 (Th-type1) immune reactions may be hurtful in gestation and might produce refusal of the fetus by the mother. Lowered circulating HSPS70 level results in maintenance of immunological attitude to the fetus; conversely, increased circulating HSPS70 levels might increase the risk of gestation complications. Higher HSPS70 concentration in normal pregnancy may also influence the beginning of parturition. During pregnancy toxemia, HSPS70 levels in the blood are sharply elevated, and might predispose to oxidation stress, systematic inflammation and hepatocellular damage. Moreover, serum HSPS70 ratios are considerably increased in patients with hemolysis, low platelet count (HELLP syndrome) and higher liver enzymes than in patients with severe preeclampsia [2]. In HELLP syndrome “A combination of the breakdown of red blood cells (hemolysis; the H in the acronym), elevated liver enzymes (EL), and low platelet count (LP)”, higher serum HSPS70 level denotes tissue injury and severity of the disease. Greater circulating HSPS70 level may not only be an indicator of these illnesses, but may also participate in their pathogenesis [28]. Extracellular HSPS70 originated from exhausted, damaged, and necrotic cells evolve a pro-inflammatory (Th1) immune response that involved in development of systemic inflammatory reaction of the mother. Thus, damage of endothelial tissue occurred in HELLP and syndrome preeclampsia. Circulating HSPS70 standard supreme in pre-term delivery high-risk mammals, and represent useful signs for estimating the therapeutic effects of treatment for pre-term parturition. Furthermore, elevated serum HSPS70 levels detected in asthmatic gravid mammals considered a link line in the pathogenesis of asthmatic inflammation and gynecological/prenatal problems [2].

### 3.2.1 Heat Shock Proteins in Normal Pregnancy

HSPS70 levels are safely lesser in normal pregnancy than in lack of pregnancy. The fetus considered a semi-allograft, its main antigens of histocompatibility complex are obtained from the father. Numerous theories have been engaged for preservation of immunological lenience to the embryo. During normal gestation, the immune response of the mother is designated for humoral immunity, while cell-mediated immunity (cytotoxic) is restrained. Extracellular HSPS70 may be eliminated by innate immune mechanisms in response to tolerogenic changes of the immune system during physiological pregnancy. The ability of extracellular HSPS70 to extract innate and adaptive pro-inflammatory immune responses including the diversion of dendritic cells (DCs, antigen-presenting cells) from tolerogenic to immunogenic and the energizing of the cytolytic action of natural killer (NK) cells and γ/δ T cells might be hurtful in gestation period, and might manage the maternal immune repudiation of the fetus. Furthermore, exosomes exist in the sera as well as inside the amniotic fluid of pregnant mammals had been assumed to be a barrier against maternal immune system. It is Interesting that, exosomes within amniotic fluid have recently been explained to contain HSPS70 [28]. Extracellular HSPS70 may be detached by innate immune reactions during normal gestation as a result of tolerogenic alterations of the immune system. Lowered serum HSPS70 level enhances maintaining the immunological tolerance to the fetus [28].

### 3.2.2 Heat Shock Proteins in Preeclampsia and Abortion

Preeclampsia, is recognized by proteinuria and hypertension activating after middle of gestation, and considered serious complexity of pregnancy with a global occurrence. Although, powerful research attempts, the causes and pathogenesis of preeclampsia are not completely comprehended. Systemic inflammatory response of mother to the gestation with oxidative stress and endothelial impairment play a critical role in the pathogenicity of this illness. Intracellular expression of HSPS70 can be created by induction of inflammatory cytokines, reactive oxygen species, ischemia and hemodynamic stress (acute hypertension). The pathogenesis of preeclampsia is systemic oxidative stress, excessive maternal systemic inflammatory response, placental ischemia and hemodynamic stress [2, 38]. In-vitro ischemia – reperfusion injury, an example for oxidative stress in preeclampsia, HSPS70 level was elevated in tissues of the placenta. Furthermore, apoptosis triggered by hypoxia was associated with elevated expression of HSPS70 in trophoblast cells. Undoubtedly, a notable up-regulation of HSPS70 was obvious in mRNA and in protein level of tissues of placenta of mammals affected by vascular illness. Acute hypertension encouraged HSPS70 gene expression in rat aorta. Moreover, in hypertensive humans, heat stress promoted HSPS70 expression in a greater level in the lymphocytes of peripheral blood in comparison to normal humans. However, HSPS70 are discharged not only from intact cells by active mechanisms, but also it may be

released from necrotic or damaged cells in a passive method. Apoptosis has been engaged in both preeclampsia and labor. In the apoptotic pathway, HSPs inhibit cell death commenced with stress-triggered injury. For instance, HSP70 prevent creation of caspase 3 and 9. Therefore, it is likely that HSP70 controls the degree of apoptosis. Released HSPs, containing HSP70, can participate in immune surveillance. They can hold antigens and react with receptors on antigen displaying cells. HSP70 bind and stimulate the mammals' monocytes, preventing the excretion of inflammatory cytokines, for example TNF-a, IL-1b, IL-6 and IL-10. HSP70 is cleared in the placenta with a spatial manner. Changes in HSP70 expression happened during parturition and preeclampsia but with variable regions in the placenta. Extracellular HSP70 originated from necrotic, damaged and stressed cells can potentiate the innate and adaptive pro-inflammatory (Th1) immunological reactions. The systemic inflammatory reaction of the mother, which is responsible for the symptoms of preeclampsia, includes activation and an increased number of leukocytes (granulocytes and monocytes) with creation of pro-inflammatory cytokines resulting in activation of the immune system as well as the secretion of acute phase proteins. Sera HSPS70 could be engaged in developing the systemic inflammatory reaction of the pregnant mother in preeclampsia. Higher serum HSPS70 levels have been detected accompanying the inflammatory responses in many pathological illnesses, such as post-liver resection and coronary artery bypass grafting, acute infections, and next myocardial infarction [2].

### 3.2.3 Heat Shock Proteins in Parturition and Dystocia

Preterm delivery, is defined as delivery before completing the full period of gestation. It is a principal problem of obstetrics among all mammals causing perinatal morbidity and mortality. Many factors engaged in the causes and pathogenesis of obstetrical complication; all induce HSPS70 expression. Infection of the amniotic fluid, term parturition and histologic chorioamnionitis have recently been revealed to be linked with increased levels of HSPS70 in the amniotic fluid. Existence of serum anti-Hsp60 and anti-HSP70 antibodies and production of HSP60 and HSP70 immune complexes within the placental tissues were related with preterm birth as well as exploring the expression of HSP60, HSP70, and HSP90 in tissues of decidua and placenta. Both intracellular expression and extracellular standards of HSPS70, in addition to immunological reactivity towards this protein, have been accelerated in preterm delivery and predisposing conditions. Vaginal HSPS70 was linked with bacterial vaginosis as a result of abnormal microflora of the vagina which may elicit secretion of nitric oxide for minimizing the pathological consequences of this alteration. Furthermore, amniochorionic membranes fundamentally express HSPS70 mRNA in human, and the bacterial lipopolysaccharide strikingly promoted HSPS70 gene transcription in the extraembryonic fetal membranes. HSPS70 was produced from cells in amniotic fluid at mid-trimester as a result of stimulation of Toll-like receptor 2, and exogenous HSPS70 encouraged TNF-α release by cells of amniotic fluid. Circulating HSPS70 in other pregnancy complications are believed to

play a critical role in the commencement and modulation of the asthmatic immune reaction and the conservation of chronic bronchial inflammation in asthma. Furthermore, serum HSPS70 concentrations are elevated and assemble with glycated hemoglobin (HbA1c) levels in mammals with gestational diabetes mellitus, oxidative stress and inflammation. Distinctive characters of diabetes mellitus have been proposed to cause an increase in serum HSPS70 levels in these disorders. Hyperglycemia is an additional cause to be accountable for elevation in circulating HSPS70 levels in pregnant mammals with pre-gestational diabetes. Surely, serum HSPS70 levels matched with the alterations in serum glucose levels in diabetic ketoacidosis, pointing out that hyperglycemia may reinforce the production of HSPS70 from hepato-splanchnic organs. Extracellular HSPS70 originated from necrotic, damaged and stressed cells might act as an intercellular stress signaling molecule, denoting an inherited risk signal of a non-physiological circumstances, as damage or cellular stress, to provoke adaptive and innate pro-inflammatory immune reactions. Extracellular HSPS70 work by uniting with surface receptors (CD14, CD36, CD40, CD91, LOX-1, and Toll-like receptors 2 and 4) on antigen-exhibiting cells, inducing their pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and chemokine secretion, in addition to costimulatory molecule expression.

## 4 Role of Heat Shock Proteins During Assisted Reproductive Technology (ART)

Heat shock proteins could be classified into diverse categories according to their molecular weight measured in kDa rather than by their function. There are 27, 60, 70 and 90 kDa HSPs. In reproductive performance, the HSP60 and HSP70 families are the extremely essential ones, possessing two main roles as a molecular chaperone and reacting to cell stress for instance temperature alterations, bacterial and viral infection, heavy metals, as well as free oxygen radical [30].

Heat shock proteins are autonomously upregulated not only in response to heat shock but also possess fundamental roles during juvenile stages or embryonic development of mammals, teleost fish and some lower vertebrates. HSP27 appears to be released during stress and during the development of embryo, mid and hindbrain, somites, lens and heart in zebrafish [23]. Small HSPs are proteins of low-molecular weight present in all species studied to date. Almost all small HSPs are considered molecular chaperones. Besides the chaperone activity of several proteins, they also have other specific roles during development as they are expressed in specific spatiotemporal manners during juvenile and/or embryonic stages.

### 4.1 *Role of Heat Shock Proteins During* In-Vitro *Embryo Production*

Assisted reproductive technology comprises medical techniques used principally to address infertility problems. It includes measures such as intracytoplasmic sperm injection (ICSI), *in-vitro* fertilization (IVF), and cryopreservation of gametes or embryos.

During *in-vitro* fertilization, the ova are fertilized and cultured *in-vitro* for several days before the freshly formed embryos being transmitted into the uterus. These treatments increase the oxidation stress for the gametes and induce production of heat shock proteins. More importantly, HSPs have fundamental roles during embryogenesis. Besides the chaperone activity of HSPs involved in assisting protein folding and assembly as well as acting as stabilizer of damaged proteins, recently the capability to prevent apoptosis has become a novel function as this might participate in their defensive effects on cells [33]. Though the machinery is not clear, recent evidence suggests an essential role of HSPs in in-vitro fertilization and early embryonic development [22, 30].

It has been reported that HSP70 is found on the acrosomal cap of bull spermatozoa and re-localized to the equator during the sperm capacitation and acrosomal reaction [17]. This suggests that HSP70 not only acts as stress protector but also has a specific importance with other extracellular proteins during sperm-oocyte interaction in IVF.

Deprivation of culture medium from HSPs during IVF culture systems deteriorates the embryonic growth and morphology. Surprisingly, in the existence of HSPs antibodies, the zygotes became shrunk, degenerated and their growth was arrested. Morphological examination of these IVF embryos showed uneven sized and degenerated blastomeres as well as several cellular fragments within the zona pellucida similar to apoptotic cells [24, 30]. Supplementation of the culture medium with monoclonal antibodies to hsp60 or hsp70 inhibits the mouse embryonic development in-vitro and the fertilization presumably by reducing sperm-joining with the zona pellucida or sperm membrane fusion with oolemma possibly by binding with receptors on the zona pellucida, embryonic cell membrane or cell membrane of oviduct. Moreover, HSP70 in bull spermatozoa might perform an important role in post-fertilization processes as a stress protector during early cleavages stages. Consequently, there is a great tendency that the stresses concomitant with early embryonic development and differentiation induce the production of hsp70 to enable ideal protein activity. Further studies have demonstrated that HSP70 expression during embryonic development correlates with resistance to apoptosis as a result of stress in blastoderm of poultry [4]. Mouse embryos cultured in-vitro had greater levels of hsp70 than in-vivo ones [39]. Taken together, all of these studies suggest that HSPs play essential roles in differentiation, fertilization and protective roles against apoptosis during early embryonic development and IVF.

Some functions of HSP70 include folding, unfolding, transportation of proteins, differentiation and controlling the embryonic cell cycle [5]; thus, HSP70 plays a

protective role during embryogenesis. The HSP70 is the most prevalent gene during the early two-cells to the blastocyst stage of embryo. The HSPs production is extremely induced during one- or two-cells stage until blastocyst stage by heat stress [22]. Therefore, HSPA14 gene, the major inducible heat shock gene, is secreted by embryonic cells for protecting the embryos from cellular stress. High temperatures, oxygen stress, and free radicals deteriorates embryonic viability and morphogenesis during early embryonic development. The HSPA14 evoked thermotolerance against variable stressors and helps to conserve the cell functions by performing the role of molecular chaperones for stabilizing and/or refolding proteins damaged by heat stress, and by hindering apoptotic pathway [34].

HSPA2 is a testis-enriched 70 kDa HSP that stimulates the folding, transportation, and assemblage of protein complexes and had been directly associated with successful IVF. Moreover, decreased expression of HSPA2 in the human sperm proteome causes a diminished capability for cumulus substance dispersion, sperm-egg perception and fertilization subsequent to both IVF and ICSI [31].

In conclusion, HSPs play an essential or indispensable role in mammalian fertilization, sperm-oocyte interaction and embryonic development. Besides, they also have protective roles against cellular apoptosis since their inhibition caused cellular fragmentation, shrinkage and decline in development of blastocyst that might be intermediated by intensified cellular death.

## *4.2 Role of Heat Shock Proteins During Cryopreservation of Semen, Oocytes and Embryos*

Cryopreservation of semen is widely used as it enables hereditary improvement of valuable species and restraining the prevalence of sexually transmitted diseases. However, thawing process caused variable kind of stress for cryopreserved spermatozoa by deteriorating their movement, plasma membrane integrity and survivability. Therefore, heat shock proteins are produced for repairing those impaired proteins and hinder the aggregates from misfolded proteins elicited by many stresses.

Cryopreservation of gametes or embryos alters the functional status of many proteins associated with cellular metabolism, membrane, structure and apoptosis. Moreover, the cryoprotectants influence the sperm membrane permeability which reduces the osmotic volume of the sperm cell cytoplasm. These changes, altogether affect the biological processes, structural integrity, and function of sperms or oocytes, eventually decreasing their fertilizing capability. The survivability of sperms following freezing-thawing procedure relies on their plasma or cell membrane as it is the most fundamental and the principal locus of cryodamage [36].

HSP70 might preserve the protein integrity, stabilize the unfolded proteins before the assemblage into macromolecular complexes, and contribute in transmission of proteins through the intracellular membranes. It has an essential role as a marker for thermotolerance and as a molecular chaperone that participates in repairing and

aiding in protein synthesis. The expression level of HSP70 gene might be strikingly related to the quality of the sperms, especially on their capacitation efficiency. Its expression level gradually decreases in spermatozoa subsequent to the freezing-thawing process, and could be correlated with sperm movement and acrosomal sturdiness.

A recent study stated that expression of HSP70 gene in bull spermatozoa was considerable at 37 °C for 30 s and at 60 °C for 6 s with $p < 0.05$ with less capacitated sperms. Furthermore, as the temperature exceeds 37 °C, protein denaturation will begin to occur and ultimately leads to the disintegration of this protein [27]. Expression levels of HSPs were elevated in sperms with superior cryotolerance and were considerably reduced in sperms with lesser cryotolerance. It was assumed that lower concentrations of HSPA8, an extremely conserved member of HSP70 family, in cryopreservation medium result in reduced post-thawing sperm survivability, while greater concentrations enhance the integrity of plasma membrane [14].

Inhibition of apoptosis by HSPs helps to maintain the cell survival during cryopreservation. HSPs can control the intrinsic apoptotic (mitochondrial-dependent) pathway and the extrinsic apoptotic (death receptor-mediated) pathway. Therefore, it is proposed that thawing of cryopreserved semen at 37 °C for 30 s, can help the HSPs to properly act as a molecular chaperon and inhibit cellular apoptosis.

The efficacy of oocyte cryopreservation in some species remains low due to high sensitivity to low temperature and low membrane permeability. Cryopreservation might cause DNA damage in the oocyte and embryos and this could be one of the reasons for reduced developmental capacity of cryopreserved oocytes or embryos.

In conclusion, HSPs are released in response to cryopreservation-triggered stress for thermotolerance and to maintain cellular integrity and repair damaged proteins. Moreover, they have a pivotal role in inhibiting apoptosis during exposure to cellular stress.

## 5 Conclusions

Many hurdles in clinical application still need to be effectively addressed, such as undesirable drug toxicity and off target effects; narrow therapeutic window; poor PK/PD profiles, etc. Recent reports on synergistic drug combination, advanced prodrug design, smart nanoparticle packaging, and RNA aptamer selection offer promising solutions to overcome these challenges. Future advancements in this fast-growing area can potentially lead to the next-generation cancer therapeutics.

**Acknowledgments** All authors thank their universities for their help in accomplishing this work.

**Disclosure of Interests** All authors declare they have no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Funding** This study did not receive any fund.

## References

1. Abane R, Mezger V (2010) Roles of heat shock factors in gametogenesis and development. FEBS J 277(20):4150–4172. https://doi.org/10.1111/j.1742-4658.2010.07830.x
2. Abdulsid A, Hanretty K, Lyall F (2013) Heat shock protein 70 expression is spatially distributed in human placenta and selectively upregulated during labor and preeclampsia. PLoS One 8: e54540. https://doi.org/10.1371/journal.pone.0054540.g001
3. Adly MA, Assaf HA, Hussein MRA (2008) Heat shock protein 27 expression in the human testis showing normal and abnormal spermatogenesis. Cell Biol Int 32(10):1247–1255
4. Bloom SE, Muscarella DE, Lee MY, Rachlinski M (1998) Cell death in the avian blastoderm: resistance to stress induced apoptosis and expression of anti-apoptotic genes. Cell Death Differ 5:529–538
5. Cushman A (2013) Physiology and Endocrinology Symposium: the current status of heat shock in early embryonic survival and reproductive efficiency. J Anim Sci 91(3):1141–1142
6. Daugaard M, Rohde M, Jäättelä M (2007) The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. FEBS Lett 581(19):3702–3710
7. Dun MD, Aitken RJ, Nixon B (2012) The role of molecular chaperones in spermatogenesis and the post-testicular maturation of mammalian spermatozoa. Hum Reprod Update 18(4):420–435
8. Eddy EM (1999) Role of heat shock protein HSP70-2 in spermatogenesis. Rev Reprod 4 (1):23–30. https://doi.org/10.1371/journal.pone.0054540.t001
9. Erata GÖ, Toker NK, Durlanık Ö, Kadıoğlu A, Aktan G, Toker GA (2008) The role of heat shock protein 70 (Hsp 70) in male infertility: is it a line of defense against sperm DNA fragmentation? Fertil Steril 90(2):322–327
10. Ferlin A, Speltra E, Patassini C, Pati MA, Garolla A, Caretta N, Foresta C (2010) Heat shock protein and heat shock factor expression in sperm: relation to oligozoospermia and varicocele. J Urol 183(3):1248–1252
11. Flores E, Cifuentes D, Fernández-Novell JM, Medrano A, Bonet S, Briz MD et al (2008) Freeze-thawing induces alterations in the protamine-1/DNA overall structure in boar sperm. Theriogenology 69(9):1083–1094
12. Govin J, Caron C, Escoffier E, Ferro M, Kuhn L, Rousseaux S et al (2006) Post-meiotic shifts in HSPA2/HSP70. 2 chaperone activity during mouse spermatogenesis. J Biol Chem 281 (49):37888–37892
13. Hiyama G, Matsuzaki M, Mizushima S, Dohra H, Ikegami K, Yoshimura T et al (2014) Sperm activation by heat shock protein 70 supports the migration of sperm released from sperm storage tubules in Japanese quail (Coturnix japonica). Reproduction 147(2):167–167
14. Holt WV, Del Valle I, Fazeli A (2015) Heat shock protein A8 stabilizes the bull sperm plasma membrane during cryopreservation: effects of breed, protein concentration, and mode of use. Theriogenology 84:693–701
15. Jha KN, Coleman AR, Wong L, Salicioni AM, Howcroft E, Johnson GR (2013) Heat shock protein 90 functions to stabilize and activate the testis-specific serine/threonine kinases, a family of kinases essential for male fertility. J Biol Chem 288(23):16308–16320
16. Ji ZL, Duan YG, Mou LS, Allam JP, Haidl G, Cai ZM (2012) Association of heat shock proteins, heat shock factors and male infertility. Asian Pac J Reprod 1(1):76–84

17. Kamaruddin MB (1998) Heat shock protein 70 in bovine spermatogenesis and fertilization. Ph. D. thesis, University of Guelph, Ontario, Canada
18. Kamaruddin M, Kroetsch T, Basrur PK, Hansen PJ, King WA (2004) Immunolocalization of heat shock protein 70 in bovine spermatozoa. Andrologia 36(5):327–334
19. Mestril R, Dillmann WH (1995) Heat shock proteins and protection against myocardial ischemia. J Mol Cell Cardiol 27(1):45–52
20. Kregel KC (2002) Invited review: heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 92(5):2177–2186
21. Légaré C, Thabet M, Sullivan R (2004) Expression of heat shock protein 70 in normal and cryptorchid human excurrent duct. Mol Hum Reprod 10(3):197–202
22. Li H, Pang Q, Qin L, Jin S, Zeng X, Bai Y, Li W (2015) HSP70 overexpression may play a protective role in the mouse embryos stimulated by CUMS. Reprod Biol Endocrinol 16(13):125
23. Marvin M, O'Rourke D, Kurihara T, Juliano CE, Harrison KL, Hutson LD (2008) Developmental expression patterns of the zebrafish small heat shock proteins. Dev Dyn 237:454–463
24. Matwee C, Kamaruddin M, Betts DH, Basrur PK, King WA (2001) The effects of antibodies to heat shock protein 70 in fertilization and embryo development. Mol Hum Reprod 7(9):829–837
25. Menon R, Gerber S, Fortunato Stephen J, Witkin Steven S (2001) Lipopolysaccharide stimulation of 70 kilo Dalton heat shock protein messenger ribonucleic acid production in cultured human fetal membranes. J Perinat Med 29:133
26. Moein-Vaziri N, Phillips I, Smith S, Alminana C, Maside C, Gil MA et al (2014) Heat-shock protein A8 restores sperm membrane integrity by increasing plasma membrane fluidity. Reproduction 147(5):719–732
27. Mohamad SFS, Ismail NH, Zuraina F, Yusof M, Ibrahim SF, Jaafar FHF, Nang CF, Osman K (2018) Quantification of HSP70 gene expression and determination of capacitation status of magnetically separated cryopreserved bovine spermatozoa at different thawing temperature and time. Sains Malaysiana 47(6):1101–1108
28. Molvarec A et al (2010) Circulating heat shock protein 70 (HSPA1A) in normal and pathological pregnancies. Cell Stress Chaperones 15(3):237–247
29. Naaby-Hansen S, Herr JC (2010) Heat shock proteins on the human sperm surface. J Reprod Immunol 84(1):32–40
30. Neuer A, Spandorfer SD, Giraldo P, Dieterle S, Rosenwaks Z, Witkin SS (2000) The role of heat shock proteins in reproduction. Hum Reprod Update 6(2):149–159. https://doi.org/10.1093/humupd/6.2.149
31. Nixon B, Bromfield EG, Dun MD, Redgrove KA, McLaughlin EA, Aitken RJ (2015) The role of the molecular chaperone heat shock protein A2 (HSPA2) in regulating human sperm-egg recognition. Asian J Androl 17:568–573
32. Pratt WB, Galigniana MD, Harrell JM, DeFranco DB (2004) Role of hsp90 and the hsp90-binding immunophilins in signalling protein movement. Cell Signal 16(8):857–872
33. Samali A, Orrenius S (1998) Heat shock proteins: regulators of stress response and apoptosis. Cell Stress Chaperones 3:228–236
34. Sharma T, Nath A, Prasad S, Singhal S, Singh N, Gade E, Dubey K, Saikumar G (2012) Expression and characterization of constitutive heat shock protein 70.1 (HSPA-1A) gene in in vitro produced and in vivo-derived buffalo (Bubalus bubalis) embryos. Reprod Domest Anim 47(6):975–983
35. Shiber A, Ravid T (2014) Chaperoning proteins for destruction: diverse roles of Hsp70 chaperones and their co-chaperones in targeting misfolded proteins to the proteasome. Biomol Ther 4:704–724
36. Söderquist L, Madrid-Bury N, Rodriguez-Martinez H (1997) Assessment of ram sperm membrane integrity following different thawing procedures. Theriogenology 48(7):1115–1125
37. Spinaci M, Volpe S, Bernardini C, De Ambrogi M, Tamanini C, Seren E, Galeati G (2005) Immunolocalization of heat shock protein 70 (Hsp 70) in boar spermatozoa and its role during fertilization. Mol Reprod Dev Incorpor Gamete Res 72(4):534–541

38. Tan H et al (2007) Association of increased heat shock protein 70 levels in the lymphocyte with high risk of adverse pregnancy outcomes in early pregnancy: a nested case-control study. Cell Stress Chaperones 12:230–236
39. Tan K, Wang Z, Zhang Z, An L, Tian J (2016) IVF affects embryonic development in a sex-biased manner in mice. Reproduction 151:443–453
40. Vydra N, Malusecka E, Jarzab M, Lisowska K, Glowala-Kosinska M, Benedyk K et al (2006) Spermatocyte-specific expression of constitutively active heat shock factor 1 induces HSP70i-resistant apoptosis in male germ cells. Cell Death Differ 13(2):212–222
41. Walsh A, Whelan D, Bielanowicz A, Skinner B, Aitken RJ, O'Bryan MK, Nixon B (2008) Identification of the molecular chaperone, heat shock protein 1 (chaperonin 10), in the reproductive tract and in capacitating spermatozoa in the male mouse. Biol Reprod 78(6):983–993
42. Wang P, Wang YF, Wang H, Wang CW, Zan LS, Hu JH et al (2014) HSP90 expression correlation with the freezing resistance of bull sperm. Zygote 22(2):239–245
43. Widlak W, Vydra N (2017) The role of heat shock factors in mammalian spermatogenesis. In: The role of heat shock proteins in reproductive system development and function. Springer, Cham, pp 45–65
44. Witkin SS, Linhares IM (2010) Heat shock proteins and fertility. In: Asea AAA, Pedersen BK (eds) Heat shock proteins and whole body physiology. Springer Netherlands, Dordrecht, pp 151–162
45. Xun W, Shi L, Cao T, Zhao C, Yu P, Wang D et al (2015) Dual functions in response to heat stress and spermatogenesis: characterization of expression profile of small heat shock proteins 9 and 10 in goat testis. BioMed Res Int 2015:686239
46. Yang K, Meinhardt A, Zhang B, Grzmil P, Adham IM, Hoyer-Fender S (2012) The small heat shock protein ODF1/HSPB10 is essential for tight linkage of sperm head to tail and male fertility in mice. Mol Cell Biol 32(1):216–225
47. Zhang XG, Hong JY, Yan GJ, Wang YF, Li QW, Hu JH (2015) Association of heat shock protein 70 with motility of frozen-thawed sperm in bulls. Czeh J Anim Sci 60(6):256–262

# Heat Shock Proteins 70 in Cellular Stress: Fight or Flight


**Mohamed M. A. Hussein, Islam M. Saadeldin, and Mona M. Ahmed**

## Abstract

**Introduction** Heat shock proteins (Hsp) are a family of proteins and the major chaperons of the cells. Hsp70 assists in folding and correct misfolded protein, improve the cellular transportation of proteins, reducing toxic proteins aggregation and act as a buffering system against cellular stressors either extrinsic or intrinsic stimuli.

**Methods** A literature-based collection of articles in the available search engines (PubMed and Google Scholar).

**Results** We show the critical roles of Hsp70 in balancing cell survival through corrections of misfolded proteins that are associated with apoptosis and toxicological effects on the cells.

**Conclusion** Hsp70 is a cornerstone not only for chaperones but also for several essential functions of the cells and further investigations are required for developing therapeutic agents targeting this critical molecule.

**Keywords** Apoptosis · Cells · Chaperons · Hsp · Immunity · Toxicology

M. M. A. Hussein (✉)
Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

I. M. Saadeldin
Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

Department of Animal Production, College of Food and Agriculture Science, King Saud University, Riyadh, Saudi Arabia

M. M. Ahmed
Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

Heat Shock Proteins (2021) 22: 429–446
https://doi.org/10.1007/7515_2020_37

Published Online: 6 December 2020

## Abbreviations

| | |
|---|---|
| AIF | Apoptosis-inducing factor |
| Apaf1 | Apoptosis protease activating factor-1 |
| APC | Antigen-presenting cell |
| Ask-1 | Apoptosis signal regulatory kinase -1 |
| BAD | Bcl-2 associated death promoter protein |
| CARD | Caspase recruitment domain |
| CD | Cluster of differentiation |
| CTL | Cytotoxic T-cell |
| Cytc | Cytochrome c |
| DC | Dendritic cell |
| DISC | Death inducing signaling complex |
| ER | Endoplasmic reticulum |
| ERK | Extracellular signal-regulated kinase |
| FADP | Fas-associated death domain protein |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| GPI | Glycosyl phosphatidylinositol |
| GRP | Glucose regulatory protein |
| HSBP | Heat shock binding protein |
| HSF | Heat shock factor |
| HSP | Heat shock protein |
| IKK-β | Inhibitor of nuclear factor kappa-B kinase subunit beta |
| IRS-1 | Insulin receptor substrate -1 |
| JAK | Janus kinase |
| JNK | c-Jun N-terminal kinase |
| LBs | Lewy bodies |
| LIF | Leukemia inhibitory factor |
| MAPK | Mitogen-activated protein kinase |
| MCP | Monocyte chemoattractant protein |
| MDscs | Myeloid-derived suppressor cells |
| MHC | Major histocompatibility cell |
| MIP | Macrophage inflammatory protein |
| MKK4 | Mitogen-activated protein kinase -4 |
| MOMP | Mitochondrial outer membrane permeabilization |
| MyD88 | Myeloid differentiation primary response |
| NEF | Nucleotide exchange factor |
| NF-IL6 | Nuclear factor interleukin-6 |
| NF-kB | Nuclear factor kappa light chain enhancer of activated B cells |
| PD | Parkinson's disease |
| PKC | Protein kinase -c |
| STAT | Signal transducer and activator transcription |
| TLR | Toll-like receptor |
| TNF | Tumor necrotic factor |
| α Syn | Alfa synuclein |

## 1 Introduction

In eukaryotic cells, the heat shock proteins (Hsps) represent one of the very important molecular chaperones. The discovery of Hsps in response to different stressful conditions including cancer cell and cystic fibrosis made them a target point of research in medical an biological studies due to their different functions in both physiological and pathological status.Hsps are expressed in unstressed cells with a level of 1–2% of total proteins indicate their role in the homeostasis of cells [93]. The chaperone function of Hsps concerns the folding and corrects the misfolded proteins [7]. The nomenclature was owed to their higher expression following hyperthermia [34]. Interestingly**,** Hsps are induced in response to other stressful stimuli such as heavy metals, hypoxia, starvation, and oxidative stress. The level of Hsps may rise to >15% in response to different stimuli [75]. The backbone of their classification depends on their molecular weight which are high-molecular-mass Hsps ($\geq$100kD), Hsp90), Hsp70, Hsp 60, Hsp 40, and small Hsps ($\leq$34kD) [27, 67]. Hsp70 expression is present in a constitutive and inducible form which activated by stressful conditions [33]. Hsp70and Hsp90 genes are among the earlier embryonic genes to be induced and confirm the dual effect of Hsp in both normal and stressful cells [87].

## 2 Location and Functions of Hsps

Hsps are located in either extracellular or intracellular where the main location in prokaryotic is cytosol meanwhile, in eukaryotic cells their presence in the cytosol, nuclei, mitochondria, and chloroplasts [44]. Moreover, Hsps initiate immunological responses through their binding of antigenic peptides to the extracellular plasma membrane of malignant cells or microbial infected cells [24, 54]. Hsps trigger both adaptive and innate immune responses which make them a good target for cancer therapy [16, 71].

### *2.1 The Chaperone Function of Hsps*

The chaperone functions of Hsps involved protein folding and correct misfolded proteins [25], the decay of proteins and polypeptides [63], the transmission of proteins to different cell compartments [14] and antigen-presenting processes [72].

Accumulation of unfolded proteins in stressful cells elicits Hsps expression through participating in heat shock factor (HSF)-1. The cytoplasmic HSF-1 is kept inactive form and binds with Hsp90 (Fig. 1). The aggregation of misfolded protein triggers the dissociation of HSF-1 fromHSF1-Hsp90 complex with adenosine triphosphate hydrolysis (ATP) forming trimers, which are phosphorylated to nucleus and bind to heat shock elements (HSE) inducing Hsp70, Hsp40 and small Hsp

release [49, 65]. However, different stimuli may allow Hsp27 phosphorylation (P) and reorganize into low-molecular-weight dimers or oligomers that bind misfolded proteins. Upon return to favorable conditions, the misfolded or unfolded proteins are refolded either spontaneously or with the assistance of Hsp70 and Hsp60 [45].

When the chaperones cannot repair the misfolded protein, they target the abnormal protein for degradation by the ubiquitin (Ubq)-proteasome pathway or by lysosome-mediated autophagy. These quality control mechanisms of proteostasis prevent the accumulation of misfolded proteins that would lead to aggregation and accumulation in the form of inclusions composed of amyloid fibrils [9].

### *2.2 Role of Hsps in Immunity*

The dual immune responses of Hsps relay on the Hsp-antigen-presenting cell (APC) interaction. The binding of the Hsp-peptide complex and CD91 receptor elicits specific $CD8^+$ and $CD4^+$ T lymphocyte replay [93]. The direct interaction of Hsps with CD40, CD36 induces cytokines, chemokines expression with the maturation of dendritic cell (DC).

## 3 Factors Participate in Hsps Induction

### *3.1 HSF Family*

There are 4 types of HSFs (HSF1–4) have been characterized as activators for Hsps induction in response to various stimuli. The release of Hsps in response to hyperthermia relays on HSF1 and HSF3 activation. Meanwhile, HSF2 and HSF4 are included in Hsp induction in normal cells and various biological processes [2]. The main connection between HSF-1and p53 activity relays on the ability of HSF1 to interact with a stress-responsive activator of p300 (Strap) which controls DNA damage through its regulation on p53 activity. Moreover, strap increases histone acetylation in the Hsps gene through its histone acetylation activity [91].

