ISSN 0026-8933, Molecular Biology, 2019, Vol. 53, No. 2, pp. 176–191. © Pleiades Publishing, Inc., 2019.
Russian Text © D.G. Garbuz, O.G. Zatsepina, M.B. Evgen'ev, 2019, published in Molekulyarnaya Biologiya, 2019, Vol. 53, No. 2, pp. 200–217.

REVIEWS

UDC 577.22;577.27

The Major Human Stress Protein Hsp70 as a Factor of Protein Homeostasis and a Cytokine-Like Regulator

D. G. Garbuz[a], *, O. G. Zatsepina[a], and M. B. Evgen'ev[a]

[a] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 119991 Russia*

**e-mail: dgarbuz@yandex.ru*

Received September 7, 2018; revised October 10, 2018; accepted October 22, 2018

Abstract—Heat shock proteins (HSPs) are important factors of protein homeostasis and possess chaperone properties, providing for a folding and intracellular transport of proteins and facilitating the recovery or utilization of proteins partly denatured on exposure to various stress factors. Proteins of the Hsp70 family are the most universal molecular chaperones and interact with the greatest number of protein substrates. Several proteins of the Hsp70 family are released into the extracellular space, where they play an important role in intercellular communications and act as alarmins, or "danger signals," to modulate the immune response. The secreted Hsp70 can additionally act as an effective neuroprotector, increasing the survival of neurons in various proteinopathies, as has been demonstrated in Alzheimer's and Parkinson's disease models. In this regard, recombinant Hsp70 and inducers of endogenous Hsp70 synthesis may be considered as candidate therapeutics with immune-modulating and neuroprotective properties.

Keywords: recombinant Hsp70, stress, neuroprotection, protein homeostasis

DOI: 10.1134/S0026893319020055

INTRODUCTION

Every stress factor (heat shock (SH), hypoxia, oxidative stress, heavy metal salts, etc.) increases the cell concentration of denatured proteins, which then aggregate to alter the cytoplasm structure and to cause cell death. The proteins that prevent aggregation of denatured proteins and facilitate their refolding and a folding of proteins synthesized de novo are collectively known as molecular chaperones [1, 2]. The levels of certain chaperones substantially increase in stress (e.g., SH). These proteins are known as heat shock proteins (Hsps), or stress proteins. Hsps form the most ancient universal defense system, which helps cells and the total body to survive at higher temperatures and on exposure to other stress factors [3–7]. According to a commonly accepted classification, the Hsp proteins are grouped by molecular weight: small Hsps (sHsps, 10–30 kDa), Hsp40 (40 kDa), Hsp60 (chaperonines), Hsp70 (68–78 kDa), Hsp90 (82–96 kDa), and Hsp110 (a group of proteins broadly varying in molecular weight from 80 to 170 kDa). Each group includes up to several tens of individual proteins, which are similar in both structure and function [8]. Based on the expression pattern, the Hsp proteins are classified into two large groups, inducible, which substantially increase in synthesis in stress, and constitutive (Hscs), which are normally expressed to a high level and are only slightly, if at all, upregulated in stress [4, 9].

The Hsp70 protein is thought to act as a key component of the system responsible for the folding of proteins synthesized de novo and the refolding of partly denatured proteins in stress because Hsp70 is capable of interacting with the broadest range of protein substrates as compared with other chaperones [10]. The Hsp70 capability of preventing protein aggregation in stress determines, to a great extent, how efficiently the organism adapts to adverse environmental conditions, such as frequent changes in temperature, dehydration, hypoxia, exposure to various toxins, and others [4, 7]. Species adapted to extreme biotopes were found to have far higher intracellular Hsp70 levels as compared with related species from biotopes with more favorable conditions [5, 7, 11].

