Double Face of eHsp70 in Front of Different Situations

Multiple Role of eHsp70

Maria M. Barreca and Fabiana Geraci

Abstract The Hsp70 family is one of the best conserved and abundant member of the heat shock proteins (HSP). This family includes several members and in particular one constitutively expressed member (Hsc70) and another one inducibly expressed under several stress conditions (Hsp70). To date, the intracellular functions of Hsp70 are well defined, and increasing evidences establish its roles in the extracellular environment, such as cytoprotection and immunomodulation. Increasing evidences suggest that several cell types are able to release Hsp70 in the extracellular environment, both under physiological and stress conditions. At the same time many release mechanisms have been identified. This chapter briefly reviews recent advances in our understanding on extracellular Hsp70 role in both physiological and pathological conditions. A better comprehension will be useful to take advantage of its potential as a therapeutic target.

M. M. Barreca
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy

F. Geraci (✉)
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy

Euro-Mediterranean Institute of Science and Technology, Palermo, Italy
e-mail: fabiana.geraci@unipa.it

Abbreviations

APC	antigen presenting cell
CNS	central nervous system
CSF	cerebrospinal fluid
CTL	cytotoxic T lymphocyte
DC	dendritic cells
EAE	experimental autoimmune encephalomyelitis
ECM	extracellular matrix
ER	endoplasmic reticulum
EV	extracellular vesicle
HSP	heat shock protein
HUVEC	human umbilical vein endothelial cell
LPS	lipopolysaccharide
MBP	myelin basic protein
MHC	major histocompatibility complex;
MS	multiple sclerosis
NF-kB	nuclear factor kB
NK	natural killer
PBL	peripheral blood lymphocyte
ROS	radical oxygen species
TLR	toll like receptor

Introduction

Heat shock proteins are highly conserved molecular chaperones involved in proper folding of newly synthesized proteins. They are also able to interact with naïve and denatured proteins to avoid inappropriate interactions and the formation of protein aggregates of aberrantly folded proteins (Liberek et al. 2008). Furthermore, HSP facilitate protein translocation, exhibit cytoprotective (Sharp et al. 1999; Giffard et al. 2004) and antiapoptosis functions (Martin et al. 1992; Aquino et al. 1993; Gao et al. 1995; Aquino et al. 1997), directly interacting with various components of the tightly regulated programmed cell death machinery, upstream and downstream of the mitochondrial events (Garrido et al. 2001; Beere 2004; Madden et al. 2008). More recently, it has been demonstrated that HSP are also involved in controlling cell signaling (Calderwood et al. 2007), in modulation of both immune response (Johnson and Fleshner 2006) and chronic disease conditions (Kampinga et al. 2007). Furthermore, HSP has cytostimolatory functions (Asea 2008). Almost all the HSP families consist of constitutively expressed members playing housekeeping

roles and stress-induced members, which display a crucial role in recovery after different cellular stresses (e.g. heat shock, ultraviolet radiation, viral or bacterial infections, oxidative stress, ischemia, exercise, metabolic stress, heavy metals and so on) (Collins and Hightower 1982; Lindquist 1986; Patel et al. 1995; Richard et al. 1996; Yang et al. 1996; Feder and Hofmann 1999; Said Ali et al. 2010; Pierzchalski et al. 2014). For many years heat shock proteins were thought to be exclusive cytoplasmic proteins, acting only in intracellular compartments. The biology of both intracellular constitutive and inducible HSP has been extensively summarized by a large number of reviews and it will be not treated anymore (Lindquist and Craig 1988; Morimoto 1991; Bukau et al. 2006; Hartl and Hayer-Hartl 2009).

A new twist in the stress field was given by the finding that HSP could be also extracellular protein. In fact, it is now widely accepted that almost all the HSP are released in the extracellular environment, exerting multiple effects on different cell types (Tytell 2005). For example, in 1986 Tytell and coworkers identified an HSP-like protein as a glia-axon transfer protein in squid giant axon (Tytell et al. 1986). In addition, Hightower and Guidon found that Hsp70 is released extracellularly by a mechanism different from the classical secretion endoplasmic reticulum (ER)-Golgi pathway (Hightower and Guidon Jr 1989), as later confirmed by Hunter-Lavin et al. (Hunter-Lavin et al. 2004). Therefore, HSP could have a dual function depending on their intracellular or extracellular location. In the past years heat shock proteins were classified into different families depending on their molecular mass (i.e., Hsp110, Hsp90, Hsp70, Hsp60, Hsp40 and the small HSP). They comprise members with different inducibility and intracellular localization (Feder and Hofmann 1999). In 2009, Kampinga and coworkers proposed new guidelines for human HSP nomenclature (Kampinga et al. 2009). However, as most literature refers to HSP with their old name we decided to maintain the old nomenclature.

Hsp70 Family

One of the most conserved and abundant subsets of HSP is the Hsp70 family (Muchowski and Wacker 2005; Noble et al. 2008), consisting of at least four distinct proteins, two are cytosolic/nuclear, whereas the other two are localized within mitochondria and endoplasmic reticulum (ER) (Kregel 2002; Voos 2013). Furthermore, cytosolic Hsp70 includes the stress inducible form Hsp70 (Voellmy 2004) and the constitutively expressed Hsc70. Although Hsp70 synthesis is induced by several types of stressors (Wu et al. 1985; Milarski and Morimoto 1989), it is now well accepted that a basal concentration of Hsp70 is present in many tissues without any stress. Indeed, in addition to their cytoprotective properties member of the Hsp70 families are involved in physiological processes, such as cell differentiation, cell maturation and proliferation (Luft and Dix 1999; Lui and Kong 2007).

Extracellular Hsp70 and Its Release Mechanisms

As for other HSP (Chabas et al. 2001; Stadelmann et al. 2005), it has been demonstrated that Hsp70, in both basal and stress-induced conditions, is not only an intracellular (iHsp70) but also an extracellular protein (eHsp70), functioning as intercellular-signaling ligand (Asea et al. 2000; Asea 2003; Calderwood et al. 2007; Turturici et al. 2011). Indeed, it has been demonstrated that a variety of cell types, including neural cells (Guzhova et al. 2001; Taylor et al. 2007), epithelial cells (Broquet et al. 2003), embryo cells (Hightower and Guidon Jr 1989), B lymphocytes and dendritic cells (Théry et al. 1999, Clayton et al. 2005), maturing erythrocytes (Mathew et al. 1995) and tumor cells (Gastpar et al. 2005) is able to release Hsp70 in the culture medium.

Early studies on eHsp70 hypothesized that this protein was released by necrotic or dead cells, due to its aminoacidic sequence, and in particular to its lack of any exocytosis signals, (Gallucci et al. 1999, Basu et al. 2000; Saito et al. 2005). On the contrary, it is now well known that eHsp70 is also actively released by mammalian cultured cells through non classical secretory pathways, excluding the ER/Golgi compartment (Hightower and Guidon Jr 1989; Broquet et al. 2003), as already demonstrated for other proteins that lack secretion leader sequence. eHsp70 was also detected in the peripheral circulation of either healthy subjects or in several pathological states (Pockley et al. 1998, 2002; 2003; Asea 2007; Giraldo et al. 2008). All these data demonstrated that there are at least two different methods of Hsp70 release: one active due to an unconventional secretory pathway (Nickel and Seedorf 2008), and the other one passive, due to cell death and subsequent lysis. One of the possible active Hsp70 release pathway is similar to that identified for IL-1β (Eder et al. 2008). Specifically, Mambula and Calderwood demonstrated the involvement of a lysosome-endosome pathway, requiring the ABC-1 transporter (Mambula and Calderwood 2006). Hsp70 has also been proposed to be released by secretory-like granules (Evdonin et al. 2006), typical of specialized endocrine and exocrine cells, but already identified in non-secretory cells (Beuret et al. 2004). Another interesting idea images that Hsp70 release was dependent on its initial insertion into plasma membrane, depending on phosphatidylserine presence, membrane fluidity and, for certain cell types, lipid raft integrity (Triantafilou et al. 2002; Broquet et al. 2003; Arispe et al. 2004; Hunter-Lavin et al. 2004; Chen et al. 2005; Wang et al. 2006a; Horvath et al. 2008; Vega et al. 2008; Evdokimovskaya et al. 2010). The importance of Hsp70 insertion in cellular membrane is confirmed by the finding of Hsp70 containing extracellular vesicles (EVs) in cell culture medium, even in the absence of detectable cell death (Hunter-Lavin et al. 2004; Gastpar et al. 2005; Vega et al. 2008). Two principal EV types, i.e. exosomes and membrane vesicles, with different cellular origins have been demonstrated to be involved in Hsp70 release in the extracellular environment. Exosomes are nanovesicles derived from the multive-
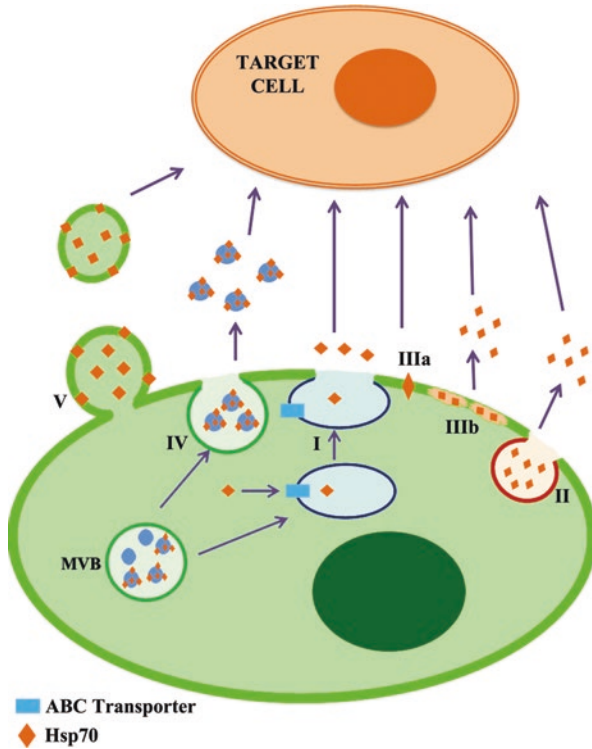
Fig. 1 Mechanism of Hsp70 release from cells. Hsp70 is able to leave cells using different way: *I* through endolysosome pathway; *II* by using secretory-like pathways; *IIIa* it can penetrate through the lipid bilayer via phospatidylserine *IIIb* or through of lipid raft interaction; *IV* it can be released inside exosomes; *V* or within membrane vesicles. In the extracellular milieu Hsp70 can interact with several cell types (target cells)

sicular endosomal cell compartment (Heijnen et al. 1999; Fevrier and Raposo 2004; Théry 2011). Several authors have demonstrated that *in vitro* different cell types are able to release Hsp70 in the extracellular environment by an unconventional pathway involving exosomes (Bausero et al. 2005; Gastpar et al. 2005; Lancaster and Febbraio 2005; Zhan et al. 2009). Indeed, for many years Hsp70/Hsc70 have been considered as exosome markers (Mathivanan and Simpson 2009). Contrary to this idea, for the first time Barreca et al. in 2017 demonstrated that mesoangioblasts, mouse vessel associated progenitor stem cells, are able to release Hsp70 in the extracellular milieu, even under basal growth conditions, by EVs originating directly from the plasmatic membrane (Barreca et al. 2017). Schematic model of different Hsp70 release mechanisms is represented in Fig. 1.