### *3.2 STAT1*

The expression pattern of Hsp70 and Hsp90 in mice lacking HSF1 is analogous to wild-type cells showed a defect in HSE after heat stress exposure [88]. These results suspect that other factors may replace HSF1abscence and allow Hsp70 and Hsp90 release under normal and abnormal growth conditions. These factors include signal

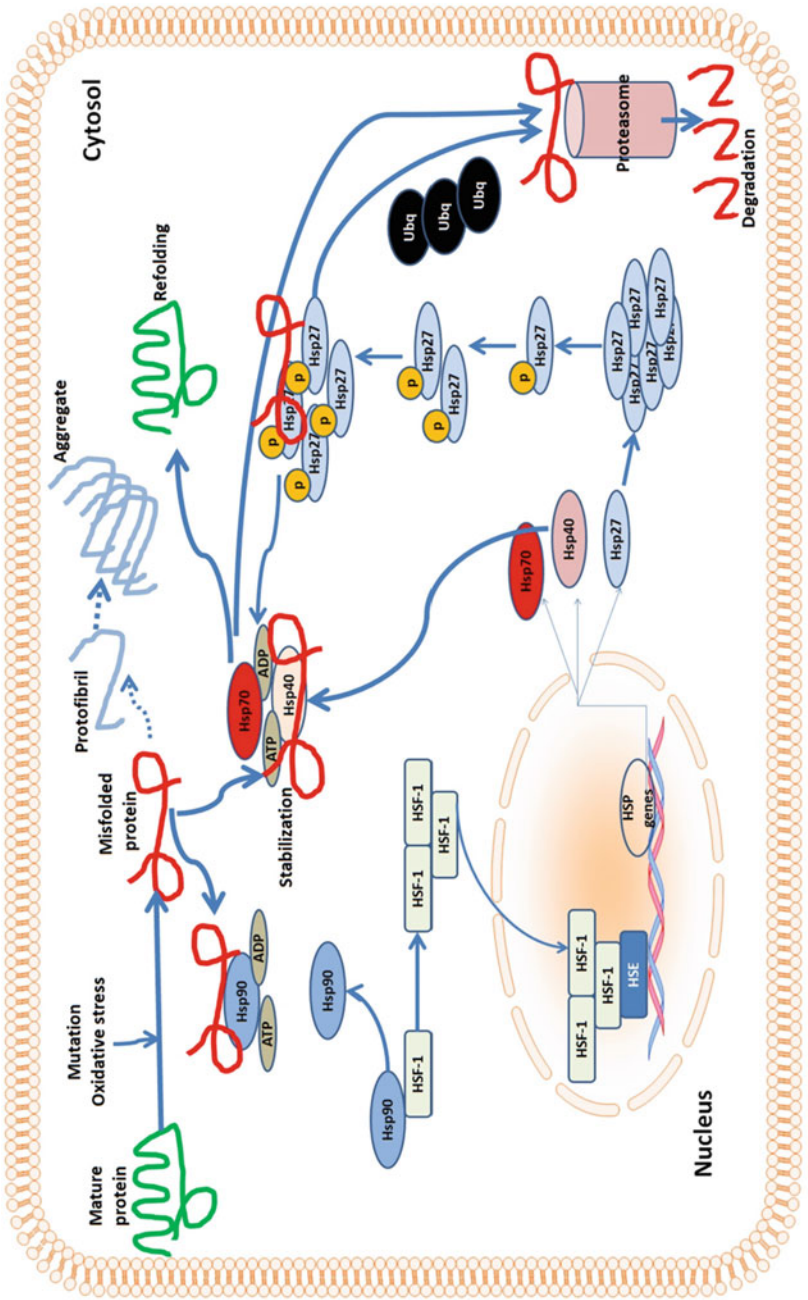



**Fig. 1** Mechanism of Hsps as a molecular chaperone

transducers and activators of transcription (STAT1, STAT3) and nuclear factor interleukin-6 (NF-IL6) [79].

STATs are a group of transcription factors that allow intracellular signaling at cytokine cell receptors to be transferred to the nucleus. They located in the cytoplasm that activated and transmitted to the nucleus after phosphorylation of tyrosine and serine residues on their C-terminal domains by Janus kinases (JAKs) and mitogen-activated protein kinase (MAPK) families, respectively. The main activator for STAT1 is Interferon- γ (IFN- γ) while interleukin-6 (IL-6) family members activate STAT3 [31]. Moreover, STAT1 involved in apoptotic cascade through its acting as a coactivator for p53 [82].

Once, STAT1 activated by (IFN-γ), the activities of Hsp70 and HSP90 are enhanced and these effects were absent in the STAT1-deficient cell line confirmed the direct link between HSP expression and STAT1 [77]. The synergetic role of IFN-γ/STAT1 and HSF1 via a protein-protein interaction on Hsps expression was confirmed through their ability to bind in the same region in Hsp-70/Hsp-90 promoters [78].

### *3.3 STAT3*

There is an antagonist relationship between STAT-3 and HSF-1 on binding site of Hsp70 and Hsp90 gene promoters and this indicates that STAT-1or STAT-3 interact differently on Hsp promoter activity [79].

### *3.4 NF-IL6*

NF-IL6 is a transcription factor that contains binding sites for IL-6 inducible genes. Hsp90 overexpressed in IL-6 exposed cells due to the presence of a short region of HSP90 promoter-specific region for NF-IL6 [39]. This confirms the suggestion of hsp90 as one of the IL-6 inducible genes [76]. Moreover, overexpression of NF-IL6 and STAT3 have the same effect on Hsp90 promoter and both signaling pathways were needed for Hsp90 promoter activation via IL-6 [77, 79].

## 4 Hsp70 FAMILY

This family of heat shock proteins is a molecular chaperone with a molecular weight of 70 kDa in size that serves important roles in protein stability. Hsp70 assist in folding and correct misfolded protein, improve the cellular transportation of proteins, reducing toxic protein aggregation and act as a buffering system against cellular stressors either extrinsic or intrinsic stimuli [57, 68]

## 4.1 Structure and Localization of Hsp70

The Hsp70 chaperones family consists of eight unique members that differ from each other in their expression pattern, amino acid sequences, and location inside the cell (Table 1). The pattern of cellular localization attributed to the specificity for their specific protein or to the chaperone-independent particular functions [81].

Hsp70 gene size is 2440 bp with 2 regulatory elements in 5′ end that bind with HSFs and a 242 bp of the untranslated region at 3′ end [90]. Additionally to hyperthermia, various stimuli responsible for Hsp70 induction which includes oxygen deprivation, acidosis, ischemia-reperfusion, oxidative stress, and microbial infection [40]. Hsp70 protein has highly conserved amino acids and domain sequences which include conserved ATPase domain, the central region with protease-sensitive sites, peptide-binding domain**,** and G/P-rich C-terminal region containing an EEVD-motif that allow co-chaperone of other proteins [17].

Hsp70s have good flexibility to make conformational changes in their polypeptide substrate with the help of the J domain of co-chaperones and the nucleotide exchange factors that regulate the lifetime of the Hsp70–substrate complexes [51]. The conserved ATPase domain of Hsp70 proteins maintain their functions and enable the release of hydrophobic amino acids exposed by misfolded globular proteins [13, 67] (Fig. 2).

## 4.2 Mechanisms of Hsp70 Release from Cells

The release of HSP70 from a variety of cells in response to a myriad of stimuli and the mechanisms by which Hsp70 is proposed in four different ways [21] Firstly, necrosis leads to Hsp70 release in a passive manner [47]. Secondly, it can be released within vesicles in a similar way to IL- 1β which is subsequently lysed in the extracellular environment [3]. Thirdly, it involves their secretion through endolysosomes that fuse with the cell membrane and release Hsp70 with cathepsin D, a lysosomal marker [48]

**Table 1** Summary of Hsp70 family and their localization

| Protein | Alternative names | Cellular localization and stress-induced |
|---|---|---|
| HSP70-1a | Hsp70, Hsp72, Hsp70-1 | Cytosol, Nucleus, Lysosome (Induced) |
| HSP70-1b | Hsp70, Hsp72, Hsp70-2 | Cytosol, Nucleus, Lysosome (Induced) |
| HSP70-1L | Hsp70-hom, Hsp 70t | Cytosol, Nucleus (Not induced) |
| HSP70-2 | Hsp70-3, HspA2 | Cytosol, Nucleus (Not induced) |
| HSP70-5 | Bib, Grp78 | Endoplasmic reticulum (Not induced) |
| HSP70-6 | Hsp70B | Cytosol, Nucleus (Induced) |
| HSC70 | Hsp70-8, Hsp73 | Cytosol, Nucleus (Not induced) |
| HSP70-9 | Grp75, mtHsp75, Mortalin | Mitochondria (Not induced) |

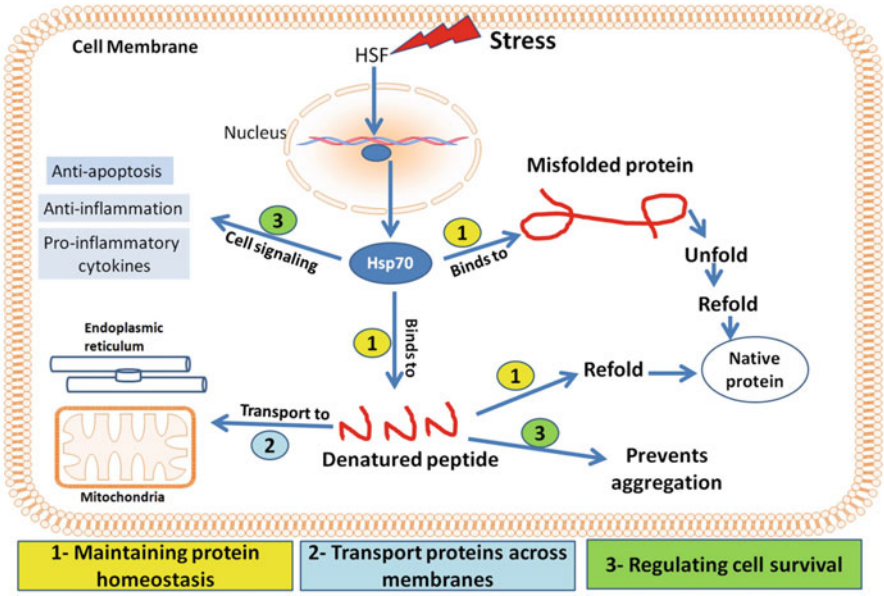



**Fig. 2** The major intracellular functions of Hsp70 proteins (Modified after [26])

Fourthly, Hsp70 may be released by exosomes that are lipid-bounded or may be released through specialized membrane domains known as lipid rafts which are specific sphingolipid and cholesterol-rich areas within the cell membrane which allow for transport of proteins [84].

## *4.3 Multidimensional Applications of Hsp70*

### 4.3.1 Hsp70 as Cellular Lifeguards: Inhibitor of Apoptosis

Caspase role in apoptosis appeared in two main categories: intrinsic and extrinsic pathwasys. Intrinsic pathway is usually active mechanism that cells counteract mitochondrial activity in response to different stressors such as DNA breaks, misfolded proteins, oxygen and growth factor deprivation [38]. The different functions of Bcl-2 families are activated in response to apoptotic signals where intrinsic apoptosis is known to stimulate the opening of mitochondrial-membrane permeability with the release of cytochrome c (a pro-apoptotic factor that showed main role in intrinsic pathway) into the cytoplasm. The presence of cytochrome c in cytosol allowed its binding with apoptotic protease activating factor that changes pro-caspase-9 to caspase-9 and forming apoptosome. The activation of caspase-9 stimulate the release of caspase-3, which stimulate the process of apoptosis of target molecules [59].

The intrinsic and extrinsic pathways of apoptosis have been proposed. The hallmark of the intrinsic route involves apoptosome formation. Cell death signals allow mitochondrial cytochrome C (Cyt c) to be released and bind to apoptosis protease activating factor-1 (Apaf-1), forming oligomerization and procaspase-9 release (Fig. 3). Apoptosome induction activates caspase-9 which stimulates downstream caspase-3 activation [80].

The anti-apoptotic role of Hsp70 depends on hinder apoptosome formation through direct engagement with caspase- recruitment domain (CARD) of an Apaf-1.The interaction of Hsp70 and Apaf-1 prevents its association with procaspase-9 and prevent assembly of functional apoptosome [8]. Moreover, HSP70 acetylation has an anti-apoptotic effect through Apaf-1 and apoptosis-inducing factor (AIF) on tumor cells and decreases autophagic cell death through autophagosome formation and increases autophagy associated genes [62].

The extrinsic way involves the attachment of death ligands to cell surface receptors (e.g., FasICD95/Apo-1 or TNF receptor) leading to Fas Associated Death Domain (FADD) or TNF Receptor Associated Death Domain (TRADD) recruitment to the cytosolic end of the receptor with the induction of Death Inducing Signaling Complex (DISC) at the plasma membrane and resultant activation of procaspase-8 and thereby procaspase-3 [59]. Hsp70 cannot modulate the extrinsic apoptosis pathway [15]. Hsp70 controls apoptosis at a pre mitochondrial stage by suppressing stress-induced signaling (for instance mediated by JNK kinases) or by stabilizing lysosomal membranes. Additionally, at the mitochondrial level, by preventing MOMP although the blockade of BAX translocation; finally, at the post mitochondrial stage by interacting with AIF (apoptosis-inducing factor) and Apaf-1 [23].

The over release of Hsp70 maintains the propagation and proliferation of tumor cells and inhibition or downregulation of Hsp70 release in different malignant tumors increase the susceptibility of tumor cells to chemotherapy, confirming the

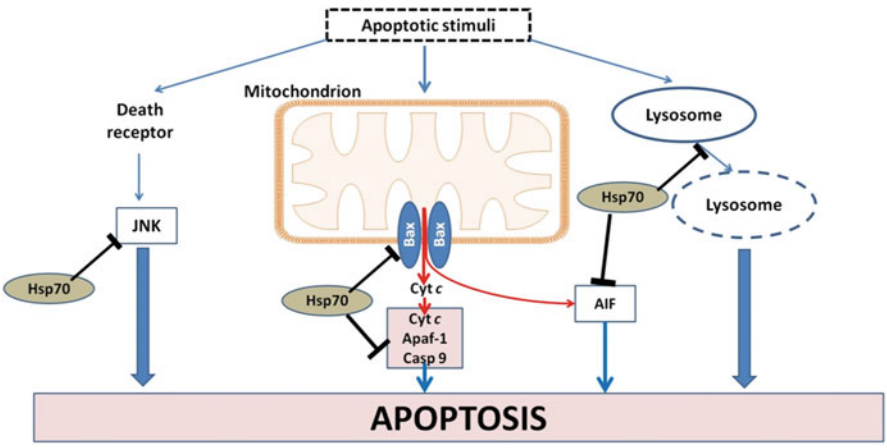



**Fig. 3** Hsp70 modulation of apoptosis

suppression roles of Hsp70 in the metastasis of cancerous cells [8]. The main role of Hsp70 as anti-apoptotic marker in cancerous cell depends on its effect on inhibiting TNF-α mediated-apoptotic cell death, thus promoting cancer cell to escape from immune response. Another mechanism of anti-apoptotic role of Hsp70 through its block the activation of apoptotic protease activating factor 1(Apaf-1) and inhibit activation of caspases as well as stimulates cell survival [62].

Collectively Hsp70 has an anti-apoptotic effect through inhibition of mitochondrial outer membrane permeabilization (MOMP) and blocking caspase activation and DNA fragmentation [38]. The high expression level of Hsp70 in cancer cells associated with a poor prognosis due to its ability to prevent apoptosis and senescence. Moreover, Hsp70 has been linked with therapeutic resistance as a result of a defect in activation of Extracellular signal-regulated kinases (ERK), nuclear factor κB (NF-κB), and JNK pathways [37]

### 4.3.2 Hsp70 Chaperone Machine: Fight or Flight

Under normal physiological status, the risk form of protein with conformational changes is polypeptide state and these changes improper their function and/or cause aggregate them into large toxic complexes. The chaperone function of Hsp70 includes maintaining polypeptide folding, transportation of misfolded protein to be degraded [36]. The significance of the molecular chaperone of Hsp70 is to maintain and control the protein conformation during their lifetime after their production by the ribosome [28]. Exposure of cells to different stressors leads to exacerbating in protein conformational changes such as protein unfolding on exposure to acute and chronic heat stress or oxidative distress induced by free radical exposure [69]. The molecular chaperone function in the stressful condition is to refolding the misfolded protein or entered this protein to proteolytic process [36]. Hsp70s never function alone as they require J protein and nucleotide exchange factor (NEF) during chaperone function. They regulate Hsp70's binding to target proteins [50]. The J proteins linked to target proteins and Hsp70 through peptide-binding domain and J domain respectively.

Under ATP hydrolysis, the target protein quickly, but momentarily, attached with the open peptide-binding domain of Hsp70 causing a structural change in HSP70 that closes the helical cover over the cleft and maintains the protein target interaction with the J protein in a complex manner. The NEF, which has a higher binding capability for Hsp70-ADP than Hsp-70 ATP, linked with Hsp70. The ADP dissociates through the mutilation of the ATP-binding site. The target protein is released due to its lower ability for HSP-ATP [35, 36]. The protection of neurons from aggregated misfolded protein during stress relay on Hsp70 family [46]. Therefore, Hsp70 is related to some neurodegenerative and conformational changes such as Parkinson's disease(PD) [83, 89].

Interestingly, Hsp70 members have been expressed with α-synuclein (αSyn), the main structure of Lewy bodies (LBs) [74], and inside the intraneuronal inclusions in PD brains [53] as well as in *the substantia nigra pars compacta* (SN) of the brain

[29]. The main way for neurodegerative disorder treatment depends on the ability of Hsp70 to prevent α Syn aggregation and toxicity [89].

### 4.3.3 Hsp70 Modulate the Immune Response

Hsp70 and Hsp60 represent members of auto-antigen complex which induce immunoregulatory changes and decreasing the immune responses in different auto immune diseases such as Type 1 diabetes and rheumatic arthritis [75]

The significance of Hsp70 as modulator for innate immunity owed to its ability to interact with specific receptors such as DCs, monocytes and myeloid-derived suppressor cells [12]. The immunological role of Hsp could be attributed to their ability to trigger immune system to infectious area, interacting with antigen presenting cells and refold of misfolded proteins, thus maintain their survival under stressful status.

The chaperone function of extracellular Hsp70 isn't related to correct the misfolded proteins but the ability to act as a signal for stimulation of a potent and chronic immune response [4].

Hsp70 has a dual effect on immune response. The pro-inflammatory effect of Hsp70 through its binding with antigen presenting cells, improve cytokine production and regulate co-stimulatory molecule release [6, 54]. The anti-inflammatory role relay on decreasing the inflammatory mediators secretions in endotoxemic model [30].

Extracellular Hsp70 induces a plethora of immune responses and the list continues to grow**.** The cytokine-induced effects of Hsp70 rely on the interaction of peptide-free Hsp70 with CD14 and Toll-like receptor (TLR2/4) on APCs initiates the production of proinflammatory cytokines, including TNF-α, IL-1β, IL-12, IL-6 [42, 71]; GM-CSF [75]; nitric oxide, a potent apoptogenic mediator [60]; chemokines including macrophage inflammatory protein (MIP-1) and monocyte chemoattractant protein-1 (MCP-1) [41].

Hsp70 also competes with CD40 ligand for binding to APCs. Furthermore, Hsp70s participated in the migration and maturation of DCs [5, 58].

A possible mechanism of Hsp70 action on innate immune cells through direct interaction with an endocytotic receptor on DCs that showed downregulates CD86 and MHC class II expression and inhibits TNF-α production [10, 55]. Also, Hsp70 can inhibit INF-γ production by monocytes and upon binding to endocytic, Hsp70 signals through TLR 2, resulting in activation of myeloid differentiation primary response (MyD88) that allow phosphorylation of extracellular signal-regulated kinase that triggers transcription factor which binds to IL-10 gene promoter leading to IL-10 production and consequently immunosuppressant [12].

Additionally, Hsp70-peptide complexes extracted from sarcomas could act as a spark for cytotoxic T cell (CTL) release [85]. The immune mechanism of Hsp70 through its ability to attach to receptor in APCs and get through the internal route of antigen producing in major histocompatibility (MHC) class I [86]. Moreover, Hsp70 conserve cytoplasmic peptides until binding with MHC class I molecules [32].

### 4.3.4 Hsp70 as a Biomarker for Heavy Metal Toxicity

The cellular response to continuous mitochondrial oxidative reactions and non-cellular responses such as inflammatory response, exercise, heavy metal toxicity, and environmental pollution includes the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which disrupt the cellular homeostasis and induce oxidative stress [66].

These radicals can be classified according to oxygen-containing capacity into superoxide anion hydroxyl anion, alkoxyl molecule, peroxyl molecule, nitric oxide molecule, nitrogen oxide. These radicals are very active and causing deleterious impacts to living system. The significance of low or moderate level of ROS relay on its role as secondary messenger in biosignaling, immune response, vascular tone and apoptosis of cancer cells [19].

The body respond to oxidative stress induced by ROS through antioxidant system to counteracts the cellular damage by changing these radicals to less harmful substances under normal condition; however, the imbalance between ROS release and antioxidant system leading to activate many inflammatory cascades. The detrimental impacts of free radical release allow body to increase the expression of Hsps which have cytoprotective role in different cellular stressors through their chaperoning functions which included corrected misfolding proteins and their assembly [1].

The adaptive response of organisms to oxidative stress induced by tested chemical depends on the measurement of reactive oxygen species and antioxidant enzymes disturbances [43]. The role of free radicals can be appeared as a double edged sword. Under normal status, ROS –induced apoptosis and performs beneficial role, but the abnormal activation of this role under stressful status leading to huge cellular destructions.

Changes in protein expression, including Hsp70, may act as guide to natural and synthetic stressors. However, The expression of some Hsp showed pro-inflammatory and apoptotic functions but most of them showed anti-apoptotic and anti-inflammatory potential through interference in nuclear factors and caspases activation. Tissue-specific changes in protein expression may represent a toxicological signature for exposure where ROS release by heavy metal may induce HSP expression. The signaling pathways that involved on metal exposure are MAPk/AP-1 and NF-kB [20].

Attachment of Hsp70 to JNK - a member of the MAP kinase family and a key component of a stress-activated protein kinase signaling pathway- inhibits its phosphorylation by MKK4 (mitogen-activated protein kinase -4). Moreover, Hsp70 prevents MKK4 activation through binding with MAP3kinase Ask1 (apoptosis signal regulatory kinase-1). Hsp70 also prevents the arsenite-induced aggregation of phosphatase leads to prevent JNK activation [56]. All these effects lead to inhibit apoptosis of cells and the increased levels of Hsp70 have been measured in tissues of fish exposed to industrial effluents and polycyclic aromatic hydrocarbons; heavy metals such as copper, zinc, and mercury; pesticides and arsenite confirm its role as a biomarker for heavy metal pollution [70].

### 4.3.5 Hsp70 and Insulin Resistance

The pathogenesis of obesity and type II diabetes may relate to insulin resistance which has been associated with hyperlipidemia [64]. Phosphorylation of insulin receptor substrate-1 (IRS-1) through classic and novel PKC isoforms leads to improper binding with insulin receptor and decreases insulin action [11]. This phosphorylation induced through kinase-beta (IKK -beta) and/or JNK [73]. The activation of these kinases in response to cytokines can promote insulin resistance in different tissues [18]. Attachment of Hsp70 with JNK, inhibit its activation by JNK kinases [61]. Moreover, Hsp70 maintain JNK in the form of in active one [22]. Additionally, Hsp70 will directly inhibit PKC which activates JNK [92]. Collectively, the inducible form of Hsp70 will decrease insulin resistance through decreasing the activation of IKK- beta and of JNK [52].

## 5 Conclusion

Hsp70 is a cornerstone not only for chaperones but also for several essential functions of the cells and further investigations are required for developing therapeutic agents targeting this critical molecule.

**Acknowledgements** None.

**Disclosure of Interests** None.

**Ethical Approval for Studies Involving Humans** No human studies are involved in this article.

**Ethical Approval for Studies Involving Animals** This study not included animal studies.

## References

1. Ahmad I, Pacheco M, Santos MA (2006) Anguilla anguilla L. oxidative stress biomarkers: an in situ study of freshwater wetland ecosystem (Pateira de Fermentelos, Portugal). Chemosphere 65 (6):952–962. https://doi.org/10.1016/j.chemosphere.2006.03.042
2. Akerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 11(8):545–555. https://doi.org/10.1038/nrm2938
3. Arai M, Kuwajima K (2000) Role of the molten globule state in protein folding. Adv Protein Chem 53:209–282. https://doi.org/10.1016/s0065-3233(00)53005-8
4. Asea A (2005) Stress proteins and initiation of immune response: chaperokine activity of hsp72. Exerc Immunol Rev 11:34–45
5. Asea A, Kabingu E, Stevenson MA, Calderwood SK (2000) HSP70 peptidembearing and peptide-negative preparations act as chaperokines. Cell Stress Chaperones 5(5):425–431. https://doi.org/10.1379/1466-1268(2000)005

6. Basu S, Srivastava PK (2000) Heat shock proteins: the fountainhead of innate and adaptive immune responses. Cell Stress Chaperones 5(5):443–451. https://doi.org/10.1379/1466-1268(2000)005
7. Beck FX, Neuhofer W, Muller E (2000) Molecular chaperones in the kidney: distribution, putative roles, and regulation. Am J Physiol Renal Physiol 279(2):F203–F215. https://doi.org/10.1152/ajprenal.2000.279.2.F203
8. Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Green DR (2000) Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nat Cell Biol 2(8):469–475. https://doi.org/10.1038/35019501
9. Benarroch EE (2011) Heat shock proteins: multiple neuroprotective functions and implications for neurologic disease. Neurology 76(7):660–667. https://doi.org/10.1212/WNL.0b013e31820c3119
10. Binder RJ, Anderson KM, Basu S, Srivastava PK (2000) Cutting edge: heat shock protein gp96 induces maturation and migration of CD11c+ cells in vivo. J Immunol 165(11):6029–6035. https://doi.org/10.4049/jimmunol.165.11.6029
11. Boden G, Carnell LH (2003) Nutritional effects of fat on carbohydrate metabolism. Best Pract Res Clin Endocrinol Metab 17(3):399–410. https://doi.org/10.1016/s1521-690x(03)00032-0
12. Borges TJ, Wieten L, van Herwijnen MJ, Broere F, van der Zee R, Bonorino C, van Eden W (2012) The anti-inflammatory mechanisms of Hsp70. Front Immunol 3:95. https://doi.org/10.3389/fimmu.2012.00095
13. Bukau B, Weissman J, Horwich A (2006) Molecular chaperones and protein quality control. Cell 125(3):443–451. https://doi.org/10.1016/j.cell.2006.04.014
14. Castelli C, Ciupitu AM, Rini F, Rivoltini L, Mazzocchi A, Kiessling R, Parmiani G (2001) Human heat shock protein 70 peptide complexes specifically activate antimelanoma T cells. Cancer Res 61(1):222–227
15. Clemons NJ, Anderson RL (2006) TRAIL-induced apoptosis is enhanced by heat shock protein 70 expression. Cell Stress Chaperones 11(4):343–355. https://doi.org/10.1379/csc-206.1
16. Das JK, Xiong X, Ren X, Yang JM, Song J (2019) Heat shock proteins in cancer immunotherapy. J Oncol 2019:3267207. https://doi.org/10.1155/2019/3267207
17. Daugaard M, Rohde M, Jaattela M (2007) The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. FEBS Lett 581(19):3702–3710. https://doi.org/10.1016/j.febslet.2007.05.039
18. de Alvaro C, Teruel T, Hernandez R, Lorenzo M (2004) Tumor necrosis factor alpha produces insulin resistance in skeletal muscle by activation of inhibitor kappaB kinase in a p38 MAPK-dependent manner. J Biol Chem 279(17):17070–17078. https://doi.org/10.1074/jbc.M312021200
19. Del Razo LM, Quintanilla-Vega B, Brambila-Colombres E, Calderon-Aranda ES, Manno M, Albores A (2001) Stress proteins induced by arsenic. Toxicol Appl Pharmacol 177(2):132–148. https://doi.org/10.1006/taap.2001.9291
20. Donaldson K, Stone V, Borm PJ, Jimenez LA, Gilmour PS, Schins RP, MacNee W (2003) Oxidative stress and calcium signaling in the adverse effects of environmental particles (PM10). Free Radic Biol Med 34(11):1369–1382. https://doi.org/10.1016/s0891-5849(03)00150-3
21. Evdonin AL, Martynova MG, Bystrova OA, Guzhova IV, Margulis BA, Medvedeva ND (2006) The release of Hsp70 from A431 carcinoma cells is mediated by secretory-like granules. Eur J Cell Biol 85(6):443–455. https://doi.org/10.1016/j.ejcb.2006.02.008
22. Gabai VL, Meriin AB, Yaglom JA, Wei JY, Mosser DD, Sherman MY (2000) Suppression of stress kinase JNK is involved in HSP72-mediated protection of myogenic cells from transient energy deprivation. HSP72 alleviates the stewss-induced inhibition of JNK dephosphorylation. J Biol Chem 275(48):38088–38094. https://doi.org/10.1074/jbc.M006632200
23. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G (2006) Mechanisms of cytochrome c release from mitochondria. Cell Death Differ 13(9):1423–1433. https://doi.org/10.1038/sj.cdd.4401950

24. Georgopoulos C, Welch WJ (1993) Role of the major heat shock proteins as molecular chaperones. Annu Rev Cell Biol 9:601–634. https://doi.org/10.1146/annurev.cb.09.110193.003125
25. Gething MJ, Sambrook J (1992) Protein folding in the cell. Nature 355(6355):33–45. https://doi.org/10.1038/355033a0
26. Haak J, Kregel KC (2008) 1962–2007: a cell stress odyssey. Novartis Found Symp 291:3–15; discussion 15–22, 137–140. https://doi.org/10.1002/9780470754030.ch2
27. Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381(6583):571–579. https://doi.org/10.1038/381571a0
28. Hartl FU, Hayer-Hartl M (2009) Converging concepts of protein folding in vitro and in vivo. Nat Struct Mol Biol 16(6):574–581. https://doi.org/10.1038/nsmb.1591
29. Hauser MA, Li YJ, Xu H, Noureddine MA, Shao YS, Gullans SR, Vance JM (2005) Expression profiling of substantia nigra in Parkinson disease, progressive supranuclear palsy, and frontotemporal dementia with parkinsonism. Arch Neurol 62(6):917–921. https://doi.org/10.1001/archneur.62.6.917
30. Hayashi Y, Sawa Y, Fukuyama N, Nakazawa H, Matsuda H (2002) Preoperative glutamine administration induces heat-shock protein 70 expression and attenuates cardiopulmonary bypass-induced inflammatory response by regulating nitric oxide synthase activity. Circulation 106(20):2601–2607. https://doi.org/10.1161/01.cir.0000035651.72240.07
31. Ihle JN (2001) The Stat family in cytokine signaling. Curr Opin Cell Biol 13(2):211–217. https://doi.org/10.1016/s0955-0674(00)00199-x
32. Javid B, MacAry PA, Lehner PJ (2007) Structure and function: heat shock proteins and adaptive immunity. J Immunol 179(4):2035–2040. https://doi.org/10.4049/jimmunol.179.4.2035
33. Jolly C, Morimoto RI (2000) Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J Natl Cancer Inst 92(19):1564–1572. https://doi.org/10.1093/jnci/92.19.1564
34. Kalmar B, Greensmith L (2009) Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev 61(4):310–318. https://doi.org/10.1016/j.addr.2009.02.003
35. Kampinga HH, Andreasson C, Barducci A, Cheetham ME, Cyr D, Emanuelsson C, Marszalek J (2019) Function, evolution, and structure of J-domain proteins. Cell Stress Chaperones 24(1):7–15. https://doi.org/10.1007/s12192-018-0948-4
36. Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11(8):579–592. https://doi.org/10.1038/nrm2941
37. Kasioumi P, Vrazeli P, Vezyraki P, Zerikiotis S, Katsouras C, Damalas A, Angelidis C (2019) Hsp70 (HSP70A1A) downregulation enhances the metastatic ability of cancer cells. Int J Oncol 54(3):821–832. https://doi.org/10.3892/ijo.2018.4666
38. Kim LS, Kim JH (2011) Heat shock protein as molecular targets for breast cancer therapeutics. J Breast Cancer 14(3):167–174. https://doi.org/10.4048/jbc.2011.14.3.167
39. Kinoshita S, Akira S, Kishimoto T (1992) A member of the C/EBP family, NF-IL6 beta, forms a heterodimer and transcriptionally synergizes with NF-IL6. Proc Natl Acad Sci U S A 89(4):1473–1476. https://doi.org/10.1073/pnas.89.4.1473
40. Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol (1985) 92(5):2177–2186. https://doi.org/10.1152/japplphysiol.01267.2001
41. Lehner T, Bergmeier LA, Wang Y, Tao L, Sing M, Spallek R, van der Zee R (2000) Heat shock proteins generate beta-chemokines which function as innate adjuvants enhancing adaptive immunity. Eur J Immunol 30(2):594–603. https://doi.org/10.1002/1521-4141(200002)30:2<594::AID-IMMU594>3.0.CO;2-1
42. Lehner T, Wang Y, Whittall T, McGowan E, Kelly CG, Singh M (2004) Functional domains of HSP70 stimulate generation of cytokines and chemokines, maturation of dendritic cells and adjuvanticity. Biochem Soc Trans 32(Pt 4):629–632. https://doi.org/10.1042/BST0320629

43. Li X, Liu Y, Song L, Liu J (2003) Responses of antioxidant systems in the hepatocytes of common carp (Cyprinus carpio L.) to the toxicity of microcystin-LR. Toxicon 42(1):85–89. https://doi.org/10.1016/s0041-0101(03)00104-1
44. Lindquist S, Craig EA (1988) The heat-shock proteins. Annu Rev Genet 22:631–677. https://doi.org/10.1146/annurev.ge.22.120188.003215
45. Liu Q, Liang C, Zhou L (2020) Structural and functional analysis of the Hsp70/Hsp40 chaperone system. Protein Sci 29(2):378–390. https://doi.org/10.1002/pro.3725
46. Lu TZ, Quan Y, Feng ZP (2010) Multifaceted role of heat shock protein 70 in neurons. Mol Neurobiol 42(2):114–123. https://doi.org/10.1007/s12035-010-8116-6
47. Mambula SS, Calderwood SK (2006) Heat induced release of Hsp70 from prostate carcinoma cells involves both active secretion and passive release from necrotic cells. Int J Hyperth 22 (7):575–585. https://doi.org/10.1080/02656730600976042
48. Mambula SS, Calderwood SK (2006) Heat shock protein 70 is secreted from tumor cells by a nonclassical pathway involving lysosomal endosomes. J Immunol 177(11):7849–7857. https://doi.org/10.4049/jimmunol.177.11.7849
49. Mathew A, Morimoto RI (1998) Role of the heat-shock response in the life and death of proteins. Ann N Y Acad Sci 851:99–111. https://doi.org/10.1111/j.1749-6632.1998.tb08982.x
50. Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62(6):670–684. https://doi.org/10.1007/s00018-004-4464-6
51. Mayer MP, Gierasch LM (2019) Recent advances in the structural and mechanistic aspects of Hsp70 molecular chaperones. J Biol Chem 294(6):2085–2097. https://doi.org/10.1074/jbc.REV118.002810
52. McCarty MF (2006) Induction of heat shock proteins may combat insulin resistance. Med Hypotheses 66(3):527–534. https://doi.org/10.1016/j.mehy.2004.08.033
53. McLean PJ, Kawamata H, Shariff S, Hewett J, Sharma N, Ueda K, Hyman BT (2002) TorsinA and heat shock proteins act as molecular chaperones: suppression of alpha-synuclein aggregation. J Neurochem 83(4):846–854. https://doi.org/10.1046/j.1471-4159.2002.01190.x
54. Milani V, Noessner E, Ghose S, Kuppner M, Ahrens B, Scharner A, Issels RD (2002) Heat shock protein 70: role in antigen presentation and immune stimulation. Int J Hyperth 18(6):563–575. https://doi.org/10.1080/02656730210166140
55. Morelli AE, Thomson AW (2007) Tolerogenic dendritic cells and the quest for transplant tolerance. Nat Rev Immunol 7(8):610–621. https://doi.org/10.1038/nri2132
56. Mosser DD, Morimoto RI (2004) Molecular chaperones and the stress of oncogenesis. Oncogene 23(16):2907–2918. https://doi.org/10.1038/sj.onc.1207529
57. Murphy ME (2013) The HSP70 family and cancer. Carcinogenesis 34(6):1181–1188. https://doi.org/10.1093/carcin/bgt111
58. Noessner E, Gastpar R, Milani V, Brandl A, Hutzler PJ, Kuppner MC, Issels RD (2002) Tumor-derived heat shock protein 70 peptide complexes are cross-presented by human dendritic cells. J Immunol 169(10):5424–5432. https://doi.org/10.4049/jimmunol.169.10.5424
59. Nosareva OL, Ryazantseva NV, Stepovaya EA, Shakhristova EV, Stepanova EA, Gulaya VS (2016) The role of heat shock proteins 27 and 70 in redox-dependent regulation of apoptosis in Jurkat tumor cells. Biomed Khim 62(6):670–673. https://doi.org/10.18097/PBMC20166206670
60. Panjwani NN, Popova L, Srivastava PK (2002) Heat shock proteins gp96 and hsp70 activate the release of nitric oxide by APCs. J Immunol 168(6):2997–3003. https://doi.org/10.4049/jimmunol.168.6.2997
61. Park HS, Lee JS, Huh SH, Seo JS, Choi EJ (2001) Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. EMBO J 20(3):446–456. https://doi.org/10.1093/emboj/20.3.446
62. Park YH, Seo JH, Park JH, Lee HS, Kim KW (2017) Hsp70 acetylation prevents caspase-dependent/independent apoptosis and autophagic cell death in cancer cells. Int J Oncol 51 (2):573–578. https://doi.org/10.3892/ijo.2017.4039