Apart from acting as a molecular chaperone in the cell cytoplasm, Hsp70 is secreted into the extracellular medium, released into the circulation, and performs functions similar to those of cytokines; i.e., Hsp70 plays a role in intercellular signaling. Several receptors were identified to recognize secreted Hsp70, and certain specific effects were described to result from an increase in extracellular Hsp70. For example, extracellular (exogenous) Hsp70 affects activities of several immune cells (primarily, macrophages and neutro-

Abbreviations: ROS, reactive oxygen species; LPS, lipopolysaccharide; APP, amyloid precursor protein; eHsp70, exogenous Hsp70; Hsp, heat shock protein; TLR, Toll-like receptor; AD, Alzheimer's disease; HS, heat shock.

phils) by binding to Toll-like receptors 2 and 4 (TLR2 and TLR4) [12–14]. Secreted Hsp70 additionally possesses high neuroprotective activity, improves the neuronal survival on exposure to a number of stress factors, and decreases the level of neuronal apoptosis in models of certain neurodegenerative disorders [15–19]. Intense studies consequently focus on inductors of Hsp70 synthesis and recombinant Hsp70 as promising immunomodulating and cytoprotective agents.

INTRACELLULAR Hsp70 AS A MAIN MOLECULAR CHAPERONE

The human genome harbors 13 genes characterized to code for Hsp70-group proteins, which are designated HSPAs. HSPA1A, HSPA1B, and HSPA6 are upregulated to the greatest extent in HS. The tissue-specific proteins HSPA1L and HSPA2 are constitutively produced to high levels in the testis and several other tissues. HSPA5 (earlier known as GRP78, or the binding immunoglobulin protein (BiP)) is localized in the endoplasmic reticulum. HSPA5 is necessary for the folding of almost all proteins occurring in the endoplasmic reticulum, including immunoglobulins. The *HSPA7* gene was thought to be a pseudogene for a long time. However, the available data indicate that *HSPA7* is a functional gene and is highly homologous to *HSPA6* [8]. HSPA8, initially described as HSC70 and HSP73, occurs in the cytosol and is involved in cotranslational protein folding and protein transport across intracellular membranes. HSPA9 is a mitochondrial protein; HSPA13 (or Stch) is localized in microsomes. HSPA12A, HSPA12B, and HSPA14 are evolutionarily more distant members of the Hsp70 family, and reliable information on their functions is currently unavailable [8]. *HSPA1A*, *HSPA1B*, and *HSPA1L* are tightly clustered in the human and other mammalian genomes, while the other genes of the group are scattered through the genome [20–24].

As was found by X-ray crystallography, an HSPA1A molecule consists of a conserved N-terminal domain of approximately 450 amino acid residues and a variable C-terminal domain of approximately 200 residues. The N-terminal domain possesses ATP-binding and, in the presence of certain protein cofactors, ATPase activities. The domain is similar in tertiary structure to the ATP-binding domains of actin and hexokinases. The C-terminal domain forms a substrate-binding site, which interacts with target proteins [25, 26]. Because Hsp70 has high affinity for ATP, its isolation by affinity chromatography on ATP-Sepharose is efficient and is broadly used in laboratory research [27].

Various functions are performed in the cell by the Hsp70 family proteins, and the majority of the functions are associated with their chaperone activity. The effect of Hsp70 is based on its capability of interacting with other cell proteins to prevent their aggregation and to facilitate the recovery of native structures by proteins partly denatured on exposure to various stress factors [10, 28, 29]. In addition, Hsp70 binds with de novo synthesized proteins during polypeptide chain elongation and facilitates their folding (Fig. 1a). The interaction of Hsp70 with partly denatured proteins is determined by the features of its C-terminal domain, which contains a hydrophobic pocket structurally similar to the peptide-binding site of main histocompatibility complex (MHC) molecules, especially those of MHC type II. The structure of the peptide-binding pocket in Hsp70 is more open than in MHC molecules, thus allowing interactions not only with peptides, but with full-size proteins as well [26]. Hsp70 recognizes the sequences that are enriched in hydrophobic amino acid residues. Such hydrophobic regions are predominantly localized within protein globules in normal conditions and their exposure on the protein surface usually suggests damage to the protein structure. Hydrophobic sequences recognized by Hsp70 were found to occur in every 40 amino acid residues on average [30]. To interact with substrate proteins, the Hsp70 family chaperones need several cofactor proteins, which regulate ATPase activity of Hsp70, exchange of ADP for ATP, and a transfer of the substrate protein from Hsp70 to another chaperone. To interact with Hsp70, a substrate protein must be in complex with Hsp40 (DnaJ), which acts both as a molecular chaperone capable of recognizing the proteins lost their native conformations and as a cofactor of Hsp70. Then ATP-bound Hsp70 interacts with the DnaJ–substrate protein complex. DnaJ subsequently stimulates ATP hydrolysis to ADP and stabilizes the interaction of Hsp70 with the substrate protein. DnaK has higher affinity for ADP than for ATP, and ADP-for-ATP exchange consequently requires a nucleotide exchange factor as an additional accessory protein. Several cofactors are known to stimulate dissociation of the eukaryotic Hsp70–ADP complex; Hsp110 is the main of them. Once the Hsp70–ADP complex dissociates, the substrate protein is released and a new ATP molecule bound. Several association–dissociation cycles accompanied by ATP hydrolysis are usually necessary for Hsp70-assisted protein folding. The energy of ATP hydrolysis is utilized to stabilize the intermediate conformation of a protein during the restoration of its native structure [31]. In addition, chaperones decrease the entropy of a client protein by reducing the number of possible intramolecular interactions [32]. To complete their folding into native structures after dissociating from Hsp70, many proteins smaller than 60 kDa interact with other chaperones, namely, Hsp60 (chaperonines) or Hsp90. Irreversibly denatured proteins, which are incapable of restoring their native conformations, are ubiquitinated after their interaction with Hsp70 and are then degraded in proteasomes [33]. CHIP ubiquitin ligase and proteins of the Bag family (alternative nucleotide exchange factors of Hsp70) play a key role in choosing the pathway between a refolding or degradation of the