Extracellular Hsp70 Roles

As several mechanisms for extracellular Hsp70 release have been identified, there should be also several physiological roles for this protein in the external environment. In particular, its passive release from dying cells is considered as a danger signal. On the contrary, Hsp70 active release from living cells could indicate a successful stress response, and eHsp70 could have an active signaling role. An intriguing aspect of Hsp70 roles is its ability to induce antagonistic events, depending on its localization (Rodrigues-Krause et al. 2012). For example, iHsp70 exerts a strong anti-inflammatory effects through the interaction with the nuclear factor kB (NF-kB), blocking its activation (Jones et al. 2011), whereas eHsp70 has the opposite role, inducing the activation of several proinflammatory pathways. To date, there is evidence that eHsp70 can be internalized by several cell types, localizing both in the cytoplasm and in the nucleus, often promoting cell survival (Guzhova et al. 2001; Novoselova et al. 2005; Tytell 2005).

eHsp70 and Immune Responses

eHsp70 possesses powerful immunological properties. Two are the possible origins of eHsp70: pathogen derived Hsp70, which signals a local infection, and mammalian Hsp70, usually indicating an intracellular trauma. Hsp70, as other HSP associates itself with a broad variety of peptides, generated within the cells (Zhu et al. 1996). These peptides consist of normal self-peptides as well as antigenic peptides (i.e., tumor, bacterial and viral antigens) (Nieland et al. 1996; Castelli et al. 2001; Zugel et al. 2001). Several studies have demonstrated that eHsp70 is able to initiate both innate and adaptive immunity response as illustrated in Fig. 2 (Johnson and Fleshner 2006; Tsan and Gao 2009). For example, it is involved in the activation of cells of the innate immune pathway, such as macrophages, monocytes, which are directly induced in cytokine synthesis, neutrophils, dendritic cells (DCs), natural killer (NK) cells (Asea et al. 2000; Basu et al. 2001; Vabulas et al. 2002a; Gastpar et al. 2004; Aneja et al. 2006; Kovalchin et al. 2006; Wang et al. 2006a; Vega et al. 2008). In addition, eHsp70 also increases microbicidal capacity and chemiotaxis of neutrophils (Ortega et al. 2006, 2009) and phagocytosis (Wang et al. 2006a).

Phagocytosis characterizes the innate immune response and is initiated and executed by the antigen presenting cells (APCs). Some of these cells, including macrophages, are activated either by the presence of pathogens, or by physical, chemical stress, trauma and so on. All these conditions are responsible for HSP, and especially Hsp70, release in the extracellular environment. Immunologically, eHsp70 binds different receptors on macrophages membrane regulating several functions, such as cytokine release, phagocytosis, tumor-rejection and upregulation of co-stimulatory molecules (Basu et al. 2001; Delneste et al. 2002; Srivastava et al. 2002; Vabulas et al. 2002b; Asea 2005). Wang et al. demonstrated that eHsp70 is able to
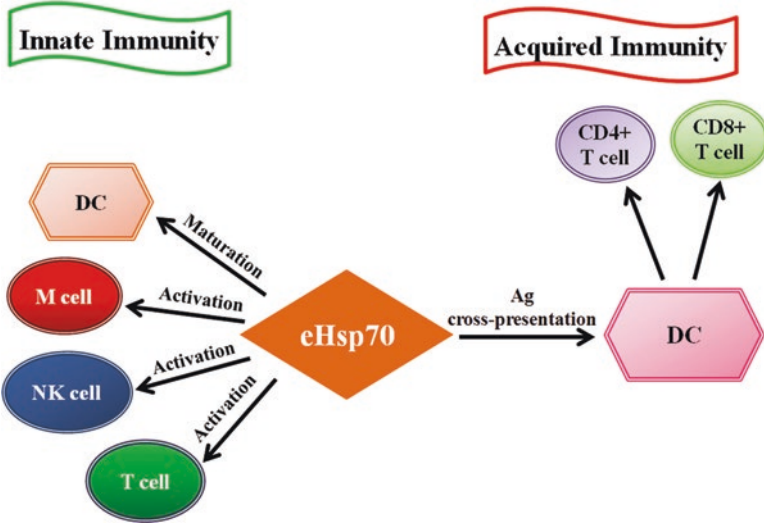
Fig. 2 Schematic model of eHsp70 immune response activation. eHsp70 is able to initiate both innate and adaptive immunity. On the one end, eHsp70 is involved in the activation of cells belonging to the innate immune pathway, such as macrophages, DCs and NK cells, directly induced in cytokine synthesizing (chaperokine role). On the other end, eHsp70 can acts as chaperone of antigens, which are transferred to APCs, processed, and cross-presented to T cells, inducing a strong T cell activation (chaperone role)

enhance murine macrophage-mediated antigen uptake. This process is concentration and time dependent. They also showed that eHsp70 mediated macrophage activation depends on actin cytoskeleton reorganization, and is not influenced by de novo protein synthesis. All these observations have suggested to the authors that the process of eHsp70 mediated phagocytosis occurs through the activation of a short signaling pathway, not involving gene upregulation. Moreover, they proved that eHsp70 and macrophages interaction occurs on lipid raft microdomains of macrophage plasmamembrane. Indeed, lipid raft disruption by cholesterol removal partially abrogates eHsp70 effects on phagocytosis. Finally, eHsp70 mediated phagocytosis enhances antigenic processing and presentation to CD4$^+$ T lymphocytes in a MHC II limited manner (Wang et al. 2006a). The involvement of eHsp70 in macrophage activation was also demonstrated by Vega and collaborators, confirming the paracrine role in cytokine expression and secretion by macrophages, already observed by Svensson and coworkers (Svensson et al. 2006). In particular they demonstrated that extracellular membrane bound Hsp70 activated macrophages that produced higher level of TNF-α in comparison with unstimulated cells. Furthermore, TNF-α production after stimulation with membrane positive eHsp70 was much higher than that observed with recombinant or purified Hsp70. According to these results the authors hypothesized that the membrane environment makes eHsp70 a better target to be recognized by macrophages that can engulf it via phagocytosis. On the other end, it is possible that eHsp70 insertion within extracellular membrane

is responsible for its multimerization, making it more active in triggering a response by macrophages (Vega et al. 2008).

In vivo wound healing experiments carried out by injecting into naïve mice mimicking a wounding model, macrophages pretreated with Hsp70 showed an enhanced macrophage wound closure ability, compared to buffer treated cells (Kovalchin et al. 2006). It is well known that macrophage-mediated phagocytosis plays a fundamental role in wound healing by clearing debris and neutrophils. Kovalchin's group results indeed demonstrated that eHsp70 mediates some of its action on wound closure through the regulation of macrophage phagocytic activity (Kovalchin et al. 2006). According to HSP capability to interact with other cell types, such as neutrophils and platelets (Hilf et al. 2002; Radsak et al. 2003), it is also possible that eHsp70 stimulates wound closure not only by stimulating macrophage-mediated phagocytosis, but also altering the wound milieu via cytokine release.

The extracellular chaperone is also responsible for DC activation through their surface binding. In their immature form these cells are prevalently involved in antigen capture and processing, whereas mature DCs are potent APCs. Indeed, when their maturation is induced, they become potent stimulator of naïve T cells (Banchereau and Steinman 1998). According to this dual role, DCs represent a connection between the adaptive and the innate immune system. Therefore, in its function of DC activation, eHsp70 may stimulate a cross-talk between the two immune responses (Srivastava 2002). In particular, Kuppner et al. have observed that eHsp70 bound and stimulated maturation of immature differentiated DCs, but reduced their maturation from monocyte precursor cells, probably due to its incapacity to interact with these cells. In addition, DCs matured by eHsp70 increased cell proliferation level of PBL and showed an enlarged capability to stimulate IFN-γ synthesis from specifically stimulated CTL (Kuppner et al. 2001). DCs are also able to bind tumor eHsp70-peptide complex, which is endocyted through a receptor mediated process and cross-presented to MHC I (Noessner et al. 2002). Milani et al. demonstrated that eHsp70-peptide complex is not only involved in providing to DCs the peptide for presentation for T cell stimulation, but also stimulates DCs to release TNF-α inducing by an autocrine loop their maturation (Milani et al. 2002).

All the presented data indicate that eHsp70, acting as a danger signal to the innate immune system, on one hand induces: a cascade of proinflammatory cytokines (e.g. TNF-α, IL-1-IL-6, Il-12); NO and C-C chemokines release by monocytes, macrophages and DCs (Asea et al. 2000, 2002; Chen et al. 2002; Panjwani et al. 2002; Asea 2006); the stimulation of DCs and the activation of NK cells. Qiao et al. demonstrated a positive switch of the DC-NK cells cross activation. In particular, they found that soluble Hsp70, which *in vivo* is released as results of pathological cell stress and death (Basu et al. 2000), could amplify DC-NK cell interaction, leading to a higher IFN-γ response (Qiao et al. 2008). On the other hand, eHsp70 acts as chaperone of bound peptides, which are transferred to DCs, also induced to mature, and cross-presented to T cells, inducing stronger cell activation compared with antigenic peptide alone (Li et al. 2002; Bendz et al. 2007).

In the last years various papers have demonstrated that HSP-reactive T cells have an immunoregulatory phenotype, suggesting that these proteins, and in particular

Hsp60 and Hsp70, could activate immunoregulatory pathways, which can suppress the immune response occurring in human inflammatory diseases. In fact, several data highlighted that in various inflammatory diseases, eHsp70 exerts under certain circumstances also immunoregulatory and suppressive functions. (Wieten et al. 2007). In various autoimmune models, such as rheumatoid arthritis and diabetes, eHsp70 can also downregulate the immune response through its ability to stimulate T cells (Th2 and Tregs) to produce IL-10, the main anti-inflammatory and immunosuppressive cytokine (Moore et al. 2001; Borges et al. 2012; Stocki and Dickinson 2012). This process, depending on the form of eHsp70 (i.e., associated with peptide, with membranes, with nucleotides, peptide free) and the receptor activated (Li et al. 2012) shifts cell phenotype towards a tolerogenic one exhibiting anti-inflammatory properties. It has been demonstrated that several adaptive mechanisms could be involved in eHsp70 specific induced Tregs. In particular, peptides derived from eHsp70, either pathogen associated or secreted endogenous Hsp70, can be presented to MHC II on IFN stimulated APCs or non APCs. Most Tregs subset induce IL-10, responsible for inflammatory response suppression (Wendling et al. 2000; Prakken et al. 2001; Bluestone 2005). Another example of the anti-inflammatory eHsp70 role was observed in a mouse model of allergic bronchial asthma inflammatory process. eHsp70 treated mice showed a significant decrease in Th2 associated cytokines IL-4, IL-5 and IL-13 secretion compared to untreated mice. In addition, it has been observed that eHsp70 administration promoted survival of neutrophils at the site of inflammation, and reduced the influx of the eosinophils to the lungs (Shevchenko et al. 2016).

Under certain circumstances also crossreaction with self-Hsp70 induce T cells to synthesize of IL-10 (Tanaka et al. 1999; Wendling et al. 2000). Indeed, in animal models of experimental arthritis T cell reactivity to self Hsp70 downregulates inflammation developing Th2 CD4$^+$ T cells producing the regulatory cytokines IL-4 and IL-10 (Tanaka et al. 1999; Wendling et al. 2000; Wieten et al. 2007). Another anti-inflammatory role for eHsp70 was observed in rheumatoid arthritis. In particular, eHsp70 exerts an anti-inflammatory role on fibroblast-like synoviocytes inhibiting TNF-α induced IL-6, IL-8 and MCP-1 secretion. The silencing of this inflammatory response is due to the downregulation of NF-kB nuclear translocation (Luo et al. 2008). Immunization with the recognized bacterial Hsp70 peptide protects rats against adjuvant-induced arthritis (Tanaka et al. 1999). A protective effect from experimentally induced arthritis was also observed in rats pre-immunized with mycobacterial Hsp70 (Wendling et al. 2000; Prakken et al. 2001).

eHsp70 Role in Cancer Immunity

The eHsp70 or the one expressed on the tumor cell surface have been shown to elicit a strong anti-tumor immune response mediated by T cells, APCs and NK cells (Multhoff et al. 1997; Asea et al. 2000; Dressel et al. 2000; Clark and Ménoret 2001). It is by now demonstrated that many cancer cells contain a high level of

chaperones, especially Hsp70 (Ciocca and Calderwood 2005). Indeed, contrary to normal cells, cancer cells overexpress Hsp70 in the cytoplasm, and also display the chaperone on their plasma membrane (mHsp70) (Multhoff et al. 1997; Gehrmann et al. 2003). This overexpressed Hsp70 has been found to stimulate an immune response specific to cancer cells (Suto and Srivastava 1995). Literature data highlighted that eHsp70 exerts a dual role in cancer, promoting survival of tumor cells, as well as contributing to anti-tumor immunity. In fact, in tumor environment eHsp70 can inhibit early-stage tumor growth by activating the immune response, whereas it stimulates late-stage tumor growth by inhibiting the immune system (Wu et al. 2012). Thus some eHsp70 features help cancer cells to escape from cytotoxic cells, while others facilitate an immune attack. According to the mechanism of Hsp70 release in the extracellular environment, i.e. as a free soluble molecule or in association with extracellular vesicles, it mediates distinct functions through the interaction with different target cells (e.g., immune cells, endothelial cells), and the activation of different intracellular pathways. Three different mechanisms could be involved in Hsp70 anti-tumor immunity:

(a) Hsp70, due to its chaperone role can act as carrier for tumor derived peptides which are re-presented by APCs, such as macrophages and DCs, the most powerful APC. This complex is recognized by specific receptors, internalized within the APCs, and cross-presented to CD4+ T cells on MHC II (Tamura et al. 1997; Li et al. 2002), initiating an adaptive tumor-specific immune response (Arnold-Schild et al. 1999; Schild et al. 1999; Noessner et al. 2002; Enomoto et al. 2006). DCs are also able to efficiently transfer tumor and self-antigens on to MHC I (cross-presentation) (Heath and Carbone 2001). eHsp70 has also immunogenic functions related to its C-terminal substrate-binding domain, which is involved in interaction with APCs and consequently in antigen cross-presentation (MacAry et al. 2004).