63. Parsell DA, Lindquist S (1993) The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu Rev Genet 27:437–496. https://doi.org/10.1146/annurev.ge.27.120193.002253
64. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 350 (7):664–671. https://doi.org/10.1056/NEJMoa031314
65. Pockley AG (2003) Heat shock proteins as regulators of the immune response. Lancet 362 (9382):469–476. https://doi.org/10.1016/S0140-6736(03)14075-5
66. Prieto AI, Jos A, Pichardo S, Moreno I, Camean AM (2006) Differential oxidative stress responses to microcystins LR and RR in intraperitoneally exposed tilapia fish (Oreochromis sp.). Aquat Toxicol 77(3):314–321. https://doi.org/10.1016/j.aquatox.2005.12.012
67. Rosenzweig R, Nillegoda NB, Mayer MP, Bukau B (2019) The Hsp70 chaperone network. Nat Rev Mol Cell Biol 20(11):665–680. https://doi.org/10.1038/s41580-019-0133-3
68. Saadeldin IM, Swelum AA, Elsafadi M, Mahmood A, Osama A, Shikshaky H, Magdeldin S (2020) Thermotolerance and plasticity of camel somatic cells exposed to acute and chronic heat stress. J Adv Res 22:105–118. https://doi.org/10.1016/j.jare.2019.11.009
69. Saadeldin IM, Swelum AA, Zakri AM, Tukur HA, Alowaimer AN (2020) Effects of acute hyperthermia on the thermotolerance of cow and sheep skin-derived fibroblasts. Animals (Basel) 10(4). https://doi.org/10.3390/ani10040545
70. Sanders BM (1993) Stress proteins in aquatic organisms: an environmental perspective. Crit Rev Toxicol 23(1):49–75. https://doi.org/10.3109/10408449309104074
71. Schmitt E, Gehrmann M, Brunet M, Multhoff G, Garrido C (2007) Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J Leukoc Biol 81(1):15–27. https://doi.org/10.1189/jlb.0306167
72. Sherman M, Multhoff G (2007) Heat shock proteins in cancer. Ann N Y Acad Sci 1113:192–201. https://doi.org/10.1196/annals.1391.030
73. Shoelson SE, Lee J, Yuan M (2003) Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. Int J Obes Relat Metab Disord 27(Suppl 3):S49–S52. https://doi.org/10.1038/sj.ijo.0802501
74. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) Alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 95(11):6469–6473. https://doi.org/10.1073/pnas.95.11.6469
75. Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev Immunol 2(3):185–194. https://doi.org/10.1038/nri749
76. Stephanou A, Amin V, Isenberg DA, Akira S, Kishimoto T, Latchman DS (1997) Interleukin 6 activates heat-shock protein 90 beta gene expression. Biochem J 321(Pt 1):103–106. https://doi.org/10.1042/bj3210103
77. Stephanou A, Isenberg DA, Akira S, Kishimoto T, Latchman DS (1998) The nuclear factor interleukin-6 (NF-IL6) and signal transducer and activator of transcription-3 (STAT-3) signalling pathways co-operate to mediate the activation of the hsp90beta gene by interleukin-6 but have opposite effects on its inducibility by heat shock. Biochem J 330(Pt 1):189–195. https://doi.org/10.1042/bj3300189
78. Stephanou A, Isenberg DA, Nakajima K, Latchman DS (1999) Signal transducer and activator of transcription-1 and heat shock factor-1 interact and activate the transcription of the Hsp-70 and Hsp-90beta gene promoters. J Biol Chem 274(3):1723–1728. https://doi.org/10.1074/jbc.274.3.1723
79. Stephanou A, Latchman DS (2011) Transcriptional modulation of heat-shock protein gene expression. Biochem Res Int 2011:238601. https://doi.org/10.1155/2011/238601
80. Takayama S, Reed JC, Homma S (2003) Heat-shock proteins as regulators of apoptosis. Oncogene 22(56):9041–9047. https://doi.org/10.1038/sj.onc.1207114
81. Tavaria M, Gabriele T, Kola I, Anderson RL (1996) A hitchhiker's guide to the human Hsp70 family. Cell Stress Chaperones 1(1):23–28. https://doi.org/10.1379/1466-1268(1996)001

82. Townsend PA, Scarabelli TM, Davidson SM, Knight RA, Latchman DS, Stephanou A (2004) STAT-1 interacts with p53 to enhance DNA damage-induced apoptosis. J Biol Chem 279 (7):5811–5820. https://doi.org/10.1074/jbc.M302637200
83. Tutar L, Tutar Y (2010) Heat shock proteins; an overview. Curr Pharm Biotechnol 11(2):216–222. https://doi.org/10.2174/138920110790909632
84. Tytell M (2005) Release of heat shock proteins (Hsps) and the effects of extracellular Hsps on neural cells and tissues. Int J Hyperth 21(5):445–455. https://doi.org/10.1080/02656730500041921
85. Udono H, Srivastava PK (1993) Heat shock protein 70-associated peptides elicit specific cancer immunity. J Exp Med 178(4):1391–1396. https://doi.org/10.1084/jem.178.4.1391
86. Ueda G, Tamura Y, Hirai I, Kamiguchi K, Ichimiya S, Torigoe T, Sato N (2004) Tumor-derived heat shock protein 70-pulsed dendritic cells elicit tumor-specific cytotoxic T lymphocytes (CTLs) and tumor immunity. Cancer Sci 95(3):248–253. https://doi.org/10.1111/j.1349-7006.2004.tb02211.x
87. Walsh D, Li Z, Wu Y, Nagata K (1997) Heat shock and the role of the HSPs during neural plate induction in early mammalian CNS and brain development. Cell Mol Life Sci 53(2):198–211. https://doi.org/10.1007/pl00000592
88. Wigmore SJ, Sangster K, McNally SJ, Harrison EM, Ross JA, Fearon KC, Garden OJ (2007) De-repression of heat shock transcription factor-1 in interleukin 6-treated hepatocytes is mediated by downregulation of glycogen synthase kinase 3beta and MAPK/ERK-1. Int J Mol Med 19(3):413–420
89. Witt SN (2010) Hsp70 molecular chaperones and Parkinson's disease. Biopolymers 93(3):218–228. https://doi.org/10.1002/bip.21302
90. Wu BJ, Kingston RE, Morimoto RI (1986) Human HSP70 promoter contains at least two distinct regulatory domains. Proc Natl Acad Sci U S A 83(3):629–633. https://doi.org/10.1073/pnas.83.3.629
91. Xu D, Zalmas LP, La Thangue NB (2008) A transcription cofactor required for the heat-shock response. EMBO Rep 9(7):662–669. https://doi.org/10.1038/embor.2008.70
92. Yoshida K, Miki Y, Kufe D (2002) Activation of SAPK/JNK signaling by protein kinase Cdelta in response to DNA damage. J Biol Chem 277(50):48372–48378. https://doi.org/10.1074/jbc.M205485200
93. Zhang Y, Zheng L (2013) Tumor immunotherapy based on tumor-derived heat shock proteins (review). Oncol Lett 6(6):1543–1549. https://doi.org/10.3892/ol.2013.1616

# Indispensable Role of Protein Turnover in Autophagy, Apoptosis and Ubiquitination Pathways

**Mohammed A. Abosheasha, Eman A. M. Abd El Khalik, and Afnan H. El-Gowily**

## Abstract

**Introduction** Heat-shock proteins (HSPs) have been engaged in versatile functions including chaperone activity, protein folding, apoptosis, autophagy and Ubiquitination. The cell safeguarded by a robust protein quality control mechanism which maintains protein homeostasis, also aka as proteostasis. Recent studies have described HSPs facilitate the folding process of newly synthesized peptides and the re-folding and recruitment process of functionalized proteins that are damaged under cellular stress, but they also participate with the degradation pathway of misfolded proteins to cope the demand of the cell. Additionally, whether a protein will be subjected to the folding or degradation process is determined by the protein quality control mechanism, which considered as a network of HSPs and degradation systems. The turnover of proteins is a representation of the balance between protein synthesis and degradation and is a vital mechanism for preserving the cellular protein pool. Protein folding, refolding of misfolded proteins or the degradation of

Afnan H. El-Gowily and Mohammed A. Abosheasha contributed equally to this study.

M. A. Abosheasha (✉)
Cellular Genetics Laboratory, Graduate School of Science, Tokyo Metropolitan University, Tokyo, Japan
e-mail: mohammed-abosheasha@ed.tmu.ac.jp; abosheasha@gmail.com

E. A. M. Abd El Khalik
Biochemistry Division, Chemistry Department, Faculty of science, Tanta University, Tanta, Egypt

A. H. El-Gowily (✉)
Biochemistry Division, Chemistry Department, Faculty of science, Tanta University, Tanta, Egypt

Organ and Cell physiology, Juntendo University, Tokyo, Japan
e-mail: a.elgowily.mc@juntendo.ac.jp; afnan.hamdy@science.tanta.edu.eg

Heat Shock Proteins (2021) 22: 447–468
https://doi.org/10.1007/7515_2020_31

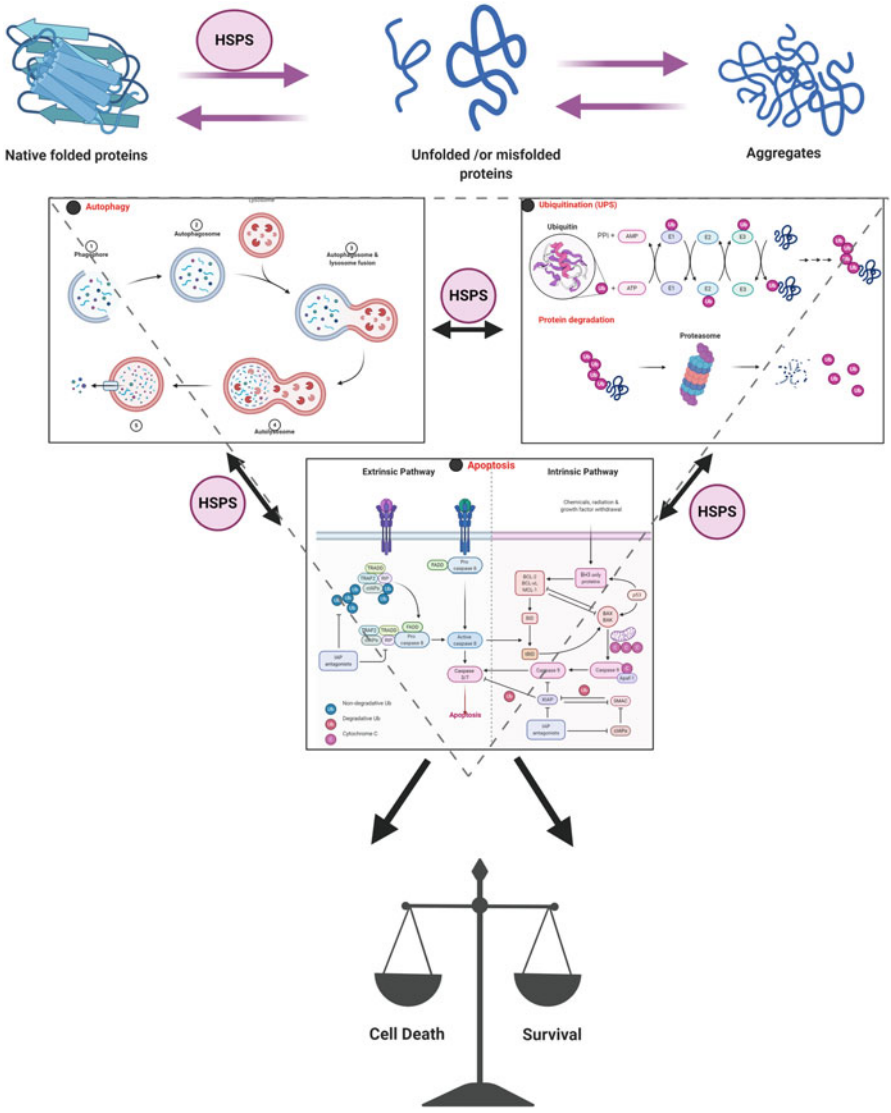
Published Online: 13 September 2020

misfolded and damaged proteins are part of protein quality control. In this chapter, we will be describing the prominent role of HSPs in protein turnover regulation; the effects of HSPs on protein degradation, apoptosis, autophagy, and the proteasome.

**Methods** The authors conducted a narrative review of most relevant papers.

**Results** Several studies also demonstrated the role of HSPs in protein turnover. As a while, there are various factors that control the correction of protein folding and degradation; the most important one is the heat shock protein family. Heat shock proteins (HSPs) are a molecular chaperone-type, which can avoid unwanted protein interactions and induce the proper folding. On the other hand, these proteins play essential roles in the pathways of protein degradation, the ubiquitin-proteasome system, apoptosis and autophagy.

**Conclusions** Regarding to the different roles of HSPs and signaling pathways in biology and medicine, we described how these systems interact to maintain proteostasis; as well as the role of the protein quality control system, which offers a novel perspective on promising therapeutic strategies based on the differential role of HSPs in protein turnover.




**Highlights**

- Protein quality control systems regulate turn over of proteins by the HSPs actions through different mechanisms such as UPS, Autophagy and Apoptosis.
- HSPs regulate autophagy and proteasomes.
- Degradation process of misfolded proteins undergoes the autophagy or proteasomal systems that fated by Ubiquitination pathway.
- Posttranslational modification mediated regulation of intracellular quality control mechanism affecting HSPs, proteasomal system, and autophagy that co-moderate apoptosis pathway to balance signaling of cell survival and death decisions.

**Keywords** Apoptosis · Autophagy · Heat shock proteins · Protein turnover · Ubiquitination

## 1 Introduction

In cells such as signaling, transportation, catalysts, membrane fusion, cell defense, and regulation, proteins have important functions [3]. Proteins must acquire their three-dimensional structure for functional purposes after ribosomal synthesis [10]. However, the cells are exposed to stress conditions such as heat, oxidative stress, inflammation, radiation, heavy metals or other toxic compounds that can cause protein production, unspecified aggregation, and protein homeostasis imbalances in their lives. Proteins are the molecules that are most vulnerable to cellular stress [29]. There are two principal ways of maintaining this balance between the synthesis, folding, and degradation of proteins [18]: (i) cell degradation machine, which targets proteins to proteolysis, and (ii) molecular chaperones which prevent aggregation and ensure the folding of proteins into their native status [17].

Heat shock proteins (HSPs) act as molecular chaperones in high cell concentrations and play a crucial role in support of cell stress protein homeostasis [29]. There are four major, ATP-dependent families classified as HSP100, HSP90, HSP70, and HSP60. There are also ATP-independent chaperones, including small HSPs [18]. The HSP60 family members act as chaperonin to ensure a proper folding of mitochondrial proteins to prevent aggregation. HSP70 and HSP90 bind in the cytoplasm to unfolded polypeptides and allow proper folding. HSP27 mediates ATP-independent client holding and protein folding. The organized function of these chaperons is essential for the efficiency of protein folding and the balance of homeostasis protein [14].

Recent studies demonstrated HSPs associate with key stress signaling and apoptotic molecules crosstalk with autophagy, thereby blocking cell death and promoting survival, proliferation or differentiation, and many stress pathways sequentially elicit autophagy, and apoptosis within the same cell [40].

As Known, Cells contain several proteolytical systems for degradation and complicated regulatory mechanisms to ensure highly selective continuous proteolytic processes. The cell portion is therefore prevented from unnecessarily breaking up. Overall, the rates of protein synthesis and degradation in each cell must be balanced precisely because even a small decrease in synthesis or a small acceleration of degradation, if sustained, can result in a marked loss of mass in the organism [44].

## 2 Structure of Heat Shock Proteins

The most crucial HSPs families include HSP100, HSP90, HSP70, HSP60 and small HSP (sHSP) with different structures. Briefly, HSP100 structure includes an N-terminal domain that is responsible for substrate binding and a small C-terminal domain. HSP100 is highly stimulated after cellular stress to solubilize proteins aggregations independently or with help of HSP70 [14]. The well-preserved cytosolic Hsp90 structure has three domains, a C-terminal domain including a dimerization motif, a drug-binding site and an N-terminal domain containing the cochaperone-binding motif and drug-binding site and an intermediate domain for binding to cochaperone and client proteins [35]. HSP70 structure involves an N-terminal ATPase domain (∼44 kDa), a binding substrate domain (∼18 kDa) and a C-terminal domain (∼10 kDa) [53]. HSP60 composed of monomers that form a complex arranged as two stacked heptameric rings. Each monomer has three domains: apical, equatorial and intermediate domain [49]. The $_{S}$HSP family members possess low molecular masses (∼ 13–43 kDa), a wide N-terminal region, a conservative α-crystallin domain (∼90 residues) and a short C-terminal extension. The α-crystalline domain forms stable dimers which constitute the construction blocks for the major oligomers of sHSP [58].

The main HSPs (HSP27, HSP60, HSP70, HSP90and HSP90) in the different apoptotic pathways. While, the pathophysiological features of HSPs in hematological malignancy may be developed as an example as defects for apoptosis and cell proliferation are at the thought of improved tumor.

Small HSPs are a group of proteins ranging in size between 15 and 30 kD and sequence homologies and homologies including phosphorylation and oligomerisation. The ATP-independent chaperone HSP27, possibly the most studies member of the family of relatives, has the major function of protecting against the aggregation of protein. Whether or not HSP27 can form a thousand kD of oligomers. The dimer HSP27 is possibly the building block of the complex.

Oligomerization HSP27 is a complex technique dependent on protein phosphorylation status and strain exposure. Human HSP27 can thus be phosphorylated in three serine residues and oligomerisation can be enhanced with its dephosphorylation. This is a reversible process which is catalyzed in reaction to various stresses, such as differentiators, mitogens, inflammatory cell cytokines including elements for tumor necrosis (TNF) and interleukin-1, hydrogen Peroxide and other oxidants via MAPKAP kinases 2 and 3. This phoryulation is an inflammable method. In many cell types and tissues, the HSP27 is expressed in unique ranges of development and differentiation [27].

In response to elevation of various stress conditions that deleterious to the cells like serum deficiency, oxidative stress, radiation or anti-cancer drugs and oxidative stress cause accumulation of lately stimulated HSP within the cells. Nonetheless, HSP27 expression is abnormally elevated and correlated to cell resistance against anticancer therapy in cancer cells. Another chaperonin identified as mammalian HSP60 is mostly included within matrix of mitochondria, also revealed in extra

sites of mitochondria including the cytosol [59]. Implying, HSP60 has essential role in the mitochondrial proteins folding and enables misfolded and/or denatured proteins amenable to proteolytic degradation with an ATP-dependent way.

HSP10 regulates the chaperone characteristic of HSP60, which links to HSP60 and regulates its substrate binding and the interest of ATPase. Two HSP10 molecules are binding in the presence of ADP with at least one HSP60. The newly imported mitochondrial proteins are seriously impaired by HSP10 inactivation [39]. Additionally, HSP60 and HSP10 don't function continuously as a single functional entity. The HSP70 family represents the highest preserved and best studied HSPs. It contains proteins from 66 to 78 kD, which can be encoded in humans as amulti-genes with at least 11 genes. Some are located especially in cytosols, such as the essentials of HSP 70 or HSP72, or in the constitutive HSC70, one in mitochondria (mtHSP70) and another in the endoplasmic reticular (GRP78/Bip). In addition, the cytosolic location of the mitochondria is important. Eukaryotic HSP70s contain two intended domains: the ATP-binding domain (ABD) of the NH2-terminal and the Peptide-binding Area (PBD) of the COOH-terminal.

HSP70 proteins, which are usually ATP-dependent molecular chaperones by helping fold newly synthesized polypeptides, meeting multi-protein complexes and delivery protein across cell membranes [33], in ordinary conditions, are co-chaperons of HSP70. The chaperone hobby of the protein can be blinded whether by PBD or ABD of HSP70. The synthesis of strain inducible HSP70 is induced quickly by several stimuli, including anticancer agents Studies of gene ablation show that inducible HSP70 plays a vital role in apoptosis. On the other hand, Mouse embryonic cells without 2 genes encoding the HSP70, hsp70.1 and hsp70.3 inducible are highly susceptible to apoptosis precipitated by a wide variety of lethal stimuli [28]. Furthermore, Disruption of HSP70 (hsp70.2) special testis results in germ cell apoptosis is another matter. HSP90and HSP90 are the main individuals of the HSP90 protein family, such two HSP90 isoforms are essential for eukaryotic cell viability. They are significantly abundant, account for 1–2% of cytosolic proteins and may be stimulated their expression level by stress [54].

HSP90 act as a friend of a variety of species by meaning associates with a number of signaling proteins including ligand-dependent transcription factors such as steroid receptors, Myoblast determination protein (MyoD) tyrosine kinases, Rous sarcoma virus (V-Src) included and serine/threonine kinases, which include rapidly accelerated fibrosarcoma-1 (Raf-1) Such proteins, known as HSP90 proteins, are assured with the aid of HSP90 for their stability. Chaperone inhibition contributes to the use of proteasome for its degradation. HSP90, for its chaperone function, binds to ATP and undergoes a conformational upon ATP binding. Co-chaperones of HSP90 include cell Division Cycle 37 (CDC37), p23, Activator of Hsp90 ATPase protein (Aha1), protein phosphatase (PP5), Hsp90-organizing protein (HOP) and C terminus of Hsc70-interacting protein (CHIP) [46].

## 3 Protein Folding and Turnover

To obtain the functional shape or conformation of protein structure, protein folding is needed. It starts when numerous molecular chaperones form transient complexes with protein substrates by hydrophobic interactions during protein synthesis in the ribosome [10]. Protein folding is performed by help of heat shock proteins under a thermodynamic process. HSPs can recognize hydrophobic amino acids of unfolded and incompletely folded proteins preventing aggregation of protein [26]. HSPs is not only responsible for quality of protein folding, also stress conditions is involved in disruption of protein folding resulting in protein misfolding then aggregation [57]. Several diseases such as Alzheimer disease, Parkinson disease, Huntington disease, Creutzfeldt–Jakob disease, or type 2 diabetes caused by protein aggregation. Also, aging and cancer are related to misfolding and aggregation of protein [27].

The protein quality control with different functions involving various groups of HSPs. Besides, folding nascent proteins and filling denatured proteins, HSP70 targets proteins for degradation if the protein cannot be adequately renatured. In addition, theBag-1 and CHIP co-chaperones helped HSP70 to degrade unfolded or misfolded proteins as shown in (Fig. 1). Alternatively, the cooperative HSP70/HSP40 system could degrade only small aggregates, but large one also required HSP104, a AAA-ATPase chaperone in the HSP110 family. Moreover, HSP104 has significant role in disaggregation of denatured proteins and thus could precisely participate in the protein turnover process. Similarly, HSP90 revealed several roles in the conformational regulation of numerous protein signaling [31].

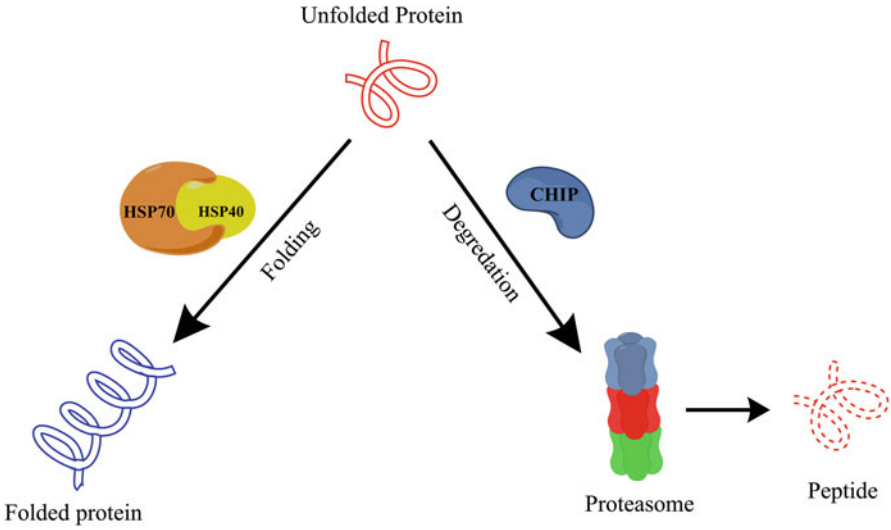



**Fig. 1** The involvement of heat shock proteins in protein turnover
Unfolded or misfolded proteins will be repaired or folded by cooperative HSP70/HSP40 binding chaperones system or switch to be degraded by the assistance of CHIP mediates ubiquitination trough the proteasome

## 4 Autophagy and HSPs

Autophagy is an intracellular system for degradation which supplies the lysosome with cytoplasmic components. This mechanism safeguards cells against several stress factors and is considered to play a pivotal role in stress prevention (Fig. 2). Hence, autophagy is implicated in the cell homeostasis as a catabolic process [51]. In mitochondrial autophagy for example, depletion of cytochrome c release and caspase-3 activation protect the cells from heat-induced apoptosis [63].

Mitochondria and endoplasmic reticulum involved in autophagy initiation by using the autophagy proteins (e.g., ATG5 and light chain-3 [LC3]). Modifications of autophagy-related genes were discovered in neurodegenerative and lysosomal storage disorders, and numerous types of cancer [21]. Mitophagy, mitochondrial autophagy, is considered as a selective form of autophagy, which is vital in preserving mitochondrial homeostasis. Recent studies revealed that Hsps involved in protection of NADH: ubiquinone oxidoreductase and NADH dehydrogenase activity under heat and oxidative stress in mitochondria [64]. Furthermore, the expression of HspB1 (HSP27) progressed during oxidative stress in acute kidney injury. Overexpression of HspB1 lowered BAX activation and $H_2O_2$-induced apoptosis and increasing of autophagic flux in renal tubular cells [42]. Mutations of mitochondrial DNA can cause some human neuromuscular diseases, including an inherited encephalomyopathy so-called as Myoclonus epilepsy associated with ragged-red fibers (MERRF). A substantial decrease in HSP27 has been observed, according to the findings, in patients with A8344 G mutation in lymphoblastoid and cytoplasmic hybrid (cybrid) cells in MERRF. An increased formation of 1A/1B-LC3-II microtubulate and autophagosomes has been found in the MERRF cybrids suggesting an autophagic pathway that is constitutively triggered. These data also

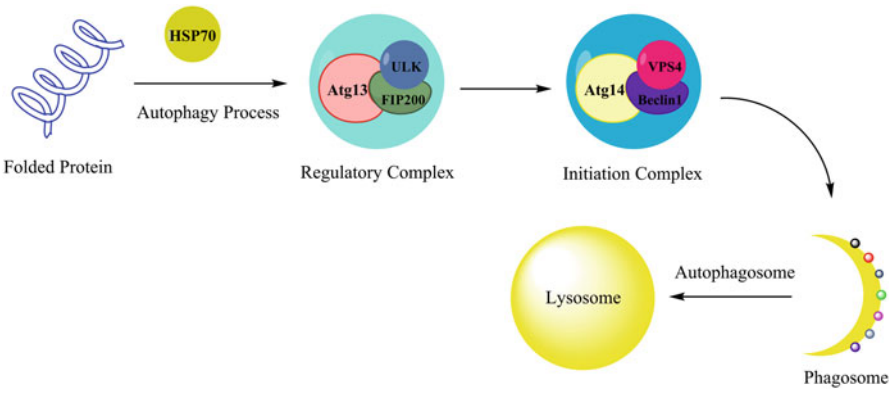



**Fig. 2** Autophagy pathway enhanced by HSP
Firstly, HSP70 binds to the protein and autophagy process begins; The regulatory complex contains ULK1, Atg13 and FIP200 activated by HSPs. Then, HSPs stabilize the initiation complex that composed of Beclin1, VPS4 and Atg14. After that, the phagosome markers collected and formed phagosome. Finally, the lysosome fuses with the completed autophagosome

reveal that the autophagic pathway can degrade HSP27 and indicate its quick turnover and protective function in MERRF cells. HSP27 and autophagic pathway regulation may thus be considered an effective approach for the treatment of MERRF syndrome [16]. The researchers noted that apoptosis could be accompanied by autophagy, increasing LC3 expression and the decreased pH value of Lysosome. Nicotinic-mycoepoxydien for example may not necessarily have an anti-tumor function, but control apoptosis and autophagy by inhibiting HSP90 [42]. Another research has shown that autophagy in a rat model contributed towards HSP72-mediated cytoprotective peritonitis in the case of lipopolysaccharide (LPS). Autophagy accompanied by apoptosis was the result of exposure of the cultured Peritoneal Mesothelial cells to LPS. The data showed that the antiapoptotic effect of HSP72 was decreased by inhibiting autophagy. HSP72 overexpression also boosted autophagy, inhibited apoptosis and attenuated peritoneal injury by phosphorylation of the JNK and by upregulation of Beclin1. Indeed, JNK suppression decreased HSP72 upregulation and autophagy mediated by Beclin-1. These findings suggested that HSP72 induction may be a possible peritonitis therapy [55].

## 5 Apoptosis and HSPs

Apoptosis is a normal physiological process of programmed cell death that leads to throwing cells into the body that may be unwanted or irreparably harmed now. It plays an important role in the development of embryo and the homeostasis of human tissue. Hence, Apoptosis deregulation has been established as a predictor of cancer progression by deactivating exact mechanisms, usually helping to cause apoptosis, most cancer cells can continue to survive and proliferate regardless of the genomic changes that cause apoptosis. The p53 tumor suppressor gene is often inactivated by cancer mutations and serves as a crucial channel in the cellular for the identification of harmful DNA and apoptosis initiation. The inactivation of p53 helps most cancer cells not to necessarily circumvent their underlying genomic aberrations by intrinsic apoptotic responses, but also by inducing apoptosis in response to the damage done to DNA by exposure to conventional medicine [36]. Therefore, agents that may restore apoptosis in cancer cells considered as a promising therapy and had been the point of interest of many preclinical drug discovery studies. Upon cytotoxic drug therapy, Apoptosis is also a highly common form of cell death.

In addition, Apoptosis is also a very common type of cytotoxic cell death after remediation. Apoptotic processes are defined in two ways: the intrinsic and Mitochondrial route, and the extrinsic or dying reception pathway, each mediates via their own familiar cysteine proteases known as caspases. Moreover, capase-3 identified as an effector protein that contributes to typical apoptotic cell morphological and biochemical changes.

The intrinsic pathway includes the production or activation by intracellular stress signals of pro-apoptotic molecules. Such molecules assembly on mitochondria to release mitochondrial apoptogenic factors under control of the Bcl-2 protein family.

Pro-apoptotic proteins Bcl-2 include anti-apoptotic components such as Bcl-2 and Bcl-xL; Bax, Bak, and a sequence of BH3 domain-only pro apoptotic proteins, along with Bid, which function upstream of Bax and Bok [15].

One of mitochondrial released molecules is cytochrome c, that interacts with apoptotic protease activating factor 1 (Apaf-1) and pro caspase 9 forms an apoptosome, the caspase3 activation complex. In particularly, other mitochondrial proteins (Smac/Diablo) and Htra2/Omi activate apoptosis by neutralization of apoptotic proteins (IAPs) inhibitory activity that support and suppress some of activated caspases.

The extrinsic apoptotic pathway is induced via the TNF receptor plasma membrane family proteins identified as death receptors and caused the direct receptor caspase-8 or 10 activation in the death-inducing signaling complex (DISC). Then, Caspase-8 either transforms into an active truncated form called tBid which connects the extrinsic to the intrinsic apoptotic routes via mitochondrial permeabilization directly onto the downstream cascade or Bid cleaves pathway [19].

Upon cytotoxic drug therapy, Apoptosis is also a highly common cell death type. The mitochondrial or intrinsic pathway and death receptor or the extrinsic pathway describe apoptotic processes, both of which are regulated by the cysteine proteases family known as caspases. Furthermore, Developmental signals or extreme cells including DNA or cytoskeletal damage are used to improve the intrinsic pathway by inducing the BH3-simplest BCL-2 proapoptotic circle of relative proteins to transcribe or activate it post-translationally. For instance, the p53 DNA damage activation results in transcriptional induction of the best proteins of BH3 "PUMA and NOXA" as showed in Fig. 3 [6].

In turn, these drive activation of other family members such as BAX and BAK. In normal healthy cells, antiapoptotic family members such as "BCL-2, BCL-XL", or myeloid cell leukemia sequence 1 (MCL-1) bind to proapoptotic members as "BAX and BAK" to prevent their activation. Only the BH3 proteins seize anti-apoptotic proteins after stimulation, thus allowing the action of BAX and BAK. Bothe of them might engage a mechanism to releasing mitochondrium factors that promote cytoplasmic caspase activation. One such factor is cytochrome c, which cooperates with Apaf-1 to activate caspase 9 that activates the effector caspases 3, 6, and 7, which carry out apoptosis.

Additionally, Mitochondria-derived caspases activator or low pI direct apoptosis binding protein inhibitor (SMAC/DIABLO) considered as another factor, which binds to inhibitor of apoptotic proteins and reversing their inhibition of several caspases cause augmenting apoptosis. Other point of view, while the Apo2L/TRAIL-associated apoptosis related to tumor necrosis factor inducible ligand binds to specific proapoptotic death membrane receptors as DR4 and DR5 thus stimulate the activation of the apoptotic extrinsic pathway [32].

On the other hand, each receptor might reform the DISC through recruiting pro-caspases forms "8 and 10" and the FADD (adapter death domain), which in turn undergoes self-processing and releases active caspase molecule through the cytoplasm. Then these enzymes undergo the cleavage process thereby effector caspases "3, 6, and 7" induced and eventually proceed apoptosis [34].

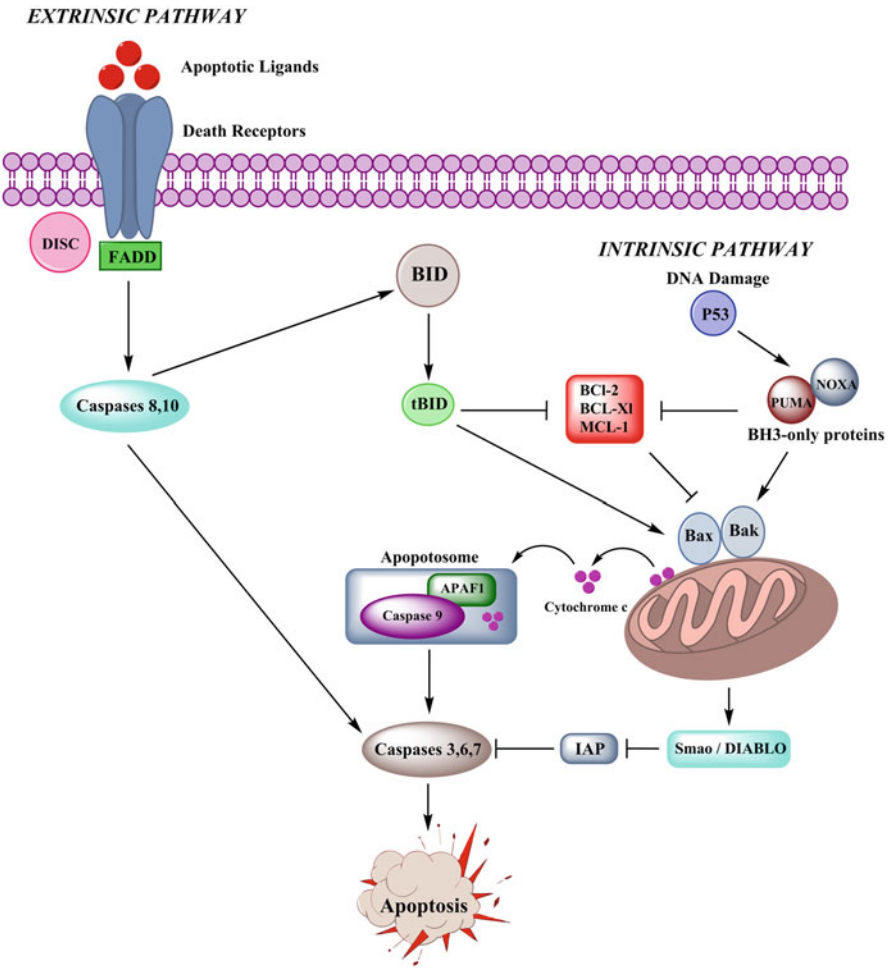



**Fig. 3** Schematic representation of the two major apoptotic pathways
The low levels of DISC formation in the cell, an amplification loop can recruit the intrinsic pathway, and appointment of mitochondria is mediated via caspase 8-dependent cleavage of the BH3-only protein Bid to its active form tBid. Furthermore, the intrinsic pathway induced by the BH3-simplest BCL-2 proapoptotic circle of relative proteins to transcribe or activate it post-translationally. For instance, the p53 DNA damage activation results in transcriptional induction of the best PUMA and NOXA proteins of BH3

The crosstalk between both the apoptotic two basic "intrinsic and extrinsic" pathways occur at the effector caspases levels. For example, in cells with low levels of DISC formation, an amplification loop can recruit the intrinsic pathway, and appointment of mitochondria attenuates caspase 8 activation and subsequent Bid cleavage to its active form tBid as described in Fig. 3. Previously study reported that The TRAIL/Apo2L pathway considered as a vital promising target for cancer

therapy because it appears to be intact and acts as p53-independently ways in many types of cancer cells. [5, 7].