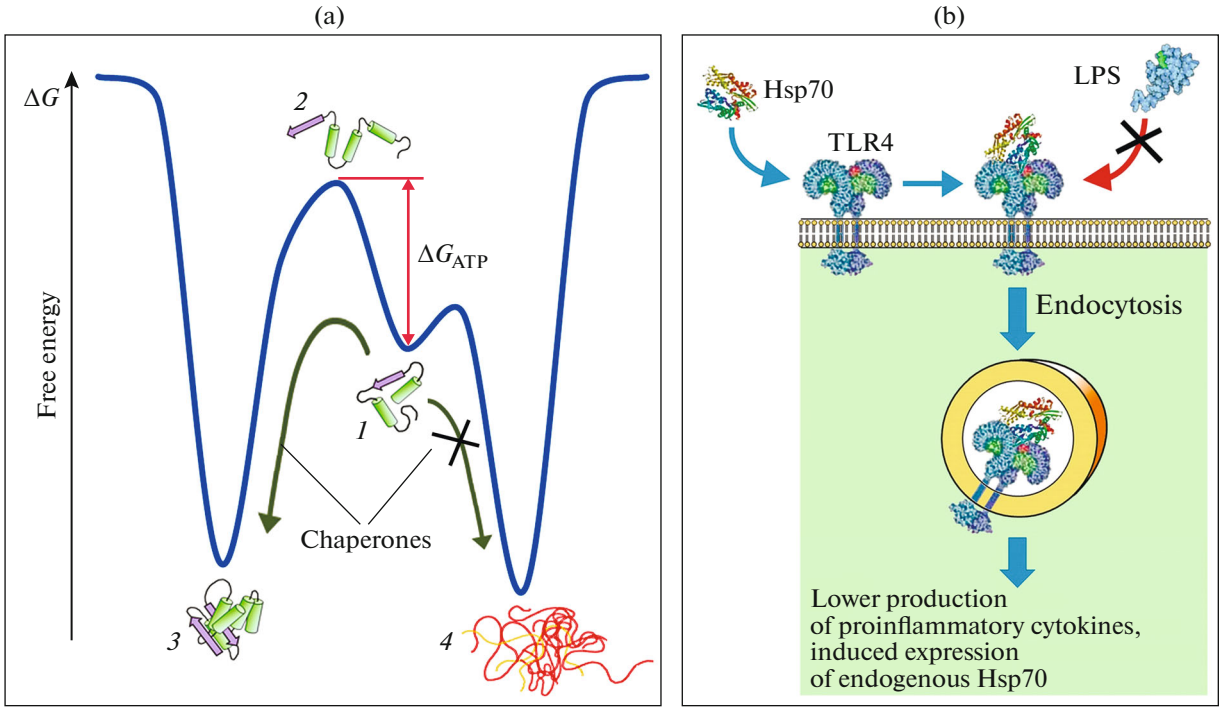

Fig. 1. (a) Mechanism of action of chaperones as intracellular proteins: (*1*) partly denatured proteins; (*2*) an intermediate conformation (a transition state); (*3*) a native conformation; (*4*) aggregates. Chaperones, including Hsp70, prevent aggregation of partly denatured proteins and facilitate their folding into native structures by stabilizing their transition states with the use of energy from ATP hydrolysis. (b) Exogenous Hsp70 as a cytokine. According to a hypothetical mechanism, exogenous Hsp70 blocks the effect of LPS by competing for TLR4.

substrate protein. Hsp70 is involved in ubiquitin-dependent degradation of many cell proteins, including actin, α-crystallins, glyceraldehyde 3-phosphate dehydrogenase, and histone 2A [34].

Apart from cell protein renaturation after stress, proteins of the Hsp70 family are necessary for cotranlational protein folding in normal conditions [30, 35, 36]. Virtually all cell proteins at least temporarily interact with HSPA8 (a constitutively expressed member of the Hsp70 family) during translation. The Hsp70 family proteins are involved in lysosomal degradation of intracellular proteins, playing a role in their transport across the membrane in complex with lysosomal-associated membrane protein 2 (LAMP2) [37]. Protein transport into the endoplasmic reticulum and mitochondria also requires the Hsp70 family proteins. Newly synthesized proteins bind with HSPA1L and HSPA2 in the cytosol. As the proteins are transported across the mitochondrial membrane, the resulting complexes dissociate, and their dissociation is accompanied by ATP hydrolysis. In the cytosol, HSPA1L and HSPA2 render the polypeptide chain unfolded and prevent the formation of a tertiary structure, which hinders protein transfer through the translocon [38]. ATP is not required for transporting proteins preliminarily denatured with urea. During the transfer across the membrane and cleavage of the N-terminal signal sequence, the polypeptide chain binds with mitochondrial Hsp70 and is then released after ATP hydrolysis. When mitochondrial Hsp70 interacts with a transported protein, ATP hydrolysis provides energy for transferring the protein across the inner mitochondrial membrane [39–41].