(b) The interaction between eHsp70 and its receptors on APCs stimulates these cells to secrete immunostimulatory cytokines, playing a proinflammatory role, responsible for enhanced programming of cytotoxic lymphocytes (Calderwood et al. 2012). For this "chaperokine" effect immunogenic peptides are not required (Asea et al. 2000, 2002; Asea 2006).

(c) The last possible mechanism of action against tumor cells is NK dependent, and is correlated to plasmamembrane localization of Hsp70 (Multhoff et al. 1999). In fact, resting NK cell activation either by eHsp70 or by mHsp70 derived from tumor, even in the absence of immunogenic peptides (Multhoff et al. 1998), leads to an increased proliferative, migratory and cytotoxic activity against transmembrane Hsp70 positive tumor cells, whereas no killing activity was observed for Hsp70 negative cells (Multhoff et al. 1997; Gastpar et al. 2004, 2005).

Schematic model of different eHsp70 roles in cancer immunity is represented in Fig. 3.

Due to the observation that preactivated CD94+ NK cells (Gross et al. 2003a, b), through an Hsp70 peptide (TDK) plus low doses of interleukin 2, were able to induce regression of Hsp70 membrane positive tumors in immunodeficient mice
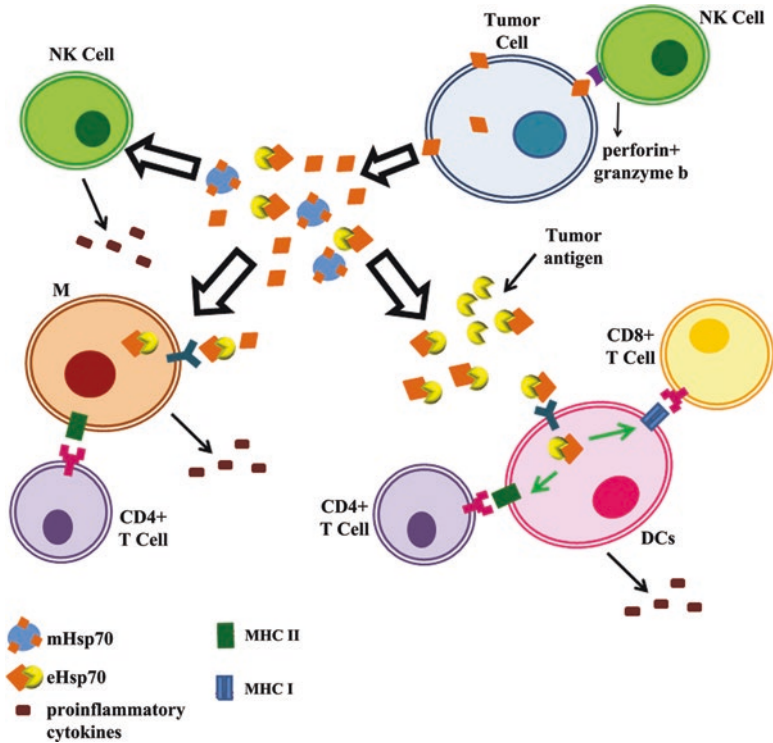
Fig. 3 Summary of Hsp70 activities in inducing anti-tumor immune responses. eHsp70 either alone or in combination with immunogenic peptides is able to induce an immune response. mHsp70 acts as a tumor-specific antigen, which is recognized by NK cells inducing their cytolytic, proliferative, and migratory capacity. eHsp70 also induces macrophages (M), NK and DCs to release proinflammatory cytokines. Moreover, eHsp70 could act as a carrier for tumor antigens and could support antigen uptake, processing, and presentation on MHC I to CD8^{+} cytotoxic T lymphocytes, or on MHC II s to CD4^{+} helper T cells

(Moser et al. 2002; Stangl et al. 2006), Hsp70 was used in clinical trials of patients with colorectal and non small lung cell cancer who failed standard therapies such as chemotherapy, radiotherapy or laser induced thermotherapy (Krause et al. 2004; Specht et al. 2015). The recognition by NK cells of fragment crystallizable region of antibodies that have bound to tumor specific antigens activate antibody-dependent cellular cytotoxicity, establishing a link between B cell and NK cell mediated immune responses.

It is now well demonstrated that increased membrane bound Hsp70 expression and its extracellular release can stimulate an anti-tumor immune response, rendering cancer cells more susceptible to immune cells. For this reason two possible methods have been tested to increase eHsp70 amount: pharmacological agents (Arispe et al. 2002), and physical factors (e.g., hyperthermia, photodynamic therapy and ionizing radiations) (Stangl et al. 2011). Some *in vivo* data indicate the prospect

of therapeutic vaccines based on Hsp70 administration (Ito et al. 2004; Geng et al. 2006; Kumar et al. 2009; Abkin et al. 2013). eHsp70 released by tumor cells can serve as autocrine and paracrine cytokine and through the interaction with receptors on tumor cells or APCs it stimulates the secretion of chemokines, and proinflammatory cytokines and nitric oxide respectively (Asea et al. 2000; Panjwani et al. 2002; Vega et al. 2008), providing an inflammatory microenvironment. This inflammation exerts anti-tumor activity at early tumor stage, whereas it support tumor growth and metastasis formation at a chronic stage. Equally, eHsp70 has an effect also on dendritic cells, which were activated, and chemoattracted from tumor tissues to secondary lymphoid organs, where they showed an improved capacity in the induction of tumor antigen-specific cytotoxic T lymphocytes (Kuppner et al. 2001; Wang et al. 2005; Chen et al. 2009).

In confirmation of the protective role of the protein in tumors, it has been demonstrated that its inhibition is lethal, and its silencing induces *in vitro* cell death as well as in tumor xenografts in mice (Wei et al. 1995; Kaur et al. 2000; Nylandsted et al. 2000). For this reason, in several cancer patients a more elevated eHsp70 level was found in blood compared to patients with non-cancerous pathologies (Gehrmann et al. 2014) (e.g., chronic myeloid leukaemia, acute leukaemia, colorectal cancer, glioblastoma, pancreatic cancer) (Yeh et al. 2009, 2010; Kocsis et al. 2010; Elstner et al. 2011; Dutta et al. 2012). Wu and coworkers demonstrated that eHsp70 encourages hepatocarcinoma growth by promoting tumor cell proliferation and apoptosis resistance (Wu et al. 2012). Both the events were induced in a dose dependent manner through the activation of the NF-kB pathway. The involvement of eHsp70/Hsp70-peptide complex in hepatocellular carcinoma cell proliferation was also confirmed by Zhe et al. (2016). Protective effect of eHsp70 on induced apoptosis by H_2O_2 on lymphoma cells was also observed. Indeed, pretreatment of U937 cells with eHsp70 inhibited hydrogen peroxide apoptosis induction (Franco et al. 2016).

eHsp70 and the Nervous System

To date it has been extensively demonstrated that Hsp70 exerts a cellular protective role under stress conditions. However, some cell types are not able to express this protein, among them there are certain types of neurons (e.g., hippocampal neurons) (Sprang and Brown 1987; Guzhova et al. 2001; Robinson et al. 2008). These mature neurons are particularly susceptible to toxic conditions due to their inability to increase Hsp70 level after stress (Morimoto et al. 1997), even if they contain Hsc70 (Brown 1991). The inability to activate the stress response is responsible for a wide range of neurodegenerative diseases (Planas et al. 1997), such as amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and polyglutamine diseases (Adachi et al. 2009). In this context, it has been demonstrated that Hsp70 released by surrounding glial cells is able to be endocyted by them (Guzhova et al. 2001; Novoselova et al. 2005; Robinson et al. 2005). The internalization of eHsp70 makes the neuronal cells resistant to cytotoxic effects and thermal stresses (Guzhova et al.

2001). A protective effect of exogenous Hsp70, on moto or sensory neurons and glial cells has been demonstrated by several authors (Edbladh et al. 1994; Guzhova et al. 2001; Tidwell et al. 2004; Robinson et al. 2005; Tytell 2005). The eHsp70 neuroprotective effect was also observed both under stress growth conditions (e.g., oxidative stress common in several neurodegenerative disease) and for those neurons undergoing natural cell death (Robinson et al. 2005).

Luo and colleagues demonstrated that eHsp70 in a dose dependent fashion is responsible for Schwann cell protection against apoptosis induced by H_2O_2. In particular, this protective effect was exerted through failure of caspase 3 and 9 activation and Bcl2 upregulation (Luo et al. 2012). Oxidative stress is the cause of several neurodegenerative diseases, as ROS induces membrane lipid peroxidation, nitration of proteins, and degradation of DNA, all inducing cell apoptosis (Fiskum 2004; Park et al. 2005). Because Schwann cells can regulate neuron survival and nerve regeneration, their preservation by eHsp70 is fundamental for peripheral nerve protection. Oxidative stress also impairs motoneurons during exercise. In fact, exercise induces ROS production, depending on the intensity, the type and the duration of the exercise. Although motoneurons during exercise are submitted to stress they still continue to function without any apparent damage, even if they are not able to increase iHsp70 synthesis (Robinson et al. 2005). This resistance can be explained by the release of eHsp70 by the liver, or by in *vitro* external addiction, and its internalization in motoneurons. eHsp70 could also act as extracellular signaling molecule, and this ability, together with its chaperone role after endocytosis, helps the cells against stress and damage (Robinson et al. 2005; Krause and Rodrigues-Krause 2011).

On the one end, many papers have demonstrated the involvement of HSP in central nervous system (CNS) diseases, such as those characterized by the presence and accumulation of misfolded proteins. Because HSP are found in protein aggregates, along with disease proteins, ubiquitin or other cellular molecules, we can hypothesize that both iHSP and eHSP have a role in refolding the misfolded proteins. In the other end, diseases in which CNS immune activation is a prominent feature, such as ischemia, immune-mediated disorders, infections, and trauma, may involve eHSP, because they are able to induce the innate immune response and to enhance the adaptive immunity. The most important HSP implicated in the immune response is eHsp70 (for a review see Turturici et al. 2011). eHsp70, through the interaction with specific cell surface receptors, are responsible for the expression of proinflammatory cytokines, chemokines and for DC activation, which are involved in antigen presentation to B and T lymphocytes.

High levels of anti-Hsp70 autoantibodies were found in the cerebrospinal fluid (CSF) of patients affected by multiple sclerosis (MS), than in that of healthy controls. In particular, the highest levels were detected in patients with progressive MS, in contrast to those with a stable disease (Chiba et al. 2006). This increase induces a higher production of IL-8 in THP-1 monocytes with consequent higher inflammatory levels (Yokota et al. 2010). eHsp70 was also found in and around MS lesions, and it may be involved in the induction or exacerbation of the immunologic response, because of its ability to act as a proinflammatory cytokine (for a review see Turturici et al. 2014). In addition, Lund and collaborators demonstrated that eHsp70 was

associated with myelin basic protein (MBP)-derived peptides in normal appearing white matter of both MS ad normal human brain. They hypothesize that part of eHsp70-MBP peptide is secreted by stressed oligodendrocytes and it can act as an adjuvant molecule that stimulates an adaptive immune response against specific autoantigens. This event could be involved in the initiation of MS, and in the subsequent immune mediated destruction of myelin (Lund et al. 2006).