Therefore, PARAs aka as Pro-apoptotic receptor agonists which target DR4 and/or DR5 have substantial therapeutic gain from destroying tumour cells that would be resistant and immunize to standard chemotherapeutic agents. Furthermore, in various preclinical models, PARA combination with chemotherapy has a synergistic antitumor effect, which can also provide a novel therapeutic treatment alternative to restore sensibility to chemotherapy.

Some of PARAs improved and investigated the potential ability of the extrinsic pathway to be the main cancer target. Apo2L/TRAIL that expressed as Recombinant human (rh) transmembrane protein identified as a soluble natural ligand dependent protein that activates both of DR4 and DR5 proapoptotic receptors. Furthermore, several monoclonal antibodies that activate either DR4 or DR5 have been developed [61], including the fully human DR5 agonistic antibody, so that, these PARAs induce selective apoptosis in cancer cells whilst detectably sparing normal cells.

Many different extrinsic and intrinsic inducible apoptotic proteins trigger the cells to produce stressed strain or HSPs. Also, it considered as a highly conserved sequence proteins that recognized in both of prokaryotes and eukaryotes, In addition, these proteins play an indispensable role as chaperones through refolding of unfolded nascent and accumulated stressed misfolded proteins and avoiding their aggregations, also these proteins have a protective role that allow the cells to survive else under lethal conditions. So that various insight mechanisms have been suggested to measure the HSPs cytoprotective functions.

A thorough understanding of several of HSPs proteins have shown their direct association with cell signaling pathways, as the tightly mediated caspases, dependent machinery programmed cell death, upstream, downstream, and at the mitochondrial levels. Also, these proteins can stimulate apoptosis caspase-independent pathway via the interaction with apoptogenic factors as AIF (apoptosis-inducing factor) or acting at lysosome level.

The Fullscope of HSPs cytoprotective effects to other cellular proteins that regarding to it conduct as molecular chaperones. Inappropriate activation of signaling pathways occurs during acute or chronic stress due to misfolding protein, protein aggregation or regulatory complex disturbances of signalers takes place at some point during acute or chronic strain. Chaperones are designed to restore equilibrium by using their protein homeostasis properties. The function of Chaperones according their properties in the proteostasis that is proposed to restore the balance.

The mammalian HSPs classified upon their size into high and small molecular weight, the first major groups include three mainly proteins families Hsp60,70,90 [48]. Some are constructively conveyed expressed, while others induced by stressful conditions. These proteins can be targeted to different subcellular compartments. High molecular weight HSPs are adenosine-5- triphosphate (ATP)-dependent chaperones and require co-chaperones to modulate their conformation and ATP binding. Otherwise, HSP27 as one of small HSPs defined as ATP-independent chaperones after it exposed to stressful conditions as stressful factors and anticancer or irradiation therapy, most of the specific HSPs, HSP27 and HSP70 are highly effective, both

of them are major expressed in cancer cells and thus proposed as important factors for prognosis in various malignant diseases [20].

On the other view side, HSPs was reported to inhibit or block apoptosis by interrupted with activation of caspase. Recently study of Seul et al., described that, the HSPs overexpression as "HSP27, HSP70, HSP60 or HSP90" caused block activation of caspases and apoptosis inhibition in many different cellular models depend on versatile cellular stresses, including misfolded proteins aggregation and accumulation, reactive oxygen species (ROS) or DNA damage. In contrast, the depletion or deficiency of these HSPs either by siRNA strategies or anti-sense constructions increases the sensitivity of cells in response to apoptotic stimuli [52].

In some cellular contexts, HSP70 depletion is adequate to promote apoptosis via caspase-3 activation, within the absence of any additional stressful stimulus. Therefore, HSPs are directly or indirectly engage in a various of caspase activities, also these proteins can block intrinsic as well as external apoptotic pathway by interacting on three levels with key proteins.: (*i*) mitochondrial upstream level, thus modulating signaling pathways; (*ii*) at the mitochondrial level, regulating apoptogenic molecules release and (*iii*) the post-mitochondrial level, with the aid of apoptosis blocking from a later phase than any known survival enhancing drug or protein [1].

## 6 Ubiquitination and HSPs

Ubiquitin (Ub)-proteasom pathway (UPP) degradation is the majore of the intracellular proteins in all tissues [14]. However, endocytoses and degradation within lysosomes are associated with extracellular proteins and certain surface cell proteins. Such organelles contain many proteases with optimum acidity, including cathepsins B, H, and D, and several more hydrolases of acid. After engulfing in autophagic vacuoles that fuse with lysosomes, certain cytosolic proteins are degraded in lysosomes [9]. Mostly, this process is activated by shortage of insulin or essential amino acids and in the glycolysis liver [24].

In mammalian cells, there are other cytosolic proteolytic system. Hence, The $Ca^{2+}$ activated (ATP-independent) proteolytic process involves the cysteine proteases termed calpains. These proteases stimulated when the cells are damaged and Ca2+ rises in cytoplasm, so they might play vital role in cell damage, autolysis and necrosis [23]. However, the caspases are recognized as a main cytosolic family protease, and after the residues of aspartic acid, cleave protein. Such enzymes are cysteine proteases which are important for cell destruction during apoptosis [50].

Recently, the protein degradation and ubiquitination were established as more mechanisms for modifying synapses structure and molecular composition. Such latest results and findings also intriguingly pose questions about how structural synaptic shift can be accomplished in the face of high molecular turnover. Whereas, the turnover of proteins is additionally induced by changes in cell usage pattern, also the physical activity that sustained to decreases or increases cause the cell to cope

and adapt, altering protein expression. The adaptive responses outcome implying the net balance between protein synthesis and protein degradation.

## 7 How Can a Cell Distinguish Proteins That Are Meant for Degradation?

### *7.1 Quality Control Mechanism*

As known the proteins have major and essential functions in cells such as signaling, transport, catalysis, membrane fusion, cell protection, and regulation, after ribosomal synthesis, proteins have to gain their three-dimensional structure to be functionable [2]. Also, Proteins are the molecules most sensitive to cellular stress conditions, these conditions may occurred to the cells during the life such as heat, oxidative stress, inflammation, irradiation, and heavy metals or other toxic compounds, which considered as the one causes of protein unfolding, nonspecific aggregation, and imbalance in protein homeostasis [29]. The balance between the synthesis, folding, and degradation of proteins controls the proteome function [4], to keep this balance there are two mainly pathways are: (i) the cellular machinery degradation pathway, which targets proteins for proteolysis, and (ii) molecular chaperones pathway, which prevent aggregation and ensure the folding of proteins to their native state [17].

Furthermore, Ubiquitin (Ub)-proteasome pathway (UPP) selectively eliminates unfolded, misfolded or disturbed proteins that have arisen by mis or non-sense mutations, biosynthetic errors, or damage by denaturation (especially at high temperatures) or reactive oxygen radicals. For example, In cystic fibrosis, the mutant form of the conductance regulator transmembrane protein (CFTR) is selectively degraded and therefore fails to reach the cell surface [62]. Because of the Ub degradation and conjugation machinery pathways are cytoplasmic, the destruction of CFTR revealed that the unfolded protein response (UPP) degrades misfolded or secreted proteins. In the machinery process of ER-associated degradation, many misfolded proteins inside the ER are retro-translocated to cytosol compartment by a sequence of ER membrane-related Ub conjugating proteins from this compartment, and then target cytosolic proteasomes [43].

## 8 Ubiquitination and Membrane Protein Turnover: Proteasomal Degradation Mechanisms

The ubiquitin-proteasome system (UPS) is mainly involve of E3 ligases and deubiquitinating enzymes (DUBs) that considered as the key regulator of the apoptosis process by controlling of the pro or antiapoptotic proteins and transcribe

the cell survival vs. death The UPS, which symbolizes the major pathway for extra cellular lysosomal protein degradation, that allow instability of metabolic conditions on a protein by temporary control and selective degradation.

At this phase, protein substrates are firstly tagged via the covalently binding of unique or multiple moieties of ubiquitin then the tagged proteins get degraded ultimately by the 26S proteasome, otherwise eventually their component amino acids recycled. Over the years many thoughts, the general definition of proteasomal degradation was the recognition of polyubiquitination chain that consist of four or more ubiquitin bounded to substrate protein through the proteasome [56], but most of studies proposes that even monoubiquitination or multi-monoubiquitination is suitable to degrade small substrates in range between 20 and 150 residues [45]. Protein ubiquitination and degradation is a three-cascade mechanism as explained in (Fig. 4).

Whereas, The Ubiquitin proteasome pathway (UPP) that consist of concerted enzymes actions that connect the chains of the co-factor polypeptide, Ub, targeted proteins to tagged them for degradation.This tagging pathway leads to their reorganization through proteasome complex that degrades these ubiquitinated proteins [22]. Three enzymatic set compartments are required to tagging the chains of Ub

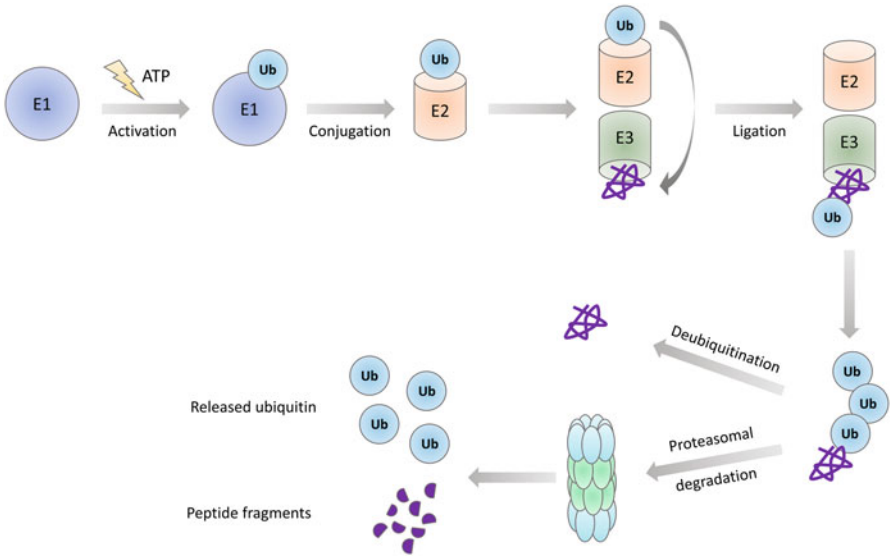



**Fig. 4** (**a**, **b**) The ubiquitin proteasome system (UPS)
UPS system as an ATP dependent reaction is regulated by a set of E1, E2, and E3 enzymes, which induce a ubiquitin molecule ligation o the lysine residues of the protein substrates. Ub is activated by the E1 "ubiquitin activating enzyme", then conjugated to protein target by consecutive action of E2 "ubiquitin conjugating enzymes" and E3 "ubiquitin ligases", the multiubiquitin chains forms, attached and recruits the proteins targeted to the 26S proteasome for the degradation. This process is reversing by the deubiquitinating enzymes activity (DUBs), that cleave ubiquitin molecules from proteins and facilitate the free ubiquitin monomers recycling the ubiquitin proteasome pathway

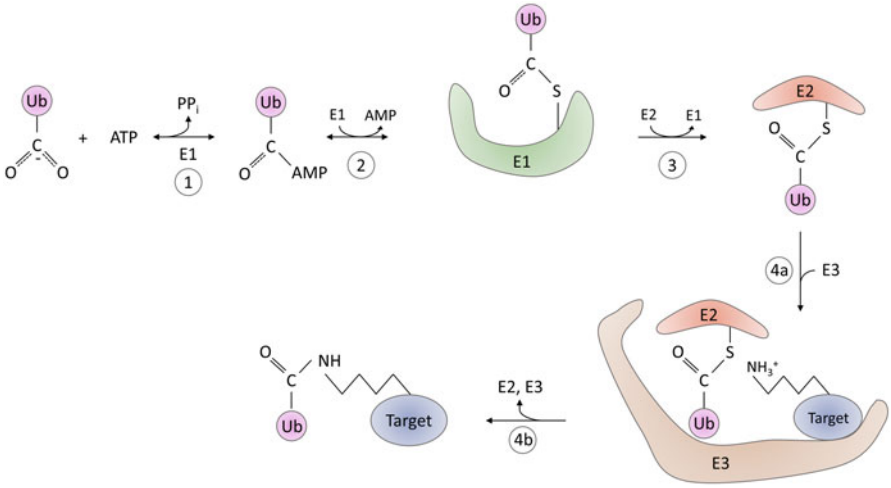



**Fig. 5** The ubiquitin-protein substrate conjugation biochemical pathway
The Ub-Protein conjugation complexes formed in ATP dependent manner, this process is repeated till Ub molecules chains is attached in dependent process, requiring three distinct enzymes. First, E1-activates Ub. Next, the active form of Ub is moved to the cysteine residue in the active site of E2- Ub conjugating enzyme. Lastly, E3-ligase allows conjugation of Ub to the substrate. Ub attached to the substrate can itself become a substrate for ubiquitination, resulting in the formation of ubiquitin chains (referred to as poly-ubiquitination). Such a Ub chain target protein substrate for proteasomal degradation.

onto proteins that are fated for degradation. E1 (Ub-activating enzyme) and E2s (Ub-carrier or conjugating pro- teins) prepare Ub for conjugation, but the vital key enzyme in the process is the E3 (Ub-protein ligase), because it identifies a specific protein substrate and catalyzes the transfer of activated Ub to it mechanism as showed in (Fig. 5).

Science the first description of UPP as a protein degradation and tagging mechanism, with various proteins exhibited to be degraded by the additionally novel functions for Ub conjugation system that being uncovered. As mean, extremely stressful conditions that cause the mitochondrial proteins denaturation that exceed the level of repair systems of chaperone capacity. Because the respiratory chain complexes close to inner mitochondrial membrane proteins, which are responsible for ROS generation, they are seemlier to be damaged by oxidative stress and to form aggregates [37]. So that, many cell types contain special quality control systems located in the inner membrane consisting of specialized chaperones. The ATPases are linked to various cellular activity HSP 100/peptidase caseinolytic-subfamily that are responsible for aggregates reactivation and are important to thermotolerance. They are recognized to co-bind with HSP70, cause unfolding protein catalyzing, disaggregation and disassembly in plants, bacteria and fungi [11].

Otherwise, Mutation Proteasome studies have shown the ubiquitin/proteasome system as its primary function in the removal of misfolded proteins, with substantial degradation and ubiquitinated protein accumulation due to the induction of canavanine protein misfolding [12]. Other story provided evidence the 20S core

proteasome not the 26S proteasome, which dissociated from the 26S proteasome, considered as the mostly responsible for the degradation of oxidized intracellular proteins. Also, this reported that HSP70 mediates dissociation and reassociation of the 26S proteasome during adaptation to oxidative stress, the activation of the nuclear 20S proteasome by poly (ADP ribose) polymerase (PARP) that has a substantial increase in proteolytic ability, thereby preventing oxidized proteins from being aggregated and accumulated.

Contradictory, The unbalance of 26S proteasome system cause severe cell metabolism problems because Ubiquitin–26S proteasome affects various processes, such as the transcription, translation, and turnover of some 100 shock/stress proteins [25]. Ultimately, it becomes clear that the 20S proteasome is deliberately de novor synthesized in order to retain oxidized protein removement when the 26S proteasome is re-constituted, HSP90 demands ATP for its in vivo functions as the 26S proteasome due to it act as an abundant molecular chaperone that indispensable for the establishment of many cellular regulation and signal transduction systems, Whether a protein is stabilized and refolded or is ubiquitinated and degraded by the proteasome depends on the binding of different cochaperones and the degree of the damage [30]. As Known, Both of proteasome systems and HSP90 are considered to play roles in protein degradation, On the other side, HSP90 can inhibit degradation of proteasomes through the inhibition of the Z-Leu-Leu-Leu-MCA degrading activity of the proteasome, this inhibition results in increased ubiquitin-dependent proteasomal degradation [47]. The explanation of this phenomena may be due the stabilization of client proteins that facilitated by HSP90. Consequently, Inhibition of HSP90 by geldanamycin and other derivatives results in destabilization, leading to the ubiquitination and proteasomal degradation of the client proteins [13]. This hypothesis CFTR protein was also demonstrated by a study on the CFTR protein [8].

Meanwhile, other study exhibited that approximately complete dissociation of 26S proteasome into its components is instigated via inactivation of HSP90 in yeast (*in vivo),* It was demonstrated that this dissociation was reversed based on HSP90 and ATP hydrolysis [41]. Additionally, the importance of HSP90 in the integrity of the 26S proteasome confirmed by the genetic link of various proteasomal Rpn (regulatory particle non-ATPase subunit) genes with HSP90 role. Other theories revealed that highly expression of HSP70 in most cancer cells occurred due to inhibition of HSP90, also it known as CHIP inhibition cause degradation reduction of HSP90 client protein, whereas CHIP over-expression enhances their degradation [38] as described in (Fig. 6). The mechanism of this study as a while when the reduction of HSP90 activity resulted in stabilization of client proteins by HSP70, then ubiquitinated by CHIP and degraded by the proteasome, so that client protein accumulated and aggregated if HSP70 inhibition occurred, furthermore, this inhibition impairs the interaction between CHIP and HSP90, which has a negatively effects on the folding or the stabilization of HSP90 client proteins [60].

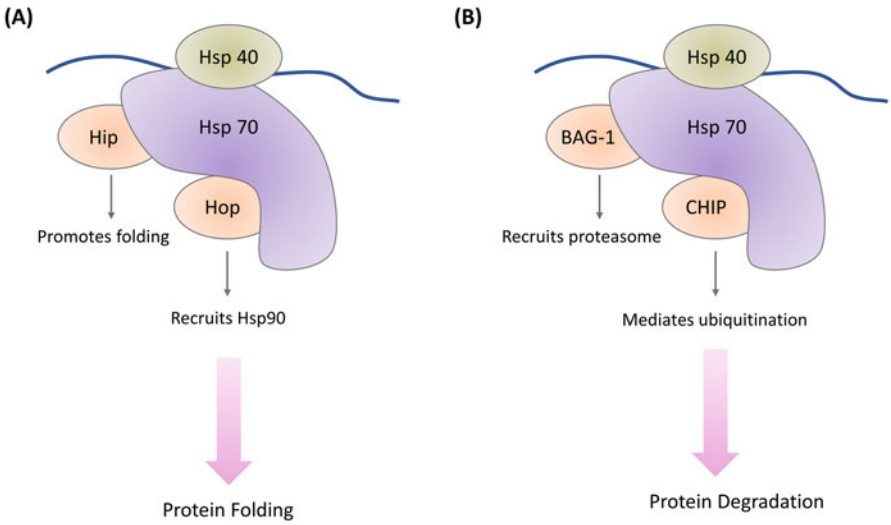



**Fig. 6** Role of HSP70 chaperone system in the folding and the degradation machinery
The chaperone system of HSP70 that controlled the folding and degradation of proteins. The fate of the unfolded/misfolded protein was decided via the binding of different cochaperones. ATPase domain bind to BAG-1 or Hip bind, and C terminal of HSP70 bind to Hop and CHIP. In case of folding machinery, Hop recruits HSP90 and Hip induces folding (**a**). Otherwise, CHIP facilitates ubiquitination and BAG-1 recruits the proteasome in the case of degradation machinery (**b**)

## 9 Conclusion

In this chapter, it will be intriguing to reveal associated alterations in the modes of cellular proteasomal, autophagy and apoptosis, with the scope of mechanistic insights in their functional articulation as well as potential new targets for therapeutic intervention, Whether the ubiquitination system has degradation fate of misfolded by the proteasomal or apoptotic and autophagic system.

**Acknowledgements** We apologize to all researchers whose crucial contributions in the field of Protein quality control system and Heat-shock proteins were not referenced in this review, due to limitations in space.

**Disclosure of Interests** The authors declare they have no conflict of interest regarding financial and/or personal relationships with other people or organizations that could inappropriately influence (bias) this work.

**Compliance with Ethical Standards** The author(s) received no specific funding for this work.

This article does not contain any studies with human participants and/or animals performed by any of the authors.

## References


1. Aghdassi A, Phillips P, Dudeja V, Dhaulakhandi D, Sharif R, Dawra R, Lerch MM, Saluja A (2007) Heat shock protein 70 increases tumorigenicity and inhibits apoptosis in pancreatic adenocarcinoma. Cancer Res 67(2):616–625. https://doi.org/10.1158/0008-5472.CAN-06-1567
2. Amm I, Sommer T, Wolf DH (2014a) Protein quality control and elimination of protein waste: the role of the ubiquitin-proteasome system. Biochim Biophys Acta 1843(1):182–196. https://doi.org/10.1016/j.bbamcr.2013.06.031
3. Amm I, Sommer T, Wolf DH (2014b) Protein quality control and elimination of protein waste: the role of the ubiquitin-proteasome system. Biochim Biophys Acta Mol Cell Res 1843 (1):182–196. https://doi.org/10.1016/j.bbamcr.2013.06.031
4. Asano J, Sato T, Ichinose S, Kajita M, Onai N, Shimizu S, Ohteki T (2017) Intrinsic autophagy is required for the maintenance of intestinal stem cells and for irradiation-induced intestinal regeneration. Cell Rep 20(5):1050–1060. https://doi.org/10.1016/j.celrep.2017.07.019
5. Ashkenazi A (2008) Targeting the extrinsic apoptosis pathway in cancer. Cytokine Growth Factor Rev 19(3–4):325–331. https://doi.org/10.1016/j.cytogfr.2008.04.001
6. Ashkenazi A, Dixit VM (1999) Apoptosis control by death and decoy receptors. Curr Opin Cell Biol 11(2):255–260. https://doi.org/10.1016/S0955-0674(99)80034-9
7. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, Blackie C, Chang L, McMurtrey AE, Hebert A, DeForge L, Koumenis IL, Lewis D, Harris L, Bussiere J, Koeppen H, Shahrokh Z, Schwall RH (1999) Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Investig 104(2):155–162. https://doi.org/10.1172/JCI6926
8. Baaklini I, de Gonçalves CC, Lukacs GL, Young JC (2020) Selective binding of HSC70 and its co-chaperones to structural hotspots on CFTR. Sci Rep 10(1):4176. https://doi.org/10.1038/s41598-020-61107-x
9. Baehrecke EH (2005) Autophagy: dual roles in life and death? Nat Rev Mol Cell Biol 6 (6):505–510. https://doi.org/10.1038/nrm1666
10. Bartlett AI, Radford SE (2009) An expanding arsenal of experimental methods yields an explosion of insights into protein folding mechanisms. Nat Struct Mol Biol 16(6):582–588. https://doi.org/10.1038/nsmb.1592
11. Bender T, Lewrenz I, Franken S, Baitzel C, Voos W (2011a) Mitochondrial enzymes are protected from stress-induced aggregation by mitochondrial chaperones and the Pim1/LON protease. Mol Biol Cell 22(5):541–554. https://doi.org/10.1091/mbc.E10-08-0718
12. Bender T, Lewrenz I, Franken S, Baitzel C, Voos W (2011b) Mitochondrial enzymes are protected from stress-induced aggregation by mitochondrial chaperones and the Pim1/LON protease. Mol Biol Cell 22(5):541–554. https://doi.org/10.1091/mbc.E10-08-0718
13. Blagg BSJ, Kerr TD (2006) Hsp90 inhibitors: small molecules that transform the Hsp90 protein folding machinery into a catalyst for protein degradation. Med Res Rev 26(3):310–338. https://doi.org/10.1002/med.20052
14. Bozaykut P, Ozer NK, Karademir B (2014) Regulation of protein turnover by heat shock proteins. Free Radic Biol Med 77:195–209. https://doi.org/10.1016/j.freeradbiomed.2014.08.012. Elsevier Inc
15. Breckenridge DG, Xue D (2004) Regulation of mitochondrial membrane permeabilization by BCL-2 family proteins and caspases. Curr Opin Cell Biol 16(6):647–652. https://doi.org/10.1016/j.ceb.2004.09.009
16. Chen CY, Chen HF, Gi SJ, Chi TH, Cheng CK, Hsu CF, Ma YS, Wei YH, Liu CS, Hsieh M (2011) Decreased heat shock protein 27 expression and altered autophagy in human cells harboring A8344G mitochondrial DNA mutation. Mitochondrion 11(5):739–749. https://doi.org/10.1016/j.mito.2011.05.014
17. Cyr DM, Höhfeld J, Patterson C (2002) Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. Trends Biochem Sci 27(7):368–375. https://doi.org/10.1016/S0968-0004(02)02125-4

18. Doyle SM, Genest O, Wickner S (2013) Protein rescue from aggregates by powerful molecular chaperone machines. Nat Rev Mol Cell Biol 14(10):617–629. https://doi.org/10.1038/nrm3660
19. Du C, Fang M, Li Y, Li L, Wang X (2000) Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 102(1):33–42. https://doi.org/10.1016/S0092-8674(00)00008-8
20. Garrido C (2002) Size matters: of the small HSP27 and its large oligomers. Cell Death Differ 9 (5):483–485. https://doi.org/10.1038/sj.cdd.4401005. Nature Publishing Group
21. Ghavami S, Shojaei S, Yeganeh B, Ande SR, Jangamreddy JR, Mehrpour M, Christoffersson J, Chaabane W, Moghadam AR, Kashani HH, Hashemi M, Owji AA, Łos MJ (2014) Autophagy and apoptosis dysfunction in neurodegenerative disorders. Prog Neurobiol 112:24–49. https://doi.org/10.1016/j.pneurobio.2013.10.004. Elsevier Ltd
22. Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82(2):373–428. https://doi.org/10.1152/physrev.00027.2001. American Physiological Society
23. Goll DE, Thompson VF, Li H, Wei W, Cong J (2003) The calpain system. Physiol Rev 83 (3):731–801. https://doi.org/10.1152/physrev.00029.2002
24. Gronostajski RM, Goldberg AL, Pardee AB (1984) The role of increased proteolysis in the atrophy and arrest of proliferation in serum-deprived fibroblasts. J Cell Physiol 121(1):189–198. https://doi.org/10.1002/jcp.1041210124
25. Grune T, Catalgol B, Licht A, Ermak G, Pickering AM, Ngo JK, Davies KJA (2011) HSP70 mediates dissociation and reassociation of the 26S proteasome during adaptation to oxidative stress. Free Radic Biol Med 51(7):1355–1364. https://doi.org/10.1016/j.freeradbiomed.2011.06.015
26. Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381(6583):571–580. https://doi.org/10.1038/381571a0
27. Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475(7356):324–332. https://doi.org/10.1038/nature10317
28. Huang L, Mivechi NF, Moskophidis D (2001) Insights into regulation and function of the major stress-induced hsp70 molecular chaperone in vivo: analysis of mice with targeted gene disruption of the hsp70.1 or hsp70.3 gene. Mol Cell Biol 21(24):8575–8591. https://doi.org/10.1128/mcb.21.24.8575-8591.2001
29. Kästle M, Grune T (2012a) Interactions of the proteasomal system with chaperones: protein triage and protein quality control. Prog Mol Biol Transl Sci 109:113–160. https://doi.org/10.1016/B978-0-12-397863-9.00004-3
30. Kästle M, Grune T (2012b) Interactions of the proteasomal system with chaperones: protein triage and protein quality control. Prog Mol Biol Transl Sci 109:113–160. https://doi.org/10.1016/B978-0-12-397863-9.00004-3
31. Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Ulrich HF (2013) Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 82(1):323–355. https://doi.org/10.1146/annurev-biochem-060208-092442
32. Kischkel FC, Lawrence DA, Chuntharapai A, Schow P, Kim KJ, Ashkenazi A (2000) Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5. Immunity 12(6):611–620. https://doi.org/10.1016/S1074-7613(00)80212-5
33. Kumar SJ, Stokes J, Singh UP, Scissum Gunn K, Acharya A, Manne U, Mishra M (2016) Targeting Hsp70: a possible therapy for cancer. Cancer Lett 374(1):156–166. https://doi.org/10.1016/j.canlet.2016.01.056. Elsevier Ireland Ltd
34. Lavrik IN, Golks A, Krammer PH (2005) Caspase: pharmacological manipulation of cell death. J Clin Invest 115(10):2665–2672. https://doi.org/10.1172/JCI26252
35. Lee C-C, Lin T-W, Ko T-P, Wang AH-J (2011) The Hexameric structures of human heat shock protein 90. PLoS One 6(5):e19961. https://doi.org/10.1371/journal.pone.0019961
36. Lee JM, Bernstein A (1995) Apoptosis, cancer and the p53 tumour suppressor gene. Cancer Metastasis Rev 14(2):149–161. https://doi.org/10.1007/BF00665797

37. Lee S, Sowa ME, Choi J-M, Tsai FTF (2004) The ClpB/Hsp104 molecular chaperone-a protein disaggregating machine. J Struct Biol 146(1–2):99–105. https://doi.org/10.1016/j.jsb.2003.11.016
38. Leu JIJ, Pimkina J, Pandey P, Murphy ME, George DL (2011) HSP70 inhibition by the small-molecule 2-phenylethynesulfonamide impairs protein clearance pathways in tumor cells. Mol Cancer Res 9(7):936–947. https://doi.org/10.1158/1541-7786.MCR-11-0019
39. Li CY, Lee JS, Ko YG, Kim JI, Seo JS (2000) Heat shock protein 70 inhibits apoptosis downstream of cytochrome c release and upstream of caspase-3 activation. J Biol Chem 275 (33):25665–25671. https://doi.org/10.1074/jbc.M906383199
40. Mariño G, Niso-Santano M, Baehrecke EH, Kroemer G (2014) Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 15(2):81–94. https://doi.org/10.1038/nrm3735. Nature Publishing Group
41. Marshall RS, Vierstra RD (2019) Dynamic regulation of the 26S proteasome: from synthesis to degradation. Front Mol Biosci 6:40. https://doi.org/10.3389/fmolb.2019.00040. Frontiers Media S.A
42. Matsumoto T, Urushido M, Ide H, Ishihara M, Hamada-Ode K, Shimamura Y, Ogata K, Inoue K, Taniguchi Y, Taguchi T, Horino T, Fujimoto S, Terada Y (2015) Small heat shock protein beta-1 (HSPB1) is upregulated and regulates autophagy and apoptosis of renal tubular cells in acute kidney injury. PLoS One 10(5):e0126229. https://doi.org/10.1371/journal.pone.0126229
43. Meusser B, Hirsch C, Jarosch E, Sommer T (2005) ERAD: the long road to destruction. Nat Cell Biol 7(8):766–772. https://doi.org/10.1038/ncb0805-766
44. Mitch WE, Goldberg AL (1996) Mechanisms of disease: mechanisms of muscle wasting: the role of the ubiquitin-proteasome pathway. N Engl J Med 335(25):1897–1905. https://doi.org/10.1056/NEJM199612193352507
45. Nakagawa T, Nakayama K (2015) Protein monoubiquitylation: targets and diverse functions. Genes Cells 20(7):543–562. https://doi.org/10.1111/gtc.12250. Blackwell Publishing Ltd
46. Nathan DF, Lindquist S (1995) Mutational analysis of Hsp90 function: interactions with a steroid receptor and a protein kinase. Mol Cell Biol 15(7):3917–3925. https://doi.org/10.1128/mcb.15.7.3917
47. Panaretou B, Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1998) ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. EMBO J 17(16):4829–4836. https://doi.org/10.1093/emboj/17.16.4829
48. Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C (2003) Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochem Biophys Res Commun 304(3):505–512. https://doi.org/10.1016/S0006-291X(03)00623-5
49. Ranford JC, Coates ARM, Henderson B (2000) Chaperonins are cell-signalling proteins: the unfolding biology of molecular chaperones. Expert Rev Mol Med 2(8):1–17. https://doi.org/10.1017/S1462399400002015
50. Salvesen GS, Dixit VM (1997) Caspases: intracellular signaling by proteolysis. Cell 91 (4):443–446. https://doi.org/10.1016/S0092-8674(00)80430-4. Cell Press
51. Santiago-O'Farrill JM, Kleinerman ES, Hollomon MG, Livingston A, Wang WL, Tsai JW, Gordon NB (2018) Phosphorylated heat shock protein 27 as a potential biomarker to predict the role of chemotherapy-induced autophagy in osteosarcoma response to therapy. Oncotarget *9* (2):1602–1616. https://doi.org/10.18632/oncotarget.20308
52. Seul-Ki C, Kam H, Kye-Young K, In Park S, Yun-Sil L (2019) Targeting heat shock protein 27 in cancer: a druggable target for cancer treatment? Cancers 11(8). https://doi.org/10.3390/cancers11081195. MDPI AG
53. Sharma D, Masison D (2009) Hsp70 structure, function, regulation and influence on yeast prions. Protein Pept Lett 16(6):571–581. https://doi.org/10.2174/092986609788490230
54. Sreedhar AS, Kalmár É, Csermely P, Shen YF (2004) Hsp90 isoforms: functions, expression and clinical importance. FEBS Lett 562(1–3):11–15. https://doi.org/10.1016/S0014-5793(04)00229-7

55. Sun Y, Xiao S, Chen J, Wang M, Zheng Z, Song S, Zhang L (2015) Heat shock protein 90 mediates the apoptosis and autophage in nicotinic-mycoepoxydiene-treated HeLa cells. Acta Biochim Biophys Sin 47(6):451–458. https://doi.org/10.1093/abbs/gmv034
56. Thrower JS, Hoffman L, Rechsteiner M, Pickart CM (2000) Recognition of the polyubiquitin proteolytic signal. EMBO J 19(1):94–102. https://doi.org/10.1093/emboj/19.1.94
57. Tyedmers J, Mogk A, Bukau B (2010) Cellular strategies for controlling protein aggregation. Nat Rev Mol Cell Biol 11(11):777–788. https://doi.org/10.1038/nrm2993
58. Sudnitsyna MV, Mymrikov EV, Seit-Nebi AS, Gusev NB (2012) The role of intrinsically disordered regions in the structure and functioning of small heat shock proteins. Curr Protein Pept Sci 13(1):76–85. https://doi.org/10.2174/138920312799277875
59. Vargas-Parada L, Solís CF, Laclette JP (2001) Heat shock and stress response of Taenia solium and T. crassiceps (Cestoda). Parasitology 122(5):583–588. https://doi.org/10.1017/S0031182001007764
60. Wang AM, Morishima Y, Clapp KM, Peng HM, Pratt WB, Gestwicki JE, Osawa Y, Lieberman AP (2010) Inhibition of Hsp70 by methylene blue affects signaling protein function and ubiquitination and modulates polyglutamine protein degradation. J Biol Chem 285 (21):15714–15723. https://doi.org/10.1074/jbc.M109.098806
61. Wang X, Chen M, Zhou J, Zhang X (2014) HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy (review). Int J Oncol 45(1):18–30. https://doi.org/10.3892/ijo.2014.2399. Spandidos Publications
62. Ward CL, Omura S, Kopito RR (1995) Degradation of CFTR by the ubiquitin-proteasome pathway. Cell 83:121–127
63. Yang Y, Xing D, Zhou F, Chen Q (2010) Mitochondrial autophagy protects against heat shock-induced apoptosis through reducing cytosolic cytochrome c release and downstream caspase-3 activation. Biochem Biophys Res Commun 395(2):190–195. https://doi.org/10.1016/j.bbrc.2010.03.155
64. Zeng L, Tan J, Lu W, Lu T, Hu Z (2013) The potential role of small heat shock proteins in mitochondria. Cell Signal 25(11):2312–2319. https://doi.org/10.1016/j.cellsig.2013.07.027

# Heat Shock Proteins in *Leishmania* Parasites


**Constanze Kröber-Boncardo, Janne Grünebast, and Joachim Clos**

## Abstract

**Introduction** Parasites of the genus *Leishmania* (Class Kinetoplastea, Order Trypanosomatida, Family Trypanosomatidae) cause a variety of clinical syndromes from localised, self-healing skin lesions to generalised, progressive and lethal systemic infections. They are early branching eukaryotes that lack gene-specific transcription regulation, RNA polymerase II promoters and trans acting transcription factors, and rely instead mostly on regulated translation and gene copy number variations for short- and long-term gene expression control.