The proteins that are secreted into the intercellular medium or expressed on the cell membrane are incapable of folding into native structures in the endoplasmic reticulum without being assisted by chaperones. HSPA5 binds to the luminal moiety of a transmembrane pore and facilitates the transfer of newly synthesized proteins from polysomes into the endoplasmic reticulum [42, 43]. In the lumen of the endoplasmic reticulum, HSPA5 is necessary for a refolding of the receptors for insulin, immunoglobulins, and other secreted proteins [44, 45]. In addition, HSPA5 is involved in endoplasmic reticulum-associated degradation (ERAD) of damaged proteins in stress [46, 47].

The Hsp70 protein is an important component of the antioxidant system, which protects the cell from free radicals. Hsp70 expression is upregulated in oxidative stress. Hsp70 plays a role in degradation of oxidized proteins and stimulates superoxide dismutase and catalase activities, thus indirectly contributing to inactivation of superoxide anions and hydrogen peroxide [33].

Vast data have accumulated to date to indicate that Hsp70 and small Hsp proteins act as efficient apoptosis inhibitors [48–55]. Hsp70 blocks p53-dependent apoptosis and apoptosis induced by TNF-α, but does not affect Fas-mediated apoptosis [56]. Hsp70 acts at various steps of the apoptotic cascade to affect apoptosis. First, Hsp70 expression substantially inhibits activation of the SAPK/JNK stress kinases, which act as main early triggers of apoptosis [57–60]. Hsp70 prevents the cytochrome *c* release from mitochondria, thus suppressing the Bax translocation [61]. There are data that Hsp70 interacts with Apaf-1 to prevent its complexation with cytochrome *c* and activation of caspase 9 [62]. To affect apoptosis, the Hsp70 family proteins are additionally capable of interacting with Bag1, which activates the Bcl-2 anti-apoptotic protein Bcl-2 [57].