Galazka et al., obtained conflicting results which demonstrated that mouse immunization with eHsp70 fraction associated with peptide complexes isolated from animals with experimental autoimmune encephalomyelitis (EAE) reduced its subsequent induction (Galazka et al. 2006). On the contrary, the disease was not induced using eHsp70-complexes isolated from healthy donors. These results suggest substantial differences in the peptide that bind Hsp70 in normal versus pathological CNS. In contrast, in EAE, Hsp70 pharmacologically induced (e.g., with geldamycin) has a protective role because it suppresses the glial inflammatory response and ameliorates clinical signs (Dello Russo et al. 2006). All the evidences according to which Hsp70 is not only an intracellular chaperone but has also extracellular functions, such as neuroprotective effects in many brain diseases, could open a new scenario in CNS disease therapy.

Other Functions of eHsp70

In recent years new roles for eHsp70 have been found. For example, several studies have demonstrated that circulating eHsp70 is a marker for cardiovascular disease (Pockley et al. 2003). In addition to its marker role, eHsp70 actively participate in the inflammatory damage typical of the cardiovascular damage through its binding to TLR4 (Yang et al. 2005). Furthermore, in 2013 Gonzáles-Ramos et al. demonstrated that eHsp70 acts as a profibrotic regulator of the ECM protein synthesis (fibronectin and type I collagen) by the vascular smooth muscle cells. Also this stimulation depends on eHsp70 binding to the membrane receptor TLR4 and the subsequent activation of the ERK and JNK pathways. These two kinases determine the activation of the transcription factor AP-1, which increased the transcriptional capacity of the TGF-β1 promoter involved in increasing fibronectin and collagen I amount (Gonzáles-Ramos et al. 2013).

In vitro and *in vivo* assays showed that eHsp70 could be also an angiogenic regulator. Indeed, experiments on HUVEC cells demonstrated that eHsp70 activates the ERK signaling pathway involved in cell migration, tube formation and microvessel formation (Kim et al. 2016). Furthermore, eHsp70 promotes osteogenic differentiation of mesenchymal stem cells. Chen et al. showed that the addition of Hsp70 to the culture medium of the stem cells stimulate the expression of the alkaline phosphatase, an early marker of osteoblastic differentiation. eHsp70 also enhanced calcium deposits in both dose and time dependent manners, via the activation of the ERK signaling pathway (Chen et al. 2015).

Additionally, eHsp70 is involved in stem cell migration as demonstrated by Barreca et al. (Barreca et al. 2017). One of the fundamental problems in stem cells based therapies is their homing to the site of injury. It is well known that MMPs play a fundamental role in mesenchymal stem cell migration (Ries et al. 2007). Barreca and coworkers demonstrated that mouse mesoangioblast release Hsp70 in membrane derived vesicles as a transmembrane protein. mHsp70 acts via an autocrine signaling pathway by increasing mesoangioblast MMP2 release and accentuating their migration and invasion capability. A predominant role in the activation of these processes was carried out by the eHsp70 receptors TLR4 and CD91 and the consequent NF-kB pathway activation (Barreca et al. 2017).

eHsp70 Receptors

As described above Hsp70 may be released in the extracellular environment by both active and passive mechanisms, it can travel through the circulation to reach cellular target cells, such as immune effector cells. HSP have two essential properties that allow them to activate the immune response. First they are chaperones of antigenic peptides produced by specific cell types and presented via MHC I and MHC II. Second HSP are capable of binding to membrane receptors on APCs. Indeed, for eHsp70 activating innate and adaptive immune responses, it must bind to specific cell receptors. In the last years many cell-surface receptors involved in eHsp70 mediated signaling have been identified, including TLR2 and TLR4 with their cofactor CD14 (Asea et al. 2000, 2002), the co-stimulatory molecule CD40 (Becker et al. 2002; Wang et al. 2001), the chemokine receptor CCR5, the scavenger receptors (SR) LOX-1 (Delneste et al. 2002; Thériault et al. 2006), SCREC-1 (Thériault et al. 2006), FEEL-1 (Thériault et al. 2006), CD94 (C-type lectin) (Gross et al. 2003a, b), CD91 (Basu et al. 2001), CD36 (Delneste et al. 2002). All these immune receptors are differentially expressed on various cell types and have different roles in eHsp70 signaling (Fig. 4). Indeed, the receptors can be divided in three groups: pattern recognition receptors (e.g., TLR2, TLR4), uptake of antigens by APCs (e.g., LOX-1, CD91), mediating co-stimulatory signals (e.g., CCR5, CD40).

The TLR family consists of several members recognizing specific ligands, e.g., TLR2 (triacyled lipoproteins) and TLR4 (LPS). Activation of TLR pathway by ligand binding is responsible for activation of the transcription factors NF-kB and AP1 (Takeda and Akira 2004). NF-kB is central to the inflammatory response by innate cells (neutrophils, macrophages and DCs). For the first time Asea et al. in 2002 demonstrated that eHsp70 induced proinflammatory cytokine by binding monocyte TLR2 and TLR4 with their cofactor CD14 and the adaptor molecule MyD88, and by activating the NF-kB pathway involved in IL-1β, IL-6 and TNF-α (Asea et al. 2000, 2002). Furthermore, LRs located on macrophages have been demonstrated to be involved in antigen uptake and phagocytosis induction (Blander and Medzhitov 2004 Science). In particular, another member of TLR family which is able to interact with eHsp70 is the lipid raft associated TLR7, generally involved in
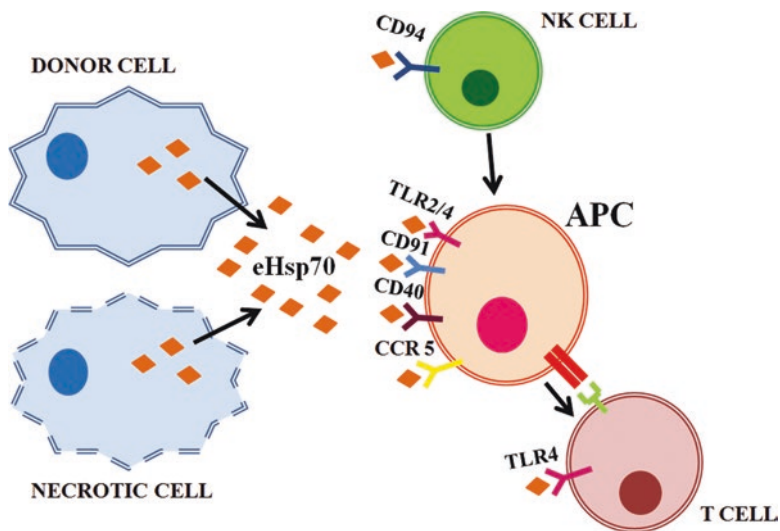
Fig. 4 Hsp70 release from the donor cells and its binding to cell surface receptors on the target cells. Several cell types release the Hsp70 into the extracellular microenvironment through different active secretion mechanism or through a passive release after necrosis. eHsp70 can be recognized by several receptors on the target cells. Among them there are TLR2, TLR4, LOX-1, CD91, CD94, CCR5 and CD40 expressed on APCs, NK cells, and T cells Their integrated function is discussed in the text

viral RNA recognition. Wang et al. demonstrated that exogenous Hsp70 binds TLR7 on the lipid raft, which is essential for this binding. The interaction between eHsp70 and this member of the TLR family stimulates phagocytosis and antigen presentation through PI3K and the p38 MAPK pathway (Wang et al. 2006b).

TLR2 and TLR4 are not only expressed on immune cells, but also on endothelial cells, and airway epithelial cells (Greene et al. 2005). In heart eHsp70 directly decreased cardiomyocyte contractility and increased cell death through the activation of the inflammatory response by the signaling pathway TLR2-MyD88-NF-kB (Mathur et al. 2011). On the other hand, eHsp70 induced inflammation in the lung is TLR4-NF-kB dependent (Chase et al. 2007).

Another receptor involved in eHsp70 binding is CD40, a member of the TNF family. This binding was competed by the cochaperone Bip, whereas it is enhanced by the antigenic peptide. As observed for other receptor, binding of eHsp70-peptide complex to CD40 increases peptide uptake and activate the p38 intracellular pathway (Becker et al. 2002). Moreover, CD40+ cells were stimulated to produce chemokines (Lehner et al. 2003). As TLRs, CD40 is not only expressed on immune cells, but also on other cell types, such as HUVEC endothelial cells, where it functions by competing CD40 ligand and decreasing tubular formation process (Futagami et al. 2008). By binding assays Multhoff's group demonstrates the involvement of the C-type lectin receptor CD94 in the interaction of NK cells with

eHsp70/mHsp70 (Gross et al. 2003b). This interaction induce both their proliferation and cytolytic activity (Multhoff et al. 1999, 2001).

Another receptor implicated in eHsp70 binding is CD91, a transmembrane scavenger receptor ubiquitously expressed in multiple tissues where it plays a key role in intracellular signaling and endocytosis. Basu et al. identified CD91 as a receptor for several HSP including eHsp70 (Basu et al. 2001). Its role in binding and internalization of HSP by APCs, necessary for HSP chaperoned peptides cross-presentation, was reported in several papers (Delneste et al. 2002; Martin et al. 2003; Tobian et al. 2004). CD91-HSP was recently also be shown to be a signaling complex regulating multiple pathways in APCs (Pawaria and Binder 2011). The signaling pathway started up by CD91 upon HSP stimulation includes the activation of NF-kB and p38 MAPK (Pawaria and Binder 2011). As a consequence of this intracellular signaling several cytokines are released by HSP stimulated APCs (such as TNF-α, IL-1β, IL-6, IL-12 and GM-CSF) (Pawaria and Binder 2011). APCs also upregulate the expression of costimulatory molecules, including CD80, CD86, CD40 and MHC II (Basu et al. 2000). The costimulatory molecules and cytokines expressed depends on both the APC stimulated and the HSP involved in stimulation. According to these data CD91 has a role in cross-presentation and in costimulation given by the APCs to T cells in response to eHSP. The receptor CD91 expressed on monocytes, after eHsp70-peptide binding stimulates antigen specific CD4^{+} T cell proliferation (Fischer et al. 2010). Although CD91 is highly expressed on macrophages, it is very low on immature DCs. Delneste and coworkers demonstrated that on these cells the scavenger receptor LOX-1 is the main eHsp70 binding receptor and is involved in antigen cross-presentation to MHC I *in vitro* and *in vivo* (Delneste et al. 2002).

One of the last eHsp70 receptor identified is the receptor for advanced glycation endproducts (RAGE), a receptor that interacts with several inflammation and stress mediators to activate the inflammatory pathway (Xie et al. 2013). The activation of RAGE by eHsp70 evokes strong proinflammatory changes in human lung carcinoma cell line. In fact, interaction between eHsp70 and RAGE trigger the typical cellular effects of this receptor activation (i.e., ERK phosphorylation and activation of NF-kB, increased expression of RAGE itself and secretion of proinflammatory cytokines) (Somensi et al. 2017).

Conclusions

Over the years several authors have detected Hsp70 in the extracellular environment. Early studies hypothesized that this protein was released consequently to cell death, as it lacks any exocytosis signals. Nowadays, it is well demonstrated that Hsp70 is released through active mechanism, involving non classical secretory pathways (e.g., extracellular vesicles, lysosome-endosome pathway and so on). Several roles have been demonstrated for eHsp70, especially at cell signaling and cell communication level, depending on its state (i.e. membrane-bound or

membrane-free). An intriguing aspect of eHsp70 biology is its ability to induce antagonist responses, such as inflammatory and anti-inflammatory events. A particularly abundant field on eHsp70 functions regards its ability to modulate the immune system. Indeed, it has been displayed that eHsp70 possesses powerful immunological properties, acting both on innate and adaptive immune system. eHsp70 activates innate immune system cells by inducing cytokine release, or stimulate the adaptive immune response via antigen cross presentation. In addition to its immunological role, eHsp70 has protective functions in nervous cells, such as motoneurons, sensory neurons, glial cells. Finally, eHsp70 has been demonstrated to be involved in cell migration and angiogenesis, and it is a marker for several diseases. A better comprehension of eHsp70 multiple roles will be useful for therapeutic applications in inflammatory diseases, cancer and autoimmunity.

References

Abkin, S. V., Pankratova, K. M., Komarova, E. Y., Guzhova, I. V., & Margulis, B. A. (2013). Hsp70 chaperone-based gel composition as a novel immunotherapeutic anti-tumor tool. *Cell Stress & Chaperones, 18*, 391–396.

Adachi, H., Katsuno, M., Waza, M., Minamiyama, M., Tanaka, F., & Sobue, G. (2009). Heat shock proteins in neurodegenerative diseases: Pathogenic roles and therapeutic implications. *International Journal of Hyperthermia, 25*, 647–654.