**Methods** The authors reviewed the literature about the roles and functions of heat shock proteins in *Leishmania* spp.

**Results** *Leishmania* spp. possess a full complement of molecular chaperones, which play crucial roles in both parts of the biphasic, parasitic life cycle, having an impact on the temperature-induced differentiation from the insect stage to the mammalian stage, but also on the intracellular survival within mammalian hosts. They are part of the signal transduction pathways regulating life cycle stage conversion and subject to modulation by protein kinases. Heat shock proteins are also implicated in the immune response to *Leishmania* infections, the modulation of the host's immune system and in the resistance of the parasites against chemotherapy.

**Conclusions** Heat shock proteins play pivotal roles in the control of the parasitic life cycle and in the survival within the mammalian hosts.

Constanze Kröber-Boncardo, Janne Grünebast and Joachim Clos contributed equally with all other contributors.

C. Kröber-Boncardo · J. Grünebast · J. Clos (✉)
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
e-mail: clos@bnitm.de

Heat Shock Proteins (2021) 22: 469–488
https://doi.org/10.1007/7515_2020_27

Published Online: 9 September 2020

**Keywords** Amastigote · Exosomes · HSP100 · HSP23 · HSP90 · *Leishmania*

# 1 Introduction

## *1.1 Gene Regulation in* Leishmania spp.*: A Farewell to Promoters*

The genus *Leishmania* is part of the family Trypanosomatidae, Order Trypanosomatida, and part of the early branching, eukaryotic phylum Euglenozoa. Due to some peculiar biochemical features, they garnered the attention of molecular biologists early on. Processes such as trans-splicing and RNA editing were first described in *Trypanosoma brucei* and are found in all the kinetoplastida [1].

Also common to the members of this order is a lack of gene-specific transcription regulation [2]. There are no gene promoters in the strict sense in *Leishmania*, and the genome projects of numerous Trypanosomatida species (e.g. *L. major, L. infantum, L. braziliensis, Trypanosoma brucei,* and *Trypanosoma cruzi*) did not yield any genes for common trans-regulatory factors of transcription. Rather, large chromosomal regions are transcribed as multicistronic precursor RNA which is subject to trans-splicing coupled to polyadenylation to create mature, monocistronic mRNA [3]. This mode of transcription alone effectively precludes gene-specific transcription control. Recent work indicates a strong role for regulated translation for ad-hoc regulation of gene expression [4, 5], but also mosaic aneuploidy and gene copy number variations for intermediate and long-term adaption to the parasite's environments [6–12].

## *1.2* Leishmania *Life Cycle and Pathogeny*

*Leishmania* infections are major health problems in large parts of the world, and they are rated among the most important poverty-related diseases. Over 10 million humans are infected with various *Leishmania* species, with up to 1.4 million new infections per year [13]. Almost 1 billion people in 88 endemic countries are at risk of an infection [14].

There are three types of *Leishmania* infections, depending on the infecting species: (i) localised, self-healing cutaneous lesions (Oriental sore, Fig. 1a) are caused by Old World parasite species, such as *L. major* and *L. tropica,* and, in South America, by *L. mexicana, L. amazonensis, L. panamensis, L. (Viannia) braziliensis, L. (Viannia) guyanensis,* and *L. (Viannia) peruviana*, to name the most important; (ii) mucocutaneous lesions (Fig. 1b) of the nasopharyngeal region which are mostly caused by the South American parasite *L. (Viannia) braziliensis*; and (iii) generalised infections (visceral leishmaniasis or Kala Azar, Fig. 1c) caused

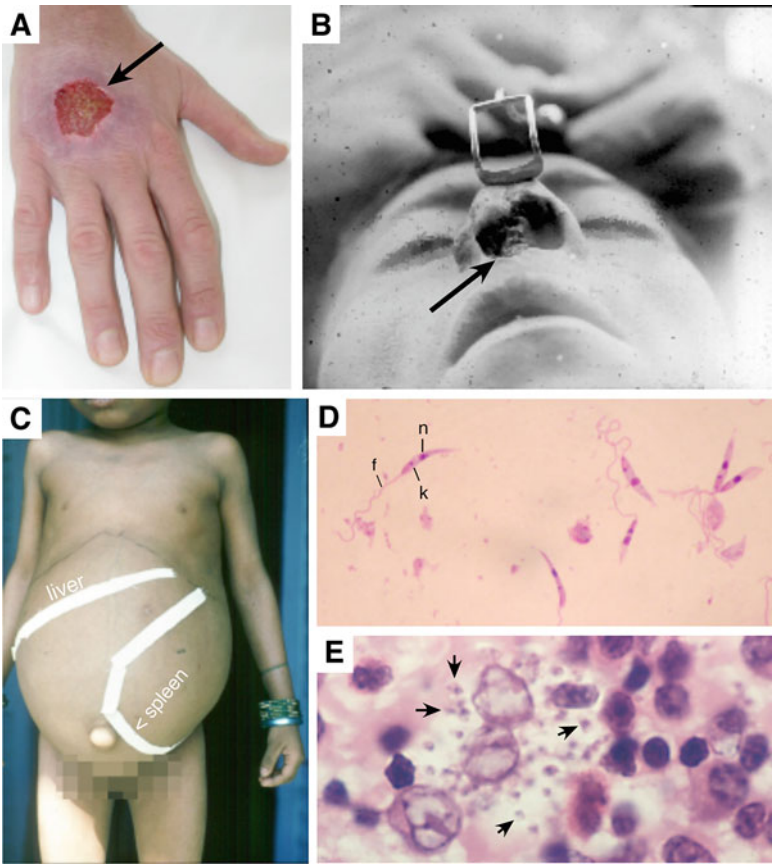



**Fig. 1** (**a**) Cutaneous leishmaniasis, CL, caused by *L. braziliensis*; (**b**) mucocutaneous leishmaniasis, MCL, caused by *L. braziliensis*; (**c**) advanced visceral leishmaniasis, VL, or Kala-Azar, the tape marks the extent of spleen and liver enlargement (splenomegaly, hepatomegaly); (**d**) *L. major* promastigotes from culture, Giemsa staining, 100X; (**e**) amastigotes (arrows) in infected spleen tissue (VL), Giemsa staining

primarily by *L. donovani* and, in infants and immune-compromised persons, by *L. infantum* (a.k.a. *L. chagasi* in South America) [15].

The mode of transmission is identical for all *Leishmania* species. When infected female sandflies of the genera *Phlebotomus* (Asia, Africa, and Europe) or *Lutzomyia* (Latin America) take a blood meal on a mammal, the slender, flagellated promastigote stages (Fig. 1d), which are abundant in the digestive tract of the sandfly, may enter the skin tissue where they are phagocytised by tissue macrophages [16]. They escape lysis in the phagolysosome and establish themselves as round amastigote (Greek: non-flagellated) stages (Fig. 1e).

Proliferation of these amastigotes results, by as yet unknown mechanisms, in the destruction of the macrophage. The free amastigotes are then phagocytised by other

macrophages, bloodstream monocytes, or dendritic cells [17]. While the spread of infections by *L. major* and *L. tropica* is limited to the draining lymph node, *L. donovani* and *L. infantum* will, in time, disseminate into the entire reticuloendothelial system, e.g. spleen, liver, and bone marrow. The local or generalised depletion of macrophages and the concomitant influx of T cells causes the overt effects of *Leishmania* infections, i.e. lesions in cutaneous and mucocutaneous infections, splenomegaly and hepatomegaly in visceral leishmaniasis [17–19].

Opportunistic infections are furthered by the depletion of the macrophage population and also by the fact that *Leishmania* can drive the immune response into a TH2-dependent direction, which is advantageous for several important intracellular pathogens, including viruses. Hence, visceral leishmaniasis is a wide-spread opportunistic disease in HIV co-infected individuals with limited therapeutic options [20].

The uptake and destruction of infected monocytes in the peripheral blood by a feeding female sandfly sets the parasites free in the lumen of the fly's gut. Within 24 h, the amastigotes will undergo stage conversion into the flagellated promastigotes. The promastigotes will attach to the gut epithelium and proliferate until they reach a stationary growth phase. This induces a change of surface molecules and the promastigotes will detach from the gut epithelium and spread into the mouth part from where they can be transmitted when the fly takes another blood meal [21].

## *1.3 Axenic Differentiation* in vitro

The transmission from a poikilothermic arthropod vector into a homeothermic mammalian host includes two major stresses: a significant increase of the ambient temperature and a drop of extracellular pH once the macrophage's phagolysosome is acidified. Both stresses are classical inducers of the cellular stress response and, indeed, an equivalent temperature increase *in vitro* is sufficient to induce heat shock protein synthesis [22–26]. Nevertheless, the stresses to which parasites are exposed during transmission are regular features of their life cycle. More importantly, the temperature range of the mammalian tissue is very limited and, under physiological conditions, does not exceed 42 °C. This means that temperature tolerance mechanisms only have to cope with pre-defined heat stress in contrast with the temperature range encountered by free-living microorganisms. We may thus expect an adaptation of the stress response to meet the specific needs of a parasite [27].

Apart from being a stress factor, the rise in ambient temperature during transmission and the acidification of the phagolysosome are both necessary and, for some species, sufficient signals for the induction of promastigote-to-amastigote stage conversion and thus key factors for parasite persistence inside the mammalian host. While cultured promastigotes of *L. mexicana* and other Central American leishmaniae will differentiate into axenic amastigote-like forms upon a mere increase of the incubation temperature to 34 °C [28, 29], the *in vitro* development of axenic amastigote-like forms of *L. donovani* and *L. infantum* requires a treatment regimen

consisting of a 24 h heat stress at 37 °C, followed by incubation at 37 °C and pH 5.5 for 3–5 days [30, 31]. Once the temperature drops back to 25 °C and the pH is shifted back to 7.0, the axenic amastigotes undergo differentiation into promastigote stages [26, 32]. Thus, it becomes clear that the elevated temperature of the mammalian host is a key trigger for the development of the mammalian stage of at least some *Leishmania* parasites.

## 2 The Heat Shock Proteins of *Leishmania*

### 2.1 *The HSP Complement of* Leishmania

Beginning in the mid-1980s, heat shock genes of *Leishmania* spp. were cloned and sequenced. Genes encoding HSP70 [33] and HSP90 (HSP83) [34–36] were found to be organised in multi-copy tandem gene clusters, with up to eighteen copies per haploid genome. In addition, genes encoding HSP70-related proteins were isolated from stage-specific cDNA libraries [37, 38].

By contrast, HSP100 (ClpB) was found to be encoded by a single-copy gene [23]. CPN60 (HSP60) was found encoded by three paralogues, but only two of them, CPN60.2 and CPN60.3, could be detected on the protein level [24, 39]. The *Leishmania* CPN10 (HSP10) homologue was found to be encoded by two copies of the same gene [25, 40].

The completion of the first *Leishmania* Genome Projects [3, 41] finally allowed a comprehensive search for heat shock genes. While the exact copy number of various heat shock genes varies between isolates [42, 43], mining the data discovered a large number of chaperone and co-chaperone genes. Table 1 shows the most important genes and their accession numbers in *L. donovani*.

### 2.2 *HSPs and the Heat Shock Response*

The induction of HSP genes under heat stress has been subject to numerous studies, starting as early as in 1984. Hunter et al. [49] were also the first to conclude that heat shock gene expression had to be regulated at a post-transcriptional level.

The latter concept was proven first by Argaman et al. for the HSP90 (HSP83) of *L. mexicana* [50], and, more generally, by others [5, 22, 51]. It was found that RNA polymerase density on various heat shock genes, as measured by nuclear run-on analysis, did not increase during heat stress, arguing against a heat-inducible transcription. Moreover, heat-inducible synthesis of three HSPs, HSP70, HSP90 (HSP83), and HSP100, was unaffected by actinomycin C1, showing that the heat shock response was independent of *de novo* RNA synthesis. Lastly, ribosome profiling analysis showed that regulation of HSP synthesis does not correlate with heat shock protein mRNA abundances [5].

**Table 1** Chaperones of Leishmania and their roles and function

| Family | Gene | Identifier | Mass | Function | Reference |
|---|---|---|---|---|---|
| CPN10 | Co-chaperonin 10 = CPN10 | LdBPK_260590.1, LdBPK_260610.1 | 10.7 K | Essential gene, interaction with CPN60, | [25, 40] |
| CPN60 | Chaperonin 60.1 = CPN60.1 | LdBPK_321940.1 | 64.3 K | Not characterised | [24] |
| | Chaperonin 60.2 = CPN60.2 | LdBPK_362130.1 | 60.5 K | Mitochondrial | [24] |
| | Chaperonin 60.3 = CPN30.2 | LdBPK_362134.1 | 59.3 K | Not characterised | [39] |
| | Chaperonin 60.4 = CPN30.4 | LdBPK_302830.1 | 58.2 K | Not characterised | |
| Small HSPs | HSP20 | LdBPK_292560.1 | 17.6 K | Not characterised | [85, 87] |
| | HSP23 | LdBPK_340230.1 | 22.9 K | Stress and drug tolerance | [26, 88] |
| HSP40 | Chaperone protein DNAJ, | Numerous putative genes | | | – |
| HSP70 | Heat shock protein 70 | LdBPK_282930 | 71.6 K | Related but not identical; constitutive expression, interaction with HSP90, MAP kinase I, casein kinase 1.2 | [22, 59, 79, 80] |
| | | LdBPK_282940 | | | |
| | | LdBPK_282950 | | | |
| | | LdBPK_282960 | | | |
| | | LdBPK_282970 | | | |
| | | LdBPK_283000 | | | |
| | Heat shock protein 70.4 | LdBPK_261220.1 | 70.4 K | Metacyclic stage | [38] |
| | HSP70-related, BiP | LdBPK_281310.1 | 71.8 K | Not characterised | |
| | Heat shock 70-related protein 1, mitochondrial precursor, putative | LdBPK_302470.1 | 71.8 K | Not characterised | [37] |
| | | LdBPK_302480.1 | | | |
| HSP90 | HSP90 = HSP83 | LdBPK_330360.1 (varying tandem repeat numbers) | 80.5 K | Proliferation, cell cycle, life cycle, interaction with MAP kinase I, casein kinase 1.2 | [34–36, 48, 52, 79] |
| | LPG3/GRP94 | LdBPK_290790.1 | 87 K | Lipophosphoglycan synthesis | [44] |
| | HSP75/TRAP1 | LdBPK_332520.1 | 72 K | Exosome component | [39] |

| | | | | | |
|---|---|---|---|---|---|
| TPR proteins | Stress-inducible protein (STI-1) | LdBPK_360080.1 | 29.0 K | Co-chaperone of HSP90, essential phosphoprotein | [60, 78, 109] |
| | HOP homologue | LdBPK_081020.1 | 62.2 K | No known function, non-essential | [45] |
| | Possible HIP homologue | LmjF29.0320 | 36.9 K | No known function, non-essential | [45] |
| | Small, glutamine-rich tetratricopeptide repeat protein, SGT | LdBPK_302740.1 | 45.8 K | Essential, part of HSP90 foldosome complex | [46] |
| P23 | Co-chaperone P23 | LdBPK_354540.1 | 21.6 K | Affects HSP90 ATPase domain | [86] |
| AHA1 | Activator of Hsp90 ATPase (AHA1) | LdBPK_180210.1 | 38.4 K | Affects HSP90 ATPase domain | [48] |
| Cyclophilins | Cyclophilin 40 | LdBPK_354830.1 | 38.5 K | Essential for intracellular parasite stages, exosome formation | [47, 69] |
| AAA ATPase | HSP100 | LdBPK_291360.1 | 96.9 K | Essential for intracellular parasite stages, exosome formation, immune modulation | [32, 39, 57, 58] |
| | Heat shock protein 78 | LdBPK_020680.1 | 90.8 K | Not characterised | |

HSP synthesis in *Leishmania* is inducible by elevated temperatures, but not by chemical stresses known to induce a stress response in other eukaryota. Ethanol, acidic pH, cadmium, copper, arsenite, and pentavalent antimony all failed to induce HSP synthesis, as measured by metabolic labelling, SDS-PAGE, and autoradiography [51]. One notable exception is the observed induction of HSP synthesis due to geldanamycin-mediated inhibition of HSP90. Although the induction is not as strong as with a true heat shock, *L. donovani*, but also *Trypanosoma cruzi*, respond to geldanamycin with elevated HSP synthesis [5, 52, 53].

## 2.3 *Stage-Specific Expression of HSPs in* Leishmania

### 2.3.1 Pre-genome Era

Stage specificity of heat shock gene expression was assumed from the start, given the peculiar life cycle of *Leishmania* spp. [54]. Early studies [33, 55], however, suffered from the lack of appropriate detection methods, relying mostly on Northern blot analyses to quantify steady-state mRNA levels. As mentioned earlier, there is no regulated transcription in *Leishmania* [49, 56].

The availability of antibodies directed against *Leishmania* heat shock proteins allowed for semi-quantitative analyses. The results indicated an elevated abundance of a few, but not all heat shock proteins.

HSP100 was found to be amastigote-specific, not only because of its preferential expression in amastigotes from infected tissue [57] and in axenically grown amastigotes [58] but also because it is dispensable in the promastigote stage [32, 57, 58].

The CPN60.2 variant of HSP60 was found to be moderately elevated in axenic amastigotes, as was its cognate co-chaperonin, CPN10 [24, 25].

### 2.3.2 Post-genome Era

Proteome analysis of *L. infantum* during its differentiation *in vitro* from the promastigote to the amastigote form provided the first comprehensive set of data. A large number of heat shock proteins were identified and their relative abundance mapped against the time course of differentiation. The results mostly match the pre-genome era data, but add a level of accuracy absent from Western blot-based studies [59].

For instance, the moderate increase of chaperonins CPN60.2 and CPN10, located in the mitochondrion of *L. donovani*, reflects the results of earlier research [24, 25], where authors also found moderate increases in the amastigote stage. The transient and relatively minor increase of HSP70 and HSP90 (HSP83) levels matches with published work from the mid-1990s and must be seen in the context of the very high basal concentration of both proteins in *Leishmania* promastigotes [22].

The ~5-fold induction of HSP100 in the amastigote was also observed using Western Blot analysis, both for *L. major* and for *L. donovani* [57, 58]. It is interesting to see that HSP70, HSP90 (HSP83), and several putative co-chaperones are found at lower concentrations in the fully differentiated amastigote. HSP90 (HSP83) is critical for proliferation and infectivity [52, 60]. The lower abundance of this important chaperone and its putative functional partners may thus be connected to the slow growth rates and even quiescence observed for amastigotes *in vitro* [61, 62].

## 3 Specific Roles in the Parasitic Life Cycle

### *3.1 HSP100*

HSP100 is encoded by a single copy gene [23] and non-essential *in vitro*. Hsp100$^{-/-}$ (Δclpb) mutants were generated in two *Leishmania* species, *L. major* [57] and *L. donovani* [32, 63]. In both species, loss of HSP100 causes no overt phenotype in cultivated promastigotes. Growth rates are comparable to wild type cells. A minor reduction of growth at elevated temperatures in *L. major hsp100*$^{-/-}$ [57] could not be reproduced with *L. donovani hsp100*$^{-/-}$ mutants, implying that thermotolerance may not be the primary function of HSP100 in the leishmaniae [32].

Indeed, the need for HSP100 appears to be restricted to intracellular amastigotes. *L. donovani hsp100*$^{-/-}$ can differentiate into viable amastigote-like cells in axenic culture, i.e. outside any host cells. The only differences observed was the reduced expression of a family of amastigote marker proteins and an accelerated differentiation back to the promastigote stage when the temperature was lowered. However, *hsp100*$^{-/-}$ mutants of both *L. major* and *L. donovani* failed to infect macrophages successfully, and *L. major hsp100*$^{-/-}$ shows attenuation of virulence in susceptible BALB/c mice, compared with wild type parasites [32, 58]. However, repeated mouse infection cycles yielded an *hsp100*$^{-/-}$ escape variant with restored infectivity and pathogenicity, the molecular basis of which remains unknown [64].

The discovery and analysis of a vesicular protein export pathway in *Leishmania* [65] identified HSP100 as a part of the payload of temperature-induced exosomes, which are shedded off the flagellar pocket membrane. They transport proteins, including known virulence markers, into the host cell cytoplasm [65–69]. The composition of the exosomal protein payload also changes drastically in the avirulent hsp100$^{-/-}$ null mutants [39], suggesting an important role for HSP100 in the sorting process, and reducing the exosome-based export of major chaperones such as HSP90, HSP70, HSP75 (TRAP1), CPN60.2, and CPN60.3. These changes in payload composition affect the observed immune-modulatory impact of exosomes on the antigen-presenting cell population [39], explaining the exclusive role of HSP100 in true, intracellular amastigotes, and its dispensability in all axenic culture forms [32].

In summary, HSP100 is overexpressed in the amastigote stage where it promotes intracellular survival within the macrophage, making it a pivotal factor for parasite virulence.

## 3.2 HSP90

HSP90 is encoded by varying numbers (up to 18) of identical, tandemly arranged gene copies, and it is among the most abundant proteins in *Leishmania* spp., accounting for 2.8% of the promastigote's extractable proteins [22, 36]. On the other hand, Grp94, a paralogue of HSP90 located in the Endoplasmatic Reticulum (ER), is only encoded by a single copy in *L. infantum* [70].

Predictably, inhibition of HSP90, using the specific inhibitors geldanamycin [71] or radicicol [72], arrests proliferating promastigotes in the G2/M phase of the cell cycle. This was observed for *L. donovani* [52] and the related parasite *Trypanosoma cruzi* [53]. Also, inhibition of HSP90 induces the elevated synthesis and/or abundance of the major heat shock proteins HSP60, HSP70, HSP90, and HSP100 in *Leishmania* and *Trypanosoma cruzi*.

HSP90 inhibition also mimics the heat stress signal in an *in vitro* stage conversion. In *L. donovani,* which transforms into amastigote-like culture forms induced by heat stress and acidic milieu, treatment of axenic promastigotes with low doses of geldanamycin or radicicol induces a morphological differentiation towards amastigote-like axenic forms [52, 60, 73]. The effect is specific since expression of a radicicol-resistant mutant of HSP90 [74] abrogates radicicol-mediated stage conversion [60, 73]. In *T. cruzi*, geldanamycin treatment fails to induce life cycle progression [53]. Obviously, the different tropism of *T. cruzi* also entails different regulatory pathways for life cycle control.

In addition to morphological differentiation, HSP90 inhibition also induces amastigote stage-specific gene expression. Given the absence of regulated transcription and the poor correlation between RNA- and protein abundance [2, 4, 5], gene expression analysis in *Leishmania* must focus on protein steady-state levels, i.e. by differential proteome analysis, and/or on proteins synthesis rate analysis, i.e. by ribosome profiling [75, 76]. A comparative proteomics analysis of the changes in protein abundance over the time course of temperature-induced axenic amastigote differentiation revealed expression kinetics for 973 of 8239 putative proteins (11.8%). Elevated synthesis during amastigote conversion occurred in the groups of molecular chaperones, calpain-like peptidases, and histones, but also included a down-regulation of the protein synthesis machinery and adaptive changes in metabolic pathway enzymes [59].

More recently, the impact of HSP90 inhibition on the protein synthesis patterns was investigated using ribosome profiling analysis. *L. donovani* promastigotes were treated with radicicol ($IC_{80}$) for 24 h before poly-ribosomes were isolated and treated with RNase. The remaining, ribosome-protected RNA fragments were recovered for RNA-Seq analysis, and the sequencing reads were aligned to the ~8300 protein-

coding genes to determine the extent and usage of all open reading frames. The coverage (>95%) allowed a high resolution look at gene expression modulation under HSP90 inhibition. This analysis, too, revealed increased synthesis rates for (i) most major chaperones and stress proteins (e.g. HSP100, DNAj, HSP23, HSP70, HSP90, mtHSP70, and the A2 stress proteins), (ii) a number of redox enzymes (glutathione, glutathione reductase, trypanothione reductase, tryparedoxin, and tryparedoxin peroxidase), (iii) proteolytic enzymes including calpain-like peptidases, (iv) the amastin-like cell surface markers typical for amastigotes [77], (v) several protein kinases, and lastly (vi) histone proteins. In turn, several fatty acid synthesis enzymes were down regulated, reflecting the changing usage of carbon sources in the amastigote stage [5].

HSP90 in *Leishmania* is also subject to stage-specific phosphorylation [78] and a substrate for at least two protein kinases, MAP kinase 1 and casein kinase 1.2 [73, 79, 80]. Phosphorylation of HSP90 impacts on proliferation, morphology and *in vitro* infectivity, linking chaperone activity and signal transduction pathways in the control of the *Leishmania* life cycle.

In summary, the activity of HSP90 is pivotal for the *Leishmania* cell fate and modulates translational control of gene expression.

### *3.3 Small Heat Shock Proteins*

In contrast to other chaperone families such as HSP70 and HSP90, small heat shock proteins (sHSPs) comprise the most evolutionarily divergent family of molecular chaperones and are highly heterogenous in both sequence and size [81]. A key characteristic feature of this protein family is the conserved α-crystallin domain (ACD) that folds into a seven- or eight-stranded β-sandwich structure and is involved in dimer formation [82]. The sHSPs usually function as holdases, maintaining their cellular substrates in a near-native state to prevent stress-induced aggregation and to facilitate the refolding via ATP-dependent chaperones [83]. Small HSPs bind a broad range of client proteins, and are thereby involved in the regulation of a variety of cellular processes and stress responses (i.e. high temperature and oxidative stress) [84].

To date, three sHSPs have been identified in the *Leishmania* genome: HSP20, P23 and HSP23 [26, 43, 85, 86].

The *Leishmania* HSP20 was first identified as immunogenic in natural infections of dogs [87]. Further results of this study, however, questioned its immunoprotective properties and implication as suitable DNA vaccines.

Biochemical characterisation of recombinant P23 and HSP23 proteins of *L. braziliensis* (named Lbp23A and Lbp23B) showed that both negatively modulate the HSP90 ATPase activity thereby being implicated as putative HSP90 interacting partners [88]. In another study, functional analysis of *L. donovani* P23 null mutants revealed hypersensitivity against the HSP90-specific inhibitors geldanamycin and radicicol confirming P23 as a HSP90 co-chaperone [86].

A reverse genetic analysis of the *L. donovani* HSP23 gene [26] provided evidence for the involvement of HSP23 in the parasite stress response. Expression of HSP23 was shown to be temperature-dependent, with a marked up regulation during axenic amastigote differentiation [5, 26, 89]. The lack of this protein in HSP23$^{-/-}$ null mutants renders the parasites non-viable at mammalian tissue temperatures and incapable of differentiating into axenic amastigotes *in vitro*. Furthermore, HSP23 null mutants show an increased sensitivity towards chemical stressors such as ethanol, acidic pH, and trivalent antimony Sb(III). The latter effect implicates HSP23 in resistance against antimony-based anti-leishmanial drugs [90].

### 3.4 *HSPs as Antigens in* Leishmania *Infections*

Many HSPs have been identified as major target antigens in several systemic autoimmune and infectious diseases [91–94] caused by pathogens such as leishmaniasis, malaria, trypanosomiasis, and Chagas' disease [95–99].

First, the *L. donovani* HSP70 was characterised as a dominant antigen targeted by the humoral immune response to *Leishmania* infections [98]. Moreover, anti-HSP70 antibodies are frequently found in sera from human and animal patients with different clinical forms of leishmaniasis [99–103].

Furthermore, members of the HSP90 family have been described as dominant antigens during infections caused by *L. donovani* [104], *L. braziliensis* [100, 103], and *L. infantum* [105]. Finally, antibodies against the parasite HSP60 have been found in sera of patients with American CL [106].

Among the HSPs, the HSP70 family comprises highly conserved molecules, which are found in all types of prokaryotic and eukaryotic cells. Despite the high sequence identity of 73% between parasite and human HSP70 [107], the host immune response elicited during this parasitic infection is directed against specific epitopes of *Leishmania* HSP70 [101]. Cellular and humoral responses against the parasite HSP70 are highly specific, while anti-self antibodies are not induced [101, 103]. Similar results were obtained with *T. cruzi* [97, 108].

Co-chaperones, too, were identified as antigens in *Leishmania* infection. The *L. major* homologue of the eukaryotic stress-inducible protein Sti1 (HOP) was recognised as an important antigen. Analyses of sera from human patients with CL, VL, and post-kala azar dermal leishmaniasis (PKDL) showed that most individuals from these three clinical groups mounted strong humoral responses against LmSTI1 [109].

In another study, *Leishmania* HSP70 and HSP90 (HSP83) behaved as potent B cell mitogens [110]. Gamma-delta T cells from peripheral blood mononuclear cells (PBMC) of a patient suffering from leishmaniasis of the mucosa responded to stimulation with recombinant 70 kDa heat shock protein of *L. chagasi* [111]. A similar effect was observed with *L. braziliensis* HSP90 and HSP70 [100, 103], indicating a T cell-proliferative potential of parasite HSPs. The relative degree of PBMC stimulation by the two HSPs varied between individuals suffering from

mucosal leishmaniasis versus self-healing cutaneous lesions. Since the outcome of *Leishmania* infections greatly depends on cell-mediated immune reactions [18], this finding may have implications on the pathology of *L. braziliensis* infections.

### 3.5 Protection Against Anti-microbial Agents

Another field of growing interest is the role of HSPs in the resistance of the parasites against anti-microbial agents and chemotherapy. *Leishmania* parasites must survive exposure to anti-microbial oxidants such as superoxide, hydrogen peroxide, and nitric oxide that are generated during phagocytosis in the host macrophages [112–114]. Toxic oxidants induce a moderate increase of HSP70 in *Leishmania* promastigotes. This response results in increased resistance to the toxic effects and increased virulence for the mammalian host [113, 115]. It was shown, too, that elevated temperature and oxidants induce an increase of antioxidant enzymes [116].

Experiments have also shown that HSP70 and mtHSP70 are implicated in the resistance to antimony in *Leishmania* [42, 117]. Also, HSP23 null mutants are highly sensitive against stresses such as high temperature, oxidative stress, antimony (III) and antimony (V), indicating a role for HSP23 in the defence against metalloid-based anti-leishmanial drugs [26]. HSP23 is part of a three-gene cluster of antimony resistance markers on chromosome 34 of Old World *Leishmania* spp., a region that is selected in genetic complementation experiments under antimony drug selection [90].

HSP90 (HSP83), too, is implicated in drug resistance in *L. donovani* [118]. It was reported that in a Sb(V)-resistant *L. donovani* field isolate, which shows cross-resistance to miltefosine (another anti-*Leishmania* drug), the expression of HSP90 (HSP83) was increased. Targeted over-expression of HSP90 also conferred protection *in vitro*. HSP90 and HSP70 may confer a first, unspecific stress protection that allows the parasite to develop more specific and efficient resistance mechanisms against anti-*Leishmania* drugs.

## 4 Conclusions

The functions of most heat shock proteins are still not fully understood in the Trypanosomatida. While reverse genetics have identified the critical *roles* played by chaperones in morphology, viability, infectivity, virulence and stress resistance, we know little about their biochemical *functions*. Assumptions based on amino acid sequence orthologies can be quite misleading given (i) the phylogenetic distances between the Trypanosomatida and established model organisms and (ii) the unorthodox gene regulation mechanisms used by these early branching eukaryotes. Therefore, primary and secondary (“moonlighting”) functions of chaperones, their client proteins and their upstream regulators or modulators need to be characterised.

Protein kinases and the impact of phosphorylation on HSP function, especially in the pathogenic amastigote stage, is another urgent question in *Leishmania* research since the lack of conventional gene regulation at the transcription level places more importance on post-translational modifications to modulate cellular responses to environmental change [119].

Comparatively little is also known about the functions of co-chaperones for the activities of the major HSPs. It is therefore time to reconstitute *Leishmania* chaperoning complexes *in vitro*, aided by *in silico* analysis, to characterise the composition and clientele of various foldosome complexes and their impact on the viability of the pathogenic intracellular amastigote stages.

The roles played by *Leishmania* HSPs in modulating the host's immune response of parasite infection also requires more insight. HSPs are known to be strong antigens eliciting both humoral and cellular responses. They are also targeted to the host cell cytoplasm via exosome-mediated secretion and modulate host and host cell immune responses to the parasite's advantage. Identification of clients/targets in the host cell may yield better insight into host-parasite interactions on the molecular level.

The wide availability of high-throughput sequencing will also aid in the phenotype analysis of HSP null mutants. Quite a number of chaperones are either essential for *in vitro* growth and therefore not accessible to targeted replacement or the null mutants have no or weak phenotypes. Inducible gene loss, e.g. by DiCre recombination [120], can unravel the roles and functions of essential chaperones. On the other hand, comparative, qualitative and quantitative genome analysis [9–11] of viable HSP null mutants and wild type parasites will point out chromosomal ploidy changes, gene copy number variations, insertions, deletions and sequence polymorphisms selected specifically in parasites lacking a particular HSP. This can be aided by functional cloning strategies for easy identification of compensatory genetic adaptions [67, 121, 122].

In *Saccharomyces cerevisiae*, HSP90 and p23 are known to be involved in chromatin packaging by keeping chromatin remodeling proteins functional [123]. In addition, HSP90, together with Hsc70, has been shown to participate in chaperone-mediated autophagy through interaction with other chromatin proteins, thereby maintaining a balance between the soluble histone pool of H3 and H4 [124]. Since the inhibition of HSP90 by radicicol in *L. donovani* causes an increased histone synthesis, the role of HSP90 and other HSPs in the regulation of chromatin structure is another worthwhile question [5].

Lastly, several HSPs in *Leishmania* have only been partially investigated due to the large number of orthologues, e.g. HSP70 and HSP40. New molecular tools for reverse genetics, such as CRISPR/Cas9, should now be utilised for the analysis of multi-member HSP families [125].

**Acknowledgements** We wish to acknowledge the contributions of our colleagues in the field, our collaborators past and present, and of former laboratory members who compiled the body of data that we presented here.

**Disclosure of Interest** The authors are employees of the Bernhard Nocht Institute Foundation, jointly funded by the Federal Republic of Germany and the State of Hamburg. They are not aware of any conflict of interest.