Several findings implicate Hsp70 in regulating apoptosis at later steps. For example, constitutive Hsp70 expression decreases the intensity of proteolysis of caspase-3 substrates, although Hsp70 does not directly affect catalytic activity of activated caspase 3 in vitro [57]. It is possible that Hsp70 binds with caspase substrates to prevent their proteolysis. The hypothesis is supported by the finding that Hsp70 is capable of protecting cells that express originally active gene-engineering caspase 3 from death [63]. Hsp70 can interact with proteins of the NF-κB family to prevent their transfer into the nucleus, thus suppressing TNF-induced apoptosis in U-937 cells. The effect is independent of the NF-κB inhibitor (IkB) because IκB is undetectable in Hsp70 complexes with p65 or c-Rel [64].

The protective effect of intracellular Hsp70 has both positive and negative aspects. On the one hand, the Hsp proteins act as protective factors by preventing apoptosis in brain cells and cardiomyocytes in ischemia. For example, induction of Hsp70 expression in the myocardium decreases the extent of cardiomyocyte death from ischemia in myocardial infarction and heart surgery. Several chemical compounds, such as the Hsp synthesis inducers bimoclomol and geranylgeranyl-acetone, were proposed as drugs to reduce risk of postoperative complications [65]. In brain tissues, Hsp70 inhibits of α-synuclein and tau protein aggregation, thus exerting a neuroprotective effect and preventing neurodegenerative disorders, such as parkinsonism and Alzheimer's disease [60, 67]. On the other hand, Hsp70 may promote carcinogenesis by exerting its high antiapoptotic activity. Constitutively high Hsp70 levels are observed in the majority of tumors compared with surrounding tissues and confer resistance to many cytotoxic factors, including apoptosis-inducing chemotherapeutics, and hypoxia due to insufficient vascularization of the tumor [53, 54, 68, 69]. A high Hsp70 level in the tumor suggests a poor prognosis in certain cancers [70]. A screening of chemical compounds is consequently performed now to identify the agents that are capable of inhibiting Hsp70 activity and can be considered as potential anticancer drugs potentiating the effect of chemotherapeutics, such cisplatin [71].

MECHANISMS OF EXOGENOUS Hsp70 SECRETION

For a period of time after their discovery, the Hsp proteins were thought to be exclusively intracellular proteins with functions restricted to maintaining protein homeostasis in the cell. However, more recent data showed that many Hsps (as well as several other proteins earlier believed to be intracellular) are released into the intercellular space in certain conditions. For example, rat embryonic cells in primary culture release several Hsps (Hsp110, Hsp70, and Hsc70) into the culture medium when exposed to HS [72]. Further studies showed that human Hsp70 circulates in the blood serum in all people free from overt disorders [73]. It was found that members of the majority of Hsp families are secreted into the intercellular space and occur in circulation. Secretion into the intercellular medium was demonstrated for Hsp27, Hsp60, Hsp70, Hsc70, Hsp90, and Hsp110 both in vitro and in vivo. The capability of Hsp secretion is not specific to a particular cell type because several research teams independently demonstrated Hsp secretion by neurons, monocytes, macrophages, B cells, endothelial cells, and tumor cells of the epithelial origin [13, 74–77]. Secretion of Hsp70 and its cofactor Hsp40 was observed not only in mammalian, but also in invertebrate (*Drosophila melanogaster*) cells [78, 79]. The mechanism of Hsp70 secretion remained unknown for a period of time. As classical housekeeping proteins, Hsps lack an N-terminal signal sequence necessary for secretion via the endoplasmic reticulum and Golgi complex. Several secretion mechanisms are known today to allow Hsp70 and several other proteins lacking an N-terminal leader sequence to find their way from the cell. The mechanisms include necrotic cell disruption by various damaging factors (a nonspecific mechanism), secretion via budding membrane vesicles (ectosomes) or classical exosomes, and exocytosis via endolysosomes [74, 80, 81]. In the last case, a mechanism of protein transfer across the membrane is required for Hsp70 secretion. There is evidence that Hsp70 transfer is mediated by ABCA1 of the ATP-binding cassette (ABC) family, which additionally includes TAP1, which is involved in transferring antigenic peptides across the endoplasmic reticulum membrane. Inhibitors of the ABC family proteins efficiently block the Hsp70 release from cells [81]. This transfer mechanism was demonstrated for several proteins that are similar to Hsp70 in lacking the N-terminal signal sequence essential for classical secretion and play their main functions outside the cell, acting as cytokines (IL-1α, IL-1β, IL-18, FGF1, and several others) [81–83]. Transmembrane transfer of a protein devoid of a proper N-terminal leader requires that the