Aneja, R., Odoms, K., Dunsmore, K., Shanley, T. P., & Wong, H. R. (2006). Extracellular heat shock protein-70 induces endotoxin tolerance in THP-1 cells. *Journal of Immunology, 177*, 7184–7192.

Aquino, D. A., Klipfel, A. A., Brosnan, C. F., & Norton, W. T. (1993). The 70-kDa heat shock cognate protein (HSC70) is a major constituent of the central nervous system and is up-regulated only at the mRNA level in acute experimental autoimmune encephalomyelitis. *Journal of Neurochemistry, 61*, 1340–1348.

Aquino, D. A., Capello, E., Weisstein, J., Sanders, V., Lopez, C., Tourtellotte, W. W., Brosnan, C. F., Raine, C. S., & Norton, W. T. (1997). Multiple sclerosis: Altered expression of 70- and 27-kDa heat shock proteins in lesions and myelin. *Journal of Neuropathology and Experimental Neurology, 56*, 664–672.

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.

Arispe, N., Doh, M., Simakova, O., Kurganov, B., & De Maio, A. (2004). Hsc70 and Hsp70 interact with phosphatideylserine on the surface of PC12 cells resulting in a decrease of viability. *The FASEB Journal, 18*, 1636–1645.

Arnold-Schild, D., Hanau, D., Spehner, D., Schmid, C., Rammensee, H. G., de la Salle, H., & Schild, H. (1999). Cutting edge: Receptor-mediated endocytosis of heat shock proteins by professional antigen-presenting cells. *Journal of Immunology, 162*, 3757–3760.

Asea, A. (2003). Chaperokine-induced signal transduction pathways. *Exercise Immunology, 9*, 25–33.

Asea, A. (2005). Stress proteins and initiation of immune response: Chaperokine activity of hsp72. *Exercise Immunology Review, 11*, 34–45.

Asea, A. (2006). Initiation of the immune response by extracellular Hsp72: Chaperokine activity of Hsp72. *Current Immunology Reviews, 2*, 209–215.

Asea, A. (2007). Hsp72 release: Mechanisms and methodologies. *Methods, 43*, 194–198.

Asea, A. (2008). Hsp70: A chaperokine. *Novartis Foundation Symposium, 291*, 173–179. discussion 179-183:221-224.

Asea, A., Kraeft, S. K., Kurt-Jones, E. A., Stevenson, M. A., Chen, L. B., Finberg, R. W., Koo, G. C., & Calderwood, S. K. (2000). HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nature Medicine, 6*, 435–442.

Asea, A., Rehli, M., Kabingu, E., Boch, J. A., Bare, O., Auron, P. E., Stevenson, M. A., & Calderwood, S. K. (2002). Novel signal transduction pathway utilized by extracellular HSP70: Role of toll-like receptor (TLR) 2 and TLR4. *The Journal of Biological Chemistry, 277*, 15028–15034.

Banchereau, J., & Steinman, R. M. (1998). Dendritic cells and the control of immunity. *Nature, 392*, 245–252.

Barreca, M. M., Spinello, W., Cavalieri, V., Turturici, G., Sconzo, G., Kaur, P., Tinnirello, R., Asea, A. A., & Geraci, F. (2017). Extracellular Hsp70 enhances mesoangioblast migration via an autocrine signaling pathway. *Journal of Cellular Physiology, 232*, 1845–1861.

Basu, S., Binder, R. J., Suto, R., Anderson, K. M., & Srivastava, P. K. (2000). Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. *International Immunology, 12*, 1539–1546.

Basu, S., Binder, R. J., Ramalingam, T., & Srivastava, P. K. (2001). CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity, 14*, 303–313.

Bausero, M. A., Gastpar, R., Multhoff, G., & Asea, A. (2005). Alternative mechanism by which IFN-gamma enhances tumor recognition: Active release of heat shock protein 72. *Journal of Immunology, 175*, 2900–2912.

Becker, T., Hartl, F. U., & Wieland, F. (2002). CD40, an extracellular receptor for binding and uptake of Hsp70-peptide complexes. *The Journal of Cell Biology, 158*, 1277–1285.

Beere, H. M. (2004). "The stress of dying": The role of heat shock proteins in the regulation of apoptosis. *Journal of Cell Science, 117*, 2641–2651.

Bendz, H., Ruhland, S. C., Pandya, M. J., Hainzl, O., Riegelsberger, S., Bräuchle, C., Mayer, M. P., Buchner, J., Issels, R. D., & Noessner, E. (2007). Human heat shock protein 70 enhances tumor antigen presentation through complex formation and intracellular antigen delivery without innate immune signaling. *The Journal of Biological Chemistry, 282*, 31688–31702.

Beuret, N., Stettler, H., Renold, A., Rutishauser, J., & Spiess, M. (2004). Expression of regulated secretory proteins is sufficient to generate granule-like structures in constitutively secreting cells. *The Journal of Biological Chemistry, 279*, 20242–20249.

Blander, J. M., & Medzhitov, R. (2004). Regulation of phagosome maturation by signals from toll-like receptors. *Science, 304*, 1014–1018.

Bluestone, J. A. (2005). Regulatory T-cell therapy: Is it ready for the clinic? *Nature Reviews. Immunology, 5*, 343–349.

Borges, T. J., Wieten, L., van Herwijnen, M. J., Broere, F., van der Zee, R., Bonorino, C., & van Eden, W. (2012). The anti-inflammatory mechanisms of Hsp70. *Frontiers in Immunology, 3*, 1–12.

Broquet, A. H., Thomas, G., Masliah, J., Trugnan, G., & Bachelet, M. (2003). Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release. *The Journal of Biological Chemistry, 278*, 21601–21606.

Brown, I. R. (1991). Expression of heat shock genes (hsp70) in the mammalian nervous system. *Results and Problems in Cell Differentiation, 17*, 217–229.

Bukau, B., Weissman, J., & Horwich, A. (2006). Molecular chaperones and protein quality control. *Cell, 125*, 443–451.

Calderwood, S. K., Mambula, S. S., Gray, P. J., Jr., & Theriault, J. R. (2007). Extracellular heat shock proteins in cell signalling. *FEBS Letters, 581*, 3689–3694.

Calderwood, S. K., Murshid, A., & Gong, J. (2012). Heat shock proteins: Conditional mediators of inflammation in tumor immunity. *Frontiers in Immunology, 3*, 1–10.

Castelli, C., Ciupitu, A. M., Rini, F., Rivoltini, L., Mazzocchi, A., Kiessling, R., & Parmiani, G. (2001). Human heat shock protein 70 peptide complexes specifically activate antimelanoma T cells. *Cancer Research, 61*, 222–227.

Chabas, D., Baranzini, S. E., Mitchell, D., Bernard, C. C., Rittling, S. R., Denhardt, D. T., Sobel, R. A., Lock, C., Karpuj, M., Pedotti, R., Heller, R., Oksenberg, J. R., & Steinman, L. (2001). The influence of the pro-inflammatory cytokine, osteopontin, on autoimmune demyelinating disease. *Science, 294*, 1731–1735.

Chase, M. A., Wheeler, D. S., Lierl, K. M., Hughes, V. S., Wong, H. R., & Page, K. (2007). Hsp72 induces inflammation and regulates cytokine production in airway epithelium through a TLR4- and NF-kappaB-dependent mechanism. *Journal of Immunology, 179*, 6318–6324.

Chen, X., Tao, Q., Yu, H., Zhang, L., & Cao, X. (2002). Tumor cell membrane-bound heat shock protein 70 elicits antitumor immunity. *Immunology Letters, 84*, 81–87.

Chen, S., Bawa, D., Besshoh, S., Gurd, J. W., & Brown, I. R. (2005). Association of heat shock proteins and neuronal membrane components with lipid rafts from the rat brain. *Journal of Neuroscience Research, 81*(4), 522–529.

Chen, T., Guo, J., Han, C., Yang, M., & Cao, X. (2009). Heat shock protein 70, released from heat-stressed tumor cells, initiates antitumor immunity by inducing tumor cell chemokine production and activating dendritic cells via TLR4 pathway. *Journal of Immunology, 182*, 1449–1459.

Chen, E., Xue, D., Zhang, W., Lin, F., & Pan, Z. (2015). Extracellular heat shock protein 70 promotes osteogenesis of human mesenchymal stem cells through activation of the ERK signaling pathway. *FEBS Letters, 589*, 4088–4096.

Chiba, S., Yokota, S., Yonekura, K., Tanaka, S., Furuyama, H., Kubota, H., Fujii, N., & Matsumoto, H. (2006). Autoantibodies against HSP70 family proteins were detected in the cerebrospinal fluid from patients with multiple sclerosis. *Journal of the Neurological Sciences, 241*, 39–43.

Ciocca, D. R., & Calderwood, S. K. (2005). Heat shock proteins in cancer: Diagnostic, prognostic, predictive, and treatment implications. *Cell Stress & Chaperones, 10*, 86–103.

Clark, P. R., & Ménoret, A. (2001). The inducible Hsp70 as a marker of tumor immunogenicity. *Cell Stress & Chaperones, 6*, 121–125.

Clayton, A., Turkes, A., Navabi, H., Mason, M. D., & Tabi, Z. (2005). Induction of heat shock proteins in B-cell exosomes. *Journal of Cell Science, 118*, 3631–3638.

Collins, P. L., & Hightower, L. E. (1982). Newcastle disease virus stimulates the cellular accumulation of stress (heat shock) mRNAs and proteins. *Journal of Virology, 44*, 703–707.

Dello Russo, C., Polak, P. E., Mercado, P. R., Spagnolo, A., Sharp, A., Murphy, P., Kamal, A., Burrows, F. J., Fritz, L. C., & Feinstein, D. L. (2006). The heat-shock protein 90 inhibitor17-allylamino-17-demethoxygeldanamycin suppresses glial inflammatory responses and ameliorates experimental autoimmune encephalomyelitis. *Journal of Neurochemistry, 99*, 1351–1362.

Delneste, Y., Magistrelli, G., Gauchat, J., Haeuw, J., Aubry, J., Nakamura, K., Kawakami-Honda, N., Goetsch, L., Sawamura, T., Bonnefoy, J., & Jeannin, P. (2002). Involvement of LOX-1 in dendritic cell-mediated antigen cross-presentation. *Immunity, 17*, 353–362.

Dressel, R., Elsner, L., Quentin, T., Walter, L., & Günther, E. (2000). Heat shock protein 70 is able to prevent heat shock-induced resistance of target cells to CTL. *Journal of Immunology, 164*, 2362–2371.

Dutta, S. K., Girotra, M., Singla, M., Dutta, A., Otis Stephen, F., Nair, P. P., & Merchant, N. B. (2012). Serum HSP70: A novel biomarker for early detection of pancreatic cancer. *Pancreas, 41*, 530–534.

Edbladh, M., Ekstrom, P. A., & Edstrom, A. (1994). Retrograde axonal transport of locally synthesized proteins, e.g., actin and heat shock protein 70, in regenerating adult frog sciatic sensory axons. *Journal of Neuroscience Research, 38*, 424–432.

Eder, K., Guan, H., Sung, H. Y., Ward, J., Angyal, A., Janas, M., Sarmay, G., Duda, E., Turner, M., Dower, S. K., Francis, S. E., Crossman, D. C., & Kiss-Toth, E. (2008). Tribbles-2 is a novel regulator of inflammatory activation of monocytes. *International Immunology, 20*, 1543–1550.

Elstner, A., Stockhammer, F., Nguyen-Dobinsky, T. N., Nguyen, Q. L., Pilgermann, I., Gill, A., Guhr, A., Zhang, T., von Eckardstein, K., Picht, T., Veelken, J., Martuza, R. L., von Deimling,

A., & Kurtz, A. (2011). Identification of diagnostic serum protein profiles of glioblastoma patients. *Journal of Neuro-Oncology, 102*, 71–80.

Enomoto, Y., Bharti, A., Khaleque, A. A., Song, B., Liu, C., Apostolopoulos, V., Xing, P. X., Calderwood, S. K., & Gong, J. (2006). Enhanced immunogenicity of heat shock protein 70 peptide complexes from dendritic cell-tumor fusion cells. *Journal of Immunology, 177*, 5946–5955.