**Ethical Approval for Studies Involving Humans** Not applicable.

**Ethical Approval for Studies Involving Animals** Not applicable.

## References


1. Liang XH et al (2003) Trans and cis splicing in trypanosomatids: mechanism, factors, and regulation. Eukaryot Cell 2(5):830–840
2. Clayton CE (2002) Life without transcriptional control? From fly to man and back again. EMBO J 21(8):1881–1888
3. Ivens AC et al (2005) The genome of the kinetoplastid parasite, Leishmania major. Science 309(5733):436–442
4. Lahav T et al (2011) Multiple levels of gene regulation mediate differentiation of the intracellular pathogen Leishmania. FASEB J 25(2):515–525
5. Bifeld E et al (2018) Ribosome profiling reveals HSP90 inhibitor effects on stage-specific protein synthesis in Leishmania donovani. mSystems 3(6)
6. Mannaert A et al (2012) Adaptive mechanisms in pathogens: universal aneuploidy in Leishmania. Trends Parasitol 28(9):370–376
7. Ubeda JM et al (2014) Genome-wide stochastic adaptive DNA amplification at direct and inverted DNA repeats in the parasite Leishmania. PLoS Biol 12(5):e1001868
8. Laffitte MN et al (2016) Plasticity of the Leishmania genome leading to gene copy number variations and drug resistance. F1000Res 5:2350
9. Dumetz F et al (2017) Modulation of aneuploidy in Leishmania donovani during adaptation to different in vitro and in vivo environments and its impact on gene expression. MBio 8(3)
10. Cuypers B et al (2018) Integrated genomic and metabolomic profiling of ISC1, an emerging Leishmania donovani population in the Indian subcontinent. Infect Genet Evol 62:170–178
11. Dumetz F et al (2018) Molecular preadaptation to antimony resistance in Leishmania donovani on the Indian subcontinent. mSphere 3(2)
12. Patino LH et al (2019) Major changes in chromosomal somy, gene expression and gene dosage driven by Sb(III) in Leishmania braziliensis and Leishmania panamensis. Sci Rep 9(1):9485
13. Alvar J et al (2012) Leishmaniasis worldwide and global estimates of its incidence. PLoS One 7(5):e35671
14. WHO (2017) Integrating neglected tropical diseases into global health and development: fourth WHO report on neglected tropical diseases
15. Burza S, Croft SL, Boelaert M (2018) Leishmaniasis. Lancet 392(10151):951–970
16. Killick-Kendrick R (1990) The life-cycle of Leishmania in the sandfly with special reference to the form infective to the vertebrate host. Ann Parasitol Hum Comp 65(Suppl 1):37–42
17. Bogdan C et al (1996) Invasion, control and persistence of Leishmania parasites. Curr Opin Immunol 8(4):517–525
18. Bogdan C, Rollinghoff M (1998) The immune response to Leishmania: mechanisms of parasite control and evasion. Int J Parasitol 28(1):121–134
19. Chang KP et al (2003) Leishmania model for microbial virulence: the relevance of parasite multiplication and pathoantigenicity. Acta Trop 85(3):375–390
20. Akuffo H et al (2018) New insights into leishmaniasis in the immunosuppressed. PLoS Negl Trop Dis 12(5):e0006375
21. Sacks DL (1989) Metacyclogenesis in Leishmania Promastigotes. Exp Parasitol 69:100–103

22. Brandau S, Dresel A, Clos J (1995) High constitutive levels of heat-shock proteins in human-pathogenic parasites of the genus Leishmania. Biochem J 310(Pt 1):225–232
23. Hübel A et al (1995) A member of the ClpB family of stress proteins is expressed during heat shock in Leishmania spp. Mol Biochem Parasitol 70(1–2):107–118
24. Schlüter A et al (2000) Expression and subcellular localization of Cpn60 protein family members in Leishmania donovani. Biochim Biophys Acta 1491:65–74
25. Zamora-Veyl FB et al (2005) Stage-specific expression of the mitochondrial co-chaperonin of Leishmania donovani, CPN10. Kinetoplastid Biol Dis 4(1):3
26. Hombach A et al (2014) A small heat shock protein is essential for thermotolerance and intracellular survival of Leishmania donovani. J Cell Sci 127(Pt 21):4762–4773
27. Berman JD, Neva FA (1981) Effect of temperature on multiplication of Leishmania amastigotes within human monocyte-derived macrophages in vitro. Am J Trop Med Hyg 30 (2):318–321
28. Bates PA (1993) Axenic amastigote culture of Leishmania amastigotes. Parasitol Today 9:143–146
29. Bates PA (1994) Complete developmental cycle of Leishmania mexicanain axenic culture. Parasitology 108:1–9
30. Barak E et al (2005) Differentiation of Leishmania donovani in host-free system: analysis of signal perception and response. Mol Biochem Parasitol 141(1):99–108
31. Zilberstein D, Nitzan Koren R (2019) Host-free systems for differentiation of axenic Leishmania. Methods Mol Biol 1971:1–8
32. Krobitsch S, Clos J (1999) A novel role for 100 kD heat shock proteins in the parasite Leishmania donovani. Cell Stress Chaperones 4(3):191–198
33. Lee MG et al (1988) Structure and expression of the hsp 70 gene family of Leishmania major [published erratum appears in Nucleic Acids Res 1988 Dec 9;16(23):11400–1]. Nucleic Acids Res 16(20):9567–9585
34. Shapira M, Pinelli E (1989) Heat-shock protein 83 of Leishmania mexicana amazonensis is an abundant cytoplasmic protein with a tandemly repeated genomic arrangement. Eur J Biochem 185:231–236
35. Hübel A, Clos J (1996) The genomic organization of the HSP83 gene locus is conserved in three Leishmania species. Exp Parasitol 82(2):225–228
36. Shapira M et al (2001) Post transcriptional control of gene expression in Leishmania. Med Microbiol Immunol (Berl) 190(1–2):23–26
37. Searle S, Campos AJR, Coulson RMR, Spithill TW, Smith DF (1989) A family of heat shock protein 70-related genes are expressed in the promastigotes of Leishmania major. Nucleic Acids Res 17(13):5081–5095
38. Coulson RM, Smith DF (1990) Isolation of genes showing increased or unique expression in the infective promastigotes of Leishmania major. Mol Biochem Parasitol 40(1):63–75
39. Silverman JM et al (2010) Leishmania exosomes modulate innate and adaptive immune responses through effects on monocytes and dendritic cells. J Immunol 185(9):5011–5022
40. Colineau L et al (2017) Leishmania donovani chaperonin 10 regulates parasite internalization and intracellular survival in human macrophages. Med Microbiol Immunol 206(3):235–257
41. Peacock CS et al (2007) Comparative genomic analysis of three Leishmania species that cause diverse human disease. Nat Genet 39(7):839–847
42. Brochu C, Haimeur A, Ouellette M (2004) The heat shock protein HSP70 and heat shock cognate protein HSC70 contribute to antimony tolerance in the protozoan parasite Leishmania. Cell Stress Chaperones 9(3):294–303
43. Folgueira C, Requena JM (2007) A postgenomic view of the heat shock proteins in kinetoplastids. FEMS Microbiol Rev 31(4):359–377
44. Descoteaux A et al (2002) Leishmania LPG3 encodes a GRP94 homolog required for phosphoglycan synthesis implicated in parasite virulence but not viability. EMBO J 21 (17):4458–4469

45. Ommen G, Lorenz S, Clos J (2009) One-step generation of double-allele gene replacement mutants in Leishmania donovani. Int J Parasitol 39(5):541–546
46. Ommen G, Chrobak M, Clos J (2010) The co-chaperone SGT of Leishmania donovani is essential for the parasite's viability. Cell Stress Chaperones 39(5):541–546
47. Yau W-L et al (2014) Cyclophilin 40-deficient Leishmania donovani fail to undergo stress-induced development of the infectious metacyclic stage. Cell Microbiol 93:80–97
48. Bartsch K, Hombach-Barrigah A, Clos J (2017) Hsp90 inhibitors radicicol and geldanamycin have opposing effects on Leishmania Aha1-dependent proliferation. Cell Stress Chaperones 22:729–742
49. Hunter KW, Cook CL, Hayunga EG (1984) Leishmanial differentiation in vitro: induction of heat shock proteins. Biochem Biophys Res Commun 125(2):755–760
50. Argaman M, Aly R, Shapira M (1994) Expression of heat shock protein 83 in Leishmania is regulated post- transcriptionally. Mol Biochem Parasitol 64(1):95–110
51. Clos J, Brandau S, Hoyer C (1998) Chemical stress does not induce heat shock protein synthesis in Leishmania donovani. Protist 149(2):167–172
52. Wiesgigl M, Clos J (2001) Heat shock protein 90 homeostasis controls stage differentiation in Leishmania donovani. Mol Biol Cell 12(11):3307–3316
53. Graefe SE et al (2002) Inhibition of HSP90 in Trypanosoma cruzi induces a stress response but no stage differentiation. Eukaryot Cell 1(6):936–943
54. van der Ploeg LHT, Giannini SH, Cantor CR (1985) Heat shock genes: regulatory role for differentiation in parasitic protozoa. Science 228:1443–1446
55. Lawrence F, Robert-Gero M (1985) Induction of heat shock and stress proteins in promastigotes of three Leishmania species. Proc Natl Acad Sci U S A 82(13):4414–4417
56. Clayton C, Shapira M (2007) Post-transcriptional regulation of gene expression in trypanosomes and Leishmanias. Mol Biochem Parasitol 156(2):93–101
57. Hübel A et al (1997) Leishmania major Hsp100 is required chiefly in the mammalian stage of the parasite. Mol Cell Biol 17(10):5987–5995
58. Krobitsch S et al (1998) Leishmania donovani heat shock protein 100: characterization and function in amastigote stage differentiation. J Biol Chem 273:6488–6494
59. Rosenzweig D et al (2008) Retooling Leishmania metabolism: from sand fly gut to human macrophage. FASEB J 22(2):590–602
60. Hombach A et al (2013) The Hsp90-Sti1 interaction is critical for Leishmania donovani proliferation in both life cycle stages. Cell Microbiol 15(4):585–600
61. Jara M et al (2017) Macromolecular biosynthetic parameters and metabolic profile in different life stages of Leishmania braziliensis: Amastigotes as a functionally less active stage. PLoS One 12(7):e0180532
62. Jara M et al (2019) Tracking of quiescence in Leishmania by quantifying the expression of GFP in the ribosomal DNA locus. Sci Rep 9(1):18951
63. Krobitsch S, Clos J (2000) Cross-species homologous recombination in Leishmania donovani reveals the sites of integration. Mol Biochem Parasitol 107:123–128
64. Reiling L et al (2006) Spontaneous recovery of pathogenicity by Leishmania major hsp100$^{-/-}$ alters the immune response in mice. Infect Immun 74(11):6027–6036
65. Silverman JM et al (2010) An exosome-based secretion pathway is responsible for protein export from Leishmania and communication with macrophages. J Cell Sci 123(Pt 6):842–852
66. Silverman JM et al (2008) Proteomic analysis of the secretome of Leishmania donovani. Genome Biol 9(2):R35
67. Reiling L et al (2010) Overexpression of a single Leishmania major gene is sufficient to enhance parasite infectivity in vivo and in vitro. Mol Microbiol 76(5):1175–1190
68. Bifeld E et al (2015) Geographical sequence variation in the Leishmania major virulence factor P46. Infect Genet Evol 30:195–205
69. Yau WL et al (2016) Phenotypic characterization of a Leishmania donovani cyclophilin 40 null mutant. J Eukaryot Microbiol 63(6):823–833

70. Larreta R et al (2000) Leishmania infantum: gene cloning of the GRP94 homologue, its expression as recombinant protein, and analysis of antigenicity. Exp Parasitol 96(2):108–115
71. Whitesell L et al (1994) Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci U S A 91(18):8324–8328
72. Schulte TW et al (1999) Interaction of radicicol with members of the heat shock protein 90 family of molecular chaperones. Mol Endocrinol 13(9):1435–1448
73. Hombach-Barrigah A et al (2019) Leishmania donovani 90 kD heat shock protein - impact of Phosphosites on parasite fitness, infectivity and casein kinase affinity. Sci Rep 9(1):5074
74. Roe SM et al (1999) Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. J Med Chem 42(2):260–266
75. Ingolia NT et al (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science 324(5924):218–223
76. Ingolia NT et al (2012) The ribosome profiling strategy for monitoring translation in vivo by deep sequencing of ribosome-protected mRNA fragments. Nat Protoc 7(8):1534–1550
77. Rochette A et al (2005) Characterization and developmental gene regulation of a large gene family encoding amastin surface proteins in Leishmania spp. Mol Biochem Parasitol 140 (2):205–220
78. Morales M et al (2010) Phosphoproteome dynamics reveals heat shock protein complexes specific to the Leishmania infectious stage. Proc Natl Acad Sci U S A 107(18):8381–8386
79. Kaur P et al (2017) MAPK1 of Leishmania donovani interacts and phosphorylates HSP70 and HSP90 subunits of foldosome complex. Sci Rep 7(1):10202
80. Martel D et al (2020) The low complexity regions in the C-terminus are essential for the subcellular localisation of Leishmania casein kinase 1 but not for its activity. bioRxiv
81. Basha E, O'Neill H, Vierling E (2012) Small heat shock proteins and alpha-crystallins: dynamic proteins with flexible functions. Trends Biochem Sci 37(3):106–117
82. Haslbeck M et al (2005) Some like it hot: the structure and function of small heat-shock proteins. Nat Struct Mol Biol 12(10):842–846
83. Ungelenk S et al (2016) Small heat shock proteins sequester misfolding proteins in near-native conformation for cellular protection and efficient refolding. Nat Commun 7:13673
84. Nakamoto H, Vigh L (2007) The small heat shock proteins and their clients. Cell Mol Life Sci 64(3):294–306
85. Fraga J et al (2013) Evolution and species discrimination according to the Leishmania heat-shock protein 20 gene. Infect Genet Evol J Mol Epidemiol Evol Gene Infect Dis 18:229–237
86. Hombach A et al (2015) Leishmania donovaniP23 protects parasites against HSP90 inhibitor-mediated growth arrest. Cell Stress Chaperones 20(4):673–685
87. Montalvo-Alvarez AM et al (2008) The Leishmania HSP20 is antigenic during natural infections, but, as DNA vaccine, it does not protect BALB/c mice against experimental L. amazonensis infection. J Biomed Biotechnol 2008:695432
88. Batista FA et al (2015) Identification of two p23 co-chaperone isoforms in Leishmania braziliensis exhibiting similar structures and Hsp90 interaction properties despite divergent stabilities. FEBS J 282(2):388–406
89. Tsigankov P et al (2014) Regulation dynamics of Leishmania differentiation: deconvoluting signals and identifying phosphorylation trends. Mol Cell Proteomics 13(7):1787–1799
90. Tejera Nevado P et al (2016) A Telomeric cluster of antimony resistance genes on chromosome 34 of Leishmania infantum. Antimicrob Agents Chemother 60(9):5262–5275
91. Kaufmann SH (1990) Heat shock proteins and the immune response. Immunol Today 11 (4):129–136
92. Young DB (1992) Heat-shock proteins: immunity and autoimmunity. Curr Opin Immunol 4 (4):396–400
93. Kaufmann SHE, Schoel B (1994) Heat shock proteins as antigens in immunity against infection and self. In: Morimoto RI, Tissières A, Georgopoulos C (eds) The biology of heat

shock proteins and molecular chaperones. Cold Spring Harbor Laboratory Press, Plainview, pp 495–531
94. Minowada G, Welch WJ (1995) Clinical implications of the stress response. J Clin Invest 95:3–12
95. Bianco AE et al (1986) A repetitive antigen of *Plasmodium falciparum* that is homologous to heat shock protein 70 of *Drosophila melanogaster*. Proc Natl Acad Sci U S A 83 (22):8713–8717
96. Engman DM et al (1989) Comparison of HSP70 genes from two strains of Trypanosoma cruzi. Mol Biochem Parasitol 37(2):285–287
97. Engman DM, Dragon EA, Donelson JE (1990) Human humoral immunity to hsp70 during Trypanosoma cruzi infection. J Immunol 144(10):3987–3991
98. MacFarlane J et al (1990) Identification and characterisation of a Leishmania donovani antigen belonging to the 70-kDa heat-shock protein family. Eur J Biochem 190:377–384
99. Wallace GR et al (1992) Mapping of a visceral leishmaniasis-specific immunodominant B-cell epitope of Leishmania donovani Hsp70. Infect Immun 60(7):2688–2693
100. Skeiky YA et al (1995) Immune responses of leishmaniasis patients to heat shock proteins of Leishmania species and humans. Infect Immun 63(10):4105–4114
101. Quijada L et al (1996) During canine viscero-cutaneous leishmaniasis the anti-Hsp70 antibodies are specifically elicited by the parasite protein. Parasitology 112(Pt 3):277–284
102. Quijada L et al (1996) Mapping of the linear antigenic determinants of the Leishmania infantum hsp70 recognized by leishmaniasis sera. Immunol Lett 52(2–3):73–79
103. Skeiky YA et al (1997) Association of Leishmania heat shock protein 83 antigen and immunoglobulin G4 antibody titers in Brazilian patients with diffuse cutaneous leishmaniasis. Infect Immun 65(12):5368–5370
104. de Andrade CR et al (1992) Recombinant Leishmania Hsp90 and Hsp70 are recognized by sera from visceral leishmaniasis patients but not Chagas' disease patients. J Clin Microbiol 30 (2):330–335
105. Angel SO et al (1996) During canine leishmaniasis a protein belonging to the 83-kDa heat-shock protein family elicits a strong humoral response. Acta Trop 62(1):45–56
106. Rey-Ladino JA, Joshi PB, Singh B, Gupta R, Reiner NE (1997) Leishmania major: molecular cloning, sequencing, and expression of the heat shock protein 60 gene reveals unique carboxy terminal peptide sequences. Exp Parasitol 85(3):249–263
107. Requena JM, Alonso C, Soto M (2000) Evolutionarily conserved proteins as prominent immunogens during Leishmania infections. Parasitol Today 16(6):246–250
108. Tibbetts RS et al (1994) Molecular cloning and characterization of the 78-kilodalton glucose-regulated protein of Trypanosoma cruzi. Infect Immun 62(6):2499–2507
109. Webb JR et al (1998) Human and murine immune responses to a novel Leishmania major recombinant protein encoded by members of a multicopy gene family. Infect Immun 66 (7):3279–3289
110. Rico AI et al (1999) Immunostimulatory properties of the Leishmania infantum heat shock proteins HSP70 and HSP83. Mol Immunol 36(17):1131–1139
111. Russo DM et al (1993) Antigen-reactive gamma delta T cells in human leishmaniasis. J Immunol 151(7):3712–3718
112. Zarley JH, Britigan BE, Wilson ME (1991) Hydrogen peroxide-mediated toxicity for Leishmania donovani chagasi promastigotes. J Clin Invest 88:1511–1521
113. Wilson ME, Andersen KA, Britigan BE (1994) Response of Leishmania chagasi promastigotes to oxidant stress. Infect Immun 62(11):5133–5141
114. Ferrari CKB (2019) Oxidative stress and antioxidants in host defense in leishmaniasis. In: Chakraborti S et al (eds) Oxidative stress in microbial diseases. Springer, Singapore, pp 245–256
115. Miller MA et al (2000) Inducible resistance to oxidant stress in the protozoan Leishmania chagasi. J Biol Chem 275(43):33883–33889

116. Levick MP et al (1998) Identification and characterisation of a functional peroxidoxin from Leishmania major. Mol Biochem Parasitol 96(1–2):125–137
117. Vacchina P et al (2016) A mitochondrial HSP70 (HSPA9B) is linked to miltefosine resistance and stress response in Leishmania donovani. Parasit Vectors 9(1):621
118. Vergnes B et al (2007) A proteomics screen implicates HSP83 and a small kinetoplastid calpain-related protein in drug resistance in Leishmania donovani clinical field isolates by modulating drug-induced programmed cell death. Mol Cell Proteomics 6(1):88–101
119. Spath GF, Drini S, Rachidi N (2015) A touch of Zen: post-translational regulation of the Leishmania stress response. Cell Microbiol 17(5):632–638
120. Duncan SM et al (2016) Conditional gene deletion with DiCre demonstrates an essential role for CRK3 in Leishmania mexicana cell cycle regulation. Mol Microbiol 100(6):931–944
121. Clos J, Choudhury K (2006) Functional cloning as a means to identify Leishmaniagenes involved in drug resistance. Mini Rev Med Chem 6(2):123–129
122. Gazanion E et al (2016) Cos-Seq for high-throughput identification of drug target and resistance mechanisms in the protozoan parasite Leishmania. Proc Natl Acad Sci U S A 113 (21):E3012–E3021
123. Echtenkamp FJ et al (2016) Hsp90 and p23 molecular chaperones control chromatin architecture by maintaining the functional pool of the RSC chromatin remodeler. Mol Cell 64 (5):888–899
124. Cook AJ et al (2011) A specific function for the histone chaperone NASP to fine-tune a reservoir of soluble H3-H4 in the histone supply chain. Mol Cell 44(6):918–927
125. Beneke T et al (2017) A CRISPR Cas9 high-throughput genome editing toolkit for kinetoplastids. R Soc Open Sci 4(5):170095

# HSF1 in RNA Polymerase II Promoter-Proximal Pausing and *HSP70* Transcription


**Heeyoun Bunch**

## Abstract

**Introduction** This review summarizes the findings regarding Heat shock factor 1 (HSF1)-mediated RNA polymerase II (Pol II) promoter-proximal pause release as a major transcriptional activation mechanism at heat shock protein 70 (*HSP70*).

**Methods** A narrative review of all the papers related to the findings was conducted.

**Results** Transcription is the biological process through which RNA molecules are synthesized from the sequence of a DNA strand. The polymerization of protein-coding RNA is mediated by Pol II, a sole, multi-subunit enzyme whose chromatin association/dissociation and enzymatic activity are regulated by many transcription factors. Critically, some transcription factors receive extracellular cues, which provoke a cascade of signaling events that prime gene activation and productive transcription. HSF1, a master regulator for *HSP70* genes, is such a transcription factor. Metazoan *HSP70* transcription is rapidly induced in response to extracellular or intrinsic stresses, mainly through a mechanism called Pol II promoter-proximal pausing. It is utilized by a large number of protein-coding and non-protein-coding genes for synchronized gene expression in metazoans. The molecular model system of HSF1-mediated pause release of Pol II at *HSP70* has been used to study this transcriptional mechanism.

**Conclusions** In this chapter, we discuss the role of HSF1 in Pol II promoter-proximal pausing for regulated *HSP70* transcription and expression.

**Keywords** Gene regulation · HSF1 · *HSP70* · RNA polymerase II promoter-proximal pausing · Transcriptional activation · Transcriptional elongation

H. Bunch (✉)
School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daeg, Republic of Korea
e-mail: hbunch@knu.ac.kr

Heat Shock Proteins (2021) 22: 489–508
https://doi.org/10.1007/7515_2021_38

Published Online: 1 June 2021

## Abbreviations

| | |
|---|---|
| AGO | Argonaute |
| ATM | Ataxia telangiectasia mutated |
| ChIP-seq | Chromatin immunoprecipitation sequencing |
| CTD | C-terminal domain |
| DDR | DNA damage response |
| DNA-PK | DNA-dependent protein kinase |
| DSB | DNA double strand break |
| DSIF | DRB sensitivity-inducing factor |
| FACT | Facilitates chromatin transcription |
| H3K4me3 | H3 trimethylated at lysine 4 |
| HSE | Heat shock element |
| HSF1 | Heat shock factor 1 |
| HSP | Heat shock protein |
| m7G | 7-methyl guanosine |
| NELF | Negative elongation factor |
| NTP | Nucleoside triphosphate |
| NURF | Nucleosome remodeling factor |
| PARP | Poly(ADP)-ribose polymerase |
| PI3 | Phosphoinositide-3 |
| Pol II | RNA polymerase II |
| P-TEFb | Positive transcription elongation factor b |
| RNA-seq | RNA sequencing |
| SWI/SNF | Switch/Sucrose non-fermentable |
| TBP | TATA box binding protein |
| TOP2B | Topoisomerase IIβ |
| TRIM28 | Tripartite motif-containing 28 |
| Trl | Trithorax-like |
| TSS | Transcription start site |

## 1 Introduction

The generation of RNA molecules complementary to one of the DNA double strands, or transcription, is the first regulatory step in gene expression [1]. Enzymes called RNA polymerases (RNA Pols) solely mediate the polymerization of ribonucleotides to synthesize RNA. Three RNA Pols in eukaryotic cells, named RNA Pol I, II, and III, were discovered about 50 years ago [2], and among these, RNA Pol II transcribes most protein-coding and long non-protein-coding genes [3, 4]. The enzymatic activity of Pol II is crucially regulated by a variety of general and gene-specific transcription factors [5]. For example, the phosphorylation of RNA Pol II by the transcription factors, general transcription factor II (TFIIH) and positive

transcription elongation factor b (P-TEFb), modulates the promoter escape and elongation processivity of RNA Pol II [6–8]. Further a gene-activation or gene-repression signals are transferred to these kinases through a cascade of signaling events involving various protein and nucleic acid factors [9, 10]. This transcription-factor-induced gene activation involves Mediator, a large protein complex, as a scaffolding protein to bridge upstream promoter or enhancer elements and the downstream Pol II complex in the transcription start site (TSS) [11, 12] (Fig. 1).

Transcription includes the steps of transcriptional initiation, elongation, and termination [13]. Previously, it was thought that gene activating signals such as heat shock, hormones, and neurotransmitters induce the recruitment of free Pol II molecules to the promoter of target genes at the onset of gene expression [14, 15] (Fig. 2). Transcription initiation consists of the recruitment of general transcription factors and Pol II, multiple rounds of abortive initiation, and promoter escape [16, 17]. Thus, transcription initiation was considered the sole rate-limiting step,

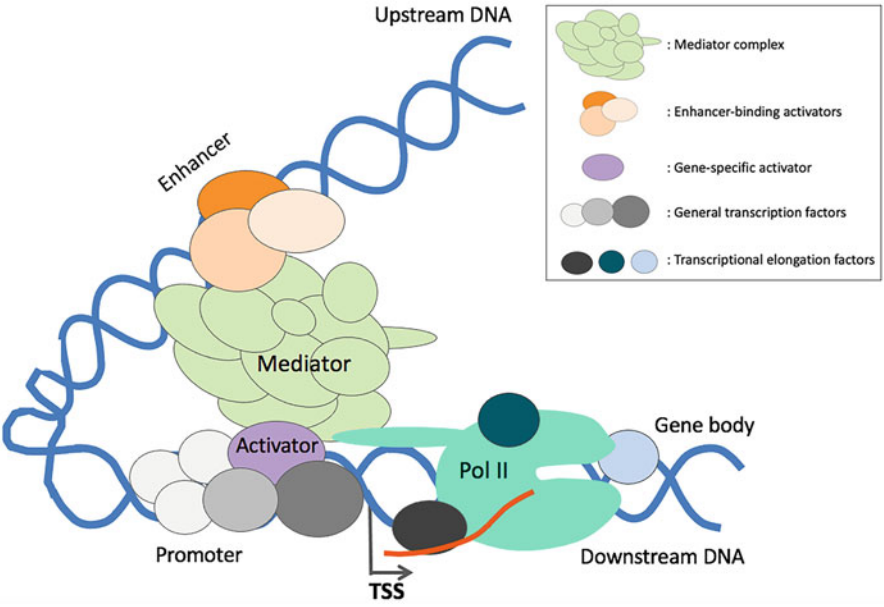



**Fig. 1 Transcription regulation by DNA binding transcription activators**. In signal-induced transcription, general and gene-specific transcription activators bind to the promoter to initiate the gene expression. Mediator, a large protein complex composed of over 20 subunits, is a central factor in signal-induced transcriptional activation as a scaffolding and coactivator protein. Mediator interacts with transcription activators, changes its conformation, and bridges the enhancer and promoter elements as well as the Pol II elongation complex in the TSS. DNA looping is considered to occur to enable these interactions. This crosstalk is known to be important for the full-forced transcriptional activation and gene expression. TSS, transcription start site. Enhancer elements in orange colored circles; RNA in orange line; general transcription factors in grey circles; gene-specific activator in purple circle; Mediator in green circles. Pol II RPB C-terminal domain is shown as a narrow, elongated structure protruding from Pol II

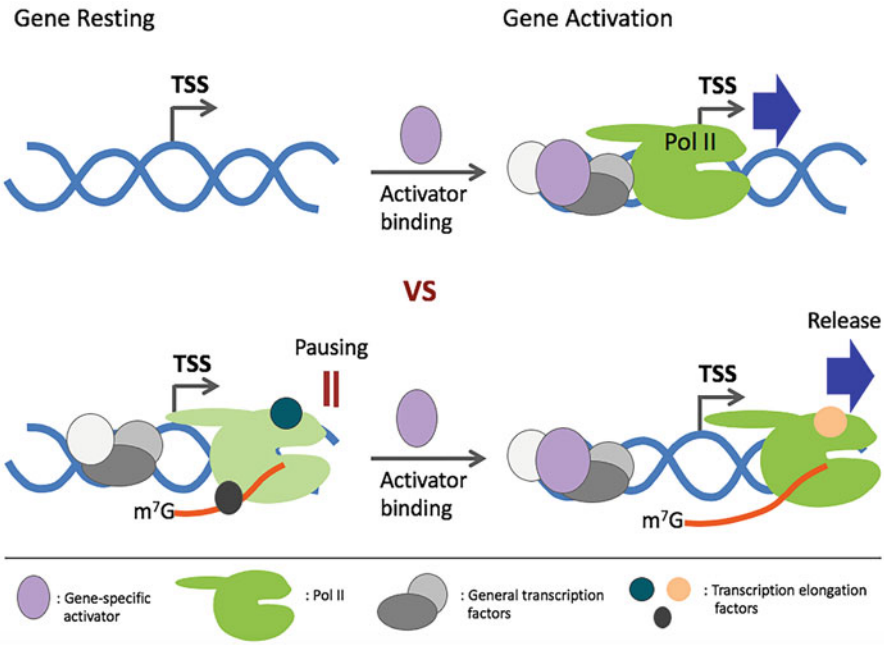



**Fig. 2 Traditional versus current models of transcriptional activation**. Previously, it was considered that the gene promoter and TSS would be vacant of Pol II in the resting state of transcription and Pol II and general transcription factors would be recruited to the promoter of targeted genes upon gene activation (upper panel). It was also thought that transcriptional initiation from the factor binding to the promoter escape (or clearance) is the rate-limiting step before processive elongation (upper panel). Currently, it has been shown that even in the resting state, the preinitiation complex of Pol II is already assembled, initiates transcription, and is paused at around +25–70 from the TSS in a large number of protein-coding and non-protein coding genes including *HSP70* (bottom panel). During the pausing, the short mRNA species engaged with Pol II is capped with 7 methyl guanosine ($m^7G$). Gene activation is thus to release the paused early elongation Pol II, which is another critical rate-limiting step of gene expression (bottom panel)

while transcriptional elongation was thought to be a continuous, processive polymerization step without energy barrier for Pol II to overcome to mature the nascent RNA molecule upon gene activation. However, about 10 years ago, along with the dramatic advancement of genomics techniques such as chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq), it was found that the majority of Pol II proteins engage with protein-coding genes, either resting or actively expressing, in the promoter-proximal region [15, 18–20]. Studies published in 2010 suggested that approximately 30% and more than 90% of protein coding genes in *Drosophila* and mice, respectively, harbor Pol II enriched in the promoter-proximal sites. This phenomenon was called Pol II promoter-proximal pausing [15, 18] (Fig. 2). The Pol II pausing site was mapped genome-wide between +20 and +60 and between +25 and +100 from the TSS in *Drosophila* and humans, respectively [10, 18].

Studies published in the decade since the discovery of genome-wide Pol II pausing have identified several players that regulate Pol II pausing and the pause release of Pol II. Many of these studies were performed using the *HSP70* gene, the first gene discovered to have promoter-proximally paused Pol II in the 1980s [21, 22]. Protein factors such as negative elongation factor (NELF), DRB sensitivity-inducing factor (DSIF), Gdown 1, and tripartite-motif containing 28 (TRIM28), and nucleic acid factors such as the G-quadruplex structure, reportedly stabilize Pol II pausing [10, 23–29]. NELF and DSIF induce Pol II pausing, and Gdown 1, G-quadruplex, and TRIM28 stabilize it (Fig. 3). In biophysics assays, artificial DNA supercoiling applied either upstream or downstream of the elongating Pol II has also been found to pause Pol II [30, 31], suggesting that topological stresses in DNA could affect the kinetics of Pol II (Fig. 3). In spite of the apparent transcription arrest, Pol II pausing is considered a prerequisite step before productive elongation [32–34]. This is supported by observations of deregulated capping and reduced productivity in mRNA synthesis upon transcriptional activation by manipulations that compromise Pol II pausing [14, 23, 35–38].

Pol II promoter-proximal pausing also enables a synchronized, rapid response to the activation signals received by the promoter [32, 34, 39]. It is well known that Pol II pausing is utilized in diverse stimulus-inducible genes including heat-, serum-, and neurotransmitter-inducible ones [32–34, 40]. It is critical that these genes be rapidly expressed upon receiving the activating signals, and Pol II pausing appears to be an important step for accumulating the potential energy to launch the processive elongation. Pol II pausing immediately downstream of TSS is likely to function as

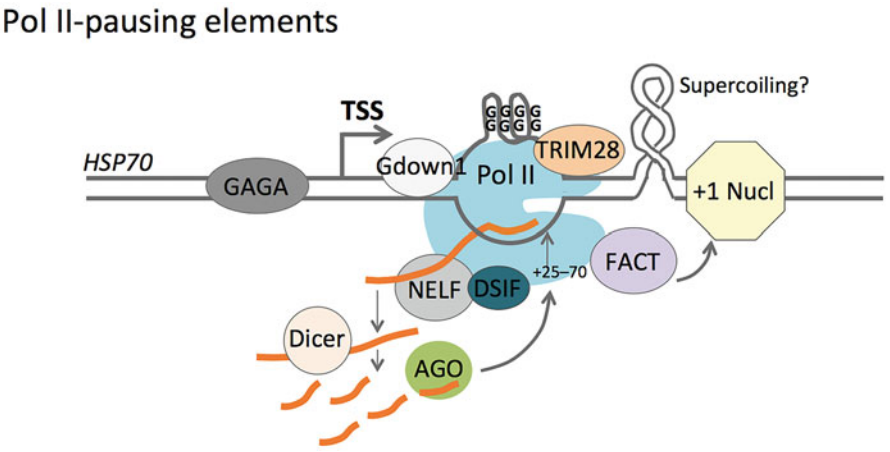



**Fig. 3 Pol II-pausing elements at *HSP70*.** The protein and nucleic acid factors that have been discovered to induce or stabilize Pol II pausing are described. The G-quadruplex formed by the non-template DNA of *HSP70* TSS was shown in a two-consecutive hairpin structure with 8 "G"s. Hypothetical positive supercoiling in advance of Pol II early elongation complex is shown. Nucl, nucleosome

an energy barrier for the tight temporal and spatial regulation of the essential genes involved in cell homeostasis and survival. Pol II pausing is observed in other organisms besides *Drosophila,* mice, and humans [10, 15, 33, 41]. For instance, in bacteriophage λ, late lytic genes are transcribed from the pR' promoter [42, 43]. The immediate downstream of the TSS includes a segment that resembles the −10 box, causing the holo-enzyme, RNA Pol and σ factor, to pause due to, the strong affinity between σ factor region 2 and the −10-like sequence [44]. This early pausing is rescued by the anti-pausing transcription factor λQ protein, upon activation of the lytic genes (https://hdl.handle.net/1813/12839) [44, 45]. In addition, recently, despite the lack of NELF homologs, the prevalence of Pol II pausing has been reported in *Arabidopsis*, and it is opposite to what had previously been thought [46–48].

Promoter-proximally paused Pol II is released when the gene is activated by different stimuli. Although various gene-specific transcription factors function in transcriptional activation, some general elements are essential in transforming the signals to Pol II and epigenetic factors to propel Pol II translocation. For example, P-TEFb is a key protein factor for Pol II pause release and transcriptional resumption that phosphorylates NELF, DSIF, and the C-terminal domain (CTD) of Pol II at serine 2 and the phosphorylation is important for Pol II to resume transcription [8, 49–51]. In addition, AFF4, ELL, MYC, topoisomerase IIβ (TOP2B), and phosphoinositide-3 (PI3) kinases modulate Pol II and epigenetic factors during Pol II pause release [10, 15, 33, 40, 52–54]. It appears that a flare of phosphorylation and ATP hydrolysis occurs upon gene activation for the energetically endothermic and anabolic pathway to synthesize macromolecules. Details of the pause release mechanisms are discussed below.

Heat shock protein 70 (*HSP70*) is a conserved gene in evolution and has been an exemplary and model gene to study Pol II pausing and pause release [1, 10, 24, 55–59]. HSP are molecular chaperones that are essential to the proper folding of cellular proteins, an important process for protein quality control [60]. Due to the thermosusceptible nature of proteins, *HSP,* including *HSP70*, are rapidly expressed upon heat shock, within a few seconds of heat induction, *in vitro*. Various other cellular stresses, including osmotic, hypoxia, and chemical stresses, activate *HSP70* expression, which depends on a potent transcription factor, named heat shock factor 1 (HSF1), a highly conserved gene in eukaryotes [60, 61]. HSF1, which is located in the cytosol, undergoes structural changes that lead to autophosphorylation and trimerization travels into the nucleus to activate the *HSP* genes including *HSP70* [1, 62]. HSF1 is a DNA-binding protein that binds to heat shock response element (HSE) as a trimer [63]. HSF1 recruitment to HSE in the promoter of *HSP70* is likely the first step to provoking a cascade of signaling events for Pol II pause release upon gene activation. The regulatory mechanisms discovered from studying the *HSP70* gene in *Drosophila* and humans are the basis for understanding Pol II pausing and pause release in diverse stress-inducible genes in metazoan cells. In this chapter, we review the regulatory mechanisms of Pol II promoter-proximal pausing and pause release in *HSP70*, the function of HSF1 in this regulation, and how these collaborate for the *HSP70* gene expression.