tertiary structure of the protein be partly destabilized to a molten globule state [82]. As molten globules, certain proteins are transported across the lipid bilayer with the help of ABC transporters or interact with phosphatidylserine in the inner lipid layer and then are transferred into the outer layer in complex with phosphatidylserine and released into the intercellular medium [81]. HS, which causes partial denaturation of cell proteins, increases secretion of not only Hsp70, but also of FGF1 and IL-1α [81]. When Hsp-overexpressing cells are affected by stress, up to 10–15% of the newly synthesized Hsp70 is incorporated into the plasma membrane and exposes a C-terminal domain fragment of 14 amino acid residues on its surface [84, 85]. The membrane-associated Hsp70 is secreted as an exosome component by certain tumor cells. Thus, two, membrane-associated and free protein, fractions of Hsp70 are released into the intercellular medium [71, 80, 86, 87].

Cell secretion of Hsp70 is stimulated both in vitro and in vivo by various stress factors [72, 74] and nonspecific bacterial antigens (such as GroEL) and lipopolysaccharide (LPS, a main endotoxin of Gram-negative bacteria). Adding purified GroEL or LPS in the culture medium increases Hsp70 secretion from cultured monocytes by a factor of 10 or 3, respectively [76]. Intravenous administration of LPS increases the serum Hsp70 level in rats [88]. An elevated extracellular ATP level is another possible inducer of Hsp70 secretion [80]. The same stimuli (extracellular ATP and LPS) increase macrophage secretion of IL-1α, which is released via the same mechanism as Hsp70 is [13]. Thus, nonspecific agents associated with an infection process or mass cell death are capable of inducing Hsp70 secretion. In addition, exogenous Hsp70 (eHsp70) increases in necrosis as Hsp70 is directly released from degrading cells.

IMMUNOREGULATORY FUNCTIONS OF eHsp70

Extracellular Hsp proteins and, in particular eHsp70, are incapable of acting as chaperones in the absence of a high ATP concentration and a set of specific cofactor proteins. Secreted Hsps, including Hsp70 and Hsp60, are recognized by the so-called pattern recognition receptors (PRRs), which are an important component of the innate immunity system. PRRs are responsible for recognizing a broad range of the molecular structures that have no analog in the body, but occur in the majority of pathogenic bacteria, fungi, and viruses (LPS, formyl peptides, teichoic and lipoteichoic acids, zymosan, dsRNA, and other pathogen associated patterns (PAMPs)) [89]. Hsp70 and Hsp60 are recognized primarily by TLR2 and TLR4 and additionally by LOX-1, SREC-1, and CLEVER-1 of the scavenger receptor (SR) group. Certain data implicate CD40 (the TNF receptor family), CD91 (a low density lipoprotein receptor), and CCR5 (a chemokine receptor) in recognizing eHsp70 [13, 90, 91]. Several receptors were found to recognize both cognate extracellular Hsps and Hsps of a bacterial origin (for example, CD40 recognizes mycobacterial Hsp70) [92]. The term chaperokine was consequently proposed for Hsp70 to emphasize its dual function as a molecular chaperone (the intracellular form) and an intercellular signal transmitter acting through specific ligand–receptor interactions, as in the case of classical cytokines (Fig. 1b) [93].

After interacting with receptors on the cell surface, eHsp70 can be internalized into the target cell. Phagocytosis of eHsp70 is probably mediated by TLR2/4, CD91, and SRs [13, 91, 94].