Evdokimovskaya, Y., Skarga, Y., Vrublevskaya, V., & Morenkov, O. (2010). Secretion of the heat shock proteins HSP70 and HSC70 by baby hamster kidney (BHK-21) cells. *Cell Biology International, 34*, 985–990.

Evdonin, A. L., Martynova, M. G., Bystrova, O. A., Guzhova, I. V., Margulis, B. A., & Medvedeva, N. D. (2006). The release of Hsp70 from A431 carcinoma cells is mediated by secretory-like granules. *European Journal of Cell Biology, 85*, 443–455.

Feder, M. E., & Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology, 61*, 243–282.

Fevrier, B., & Raposo, G. (2004). Exosomes: Endosomal-derived vesicles shipping extra- cellular messages. *Current Opinion in Cell Biology, 16*, 415–421.

Fischer, N., Haug, M., Kwok, W. W., Kalbacher, H., Wernet, D., Dannecker, G. E., & Holzer, U. (2010). Involvement of CD91 and scavenger receptors in Hsp70-facilitated activation of human antigen-specific CD4+ memory T cells. *European Journal of Immunology, 40*, 986–997.

Fiskum, G. (2004). Mechanisms of neuronal death and neuroprotection. *Journal of Neurosurgical Anesthesiology, 16*, 108–110.

Franco, L., Terrinca, J., Rodríguez, A. B., Espino, J., & Pariente, J. A. (2016). Extracellular heat shock proteins protect U937 cells from H2O2-induced apoptotic cell death. *Molecular and Cellular Biochemistry, 412*, 19–26.

Futagami, S., Hiratsuka, T., Shindo, T., Hamamoto, T., Horie, A., Ueki, N., Kusunoki, M., Gudis, K., Miyake, K., Tsukui, T., & Sakamoto, C. (2008). Extracellular HSP70 blocks CD40L-induced apoptosis and tubular formation in endothelial cells. *Journal of Gastroenterology and Hepatology, 23*, S222–S228.

Galazka, G., Stasiolek, M., Walczak, A., Jurewicz, A., Zylicz, A., Brosnan, C. F., Raine, C. S., & Selmaj, K. W. (2006). Brain-derived heat shock protein 70-peptide complexes induce NK cell-dependent tolerance to experimental autoimmune encephalomyelitis. *Journal of Immunology, 176*, 1588–1599.

Gallucci, S., Lolkema, M., & Matzinger, P. (1999). Natural adjuvants: Endogenous activators of dendritic cells. *Nature Medicine, 5*, 1249–1255.

Gao, Y. L., Brosnan, C. F., & Raine, C. S. (1995). Experimental autoimmune encephalomyelitis. Qualitative and semiquantitative differences in heat shock protein 60 expression in the central nervous system. *Journal of Immunology, 154*, 3548–3556.

Garrido, C., Gurbuxani, S., Ravagnan, L., & Kroemer, G. (2001). Heat shock proteins: Endogenous modulators of apoptotic cell death. *Biochemical and Biophysical Research Communications, 286*, 433–442.

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. *Journal of Immunology, 172*, 972–980.

Gastpar, R., Gehrmann, M., Bausero, M. A., Asea, A., Gross, C., Schroeder, J. A., & Multhoff, G. (2005). Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. *Cancer Research, 65*, 5238–5247.

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.

Gehrmann, M., Cervello, M., Montalto, G., Cappello, F., Gulino, A., Knape, C., Specht, H. M., & Multhoff, G. (2014). Heat shock protein 70 serum levels differ significantly in patients with chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. *Frontiers in Immunology, 5*, 1–7.

Geng, H., Zhang, G. M., Xiao, H., Yuan, Y., Li, D., Zhang, H., Qiu, H., He, Y. F., & Feng, Z. H. (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. *International Journal of Cancer, 118*, 2657–2664.

Giffard, R. G., Xu, L., Zhao, H., Carrico, W., Ouyang, Y., Qiao, Y., Sapolsky, R., Steinberg, G., Hu, B., & Yenari, M. A. (2004). Chaperones, protein aggregation, and brain protection from hypoxic/ischemic injury. *The Journal of Experimental Biology, 207*, 3213–3220.

Giraldo, E., Hinchado, M. D., Garcia, J. J., & Ortega, E. (2008). Influence of gender and oral contraceptives intake on innate and inflammatory response. Role of neuroendocrine factors. *Molecular and Cellular Biochemistry, 313*, 147–153.

González-Ramos, M., Calleros, L., López-Ongil, S., Raoch, V., Griera, M., Rodríguez-Puyol, M., de Frutos, S., & Rodríguez-Puyol, D. (2013). HSP70 increases extracellular matrix production by human vascular smooth muscle through TGF-β1 upregulation. *The International Journal of Biochemistry & Cell Biology, 45*, 232–242.

Greene, C. M., Carroll, T. P., Smith, S. G., Taggart, C. C., Devaney, J., Griffin, S., O'neill, S. J., & McElvaney, N. G. (2005). TLR-induced inflammation in cystic fibrosis and non-cystic fibrosis airway epithelial cells. *Journal of Immunology, 174*, 1638–1646.

Gross, C., Schmidt-Wolf, I. G., Nagaraj, S., Gastpar, R., Ellwart, J., Kunz-Schughart, L. A., & Multhoff, G. (2003a). Heat shock protein 70-reactivity is associated with increased cell surface density of CD94/CD56 on primary natural killer cells. *Cell Stress & Chaperones, 8*, 348–360.

Gross, C., Hansch, D., Gastpar, R., & Multhoff, G. (2003b). Interaction of heat shock protein 70 peptide with NK cells involves the NK receptor CD94. *Biological Chemistry, 384*, 267–279.

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 Research, 914*, 66–73.

Hartl, F. U., & Hayer-Hartl, M. (2009). Converging concepts of protein folding in vitro and in vivo. *Nature Structural & Molecular Biology, 16*, 574–581.

Heath, W. R., & Carbone, F. R. (2001). Cross-presentation, dendritic cells, tolerance and immunity. *Annual Review of Immunology, 19*, 47–64.

Heijnen, H. F., Schiel, A. E., Fijnheer, R., Geuze, H. J., & Sixma, J. J. (1999). Activated platelets release two types of membrane vesicles: Microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood, 94*, 3791–3799.

Hightower, L. E., & Guidon, P. T., Jr. (1989). Selective release from cultured mammalian cells of heat-shock (stress) proteins that resemble glia-axon transfer proteins. *Journal of Cellular Physiology, 138*, 257–266.

Hilf, N., Singh-Jasuja, H., Schwarzmaier, P., Gouttefangeas, C., Rammensee, H. G., & Schild, H. (2002). Human platelets express heat shock protein receptors and regulate dendritic cell maturation. *Blood, 99*, 3676–3682.

Horváth, I., Multhoff, G., Sonnleitner, A., & Vígh, L. (2008). Membrane-associated stress proteins: More than simply chaperones. *Biochimica et Biophysica Acta, 1778*, 1653–1664.

Hunter-Lavin, C., Davies, E. L., Bacelar, M. M., Marshall, M. J., Andrew, S. M., & Williams, J. H. (2004). Hsp70 release from peripheral blood mononuclear cells. *Biochemical and Biophysical Research Communications, 324*, 511–517.

Ito, A., Matsuoka, F., Honda, H., & Kobayashi, T. (2004). Atitumor effects of combined therapy of recombinant heat shock protein 70 and hyperthermia using magnetic nanoparticles in an experimental subcutaneous murine melanoma. *Cancer Immunology, Immunotherapy, 53*, 26–32.

Johnson, J. D., & Fleshner, M. (2006). Releasing signals, secretory pathways, and immune function of endogenous extracellular heat shock protein 72. *Journal of Leukocyte Biology, 79*, 425–434.

Jones, Q., Voegeli, T. S., Li, G., Chen, Y., & Currie, R. W. (2011). Heat shock proteins protect against ischemia and inflammation through multiple mechanisms. *Inflammation Allergy Drug Targets, 10*, 247–259.

Kampinga, H. H., Henning, R. H., van Gelder, I. C., & Brundel, B. J. (2007). Beat shock proteins and atrial fibrillation. *Cell Stress & Chaperones, 12*, 97–100.

Kampinga, H. H., Hageman, J., Vos, M. J., Kubota, H., Tanguay, R. M., Bruford, E. A., Cheetham, M. E., Chen, B., & Hightower, L. E. (2009). Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress & Chaperones, 14*, 105–111.

Kaur, J., Kaur, J., & Ralhan, R. (2000). Induction of apoptosis by abrogation of HSP70 expression in human oral cancer cells. *International Journal of Cancer, 85*, 1–5.

Kim, T. K., Na, H. J., Lee, W. R., Jeoung, M. H., & Lee, S. (2016). Heat shock protein 70-1A is a novel angiogenic regulator. *Biochemical and Biophysical Research Communications, 469*, 222–228.

Kocsis, J., Madaras, B., Tóth, E. K., Füst, G., & Prohászka, Z. (2010). Serum level of soluble 70-kD heat shock protein is associated with high mortality in patients with colorectal cancer without distant metastasis. *Cell Stress & Chaperones, 15*, 143–151.

Kovalchin, J. T., Wang, R., Wagh, M. S., Azoulay, J., Sanders, M., & Chandawarkar, R. Y. (2006). In vivo delivery of heat shock protein 70 accelerates wound healing by up-regulating macrophage-mediated phagocytosis. *Wound Repair and Regeneration, 14*, 129–137.

Krause, M., & Rodrigues-Krause, J. C. (2011). Extracellular heat shock proteins (eHSP70) in exercise: Possible targets outside the immune system and their role for neurodegenerative disorders treatment. *Medical Hypotheses, 76*, 286–290.

Krause, S. W., Gastpar, R., Andreesen, R., Gross, C., Ullrich, H., Thonigs, G., Pfister, K., & Multhoff, G. (2004). Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: A clinical phase I trial. *Clinical Cancer Research, 10*, 3699–36707.

Kregel, K. C. (2002). Heat shock proteins: Modifying factors in physiological stress responses and acquired thermotolerance. *Journal of Applied Physics, 92*, 2177–2186.

Kumar, S., Deepak, P., & Acharya, A. (2009). Autologous Hsp70 immunization induces anti-tumor immunity and increases longevity and survival of tumor-bearing mice. *Neoplasma, 56*, 259–268.

Kuppner, M. C., Gastpar, R., Gelwer, S., Nössner, E., Ochmann, O., Scharner, A., & Issels, R. D. (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. *European Journal of Immunology, 31*, 1602–1609.

Lancaster, G. I., & Febbraio, M. A. (2005). Exosome-dependent trafficking of HSP70: A novel secretory pathway for cellular stress proteins. *The Journal of Biological Chemistry, 280*, 23349–23355.

Lehner, T., Wang, Y., & Kelly, C. (2003). Heat shock protein receptors, functions and their effect on monocytes and dendritic cells. In W. van Eden & D. B. Young (Eds.), *Heat shock proteins and inflammation* (pp. 193–198). Basel: Dekker.

Li, Z., Menoret, A., & Srivastava, P. (2002). Roles of heat-shock proteins in antigen presentation and cross-presentation. *Current Opinion in Immunology, 14*, 45–51.

Li, D., Romain, G., Flamar, A. L., Duluc, D., Dullaers, M., Li, X. H., Zurawski, S., Bosquet, N., Palucka, A. K., Le Grand, R., O'Garra, A., Zurawski, G., Banchereau, J., & Oh, S. (2012). Targeting self- and foreign antigens to dendritic cells via DC-ASGPR generates IL-10-producing suppressive CD4+ T cells. *The Journal of Experimental Medicine, 209*, 109–121.

Liberek, K., Lewandowska, A., & Zietkiewicz, S. (2008). Chaperones in control of protein disaggregation. *The EMBO Journal, 23*, 27328–27335.

Lindquist, S. (1986). The heat-shock response. *Annual Review of Biochemistry, 55*, 1151–1191.

Lindquist, S., & Craig, E. A. (1988). The heat-shock proteins. *Annual Review of Genetics, 22*, 631–677.

Luft, J. C., & Dix, D. J. (1999). Hsp70 expression and function during embryogenesis. *Cell Stress & Chaperones, 4*, 162–170.

Lui, J. C., & Kong, S. K. (2007). Heat shock protein 70 inhibits the nuclear import of apoptosis-inducing factor to avoid DNA fragmentation in TF-1 cells during erythropoiesis. *FEBS Letters, 581*, 109–117.