## 2 RNA Pol II Promoter-Proximal Pausing in *HSP70*

In the early 1960s, *Hsp70* was discovered by F. Ritossa and his colleagues as they worked with *Drosophila* cells [64]. The *hsp70* DNA sequence of *Drosophila* genome and its transcription were analyzed and characterized by J. Lis' group in the 1970s and 1980s [65, 66]. In 1988, it was found that Pol II is engaged with the 5′ end of the uninduced *Drosophila hsp70* gene, transcriptionally proficient yet paused [21, 22]. This observation was novel and unexpected at the time because it had been believed then that Pol II were recruited to transcriptionally activated genes and so were void in transcriptionally inactive or unproductive genes.

In the traditional model, transcriptional activation initiates the recruitment of RNA Pol II and general transcription factors to the promoter of activated genes to form a pre-initiation complex (Fig. 2) [67]. The TATA box binding protein (TBP) of TFIID binds to and bends the promoter to unwind the double strand of DNA. Using the template DNA strand, de novo synthesis of the RNA molecule begins as RNA Pol II incorporates incoming nucleoside triphosphate (NTP) and is translocated downstream from the TSS (+1). However, the affinity between the promoter elements and RNA Pol II prevents the enzyme from being released from the promoter and requires an accumulation of energy to break the energy barrier [68]. In bacterial transcription, the kinetic energy exerted spent breaking such energy barrier is explained by the scrunching model [68]. In the model, DNA is considered to be a flexible element and the downstream DNA is scrunched into the active site of enzyme and returned to its original position. The reiteration of these actions, namely, the scrunching and relaxation of the DNA, generates small RNA fragments in the range of 2–10 nt, called abortive initiation [68, 69]. The stress energy combined with the stable RNA–DNA hybrid formation in the enzyme–nucleic acid complex permits RNA Pol to overcome the energy barrier and move downstream of the gene body. The promoter-escaped RNA Pol II complex enters transcriptional elongation, in which more processive polymerization of NTP is carried out to mature the nascent RNA molecule. In the transcriptional termination, Pol II transcribes the Poly A site, and the RNA sequence is recognized and cleaved for polyadenylation [70–72]. In another model, the Nrd1, Nab3, Sen1 complex induces the elongation complex to be dissociated [72].

In the late 2000s along with the development of genome-wide analyses, it was found that a majority of Pol II is already bound to a large number of protein-coding genes at the 5′ end, as in *Drosophila hsp70*, even before gene activation (Fig. 2) [15, 18, 34]. This phenomenon is called RNA Pol II promoter-proximal pausing. Using ChIP-seq for Pol II in *Drosophila* and mice demonstrated the enrichment of Pol II in TSS, and using RNA-seq in *Drosophila* showed that small RNA molecules are generated from the TSS-engaged Pol II [15, 18]. Since these findings, numerous studies have reported various regulatory elements that are important for controlling the paused Pol II in the TSSs.

Because *hsp70* is the first gene shown to harbor Pol II in the TSS, many findings that are important for understanding Pol II promoter-proximal pausing have been

produced from studies of the transcriptional regulation of the *Drosophila hsp70* genes (Fig. 3). Pol II pausing is mediated by DSIF and NELF [34]. In the *Drosophila hsp70* gene, NELF is co-localized with Pol II at the promoter-proximal region, causing Pol II to be paused at around +25 from the TSS and released upon *hsp70* activation [24]. NELF is composed of four subunits, NELF-A, NELF-B, NELF-C/D, and NELF-E, and it makes extensive contacts with Pol II, DSIF, and the nascent RNA [26]. For example, NELF-E and NELF-A interact with the nascent RNA and DSIF to collaborate in establishing Pol II pausing. DSIF is also known to co-localize with NELF and Pol II at the *hsp70* gene during inactive transcription [24, 32, 73]. Knock-down and immunodepletion of DSIF and NELF weaken Pol II pausing at *hsp70* gene *in vivo* and *in vitro*, respectively [24, 74]. In transcriptional activation, DSIF remains as a complex with elongating Pol II while NELF dissociates from the TSS of *hsp70* [34]. Interestingly, NELF knock-down interferes with the transcription of many genes both in humans and in *Drosophila* [23, 38, 75], which implies that Pol II promoter-proximal pausing is required for productive transcription. In fact, Pol II promoter-proximal pausing has been shown to be important for proper capping and gene activation [23, 36]. Rather than being an inhibitory step that delays transcription, it is another rate-limiting step that is preparatory and necessary for signal-induced and synchronized transcriptional activation [1, 32, 34, 39]. Supporting this, recent kinetic studies of Pol II pausing have demonstrated that Pol II pausing occurs during transcriptional initiation not only in resting genes but also in transcriptionally activated genes. In other word, transcriptional activation doesn't eliminate Pol II pausing but shortens the pausing duration [76, 77]. In addition to DSIF and NELF, TRIM28 has been shown to stabilize and regulate Pol II promoter-proximal pausing in the human *HSP70* gene (*HSPA1B*) (Fig. 3) [1, 10, 78]. In *HSPA1B*, Pol II pausing occurs at around +70, and TRIM28 is indirectly or directly associated with the non-template DNA in proximity of the pausing site [10]. *In vitro*, TRIM28 depletion alleviates Pol II promoter-proximal pausing at *HSPA1B* [10]. The G-quadruplex structure of the non-template DNA in the TSS of *HSPA1B* has also been reported to stabilize the pausing [28], suggesting that the G-C richness of TSS regulates the translocation rate of Pol II. Studies have consistently reported that Pol II is paused and tends to be backtracked to the G–C rich sequence for the formation of a more thermodynamically stable DNA–RNA hybrid [18].

Nucleosome and chromosome architecture are also important element in the regulation of Pol II promoter-proximal pausing in *HSP* genes (Fig. 3). The enrichment of nucleosome occupancy downstream of the promoter-proximally paused Pol II and the absence of nucleosome occupancy in the Pol II paused site have been reported, suggesting competition and mutually exclusive binding of Pol II with nucleosomes to DNA [23, 56, 79–81]. The nucleosome that is highly concentrated in the proximity of paused Pol II in the downstream is called the +1 nucleosome [79]. Some studies have suggested that nucleosome remodelers control Pol II pausing. Recently, facilitates chromatin transcription (FACT), a nucleosome remodeler and elongation factor, has been shown to regulate Pol II pausing in *HSP* genes [82]. In addition, GAGA, a short sequence motif in the *Drosophila hsp70* promoter, is important for establishing Pol II pausing [83]. Interestingly, GAGA is

the binding site of the trithorax-like (Trl) protein complex, which recruits NURF, a nucleosome remodeler that facilitates transcription and Pol II pausing release by decondensing nucleosomes [84, 85]. Interestingly, nucleosomes are dramatically and rapidly released from the activated *hsp70* gene in *Drosophila* and depletion of nucleosomes depends on GAGA factor, poly(ADP)-ribose polymerase (PARP), and HSF1 [56]. It has not been determined whether this is conserved in humans because studies have found evidence for the exchange of nucleosomes rather than their eviction from diverse genes that are regulated by Pol II pausing, including *HSP70* in humans. In fact, another *Drosophila* study suggested exchange rather than eviction, indicated by the accumulation of H2A.Z/v, a hyper-acetylated variant of H2A and H3 trimethylated at lysine 4 (H3K4me3) in *hsp70* upon transcriptional activation [86].

Some other interesting aspects of Pol II promoter-proximal pausing at *hsp* genes are the involvement of small interfering RNA species in the regulation of pausing (Fig. 3). In one study, Dicer and Argonaute (AGO) protein KD increased the expression of *HSP70* and *HSP68* [87]. The effects of the processing of promoter-proximal RNAs on Pol II pausing are not known. The small RNA molecules generated by Pol II pausing are about 30–50 bps, and Pol II pausing at *hsp70* in *Drosophila* has a half life of 5 min, based on a study that used GFP-tagged Pol II *in vivo* [88]. It is thought that the quite a number of aborted short transcripts could be generated during the inactive state of transcription, and their role in establishing Pol II pausing awaits further investigation.

## 3 HSF1 in Pol II Pause Release and Gene Expression at *HSP70*

HSP plays important roles in the cell. They are chaperone proteins in protein quality control, assisting the folding and refolding of other proteins [60]. In addition, they participate in various cellular pathways, including immune responses, autophagy, and transcription [89, 90]. When the protective function of these proteins becomes necessary, their induction is almost instantaneous within a few seconds. HSF1 is composed of an N-terminal DNA-binding domain, a coiled-coil domain, a regulatory domain, and a transactivation domain [62, 63]. HSF1 is present as an inactive monomer in a complex with other chaperone proteins, such as Hsp70 and Hsp90 in the cytosol [91]. The regulatory domain is heat-sensitive and becomes unfolded at higher temperatures. This structural change can induce the packing of the coiled-coil domain for trimerization [62]. Elevated temperatures in the cytosol are sensed by HSF1, resulting in the formation of an HSF1 trimer, released from Hsp70 and Hsp90, which inhibit HSF1 activation (Fig. 4). HSF1 trimer is translocated into the nucleus and binds to its binding sites on *HSP* genes (Fig. 4). Several phosphorylation sites are known, including S230 and S326, whose phosphorylation activates HSF1 [63].

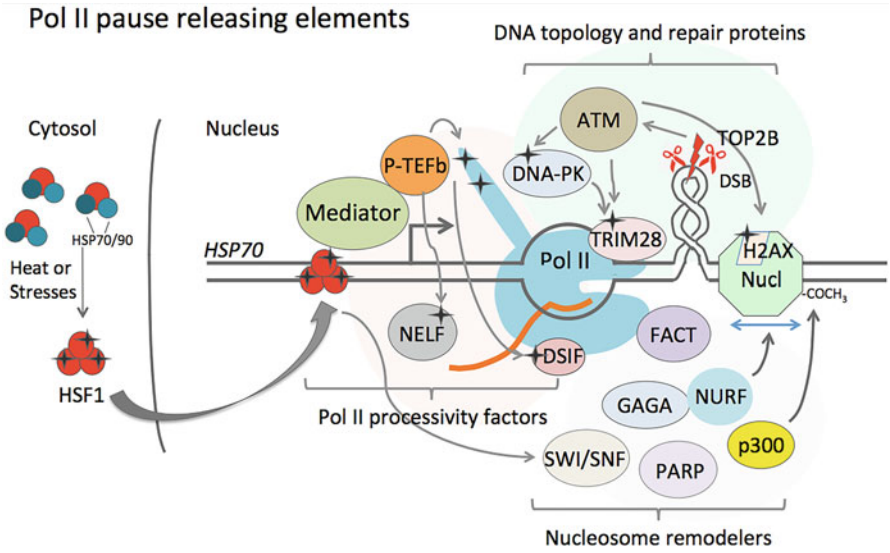



**Fig. 4 Pol II pause releasing elements at *HSP70*.** HSF1 is associated with Hsp70 and Hsp90 in the cytosol as an inactive complex. Thermal increase or various stresses can change the conformation of HSF1 to be released from Hsp70/Hsp90 for homo-trimerization and HSF1 becomes hyperphosphorylated. HSF1 trimer enters the nucleus and binds to the *HSE* of *HSP70* promoter, which triggers dramatic changes in the many elements involving diverse proteins, nucleosomes, and nucleic acids in the promoter and TSS. These factors that are important to release Pol II from the pause site of *HSP70* are depicted. TOP2B dimer is shown as a pair of scissors and DNA double strand break as a lightening sign. Nucleosome repositioning or sliding is shown in a blue bar with arrows on both ends under a nucleosome (Nucl). A protein phosphorylation is shown in a black star mark; acetylation of nucleosome by p300 in -$COCH_3$. Three categories of involved factors by main functions are shown in light pink (Pol II processivity factors: NELF, DSIF, P-TEFb), light green (DNA topology and repair proteins: TOP2B, ATM, DNA-PK, γH2AX, TRIM28), and light blue (nucleosome remodelers: FACT, SWI/SNF, GAGA, NURF, p300, PARP) background

HSF1 is a master transcription factor for the expression of *HSP* genes. Binding of an HSF1 trimer to the HSE on the promoter of *HSP70*, transcription is activated. It is now thought that the activation is mainly attributable to the reverse of Pol II promoter-proximal pausing to resume transcription (Fig. 4). The energy barrier for overcoming the pausing appears to be lowered by HSF1 binding to the promoter. It is known that HSF1 recruits diverse transcription factors to the activated *HSP70* gene, including P-TEFb, PARP1, and p300 (Fig. 4). P-TEFb is the key transcription factor to release promoter-proximally paused Pol II and is recruited by HSF1 [92]. A chemical inhibitor of HSF1, which perturbs the interaction between P-TEFb and HSF1, interferes with the transcriptional activation owing to HSF1 [93]. In *in vitro* biochemical analyses, when recombinant HSF1 purified from bacteria is incubated with the DNA segment including the promoter and early gene body of *HSP70* gene, it increases the recruitment of pre-initiation complex such as Mediator, TBP, P-TEFb, and TFIIE to the DNA [10]. In addition, HSF1 contacts TBP and TFIIB

in the pre-initiation complex [94]. P-TEFb phosphorylates the C-terminal domain of RPB1, the largest subunit of Pol II, at Ser2 (S2 Pol II), DSIF, and NELF, which is a critical event for Pol II pause release. Phosphorylated NELF dissociates from the paused Pol II complex for release, while phosphorylated DSIF remains in the Pol II elongation complex as an activator. HSF1 triggers the reformation of pausing inducing-proteins and Pol II for processive elongation through P-TEFb as well as the modulation of chromatin architecture through SWI/SNF, PARP, and p300 [89, 95, 96] (Fig. 4). P300 is a histone acetyl-transferase that modulates chromosome accessibility and acetylates HSF1 for DNA binding at *HSP70* [97]. Nucleosome remodeling factor SWI/SNF is important for the expression of human and *Drosophila hsp70*, necessary for transcriptional elongation [58, 95, 98]. HSF1 recruits SWI/SNF and mediates nucleosome rearrangement to favor processive Pol II elongation [58, 95]. PARP is a DNA repair enzyme that mediates the poly-ADP ribosylation of proteins. During transcriptional activation at *hsp70* in *Drosophila*, PARP is recruited, and the inhibition of PARP hinders nucleosome dissociation and transcription [56]. In the same organism, activated PARP recruits Mi-2 for *hsp70* transcriptional activation [99]. In humans, PARP1 is recruited by HSF1, which is important for the redistribution of PARP1 in DNA repair and transcription [96]. PARP1 catalyzes the poly-ADP ribosylation of nucleosomes and loosens them to increase accessibility [100, 101].

In addition to these protein and nucleosome factors, Pol II pause release at *HSP70* involves DNA topoisomerase IIβ (TOP2B)-mediated DNA double strand break (DSB) and DNA damage response (DDR) signaling [10, 33] (Fig. 4). TOP2B-mediated DSB and DDR signaling for transcriptional activation were first identified in estrogen- and androgen-receptor gene activation, about a decade ago, in humans [102–104]. TOP2B-mediated DSB has been mapped to the promoter of these genes upon transcriptional activation [103]. Interestingly, this TOP2B-mediated DSB is considered important for the activation of stress-inducible genes, including *HSP70* in humans [1, 10, 33, 39, 40, 105]. In the release of paused Pol II at *HSP70*, TOP2B is recruited and DDR signaling is triggered throughout the entire TSS and gene body [33]. Inhibiting TOP2B reduces the efficiency of Pol II pause release, transcriptional elongation, and DDR signaling [33, 40]. In a previous study, DDR signaling was visualized in the gene body through ChIP-seq analyses of γH2AX a phosphorylated variant (at Ser139) of histone 2A, which is indicative of DSB-induced DDR [33, 40], and phosphorylated TRIM28 at Ser824 [1, 33]. TRIM28, which stabilizes Pol II pausing in the inactive state of transcription, becomes phosphorylated at Ser824 by ATM and DNA-dependent protein kinase (DNA-PK) during Pol II pause release at *HSP70* [10, 54] (Fig. 4). In fact, TRIM28 phosphorylation at Ser824 by ataxia telangiectasia-mutated (ATM) is known to be involved in DSB repair, and it is one of the first events that follow the incident of DSB [106]. The inhibition of ATM with a small chemical interferes with Pol II translocation to the gene body and γH2AX accumulation at *HSP70* [1, 10, 33]. This signifies that DSB and DDR signaling are required for Pol II pause release and active transcription at *HSP70* [1, 33, 39]. Importantly, this TOP2B-mediated DSB and DDR signaling are required for Pol II pause release in other stress genes, including neurotransmitter- and serum-inducible genes [33, 40].

The function of TOP2B-mediated DSB and DDR signaling for transcriptional activation and elongation remains unclear and requires further investigation. However, it is speculated that DNA torsion in relation to the nature of the helical structure of double stranded DNA is generated during Pol II translocation for transcriptional activation [30, 107–109] (Fig. 3). *In vitro* biochemical studies have established that supercoiling is generated by elongating Pol II, that is, negative and positive supercoiling upstream and downstream of the motor protein, respectively [30, 108, 109] (Figs. 3 and 4). These studies have also shown that topoisomerases play an important role in resolving the torsional stresses. Consistently, a biophysical study demonstrated that RNA Pol is a torque-resistant enzyme, although it stalls over a certain threshold of torque [30, 31]. These findings suggest that torsional stresses may contribute to Pol II promoter-proximal pausing, and these must be resolved to allow Pol II processivity upon Pol II pause release (Figs. 3 and 4). It is difficult to map TOP2B on the genome, but when it is trapped in human genome using etoposide, a TOP2B poison that induces the formation of TOP2B-DNA adduct, it is enriched in the TSS of a large number of genes [110]. According to the canonical understanding, a TOP2B dimer cuts the DNA double strand, removes the positive supercoiling, and reseals the broken DNA. These three steps occur instantaneously without any need for DNA repair signaling [105, 111, 112]. It is interesting that TOP2B catalysis induces DDR signaling in the transcriptional activation of *HSP70* and other stimulus-inducible genes, because this may imply a persistent DNA break rather than the canonical catalysis of TOP2B [105, 113]. In fact, a recent study showed that TOP2B causes spontaneous DSB for abortive catalysis during the transcriptional activation of estrogen receptor genes [113, 114]. It remains to be determined whether this abortive TOP2B catalysis is responsible for DDR signaling in *HSP70* transcription, and if so, what is the cellular mechanism to repair it alongside active transcription and Pol II elongation.

Although HSF1 binding triggers dramatic changes in the protein network and nucleosomal environment, described above, whether it signals or induces TOP2B activation is not yet known. However, HSF1 binding appears to be sufficient to induce DDR signaling in the gene body presumably through TOP2B-mediated DSB [10]. The inhibition of the interaction between HSF1 and P-TEFb, for example, using a small chemical does not block the phosphorylation of TRIM28 at Ser824, indicating that TOP2B-induced DSB and DDR occur simultaneously with HSF1 binding before Pol II pause release by P-TEFb [10]. It remains to be determined how HSF1 binding to the *HSP70* promoter can regulate the catalytic activity of TOP2B.

## 4 Discussion

*HSP70* is a model gene representative for RNA Pol II promoter-proximal pausing and pause release and has been intensively studied for the phenomenon. HSF1 is almost the only transcriptional activator required to stimulate this gene *in vitro* [10, 62, 63, 91], which makes *HSP70* an ideal *in vitro* system for studying

regulations. In addition, the straightforward and controllable method to induce gene activation that is feasible to visualize (in the case of *Drosophila*) and quantify has contributed to many wonderful *in vitro* studies that have helped to elucidate transcriptional regulation in *HSP70* [10, 33, 56, 57, 115]. Therefore, it is important to take the lessons and findings from the transcriptional activation of *HSP70* to apply, test, and evaluate other signal-inducible genes utilizing Pol II pausing to understand the general and gene-specific mechanisms of Pol II pausing and pause release regulation.

In the current model, Pol II promoter-proximal pausing is induced by the central pausing factors, DSIF and NELF. These factors interact with Pol II and with nascent RNA (for NELF) to interfere with Pol II translocation downstream along the gene (Fig. 3) [24–26]. TRIM28 stabilizes Pol II pausing by directly or indirectly binding non-template DNA [10]. The G-quadruple structure of the non-template DNA in the *HSP70* TSS is a nucleic acid element that contributes to the formation of Pol II pausing [28]. In addition, the +1 nucleosome is likely to function as a roadblock by supporting a local heterochromatin structure [34, 79, 80, 116] (Fig. 3).

HSF1 is a key transcriptional activator for *HSP70*. This single-protein molecule functions as a signaling molecule from the cytosol, where it senses extracellular signals, into the nucleus where it binds to the promoters of target genes to activate them (Fig. 4). Pol II promoter-proximal pause release is initiated upon HSF1 binding. HSF1 has a variety of binding partners in the cell. Among these, P-TEFb, a master Pol II pause release factor, is also recruited by HSF1 [92, 93, 117], which recruits and interacts with Mediator protein through its transactivation domain [118]. Mediator is a large scaffolding protein that makes numerous contacts and has interactions with elements in the enhancer, promoter, and gene body (Fig. 1) [119, 120]. Transcription activator binding induces structural changes in Mediator, altering its properties and binding partners, which is crucial for productive gene activation [11, 121]. P-TEFb interacts with Mediator that accounts for the accelerated transcription [120, 121]. Pol II, DSIF, and NELF phosphorylation by P-TEFb is crucial for Pol II pause release [34, 73] (Fig. 4). The phosphorylation alters the protein dynamics and propels the Pol II early elongation complex downstream. However, the nucleosome structure must become permissive for Pol II processivity, and the nucleosome remodelers function on this matter. PARP1, p300, NURF, and SWI/SNF are involved in relaxing or rearranging nucleosomes to favor transcription [56, 97, 115, 122] (Fig. 4).

Recent findings suggest that DNA topology also helps regulate Pol II promoter-proximal pausing and pause release [33, 40, 105, 112]. *In vitro*, the DNA double helix, unwound by the transcription bubble, imposes positive and negative supercoiling ahead and behind the transcribing Pol II [30, 108]. It remains unclear whether torsional stress occurs to induce Pol II pausing *in vivo*, as has been shown *in vitro*, and if so, to what extent this happens. Setting aside this unresolved question, it has been demonstrated that TOP2B plays an important role in Pol II pause release at *HSP70* and other stimulus-inducible genes [33, 40, 105] (Fig. 4). The transcriptional activating signal that is propagated by HSF1 to express the *HSP70* gene appears to trigger the catalytic activity of TOP2B, independent of P-TEFb function

[10, 39]. This suggests that the DNA break and damage response signaling during Pol II pause release and transcriptional activation is a parallel signaling pathway to the P-TEFb-mediated one (Fig. 5). Consistent to this observation, some studies have reported that TOP2B is phosphorylated and phosphorylated TOP2B is catalytically active [123–125]. Although the nature and function of TOP2B phosphorylation is less defined and debatable, these studies suggest that TOP2B phosphorylation might be modulated during the resting state of transcription and upon HSF1 binding to the promoter. The kinases that are responsible for TOP2B phosphorylation is not clearly known because TOP2B is not co-immunoprecipitated with any particular kinases while protein kinase C, casein kinase II, and the viral lysate do phosphorylate TOP2B [124, 126–128]. The connection between HSF1 and TOP2B must be better understood as well as the exact mechanisms of TOP2B regulation during transcriptional activation.

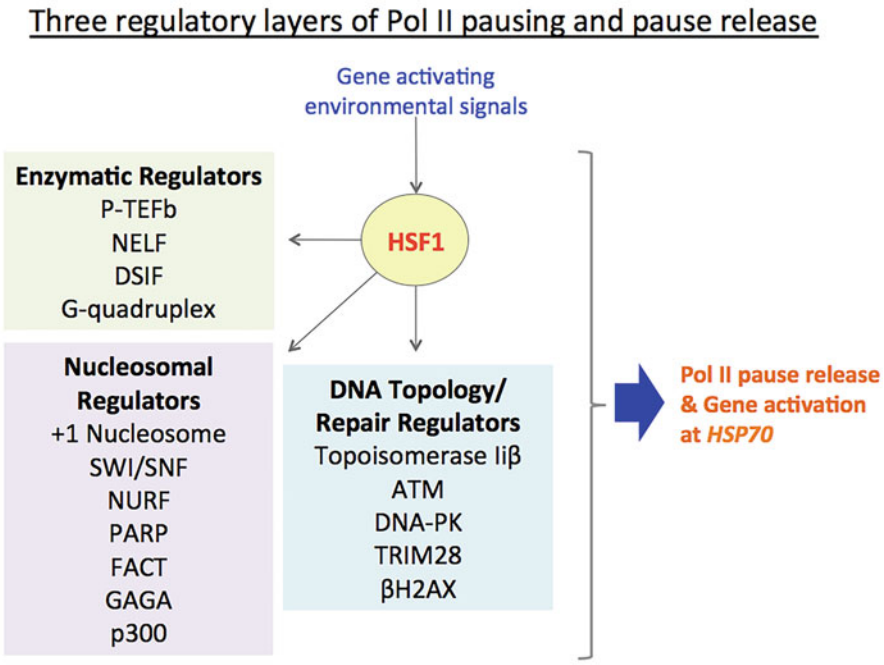



**Fig. 5 Three mechanical layers that regulate Pol II pausing and pause release at *HSP70*.** Pol II pausing and pause release are controlled by a diverse group of transcription factors and elements. They can be categorized to regulate the translocation or processivity of Pol II, the chromatin environment, or DNA topology and repair function. These three mechanical layers of regulation cooperate to coordinate transcriptional pausing and the resumption of productive transcription in the promoter-proximal site. HSF1, the central transcriptional activator of *HSP70*, drives the resumption by recruiting other transcriptional activators and triggering reversal reactions in the pre-existing players. The outcome of HSF binding to the *HSP70* promoter is Pol II pause release and an efficient production of *HSP70* mRNA

## 5 Conclusions

Pol II promoter-proximal pausing is a newly identified rate-limiting step in transcriptional regulation in metazoan cells. *HSP70* is a model gene for the study of the mechanisms of Pol II pausing and pause release. HSF1-mediated transcriptional activation provokes a series of downstream events that allow Pol II release from the pausing site, which involves regulations that modulate Pol II and nucleosome dynamics, as well as the topological state of the DNA (Fig. 5). These three elements in Pol II pause release appear to be common features in signal-induced gene activation and need to be further elucidated to understand the regulation of gene expression in metazoans.

**Acknowledgements** I appreciate S.K. Calderwood at Beth Israel Deaconess Medical Center and D.J. Taatjes at the University of Colorado for their support to study the transcriptional regulation at *HSP70*. I thank previous and current Bunch lab members in the School of Applied Biosciences at Kyungpook National University (KNU), J. Christ, John, and D.Y. Bunch for their support and loving encouragement. I also thank International Edit for the help with proofreading manuscript. This study was supported by a grant from Korea National Foundation (NRF) (NRF-2017R1D1A1B03030548 and NRF-2020R1F1A1060996) to H.B.

**Disclosure of Interest** There is no conflict of interest for the enclosed review article.

**Ethical Approval for Studies Involving Humans** This article does not contain any studies with human participants performed by any of the authors.

**Ethical Approval for Studies Involving Animals** This article does not contain any studies with animals performed by any of the authors.

## References

1. Bunch H (2017) RNA polymerase II pausing and transcriptional regulation of the HSP70 expression. Eur J Cell Biol 96:739–745
2. Roeder RG (2019) 50+ years of eukaryotic transcription: an expanding universe of factors and mechanisms. Nat Struct Mol Biol 26:783–791
3. Bunch H (2018) Gene regulation of mammalian long non-coding RNA. Mol Gen Genet 293:1–15
4. Cramer P (2004) Structure and function of RNA polymerase II. Adv Protein Chem 67:1–42
5. Hahn S (2004) Structure and mechanism of the RNA polymerase II transcription machinery. Nat Struct Mol Biol 11:394–403
6. Phatnani HP, Greenleaf AL (2006) Phosphorylation and functions of the RNA polymerase II CTD. Genes Dev 20:2922–2936
7. Buratowski S (2003) The CTD code. Nat Struct Biol 10:679–680
8. Itzen F, Greifenberg AK, Bosken CA, Geyer M (2014) Brd4 activates P-TEFb for RNA polymerase II CTD phosphorylation. Nucleic Acids Res 42:7577–7590
9. Sawadogo M, Roeder RG (1985) Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. Cell 43:165–175

10. Bunch H et al (2014) TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause release. Nat Struct Mol Biol 21:876–883
11. Ebmeier CC, Taatjes DJ (2010) Activator-mediator binding regulates mediator-cofactor interactions. Proc Natl Acad Sci U S A 107:11283–11288
12. Poss ZC, Ebmeier CC, Taatjes DJ (2013) The mediator complex and transcription regulation. Crit Rev Biochem Mol Biol 48:575–608
13. Shandilya J, Roberts SG (2012) The transcription cycle in eukaryotes: from productive initiation to RNA polymerase II recycling. Biochim Biophys Acta 1819:391–400
14. Rasmussen EB, Lis JT (1993) In vivo transcriptional pausing and cap formation on three Drosophila heat shock genes. Proc Natl Acad Sci U S A 90:7923–7927
15. Rahl PB et al (2010) c-Myc regulates transcriptional pause release. Cell 141:432–445
16. McClure WR (1980) Rate-limiting steps in RNA chain initiation. Proc Natl Acad Sci U S A 77:5634–5638
17. Henderson KL et al (2017) Mechanism of transcription initiation and promoter escape by E. coli RNA polymerase. Proc Natl Acad Sci U S A 114:E3032–E3040
18. Nechaev S et al (2010) Global analysis of short RNAs reveals widespread promoter-proximal stalling and arrest of Pol II in Drosophila. Science 327:335–338
19. Lee C et al (2008) NELF and GAGA factor are linked to promoter-proximal pausing at many genes in Drosophila. Mol Cell Biol 28:3290–3300
20. Gilmour DS (2009) Promoter proximal pausing on genes in metazoans. Chromosoma 118:1–10
21. Rougvie AE, Lis JT (1988) The RNA polymerase II molecule at the 5′ end of the uninduced hsp70 gene of D. melanogaster is transcriptionally engaged. Cell 54:795–804
22. Gilmour DS, Lis JT (1986) RNA polymerase II interacts with the promoter region of the noninduced hsp70 gene in Drosophila melanogaster cells. Mol Cell Biol 6:3984–3989
23. Gilchrist DA et al (2008) NELF-mediated stalling of Pol II can enhance gene expression by blocking promoter-proximal nucleosome assembly. Genes Dev 22:1921–1933
24. Wu CH et al (2003) NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in Drosophila. Genes Dev 17:1402–1414
25. Yamaguchi Y et al (1999) NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation. Cell 97:41–51
26. Vos SM, Farnung L, Urlaub H, Cramer P (2018) Structure of paused transcription complex Pol II-DSIF-NELF. Nature 560:601–606
27. Wada T et al (1998) DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. Genes Dev 12:343–356
28. Szlachta K et al (2018) Alternative DNA secondary structure formation affects RNA polymerase II promoter-proximal pausing in human. Genome Biol 19:89
29. Jishage M et al (2012) Transcriptional regulation by Pol II(G) involving mediator and competitive interactions of Gdown1 and TFIIF with Pol II. Mol Cell 45:51–63
30. Ma J, Bai L, Wang MD (2013) Transcription under torsion. Science 340:1580–1583
31. Ma J et al (2019) Transcription factor regulation of RNA polymerase's torque generation capacity. Proc Natl Acad Sci U S A 116:2583–2588
32. Liu X, Kraus WL, Bai X (2015) Ready, pause, go: regulation of RNA polymerase II pausing and release by cellular signaling pathways. Trends Biochem Sci 40:516–525
33. Bunch H et al (2015) Transcriptional elongation requires DNA break-induced signalling. Nat Commun 6:10191
34. Adelman K, Lis JT (2012) Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. Nat Rev Genet 13:720–731
35. Core L, Adelman K (2019) Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. Genes Dev 33:960–982
36. Nilson KA et al (2015) THZ1 reveals roles for Cdk7 in co-transcriptional capping and pausing. Mol Cell 59:576–587

37. Ebmeier CC et al (2017) Human TFIIH kinase CDK7 regulates transcription-associated chromatin modifications. Cell Rep 20:1173–1186
38. Sun J, Li R (2010) Human negative elongation factor activates transcription and regulates alternative transcription initiation. J Biol Chem 285:6443–6452
39. Bunch H (2016) Role of genome guardian proteins in transcriptional elongation. FEBS Lett 590:1064–1075
40. Madabhushi R et al (2015) Activity-induced DNA breaks govern the expression of neuronal early-response genes. Cell 161:1592–1605
41. Bunch H et al (2016) RNA polymerase II promoter-proximal pausing in mammalian long non-coding genes. Genomics 108:64–77
42. Kainz M, Roberts JW (1995) Kinetics of RNA polymerase initiation and pausing at the lambda late gene promoter in vivo. J Mol Biol 254:808–814
43. Liu X, Jiang H, Gu Z, Roberts JW (2013) High-resolution view of bacteriophage lambda gene expression by ribosome profiling. Proc Natl Acad Sci U S A 110:11928–11933
44. Petushkov I, Esyunina D, Kulbachinskiy A (2017) Possible roles of sigma-dependent RNA polymerase pausing in transcription regulation. RNA Biol 14:1678–1682
45. Grayhack EJ, Yang XJ, Lau LF, Roberts JW (1985) Phage lambda gene Q antiterminator recognizes RNA polymerase near the promoter and accelerates it through a pause site. Cell 42:259–269
46. Zhu J, Liu M, Liu X, Dong Z (2018) RNA polymerase II activity revealed by GRO-seq and pNET-seq in Arabidopsis. Nature plants 4:1112–1123
47. Hetzel J, Duttke SH, Benner C, Chory J (2016) Nascent RNA sequencing reveals distinct features in plant transcription. Proc Natl Acad Sci U S A 113:12316–12321
48. Yu X, Martin PGP, Michaels SD (2019) BORDER proteins protect expression of neighboring genes by promoting 3' Pol II pausing in plants. Nat Commun 10:4359
49. Peterlin BM, Price DH (2006) Controlling the elongation phase of transcription with P-TEFb. Mol Cell 23:297–305
50. Yamada T et al (2006) P-TEFb-mediated phosphorylation of hSpt5 C-terminal repeats is critical for processive transcription elongation. Mol Cell 21:227–237
51. Fujinaga K et al (2004) Dynamics of human immunodeficiency virus transcription: P-TEFb phosphorylates RD and dissociates negative effectors from the transactivation response element. Mol Cell Biol 24:787–795
52. Byun JS et al (2012) ELL facilitates RNA polymerase II pause site entry and release. Nat Commun 3:633
53. Liang K et al (2018) Targeting Processive transcription elongation via SEC disruption for MYC-induced Cancer therapy. Cell 175:766–779 e717
54. Bunch H, Calderwood SK (2015) TRIM28 as a novel transcriptional elongation factor. BMC Mol Biol 16:14
55. Benjamin LR, Gilmour DS (1998) Nucleosomes are not necessary for promoter-proximal pausing in vitro on the Drosophila hsp70 promoter. Nucleic Acids Res 26:1051–1055
56. Petesch SJ, Lis JT (2008) Rapid, transcription-independent loss of nucleosomes over a large chromatin domain at Hsp70 loci. Cell 134:74–84
57. Zobeck KL, Buckley MS, Zipfel WR, Lis JT (2010) Recruitment timing and dynamics of transcription factors at the Hsp70 loci in living cells. Mol Cell 40:965–975
58. Corey LL, Weirich CS, Benjamin IJ, Kingston RE (2003) Localized recruitment of a chromatin-remodeling activity by an activator in vivo drives transcriptional elongation. Genes Dev 17:1392–1401
59. de La Serna IL et al (2000) Mammalian SWI-SNF complexes contribute to activation of the hsp70 gene. Mol Cell Biol 20:2839–2851
60. Chen B, Retzlaff M, Roos T, Frydman J (2011) Cellular strategies of protein quality control. Cold Spring Harb Perspect Biol 3:a004374
61. Hammerer-Lercher A et al (2001) Hypoxia induces heat shock protein expression in human coronary artery bypass grafts. Cardiovasc Res 50:115–124