Lund, B. T., Chakryan, Y., Ashikian, N., Mnatsakanyan, L., Bevan, C. J., Aguilera, R., Gallaher, T., & Jakowec, M. W. (2006). Association of MBP peptides with Hsp70 in normal appearing human white matter. *Journal Neurology Science Novel, 249*, 122–134.

Luo, X., Zuo, X., Zhou, Y., Zhang, B., Shi, Y., Liu, M., Wang, K., McMillian, D. R., & Xiao, X. (2008). Extracellular heat shock protein 70 inhibits tumour necrosis factor-alpha induced proinflammatory mediator production in fibroblast-like synoviocytes. *Arthritis Research & Therapy, 10*, R41.

Luo, X., Tao, L., Lin, P., Mo, X., & Chen, H. (2012). Extracellular heat shock protein 72 protects schwann cells from hydrogen peroxide-induced apoptosis. *Journal of Neuroscience Research, 90*, 1261–1269.

MacAry, P. A., Javid, B., Floto, R. A., Smith, K. G., Oehlmann, W., Singh, M., & Lehner, P. J. (2004). HSP70 peptide binding mutants separate antigen delivery from dendritic cell stimulation. *Immunity, 20*, 95–106.

Madden, L. A., Sandström, M. E., Lovell, R. J., & McNaughton, L. (2008). Inducible heat shock protein 70 and its role in preconditioning and exercise. *Amino Acids, 34*, 511–516.

Mambula, S. S., & Calderwood, S. K. (2006). Heat shock protein 70 is secreted from tumor cells by a nonclassical pathway involving lysosomal endosomes. *Journal of Immunology, 177*, 7849–7857.

Martin, R., McFarland, H. F., & McFarlin, D. E. (1992). Immunological aspects of demyelinating diseases. *Annual Review of Immunology, 10*, 153–187.

Martin, C. A., Carsons, S. E., 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. *Journal of Immunology, 171*, 5736–5742.

Mathew, A., Bell, A., & Johnstone, R. M. (1995). Hsp-70 is closely associated with the transferrin receptor in exosomes from maturing reticulocytes. *The Biochemical Journal, 308*, 823–830.

Mathivanan, S., & Simpson, R. J. (2009). ExoCarta: A compendium of exosomal proteins and RNA. *Proteomics, 9*, 4997–5000.

Mathur, S., Walley, K. R., Wang, Y., Indrambarya, T., & Boyd, J. H. (2011). Extracellular heat shock protein 70 induces cardiomyocyte inflammation and contractile dysfunction via TLR2. *Circulation Journal, 75*, 2445–2452.

Milani, V., Noessner, E., Ghose, S., Kuppner, M., Ahrens, B., Scharner, A., Gastpar, R., & Issels, R. D. (2002). Heat shock protein 70: Role in antigen presentation and immune stimulation. *International Journal of Hyperthermia, 18*, 563–575.

Milarski, K. L., & Morimoto, R. I. (1989). Mutational analysis of the human HSP70 protein: Distinct domains for nucleolar localization and adenosine triphosphate binding. *The Journal of Cell Biology, 109*, 1947–1962.

Moore, K. W., de Waal Malefyt, R., Coffman, R. L., & O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annual Review of Immunology, 19*, 683–765.

Morimoto, R. I. (1991). Heat shock: The role of transient inducible responses in cell damage, transformation, and differentiation. *Cancer Cells, 3*, 295–301.

Morimoto, R. I., Kline, M. P., Bimston, D. N., & Cotto, J. J. (1997). The heat-shock response: Regulation and function of heat-shock proteins and molecular chaperones. *Essays in Biochemistry, 32*, 17–29.

Moser, C., Schmidbauer, C., Gürtler, U., Gross, C., Gehrmann, M., Thonigs, G., Pfister, K., & Multhoff, G. (2002). Inhibition of tumor growth in mice with severe combined immunodeficiency is mediated by heat shock protein 70 (Hsp70)-peptide-activated, CD94 positive natural killer cells. *Cell Stress & Chaperones, 7*, 365–373.

Muchowski, P. J., & Wacker, J. L. (2005). Modulation of neurodegeneration by molecular chaperones. *Nature Reviews. Neuroscience, 6*, 11–22.

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. *Journal of Immunology, 158*, 4341–4350.

Multhoff, G., Botzler, C., & Issels, R. (1998). The role of heat shock proteins in the stimulation of an immune response. *Biological Chemistry, 379*, 295–300.

Multhoff, G., Mizzen, L., Winchester, C. C., Milner, C. M., Wenk, S., Eissner, G., Kampinga, H. H., Laumbacher, B., & Johnson, J. (1999). Heat shock protein 70 (Hsp70) stimulates proliferation and cytolytic activity of natural killer cells. *Experimental Hematology, 27*, 1627–1636.

Multhoff, G., Pfister, K., Gehrmann, M., Hantschel, M., Gross, C., Hafner, M., & Hiddemann, W. (2001). A 14-mer Hsp70 peptide stimulates natural killer (NK) cell activity. *Cell Stress & Chaperones, 6*, 337–344.

Nickel, W., & Seedorf, M. (2008). Unconventional mechanisms of protein transport to the cell surface of eukaryotic cells. *Annual Review of Cell and Developmental Biology, 24*, 287–308.

Nieland, T. J., Tan, M. C., Monne-van Muijen, M., Koning, F., Kruisbeek, A. M., & van Bleek, G. M. (1996). Isolation of an immunodominant viral peptide that is endogenously bound to the stress protein GP96/GRP94. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 6135–6139.

Noble, E. G., Milne, K. J., & Melling, C. W. (2008). Heat shock proteins and exercise: A primer. *Applied Physiology, Nutrition, and Metabolism, 33*, 1050–1065.

Noessner, E., Gastpar, R., Milani, V., Brandl, A., Hutzler, P. J., Kuppner, M. C., Roos, M., Kremmer, E., Asea, A., Calderwood, S. K., & Issels, R. D. (2002). Tumor-derived heat shock protein 70 peptide complexes are cross-presented by human dendritic cells. *Journal of Immunology, 169*, 5424–5432.

Novoselova, T. V., Margulis, B. A., Novoselov, S. S., Sapozhnikov, A. M., van der Spuy, J., Cheetham, M. E., & Guzhova, I. V. (2005). Treatment with extracellular HSP70/HSC70 protein can reduce polyglutamine toxicity and aggregation. *Journal of Neurochemistry, 94*, 597–606.

Nylandsted, J., Rohde, M., Brand, K., Bastholm, L., Elling, F., & Jäättelä, M. (2000). Selective depletion of heat shock protein 70 (Hsp70) activates a tumor-specific death program that is independent of caspases and bypasses Bcl-2. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 7871–7876.

Ortega, E., Giraldo, E., Hinchado, M. D., Martinez, M., Ibanez, S., Cidoncha, A., Collazos, M. E., & Garcia, J. J. (2006). Role of Hsp72 and norepinephrine in the moderate exercise-induced stimulation of neutrophils' microbicide capacity. *European Journal of Applied Physiology, 98*, 250–255.

Ortega, E., Hinchado, M. D., 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.

Panjwani, N. N., Popova, L., & Srivastava, P. K. (2002). Heat shock proteins gp96 and hsp70 activate the release of nitric oxide by APCs. *Journal of Immunology, 168*, 2997–3003.

Park, C. J., Park, S. A., Yoon, T. G., Lee, S. J., Yum, K. W., & Kim, H. J. (2005). Bupivacaine induces apoptosis via ROS in the Schwann cell line. *Journal of Dental Research, 84*, 852–857.

Patel, B., Khaliq, A., Jarvis-Evans, J., Boulton, M., Arrol, S., Mackness, M., & McLeod, D. (1995). Hypoxia induces HSP 70 gene expression in human hepatoma (HEP G2) cells. *Biochemistry and Molecular Biology International, 36*, 907–912.

Pawaria, S., & Binder, R. J. (2011). CD91-dependent programming of T-helper cell responses following heat shock protein immunization. *Nature Communications, 2*, 1–11.

Pierzchalski, P., Jastrzebska, M., Link-Lenczowski, P., Leja-Szpak, A., Bonior, J., Jaworek, J., Okon, K., & Wojcik, P. (2014). The dynamics of heat shock system activation in Monomac-6 cells upon Helicobacter pylori infection. *Journal of Physiology and Pharmacology, 65*, 791–800.

Planas, A. M., Soriano, M. A., Estrada, A., Sanz, O., Martin, F., & Ferrer, I. (1997). The heat shock stress response after brain lesions: Induction of 72 kDa heat shock protein (cell types involved, axonal transport, transcriptional regulation) and protein synthesis inhibition. *Progress in Neurobiology, 51*, 607–636.

Pockley, A. G., Shepherd, J., & Corton, J. M. (1998). Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies in the serum of normal individuals. *Immunological Investigations, 27*, 367–377.

Pockley, A. G., De Faire, U., Kiessling, R., Lemne, C., Thulin, T., & Frostegård, J. (2002). Circulating heat shock protein and heat shock antibody levels in established hypertension. *Journal of Hypertension, 20*, 1815–1820.

Pockley, A. G., Georgiades, A., Thulin, T., de Faire, U., & Frostegård, J. (2003). Serum heat shock protein 70 levels predict the development of atherosclerosis in subjects with established hypertension. *Hypertension, 42*, 235–238.

Prakken, B. J., Wendling, U., van der Zee, R., Rutten, V. P., Kuis, W., & van Eden, W. (2001). Induction of IL-10 and inhibition of experimental arthritis are specific features of microbial heat shock proteins that are absent for other evolutionarily conserved immunodominant proteins. *Journal of Immunology, 167*, 4147–4153.

Qiao, Y., Liu, B., & Li, Z. (2008). Activation of NK cells by extracellular heat shock protein 70 through induction of NKG2D ligands on dendritic cells. *Cancer Immunity, 10*(8), 12.

Radsak, M. P., Hilf, N., Singh-Jasuja, H., Braedel, S., Brossart, P., Rammensee, H. G., & Schild, H. (2003). The heat shock protein gp96 binds to human neutrophils and monocytes and stimulates effector functions. *Blood, 101*, 2810–2281.

Richard, V., Kaeffer, N., & Thuillez, C. (1996). Delayed protection of the ischemic heart from pathophysiology to therapeutic applications. *Fundamental & Clinical Pharmacology, 10*, 409–415.

Ries, C., Egea, V., Karow, M., Kolb, H., Jochum, M., & Neth, P. (2007). MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: Differential regulation by inflammatory cytokines. *Blood, 109*, 4055–4063.

Robinson, M. B., Tidwell, J. L., Gould, T., Taylor, A. R., Newbern, J. M., Graves, J., Tytell, M., & Milligan, C. E. (2005). Extracellular heat shock protein 70: A critical component for motoneuron survival. *The Journal of Neuroscience, 25*, 9735–9745.

Robinson, M. B., Taylor, A. R., Gifondorwa, D. J., Tytell, M., & Milligan, C. E. (2008). Exogenous Hsc70, but not thermal preconditioning, confers protection to motoneurons subjected to oxidative stress. *Developmental Neurobiology, 68*, 1–17.

Rodrigues-Krause, J., Krause, M., O'Hagan, C., De Vito, G., Boreham, C., Murphy, C., Newsholme, P., & Colleran, G. (2012). Divergence of intracellular and extracellular HSP72 in type 2 diabetes: Does fat matter? *Cell Stress & Chaperones, 17*, 293–302.

Said Ali, K., Ferencz, A., Nemcsok, J., & Hermesz, E. (2010). Expressions of heat shock and metallothionein genes in the heart of common carp (Cyprinus carpio): Effects of temperature shock and heavy metal exposure. *Acta Biologica Hungarica, 61*, 10–23.

Saito, K., Dai, Y., & Ohtsuka, K. (2005). Enhanced expression of heat shock proteins in gradually dying cells and their release from necrotically dead cells. *Experimental Cell Research, 310*, 229–236.

Schild, H., Arnold-Schild, D., Lammert, E., & Rammensee, H. G. (1999). Stress proteins and immunity mediated by cytotoxic T lymphocytes. *Current Opinion in Immunology, 11*, 109–113.

Sharp, F. R., Massa, S. M., & Swanson, R. A. (1999). Heat-shock protein protection. *Trends in Neurosciences, 22*, 97–99.