62. Hentze N, Le Breton L, Wiesner J, Kempf G, Mayer MP (2016) Molecular mechanism of thermosensory function of human heat shock transcription factor Hsf1. elife 5:e11576
63. Vihervaara A, Sistonen L (2014) HSF1 at a glance. J Cell Sci 127:261–266
64. Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia 18:571–573
65. Lis JT, Neckameyer W, Dubensky R, Costlow N (1981) Cloning and characterization of nine heat-shock-induced mRNAs of Drosophila melanogaster. Gene 15:67–80
66. Moran L et al (1979) Physical map of two D. melanogaster DNA segments containing sequences coding for the 70,000 dalton heat shock protein. Cell 17:1–8
67. Werner F, Grohmann D (2011) Evolution of multisubunit RNA polymerases in the three domains of life. Nat Rev Microbiol 9:85–98
68. Kapanidis AN et al (2006) Initial transcription by RNA polymerase proceeds through a DNA-scrunching mechanism. Science 314:1144–1147
69. Winkelman JT, Gourse RL (2017) Open complex DNA scrunching: a key to transcription start site selection and promoter escape. BioEssays News Rev Mol Cell Dev Biol 39:1600193
70. Porrua O, Libri D (2015) Transcription termination and the control of the transcriptome: why, where and how to stop. Nat Rev Mol Cell Biol 16:190–202
71. Proudfoot NJ (2016) Transcriptional termination in mammals: stopping the RNA polymerase II juggernaut. Science 352:aad9926
72. Larochelle M et al (2018) Common mechanism of transcription termination at coding and noncoding RNA genes in fission yeast. Nat Commun 9:4364
73. Ping YH, Rana TM (2001) DSIF and NELF interact with RNA polymerase II elongation complex and HIV-1 Tat stimulates P-TEFb-mediated phosphorylation of RNA polymerase II and DSIF during transcription elongation. J Biol Chem 276:12951–12958
74. Li J et al (2013) Kinetic competition between elongation rate and binding of NELF controls promoter-proximal pausing. Mol Cell 50:711–722
75. Muse GW et al (2007) RNA polymerase is poised for activation across the genome. Nat Genet 39:1507–1511
76. Gressel S, Schwalb B, Cramer P (2019) The pause-initiation limit restricts transcription activation in human cells. Nat Commun 10:3603
77. Samarakkody A et al (2015) RNA polymerase II pausing can be retained or acquired during activation of genes involved in the epithelial to mesenchymal transition. Nucleic Acids Res 43:3938–3949
78. Bunch H, Calderwood SK (2015) TRIM28 as a novel transcriptional elongation factor. BMC Mol Biol 16:14
79. Jimeno-Gonzalez S, Ceballos-Chavez M, Reyes JC (2015) A positioned +1 nucleosome enhances promoter-proximal pausing. Nucleic Acids Res 43:3068–3078
80. Petesch SJ, Lis JT (2012) Overcoming the nucleosome barrier during transcript elongation. Trends Genet 28:285–294
81. Teves SS, Henikoff S (2011) Heat shock reduces stalled RNA polymerase II and nucleosome turnover genome-wide. Genes Dev 25:2387–2397
82. Tettey TT et al (2019) A role for FACT in RNA polymerase II promoter-proximal pausing. Cell Rep 27:3770–3779 e3777
83. Levine M (2011) Paused RNA polymerase II as a developmental checkpoint. Cell 145:502–511
84. Ohtsuki S, Levine M (1998) GAGA mediates the enhancer blocking activity of the eve promoter in the Drosophila embryo. Genes Dev 12:3325–3330
85. Tsai SY, Chang YL, Swamy KB, Chiang RL, Huang DH (2016) GAGA factor, a positive regulator of global gene expression, modulates transcriptional pausing and organization of upstream nucleosomes. Epigenetics Chromatin 9:32
86. Kusch T, Mei A, Nguyen C (2014) Histone H3 lysine 4 trimethylation regulates cotranscriptional H2A variant exchange by Tip60 complexes to maximize gene expression. Proc Natl Acad Sci U S A 111:4850–4855

87. Cernilogar FM et al (2011) Chromatin-associated RNA interference components contribute to transcriptional regulation in Drosophila. Nature 480:391–395
88. Buckley MS, Kwak H, Zipfel WR, Lis JT (2014) Kinetics of promoter Pol II on Hsp70 reveal stable pausing and key insights into its regulation. Genes Dev 28:14–19
89. Calderwood SK, Murshid A (2017) Molecular chaperone accumulation in cancer and decrease in Alzheimer's disease: the potential roles of HSF1. Front Neurosci 11:192
90. Witkin SS, Kanninen TT, Sisti G (2017) The role of Hsp70 in the regulation of autophagy in gametogenesis, pregnancy, and parturition. Adv Anat Embryol Cell Biol 222:117–127
91. Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell 94:471–480
92. Lis JT, Mason P, Peng J, Price DH, Werner J (2000) P-TEFb kinase recruitment and function at heat shock loci. Genes Dev 14:792–803
93. Yoon YJ et al (2011) KRIBB11 inhibits HSP70 synthesis through inhibition of heat shock factor 1 function by impairing the recruitment of positive transcription elongation factor b to the hsp70 promoter. J Biol Chem 286:1737–1747
94. Yuan CX, Gurley WB (2000) Potential targets for HSF1 within the preinitiation complex. Cell Stress Chaperones 5:229–242
95. Sullivan EK, Weirich CS, Guyon JR, Sif S, Kingston RE (2001) Transcriptional activation domains of human heat shock factor 1 recruit human SWI/SNF. Mol Cell Biol 21:5826–5837
96. Fujimoto M et al (2017) The HSF1-PARP13-PARP1 complex facilitates DNA repair and promotes mammary tumorigenesis. Nat Commun 8:1638
97. Stephanou A, Latchman DS (2011) Transcriptional modulation of heat-shock protein gene expression. Biochem Res Int 2011:238601
98. Mazina MY, Nikolenko YV, Krasnov AN, Vorobyeva NE (2016) SWI/SNF protein complexes participate in the initiation and elongation stages of drosophila hsp70 gene transcription. Genetika 52:164–169
99. Murawska M, Hassler M, Renkawitz-Pohl R, Ladurner A, Brehm A (2011) Stress-induced PARP activation mediates recruitment of Drosophila Mi-2 to promote heat shock gene expression. PLoS Genet 7:e1002206
100. Pinnola A, Naumova N, Shah M, Tulin AV (2007) Nucleosomal core histones mediate dynamic regulation of poly(ADP-ribose) polymerase 1 protein binding to chromatin and induction of its enzymatic activity. J Biol Chem 282:32511–32519
101. Thomas C et al (2019) Hit and run versus long-term activation of PARP-1 by its different domains fine-tunes nuclear processes. Proc Natl Acad Sci U S A 116:9941–9946
102. Haffner MC et al (2010) Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nat Genet 42:668–675
103. Ju BG et al (2006) A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. Science 312:1798–1802
104. Williamson LM, Lees-Miller SP (2011) Estrogen receptor alpha-mediated transcription induces cell cycle-dependent DNA double-strand breaks. Carcinogenesis 32:279–285
105. Pommier Y, Sun Y, Huang SN, Nitiss JL (2016) Roles of eukaryotic topoisomerases in transcription, replication and genomic stability. Nat Rev Mol Cell Biol 17:703–721
106. White D et al (2012) The ATM substrate KAP1 controls DNA repair in heterochromatin: regulation by HP1 proteins and serine 473/824 phosphorylation. Mol Cancer Res 10:401–414
107. Mondal N et al (2003) Elongation by RNA polymerase II on chromatin templates requires topoisomerase activity. Nucleic Acids Res 31:5016–5024
108. Liu LF, Wang JC (1987) Supercoiling of the DNA template during transcription. Proc Natl Acad Sci U S A 84:7024–7027
109. Crisona NJ, Strick TR, Bensimon D, Croquette V, Cozzarelli NR (2000) Preferential relaxation of positively supercoiled DNA by E. coli topoisomerase IV in single-molecule and ensemble measurements. Genes Dev 14:2881–2892

110. Dellino GI et al (2019) Release of paused RNA polymerase II at specific loci favors DNA double-strand-break formation and promotes cancer translocations. Nat Genet 51:1011–1023
111. Nitiss JL (2009) DNA topoisomerase II and its growing repertoire of biological functions. Nat Rev Cancer 9:327–337
112. McKinnon PJ (2016) Topoisomerases and the regulation of neural function. Nat Rev Neurosci 17:673–679
113. Morimoto S et al (2019) Type II DNA topoisomerases cause spontaneous double-strand breaks in genomic DNA. Genes 10:868
114. Sasanuma H et al (2018) BRCA1 ensures genome integrity by eliminating estrogen-induced pathological topoisomerase II-DNA complexes. Proc Natl Acad Sci U S A 115:E10642–E10651
115. Brown SA, Imbalzano AN, Kingston RE (1996) Activator-dependent regulation of transcriptional pausing on nucleosomal templates. Genes Dev 10:1479–1490
116. Gilchrist DA et al (2010) Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. Cell 143:540–551
117. Pan XY et al (2016) Heat shock factor 1 mediates latent HIV reactivation. Sci Rep 6:26294
118. Kim S, Gross DS (2013) Mediator recruitment to heat shock genes requires dual Hsf1 activation domains and mediator tail subunits Med15 and Med16. J Biol Chem 288:12197–12213
119. Allen BL, Taatjes DJ (2015) The mediator complex: a central integrator of transcription. Nat Rev Mol Cell Biol 16:155–166
120. Takahashi H et al (2011) Human mediator subunit MED26 functions as a docking site for transcription elongation factors. Cell 146:92–104
121. Yang Z et al (2005) Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. Mol Cell 19:535–545
122. Badenhorst P, Voas M, Rebay I, Wu C (2002) Biological functions of the ISWI chromatin remodeling complex NURF. Genes Dev 16:3186–3198
123. Chen M, Beck WT (1995) DNA topoisomerase II expression, stability, and phosphorylation in two VM-26-resistant human leukemic CEM sublines. Oncol Res 7:103–111
124. Kondapi AK, Padmaja G, Satyanarayana N, Mukhopadyaya R, Reitz MS (2005) A biochemical analysis of topoisomerase II alpha and beta kinase activity found in HIV-1 infected cells and virus. Arch Biochem Biophys 441:41–55
125. Kimura K, Nozaki N, Enomoto T, Tanaka M, Kikuchi A (1996) Analysis of M phase-specific phosphorylation of DNA topoisomerase II. J Biol Chem 271:21439–21445
126. Nakazawa N, Arakawa O, Ebe M, Yanagida M (2019) Casein kinase II-dependent phosphorylation of DNA topoisomerase II suppresses the effect of a catalytic topo II inhibitor, ICRF-193, in fission yeast. J Biol Chem 294:3772–3782
127. Ackerman P, Glover CV, Osheroff N (1985) Phosphorylation of DNA topoisomerase II by casein kinase II: modulation of eukaryotic topoisomerase II activity in vitro. Proc Natl Acad Sci U S A 82:3164–3168
128. Sahyoun N et al (1986) Protein kinase C phosphorylates topoisomerase II: topoisomerase activation and its possible role in phorbol ester-induced differentiation of HL-60 cells. Proc Natl Acad Sci U S A 83:1603–1607

# Induction of HSPA1A and Autophagy by SARS-CoV-2: Combined Potential Influence on Pregnancy Outcome

## SARS-CoV-2, HSPA1A and Pregnancy

**Ariana M. Witkin and Steven S. Witkin**

### Abstract

**Introduction** The current viral pandemic, called coronavirus infectious disease-2019 (COVID-19), is caused by the severe acute respiratory syndrome-coronavirus -2 (SARS-CoV-2). The consequences for pregnant women of becoming infected with this virus remain incomplete. There is a potential role for the inducible 70 kDa heat shock protein (HSPA1A) in the initiation of SARS-CoV-2 infection and for virus-induced pregnancy alterations. In here, we aim to present what is known about the influence of infection with known coronaviruses on pregnancy, the role of HSPA1A in coronavirus infections and speculation of how SARS-CoV-2-induction of HSPA1A could affect human gestation.

**Methods** Publications on heat shock protein involvement in coronavirus infections, pregnancy outcomes in response to past or current coronavirus epidemics and the role of HSPA1A in pregnancy progression and parturition were reviewed. The participation of HSPA1A in SARS-CoV-2-related pathology in pregnant women is proposed.

**Results** SARS-CoV-2 is the virus responsible for the worldwide pandemic of the disease called COVID-19. The virus probably originated in bats and is genetically similar to two other coronaviruses responsible for epidemics that occurred earlier in the twenty first century, Middle East Respiratory Virus (MERS)- CoV and SARS-CoV. SARS-CoV-2 as well as SARS-CoV bind primarily to angiotensin-converting

A. M. Witkin (✉)
Department of Pediatrics, Mount Auburn Hospital, Boston, MA, USA
e-mail: ariana.witkin@mah.org

S. S. Witkin
Department of Obstetrics and Gynecology, Weill Cornell Medicine, New York, NY, USA

Laboratory of Virology, Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil

Heat Shock Proteins (2021) 22: 509–517
https://doi.org/10.1007/7515_2021_39

Published Online: 4 June 2021

enzyme 2 (ACE2), a cell surface receptor that is abundantly expressed on type II lung alveolar cells. The virus has also been detected in the gastrointestinal tract and the systemic circulation, indicating that infection is not limited to the upper respiratory tract or solely to ACE2 binding. SARS-CoV-2 infection induces expression of HSPA1A. At least 2 SARS-CoV-2 proteins inhibit autophagy as a mechanism to promote its persistence and ability to proliferate. HSPA1A induction also inhibits autophagy and may further promote viral infection. In pregnant women the inhibition of autophagy by HSPA1A has been proposed as a mechanism to trigger parturition, both prematurely and at term. In addition, HSPA1A detection correlates with an increased susceptibility to pregnancy complications. The occurrence of a symptomatic SARS-CoV-2 infection in pregnant women has been associated with an elevated incidence of preterm birth. There is also evidence that SARS-CoV-2 may infrequently pass from mother to fetus prior to delivery.

**Conclusion** We propose that initiation of studies to measure levels of HSPA1A in the circulation of SARS-CoV-2-infected pregnant women may be of value in predicting the length of gestation. Similarly, detection of HSPA1A in amniotic fluid and cord blood may be useful in assessing the likelihood of transplacental passage of the virus. Based on results of these investigations, protocols to limit HSPA1A induction following SARS-CoV-2 infection might be beneficial in association with other treatments to limit adverse consequences for pregnant women.

**Keywords** Autophagy · Coronavirus · COVID-19 · Heat shock protein · HSPA1A · Pregnancy · SARS-CoV-2

## Abbreviations

| | |
|---|---|
| ACE2 | Angiotensin-converting enzyme 2 |
| COV | Coronavirus |
| COVID -19 | Coronavirus infectious disease 2019 |
| Grp78 | 78 kDa glucose-regulated protein |
| HSPA1A | The inducible 70 kDa heat shock protein |
| MERS | Middle east respiratory virus |
| mTORC1 | mammalian target of rapamycin complex 1 |
| SARS | Severe acquired respiratory syndrome |
| TLR4 | Toll-like receptor 4 |

## 1 Introduction

SARS-CoV-2 (severe acquired respiratory syndrome-coronavirus-2) is a newly identified coronavirus responsible for the 2019 pandemic disease called COVID-19. Coronaviruses are members of the *Corona virinae* subfamily, part of the

*Coronaviridae* family of enveloped single strand RNA viruses in the order *Nidovirales*. Among the four members of this subfamily, *Alpha* – and *Beta-coronaviruses* infect mammals while *Gamma* – and *Delta*-coronaviruses mainly infect birds. SARS-CoV-2, along with the two other Coronaviruses involved in earlier twenty first century epidemics – SARS-CoV and MERS-CoV (Middle East Respiratory Syndrome-Coronavirus) -are *Betacoronaviruses.* All three viruses are likely to have originated in bats, infected intermediate mammalian hosts, and then mutated to become capable of infecting humans [1]. The SARS-CoV-2 virus genome is 88% identical to that of two bat viruses, 79% identical with the SARS-CoV genome and about 50% identical with MERS-CoV [2]. The infection is initiated by binding of the viral external spike glycoprotein to receptors on host tissues. The main cell surface receptor for both SARS-CoV and SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2). It is abundantly expressed on type II alveolar cells in the lung, explaining the pulmonary manifestations of infection. ACE2 is also present on airway epithelial cells, fibroblasts, endothelial cells and macrophages [3–5]. Dipeptidyl peptidase 4 is the major receptor for MERS-CoV [4, 5]. However, it is believed that coronavirus spikes may be capable of binding to a broad range of molecules on cell surfaces that initiate their attachment and eventual entry into the cell [6].

## 2 Heat Shock Protein Expression

Two members of the 70 kDa heat shock protein family, the inducible 70 kDa heat shock protein (HSPA1A) and the 78 kDa glucose-regulated protein (GRP78), have both been shown to react with the spike protein of different coronaviruses and facilitate infection [6, 7]. Both SARS-CoV and SARS-CoV-2 have also been identified in the circulation of infected individuals [8–10] and SARS-CoV-2 replication in the gastrointestinal tract of hospitalized individuals has been reported [11].

Infection of most cells with viruses, including coronaviruses, induces the expression of heat shock proteins as a universal response of the transition to non-physiological conditions. The infection of a cell line with SARS-CoV has been shown to result in a large up-regulation of HSPA1A production [12]. HSPA1A accumulation is followed by its release into the extracellular milieu. Although HSPA1A lacks a consensus secretory signal to aid its passage through the cell membrane it is released from cells by an active mechanism involving transit through an endolysosomal compartment [13]. Once released from cells, the extracellular HSPA1A activates pro-inflammatory cytokine production [14]. In addition, HSPA1A binds to peptides present in the extracellular environment. In the case of viral infection, the peptides are derived from disrupted cells and contain both host and viral antigens. The association of HSPA1A with exogenous peptides increases the antigenicity of both the peptide and HSPA1A [15, 16]. Depending on the

particular circumstances and specificity of the antibody and cell-mediated immune response, this may either be beneficial or detrimental to the host.

## 3 Heat Shock Protein and Autophagy

Another consequence of coronavirus infection is the induction of autophagy. Autophagy is an intracellular process present in almost all cells that maintains intracellular homeostasis. Long-lived, misfolded or aggregated proteins as well as defective organelles such as mitochondria and inflammasomes become sequestered in a double-membraned structure called an autophagosome. The autophagosome fuses with a lysosome and the autosomal contents are degraded by lysosomal enzymes. The resulting amino acids, fatty acids, carbons and nitrogen are returned to the cytoplasm for reutilization by the cell [17]. Autophagy, by sequestering and removing nascent viral components from the cytoplasm, also functions as a component of the innate immune system [18]. In addition, induction of autophagy in cells capable of antigen presentation – dendritic cells, B lymphocytes, macrophages – results in the display on the cell surface of degraded peptide fragments. These are recognized by T lymphocytes and result in induction of acquired, antigen-specific cell-mediated immunity [19]. However, several viruses, including coronaviruses, have evolved mechanisms to subvert this process and, instead, to utilize autophagy for viral proliferation. Two SARS-CoV proteins, NSP6 and PLP2, have been shown to function as autophagy inducers but, concomitantly, to inhibit the fusion of autophagosomes with lysosomes and, thereby, prevent the elimination of the virus [20, 21]. This subverts the ability of host cells to prevent viral replication as well as thwarts development of acquired anti-viral immunity.

Under most conditions, the induction of the HSPA1A stress response inhibits autophagy [22, 23]. An intracellular complex, mammalian target of rapamycin complex 1 (mTORC1) that inhibits the induction of autophagy, is activated as a consequence of HSPA1A induction. The activated mTORC1 also up-regulates heat shock factor 1 to further stimulate HSPA1A gene transcription [24]. The HSPA1A-induced inhibition of autophagy has a major influence on pregnancy [25, 26]. It has been proposed that a normal trigger for parturition in a pregnancy that has proceeded to term is the up-regulation of HSPA1A and the associated inhibition of autophagy in uterine myometrial cells [26]. Excessive or abnormally high HSPA1A production as a consequence of infection can interfere with the essential housekeeping functions of autophagy during gestation and increase susceptibility to pregnancy complications such as preeclampsia, intrauterine growth restriction and premature delivery. The association between extracellular HSPA1A and an increased likelihood of adverse pregnancy outcomes has been reviewed [25].

Thus, the HSPA1A-induced inhibition of autophagy following its induction by SARS-CoV-2 infection may both facilitate viral persistence and replication and increase susceptibility to preterm birth.

## 4 SARS-COV-2 in Pregnancy and Parturition

Earlier investigations of pregnant women infected with SARS-Co-V and MERS-CoV, although limited in number, suggest the potential for virus-related adverse maternal and neonatal outcomes [27, 28]. Pregnancy-associated consequences of SARS-CoV and MERS-CoV infection have been recently summarized [29]. Among 7 pregnant women who became infected with SARS-CoV in their first trimester, 4 (57.1%) had a spontaneous abortion. Four of 5 women (80%) who were infected after 24 weeks gestation had a preterm delivery [27]. In 5 additional cases [29], 2 babies (40%) were delivered prior to term. Furthermore, pregnant women positive for SARS-CoV had worse clinical outcomes compared to women who were not pregnant [30]. Of 11 documented MERS-CoV pregnancy-associated symptomatic infections there were two fetal demises, and 3 preterm deliveries (27.3%). The combined data for pregnant women infected with SARS-CoV or MERS-CoV strongly suggests that pregnant women infected with COVID-19 and their neonates may similarly be at elevated risk for pregnancy-related complications.

Reports on the occurrence and consequences of SARS-CoV-2 infection in pregnant women have been increasing. Although many of the investigations contained only incomplete characterization and analysis of cases, the accumulating data suggests an association between SARS-CoV-2 infection and a higher rate of adverse pregnancy outcomes as compared to uninfected women. A retrospective review of outcomes in 9 symptomatic pregnant women in China, all admitted in their third trimester, identified 4 cases of preterm labor (44.4%), two of which were preceded by premature preterm rupture of membranes (PPROM) [31]. In addition, 2 mothers had pregnancy-associated hypertension or preeclampsia. A second study from China of 9 SARS-CoV-2 infected pregnant women showed similar results [32]. Preterm delivery occurred in 6 cases (66.7%), 3 of which were preceded by PPROM. In both studies, all neonates were negative for SARS-CoV-2 by throat swabs. One of the most comprehensive investigations on pregnancy-related consequences of SARS-CoV-2 infection is from the United Kingdom [33]. It involved the analysis of 427 pregnant women with a confirmed SARS-CoV-2 infection. Only 62% of the women proceeded to delivery and of these 27% had a preterm delivery. The detection of viral RNA occurred in 5% of newborns. In contrast, a study of 675 pregnant women in New York City, 10.4% of whom were SARS-CoV-2 positive, did not show an association between virus positivity and preterm birth. Furthermore, none of the babies were SARS-CoV-2 positive [34].

Although there is no evidence of trans-placental transmission of SARS-CoV or MERS-CoV from mother to baby, other coronaviruses that infect humans and cause only mild cold-like symptoms have been reported to be vertically transmitted [35]. Evidence of vertical transmission of SARS-CoV-2 from symptomatic mothers to their neonates has been reported but remains inconclusive. There have been 2 separate reports from China that identified a total of 3 neonates who were positive for IgM antibodies to SARS-CoV-2 in their cord blood [36, 37]. Since IgM is not passed from mother to baby through the placenta prior to delivery [38] the finding of

IgM anti-SARS-CoV-2 is presumptive evidence of *in utero* fetal infection. However, if the placenta had been damaged during gestation, IgM from the mother might have been transferred to the fetal circulation, providing an alternate explanation for these observations. None of the neonates were positive for the virus in throat swabs, adding to the inconclusiveness of the IgM findings. There have also been reports of a few neonates who tested positive for SARS-CoV-2 in their nasopharynx by gene amplification [39–41]. A more recent review of 179 newborns from SARS-CoV-2-infected mothers found evidence of viral transmission in 8 babies (4.5%), 5 by gene amplification and three by the presence of anti-SARS-CoV-2 IgM [42].

Mounting data suggests that pregnant women are at increased risk of morbidity, including severe respiratory complications, compared to their non-pregnant counterparts, following infection with SARS-CoV-2 [33, 34, 43]. In general, pneumonia in pregnancy is known to be associated with preterm labor, premature rupture of membranes, intrauterine fetal demise, intrauterine growth restriction and neonatal death.

## 5 Individual Variations in Manifesttions of Infection

The manifestations of SARS-CoV-2-infection vary widely. Some people remain completely asymptomatic and unaware of their infection, while others develop a wide range of symptoms from fever, muscle aches, cough, sore throat, stomach ache, diarrhea, and anosmia, to life threatening pulmonary complications. These individual differences are likely due to a combination of factors: genetic variation in the structure, prevalence and location of host receptors for SARS-CoV-2 on cell surfaces, differences in the production and activity of anti-viral immune system components, and the rapidity and specificity of innate and acquired immune responses to infection. A functional polymorphism in a gene critical for induction of autophagy *(ATG16L1)* has been shown to increase the likelihood of a preterm birth, especially when accompanied by a polymorphism in a second gene (*TLR4*) influencing the extent of a pro-inflammatory immune response [44]. The time period from labor initiation to delivery is also shortened in women with an altered *ATG16L1* gene [45]. HSPA1A, in addition to inhibiting autophagy, binds to the Toll-like receptor 4 (TLR4) protein resulting in its increased activation [46]. Strikingly, among pregnant women, the occurrence of preterm delivery is increased in those with a symptomatic SARS-CoV-2 infection [47], while asymptomatic pregnant women infected with the virus seem to have an uneventful course and deliver a healthy baby at term [48]. This dichotomy may change as more asymptomatic pregnant women are tested for SARS-CoV-2. However, presently it appears that only following a high level of virus proliferation and dissemination is the pregnancy in jeopardy.

## 6 Conclusions

Given associations between HSPA1A levels, pro-inflammatory immune activity, inhibition of autophagy and induction of preterm birth, it would be of interest to initiate investigations to compare concentrations of HSPA1A in sera from asymptomatic and symptomatic SARS-CoV-2 infected pregnant women who deliver prematurely and at term. These studies have yet to be performed. This would allow the determination as to whether HSPA1A quantitation could aid in predicting pregnancy outcome. In addition, the measurement of HSPA1A levels in amniotic fluid and cord blood may prove to be sensitive indicators of maternal-fetal viral transmission. Should HSPA1A determination be of clinical utility in predicting susceptibility to adverse pregnancy-related outcomes, inclusion of therapies to modulate HSPA1A production or its consequences may be useful adjuncts to other therapies.

**Acknowledgements** The authors acknowledge the contributions of Drs. Tomi T. Kanninen and Giovanni Sisti to our understanding of the relationship between autophagy and pregnancy and Ann Marie Bongiovanni for her supervision of our laboratory studies on heat shock proteins.

**Compliance with Ethical Standards** This study did not receive any external funding. Both authors declare that they have no conflict of interest. This article does not contain any studies with human participants performed by any of the authors.

## References

1. Lu G, Wang Q, Gao GF (2015) Bat-to human: spike features determining "host jump" of coronaviruses SARS-CoV, MERS-CoV and beyond. Trends Microbiol 23:468–478
2. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H et al (2020) Genomic characterization and epidemiology of 2019 novel coronavirus: implications for viral origins and receptor binding. Lancet 395:565–574
3. Cava C, Bertoli G, Castiglioni I (2020) In silico discovery of candidate drugs against Covid-19. Viruses 12:404
4. Weiss SR (2020) Forty years with coronaviruses. J Exp Med 217:e20200537
5. Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han T, Qin C (2019) From SARS to MERS, thrusting coronaviruses into the spotlight. Virology 11:59
6. Chu H, Chan C-M, Zhang X, Wang Y, Yuan S, Zhou J et al (2018) Middle East respiratory syndrome coronavirus and bat coronavirus HKU9 both can utilize GRP78 for attachment onto host cells. J Biol Chem 293:11709–11726
7. Zhang ZK, Yang X, Xu PW, Wu X, Zhou L, Wang HN (2017) Heat shock protein 70 in lung and kidney of specific-pathogen-free chickens is a receptor-associated protein that interacts with the binding domain of the spike protein of infectious bronchitis virus. Arch Virol 162:1625–1631
8. Chen W, Lan Y, Yuan X, Deng X, Li Y, Cai X et al (2020) Detectable 2019-Cov viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microbes Infect 9:469–473
9. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt H-R, Becker S et al (2003) Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348:1967–1976

10. Zhang W, Du R-H, Li B, Zheng X-S, Yang X-L, Hu B, Wang YY et al (2020) Molecular and serological investigation of 2019-CoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 9:386–389
11. Wolfel R, Corman VM, Guggemos W, Sellmaier M, Zange S, Muller MA et al (2020) Virological assessment of hospitalized patients with COVID-2019. Nature 581:265–269
12. Leong WF, Tan HC, Ooi EE, Koh DR, Chow VTK (2005) Microarray and real-time RT-PCR analyses of differential human gene expression patterns induced by severe acute respiratory syndrome (SARS) coronavirus infection of Vero cells. Microbes Infect 7:248–259
13. Mambula SS, Stevenson MA, Ogawa K, Calderwood SK (2007) Mechanisms for Hsp70 secretion: crossing membranes without a leader. Methods 43:168–175
14. Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK (2000) HSP70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6:435–442
15. Yang L, Danhui W, Xiutong F (2019) In vitro generation of anti- osteosarcoma cytotoxic activity using dendritic cells loaded with heat shock protein 70-peptide complexes. Fetal Pediatr Pathol 38:387–398
16. Hoos A, Levey DL (2003) Vaccination with heat shock protein-peptide complexes: from basic science to clinical applications. Expert Rev Vaccines 2:369–379
17. Mizushima N (2009) Physiological functions of autophagy. Curr Top Microbiol Immunol 335:71–84
18. Levine B (2005) Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. Cell 120:159–162
19. Levine B, Mizushima N, Virgin HW (2011) Autophagy in immunity and inflammation. Nature 469:323–335
20. Cottam EM, Whelband MC, Wileman T (2014) Coronavirus NSP6 restricts autophagosome expansion. Autophagy 10:1426–1441
21. Chen X, Wang K, Xing Y, Tu J, Yang X, Zhao Q et al (2014) Coronavirus membrane-associated papain-like proteases induce autophagy through interacting with Beclin1 to negatively regulate antiviral innate immunity. Protein Cell 5:912–927
22. Dokladny K, Zuhl MN, Mandell M, Bhattacharya D, Schneider S, Deretic V, Moseley PL (2013) Regulatory coordination between two major intracellular homeostasis systems: heat shock response and autophagy. J Biol Chem 288:14959–14972
23. Dokladny K, Myers OB, Moseley PL (2015) Heat shock response and autophagy – cooperation and control. Autophagy 11:200–213
24. Chou S-D, Prince T, Gong J, Calderwood SK (2012) mTOR is essential for the proteotoxic stress response, HSF1 activation and heat shock protein synthesis. PLoS One 7:e39679
25. Sisti G, Kanninen TT, Ramer I, Witkin SS (2015) Interaction between the inducible 70-kDa heat shock protein and autophagy: effects on fertility and pregnancy. Cell Stress Chaperones 20:753–758
26. Witkin SS, Kanninen TT, Sisti G (2017) The role of Hsp70 in the regulation of autophagy in gametogenesis, pregnancy, and parturition. In: MacPhee DJ (ed) The role of heat shock proteins in reproductive system development and function, Advances in anatomy, embryology and cell biology, vol 222. Springer, Cham, pp 117–127
27. Wong SF, Chow KM, Leung TM, Ng WF, Ng TK, Shek CC, Ng PC, Lam PW, Ho LC, To WW, Lai ST, Yan WW, Tan PY (2004) Pregnancy and perinatal outcome of women with severe acute respiratory syndrome. Am J Obstet Gynecol 191:292–297
28. Alfaraj SH, Al-Talfiq JA, Memish ZA (2019) Middle east respiratory syndrome coronavirus (MERS-CoV) infection during pregnancy: report of two cases & review of the literature. J Microbiol Immunol Infect 52:501–503
29. Rasmussen SA, Smulian JC, Lednicky JA, Wen TS, Jamieson DJ (2020) Coronavirus disease 2019 (COVID-19) and pregnancy: what obstetricians need to know. Am J Obstet Gynecol 222:415–426

30. Maxwell C, McGeer A, Tai KFY, Sermer M (2017) No. 225-management guidelines for obstetric patients and neonates born to mothers with suspected or probable severe acute respiratory syndrome (SARS). J Obstet Gynaecol Can 39:e130–e137
31. Chen H, Guo J, Wong C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q, Liao J, Yang H, Hou W, Zhang Y (2020) Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women; a retrospective review of medical records. Lancet 395:809–815
32. Zhu H, Wang L, Fang C, Peng S, Hang L, Chang G, Xia S, Zhou W (2020) Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. Transl Pediatr 9:51–60
33. Knight M, Bunch K, Vousden N, Morris E, Simpson N, Gale C, O'Brien P, Quigley M, Brocklehurst P, Kurinczuk JJ (2020) Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study. BMJ 369:m2107
34. Prabhu M, Cagino K, Mathews KC, Friedlander RL, Glynn SM, Kubiak JM, Yang YJ, Zhao Z, Baergen RN, DiPace JI, Razavi AS, Skupski DW, Snyder JR, Singh HK, Kalish RB, Oxford CM, Riley LE (2020) Pregnancy and postpartum outcomes in a universally tested population for SRS-CoV-2 in new York City: a prospective cohort study. BJOG 127:1548–1556
35. Gagneur A, Dirson E, Audebert S et al (2008) Materno-fetal transmission of human coronaviruses: a prospective pilot study. Eur J Clin Microbiol Infect Dis 27:863–866
36. Zeng H, Xu C, Fan J, Tang Y, Deng Q, Zhang W, Long X (2020) Antibodies in infants born to mothers with COVID-19 pneumonia. J Am Med Assoc 323:1848–1849
37. Dong L, Tian J, He S, Zhu C, Wang J, Liu C, Yang J (2020) Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. J Am Med Assoc 323:1846–1848
38. Woo PC, Lau SK, Wong BH, Tsoi HW, Fung AM, Chan KH, Tam VK, Peiris JS, Yuen KY (2004) Detection of specific antibodies to severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein for serodiagnosis of SARS coronavirus pneumonia. J Clin Microbiol 42:2306–2309
39. Wang S, Guo L, Chen L, Liu W, Cao Y, Zhang J, Feng L (2020) A case report of neonatal COVID 19 infection in China. Clin Infect Dis 71:853–857
40. Zeng L, Xia S, Yuan W, Yan K, Xiao F, Shao J, Zhou W (2020) Neonatal early-onset infection with SARS-CoV-2 in 33 neonates born to mothers with COVID-19 in Wuhan, China. J Am Med Assoc Pediatr 174:722–725
41. Karimi-Zarchi M, Neamatzedeh H, Dastgheib SA, Abbasi H, Mirjalili SR, Behforouz A, Ferdosian F, Bahrami R (2020) Vertical transmission of coronavirus disease 19 (COVID-19) from infected pregnant mothers to neonates: a review. Fetal Pediatr Pathol 39:246–250
42. Egloff C, Vauloup-Fellous C, Picone O, Mandelbrot L, Roques P (2020) Evidence and possible mechanisms of rare maternal-fetal transmission of SARS-CoV-2. J Clin Virol 128:104447
43. Rigby FB, Pastorek JG (1996) Pneumonia during pregnancy. Clin Obstet Gynecol 39:107–119
44. Liassides C, Papadopoulos A, Siristatidis C, Damoraki G, Liassidou A, Chrelias C, Kassanos D, Giamarellos-Bourboulis EJ (2019) Single nucleotide polymorphisms of *Toll-like receptor-4* and *autophagy-related gene 16 like 1* gene for predisposition of preterm delivery. A prospective study. Medicine 98:40
45. Doulaveria G, Orfanelli T, Benn K et al (2013) A polymorphism in an autophagy-related gene, ATG16L1, influences time to delivery in women with an unfavorable cervix who require labor induction. J Perinat Med 41:411–414
46. Mortaz E, Adcock IM, Abedini A et al (2017) The role of pattern recognition receptors in lung sarcoidosis. Eur J Pharmacol 808:44–48
47. Zaigham M, Andersson O (2020) Maternal and perinatal outcomes with COVID-19; a systematic review of 108 pregnancies. Acta Obstet Gynecol Scand 99:823–829
48. Sutton D, Fuchs K, D'Alton M, Goffman D (2020) Universal screening for SARS-CoV-2 in women admitted for delivery. N Engl J Med 382:2163–2164

# Index





A. A. Asea, P. Kaur (eds.), Heat Shock Proteins (2021) 22: 519–521
https://doi.org/10.1007/978-3-030-85168-2