Shevchenko, M. A., Troyanova, N. I., Servuli, E. A., Bolkhovitina, E. L., Fedorina, A. S., & Sapozhnikov, A. M. (2016). Study of immunomodulatory effects of extracellular HSP70 in a mouse model of allergic airway inflammation. *Biochemistry (Mosc), 81*, 1384–1395.

Somensi, N., Brum, P. O., de Miranda Ramos, V., Gasparotto, J., Zanotto-Filho, A., Rostirolla, D. C., da Silva Morrone, M., Moreira, J. C. F., & Pens Gelain, D. (2017). Extracellular HSP70 activates ERK1/2, NF-kB and pro-inflammatory gene transcription through binding with RAGE in A549 human lung cancer cells. *Cellular Physiology and Biochemistry, 42*, 2507–2522.

Specht, H. M., Ahrens, N., Blankenstein, C., Duell, T., Fietkau, R., Gaipl, U. S., Günther, C., Gunther, S., Habl, G., Hautmann, H., Hautmann, M., Huber, R. M., Molls, M., Offner, R., Rödel, C., Rödel, F., Schütz, M., Combs, S. E., & 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. *Frontiers in Immunology, 6*, 1–9.

Sprang, G. K., & Brown, I. R. (1987). Selective induction of a heat shock gene in fibre tracts and cerebellar neurons of the rabbit brain detected by in situ hybridization. *Brain Research, 427*, 89–93.

Srivastava, P. (2002). Roles of heat-shock proteins in innate and adaptive immunity. *Nature Reviews. Immunology, 2*, 185–194.

Stadelmann, C., Ludwin, S., Tabira, T., Guseo, A., Lucchinetti, C. F., Leel-Ossy, L., Ordinario, A. T., Brück, W., & Lassmann, H. (2005). Tissue preconditioning may explain concentric lesions in Balo's type of multiple sclerosis. *Brain, 128*, 979–987.

Stangl, S., Wortmann, A., Guertler, U., & Multhoff, G. (2006). Control of metastasized pancreatic carcinomas in SCID/beige mice with human IL-2/TKD-activated NK cells. *Journal of Immunology, 176*, 6270–6276.

Stangl, S., Themelis, G., Friedrich, L., Ntziachristos, V., Sarantopoulos, A., Molls, M., Skerra, A., & Multhoff, G. (2011). Detection of irradiation-induced, membrane heat shock protein 70 (Hsp70) in mouse tumors using Hsp70 Fab fragment. *Radiotherapy and Oncology, 99*, 313–316.

Stocki, P., & Dickinson, A. M. (2012). The immunosuppressive activity of heat shock protein70. *Autoimmune Disease, 2012*, 1–6.

Suto, R., & Srivastava, P. K. (1995). A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. *Science, 269*, 1585–1588.

Svensson, P. A., Asea, A., Englund, M. C., Bausero, M. A., Jernås, M., Wiklund, O., Ohlsson, B. G., Carlsson, L. M., & Carlsson, B. (2006). Major role of HSP70 as a paracrine inducer of cytokine production in human oxidized LDL treated macrophages. *Atherosclerosis, 185*, 32–38.

Takeda, K., & Akira, S. (2004). TLR signaling pathways. *Seminars in Immunology, 16*, 3–9.

Tamura, Y., Peng, P., Liu, K., Daou, M., & Srivastava, P. K. (1997). Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. *Science, 278*, 117–120.

Tanaka, S., Kimura, Y., Mitani, A., Yamamoto, G., Nishimura, H., Spallek, R., Singh, M., Noguchi, T., & Yoshikai, Y. (1999). Activation of T cells recognizing an epitope of heat-shock protein 70 can protect against rat adjuvant arthritis. *Journal of Immunology, 163*, 5560–55605.

Taylor, A. R., Robinson, M. B., Gifondorwa, D. J., Tytell, M., & Milligan, C. E. (2007). Regulation of heat shock protein 70 release in astrocytes: Role of signaling kinases. *Developmental Neurobiology, 67*, 1815–1829.

Thériault, J. R., Adachi, H., & Calderwood, S. K. (2006). Role of scavenger receptors in the binding and internalization of heat shock protein 70. *Journal of Immunology, 177*, 8604–8611.

Théry, C. (2011). Exosomes: Secreted vesicles and intercellular communications. *F1000 Biology Reports, 3*, 1–8.

Théry, C., Regnault, A., Garin, J., Wolfers, J., Zitvogel, L., Ricciardi-Castagnoli, P., Raposo, G., & Amigorena, S. (1999). Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. *The Journal of Cell Biology, 147*, 599–610.

Tidwell, J. L., Houenou, L. J., & Tytell, M. (2004). Administration of Hsp70 in vivo inhibits motor and sensory neuron de generation. *Cell Stress and Chaperones, 9*, 88–98.

Tobian, A. A., Canaday, D. H., Boom, W. H., & Harding, C. V. (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. *Journal of Immunology, 172*, 5277–5286.

Triantafilou, M., Miyake, K., Golenbock, D. T., & Triantafilou, K. (2002). Mediators of innate immune recognition of bacteria concentrate in lipids rafts and facilitate lipopolysaccharide-induced cell activation. *Journal of Cell Science, 115*, 2603–2611.

Tsan, M. F., & Gao, B. (2009). Heat shock proteins and immune system. *Journal of Leukocyte Biology, 85*, 905–910.

Turturici, G., Sconzo, G., & Geraci, F. (2011). Hsp70 and its molecular role in nervous system diseases. *Biochemistry Research International, 2011*, 1–18.

Turturici, G., Tinnirello, R., Sconzo, G., Asea, A., Savettieri, G., Ragonese, P., & Geraci, F. (2014). Positive or negative involvement of heat shock proteins in multiple sclerosis pathogenesis: An overview. *Journal of Neuropathology and Experimental Neurology, 73*, 1092–1106.

Tytell, M. (2005). Release of heat shock proteins (HSP) and the effects of extracellular HSP on neural cells and tissues. *International Journal of Hyperthermia, 21*, 445–455.

Tytell, M., Greenberg, S. G., & Lasek, R. J. (1986). Heat shock-like protein is transferred from glia to axon. *Brain Research, 363*, 161–164.

Vabulas, R. M., Ahmad-Nejad, P., Ghose, S., Kirschning, C., Issels, R., & Wagner, H. (2002a). HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. *The Journal of Biological Chemistry, 277*, 15107–15112.

Vabulas, R. M., Wagner, H., & Schild, H. (2002b). Heat shock proteins as ligands of toll-like receptors. *Current Topics in Microbiology and Immunology, 270*, 169–184.

Vega, V. L., Rodriguez-Silva, M., Frey, T., Gehrmann, M., Diaz, J. C., Steinem, C., Multhoff, G., Arispe, N., & De Maio, A. (2008). Hsp70 translocates into the plasma membrane after stress and is released into the extracellular environment in a membrane-associated form that activates macrophages. *Journal of Immunology, 180*, 4299–4307.

Voellmy, R. (2004). On mechanisms that control heat shock transcription factor activity in metazoan cells. *Cell Stress & Chaperones, 9*, 122–133.

Voos, W. (2013). Chaperone-protease networks in mitochondrial protein homeostasis. *Biochimica et Biophysica Acta, 1833*, 388–399.

Wang, Y., Kelly, C. G., Karttunen, J. T., Whittall, T., Lehner, P. J., Duncan, L., MacAry, P., Younson, J. S., Singh, M., Oehlmann, W., Cheng, G., Bergmeier, L., & Lehner, T. (2001). CD40 is a cellular receptor mediating mycobacterial heat shock protein 70 stimulation of CC-chemokines. *Immunity, 15*, 971–983.

Wang, Y., Whittall, T., McGowan, E., Younson, J., Kelly, C., Bergmeier, L. A., Singh, M., & Lehner, T. (2005). Identification of stimulating and inhibitory epitopes within the heat shock protein 70 molecule that modulate cytokine production and maturation of dendritic cells. *Journal of Immunology, 174*, 3306–3316.

Wang, R., Kovalchin, J. T., Muhlenkamp, P., & Chandawarkar, R. Y. (2006a). Exogenous heat shock protein 70 binds macrophage lipid raft microdomain and stimulates phagocytosis, processing, and MHC-II presentation of antigens. *Blood, 107*, 1636–1642.

Wang, R., Town, T., Gokarn, V., Flavell, R. A., & Chandawarkar, R. Y. (2006b). HSP70 enhances macrophage phagocytosis by interaction with lipid raft-associated TLR-7 and upregulating p38 MAPK and PI3K pathways. *The Journal of Surgical Research, 136*, 58–69.

Wei, Y. Q., Zhao, X., Kariya, Y., Teshigawara, K., & Uchida, A. (1995). Inhibition of proliferation and induction of apoptosis by abrogation of heat-shock protein (HSP) 70 expression in tumor cells. *Cancer Immunology, Immunotherapy, 40*, 73–78.

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. *Journal of Immunology, 164*, 2711–2717.

Wieten, L., Broere, F., van der Zee, R., Koerkamp, E. K., 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 Letters, 581*, 3716–3722.

Wu, B., Hunt, C., & Morimoto, R. (1985). Structure and expression of the human gene encoding major heat shock protein HSP70. *Molecular and Cell Biology, 5*, 330–341.

Wu, F. H., Yuan, Y., Li, D., Liao, S. J., Yan, B., Wei, J. J., Zhou, Y. H., Zhu, J. H., Zhang, G. M., & Feng, Z. H. (2012). Extracellular HSPA1A promotes the growth of hepatocarcinoma by augmenting tumor cell proliferation and apoptosis-resistance. *Cancer Letters, 317*, 157–164.

Xie, J., Méndez, J. D., Méndez-Valenzuela, V., & Aguilar-Hernández, M. M. (2013). Cellular signalling of the receptor for advanced glycation end products (RAGE). *Cellular Signalling, 25*, 2185–2197.

Yang, X. M., Baxter, G. F., Heads, R. J., Yellon, D. M., Downey, J. M., & Cohen, M. V. (1996). Infarct limitation of the second window of protection in a conscious rabbit model. *Cardiovascular Research, 31*, 777–783.

Yang, X., Coriolan, D., Murthy, V., Schultz, K., Golenbock, D. T., & Beasley, D. (2005). Proinflammatory phenotype of vascular smooth muscle cells: Role of efficient Toll-like receptor 4 signaling. *American Journal of Physiology. Heart and Circulatory Physiology, 289*, H1069–H1076.

Yeh, C. H., Tseng, R., Zhang, Z., Cortes, J., O'Brien, S., Giles, F., Hannah, A., Estrov, Z., Keating, M., Kantarjian, H., & Albitar, M. (2009). Circulating heat shock protein 70 and progression in patients with chronic myeloid leukemia. *Leukemia Research, 33*, 212–217.

Yeh, C. H., Tseng, R., Hannah, A., Estrov, Z., Estey, E., Kantarjian, H., & Albitar, M. (2010). Clinical correlation of circulating heat shock protein 70 in acute leukemia. *Leukemia Research, 34*, 605–609.

Yokota, S., Chiba, S., Furuyama, H., & Fujii, N. (2010). Cerebrospinal fluids containing anti-HSP70 autoantibodies from multiple sclerosis patients augment HSP70-induced proinflammatory cytokine production in monocytic cells. *Journal of Neuroimmunology, 218*, 129–133.

Zhan, R., Leng, X., Liu, X., Wang, X., Gong, J., Yan, L., Wang, L., Wang, Y., Wang, X., & Qian, L. J. (2009). Heat shock protein 70 is secreted from endothelial cells by a non-classical pathway involving exosomes. *Biochemical and Biophysical Research Communications, 387*, 229–233.

Zhe, Y., Li, Y., Liu, D., Su, D. M., Liu, J. G., & Li, H. Y. (2016). Extracellular HSP70-peptide complexes promote the proliferation of hepatocellular carcinoma cells via TLR2/4/JNK1/2MAPK pathway. *Tumour Biology, 37*, 13951–13959.

Zhu, X., Zhao, X., Burkholder, W. F., Gragerov, A., Ogata, C. M., Gottesman, M. E., & Hendrickson, W. A. (1996). Structural analysis of substrate binding by the molecular chaperone DnaK. *Science, 272*, 1606–1614.

Zügel, U., Sponaas, A. M., Neckermann, J., Schoel, B., & Kaufmann, S. H. (2001). Gp96-peptide vaccination of mice against intracellular bacteria. *Infection and Immunity, 69*, 4164–4167.