Several studies of how Hsp70 affects activities of immune system cells showed that an increase in eHsp70 triggers production of proinflammatory cytokines (TNF-α, IL-1β, and IL-6), reactive oxygen species (ROS), and nitric oxide (NO) in macrophages and neutrophils by activating TLR2 and TLR4. Like PAMP, eHsp70 was described as a nonspecific marker that suggests stress or various pathological processes and facilitates activation of immune system cells. It was thus assumed that the interaction of Hsp70 with TLR4 is aimed at stimulating nonspecific immune responses (activating innate immunity) [95–97]. Endotoxins (LPSs) of Gram-negative bacteria are known to exert a similar effect by activating TLR2/4, that is, the same TLR group. The LPS–TLR4 interaction activates NF-κB, MAPK/JNK, and interferon regulatory factor 3 (IRF3), thus leading to excess production of several proinflammatory cytokines, ROS, and NO [98]. It was therefore assumed that the above effects, which were observed using recombinant Hsp70 synthesized in *Escherichia coli* cells, are due to contamination with LPS, trace amounts of which are already capable of inducing a response in macrophages and neutrophils [99, 100]. More recent studies utilized Hsp70 that was synthesized in eukaryotic expression systems (e.g., a baculovirus system) and was free from LPS and bacterial protein contamination, and eHsp70 was found to exert an opposite effect, suppressing activation of nonspecific immunity [14, 101]. LPS tolerance was induced in cultured monocytes by eHsp70 [102]. Both in vitro and in vivo, eHsp70 was observed to suppress the LPS-induced translocation of the NF-κB (p65) transcription factor into the nucleus, MAPK/JNK protein kinase activity, and ROS production [103–105]. Purified endotoxin-free Hsp70 suppressed allergic inflammation of the airways in mice [106]. Thus, eHsp70 acted as a LPS antagonist in many experiments, blocking NF-κB activation through TLR2 and TLR4. On the other hand, eHsp70 produced in an eukaryotic cell culture and, therefore, LPS free increased the production of several proinflammatory cytokines and activated the inflammatory response in other studies [97]. The free and membrane-associated eHsp70 fractions were found to dramatically differ in effect on immune sys-

tem cells. Membrane-associated eHsp70, which is secreted as an exosome component, activates macrophages and acts as a proinflammatory agent, while free eHsp70 exerts an opposite effect and acts as a LPS antagonist, competing with LPS for TLR4 [14, 71]. It should be noted that Hsp70 and other chaperones (including recombinant chaperones use in experiments on immunity modulation) isolated from cells may occur in various forms, e.g., in complex with ATP, ADP, or target peptides. Different Hsp forms may be recognized by different receptors and may differently affect activities of immune system cells.

The interaction of eHsp70 with TLR4 was confirmed in further experiments [107]. Unlike with LPS, Hsp70 binding to TLR4 does not activate the NF-κB, MAPK/JNK, and IRF3 cascades, which lead to production of proinflammatory cytokines [97, 98]. Various ligands are known to differently interact with TLR4, and only some of them hyperactivate the TLR4–NF-κB signaling pathway [108]. The findings support the hypothesis that Hsp70 competes with LPS for TLR4 and thereby acts as a LPS antagonist.

High LPS concentrations (in sepsis, that is, mass penetration of Gram-negative bacteria in circulation) cause a set of signs and symptoms that is known as endotoxin, or septic, shock. Complexation of LPS with TLR2/4 expressed on neutrophils and macrophages plays a key role in septic shock [109]. ROS, NO, several proinflammatory cytokines, histamine, and serotonin are produced in excessive amounts as a result to cause the signs and symptoms of endotoxin (septic) shock, including an abrupt drop in blood pressure, an increase in blood clotting, consequently poor tissue oxygenation, and multiple organ dysfunction syndrome. Septic shock developing in generalized infections, especially those caused by antibiotic-resistant hospital strains, still presents an important medical problem. Sepsis is among the leading causes of death in patients admitted to intensive care units [110, 111].

Recent studies yielded several indications that implicate both intracellular and secreted Hsp70 forms in regulating the body response to LPS. Intraperitoneal LPS administration caused macrophage Hsp70 induction in mice [112]. Agonists of TLR4 (LPS), TLR2 (Pam3Cys), and TLR3 (poly(IC)) were found to induce Hsp70 in vitro and to act synergistically [113]. In pediatric patients treated in intensive care units after surgery, the serum levels of secreted Hsp70 showed a sixfold increase in septic shock as compared with levels in patients without sepsis [114]. An increase in both intracellular and secreted Hsp70 concentrations decreased the response to LPS in the patients. It was observed that eHsp70 suppresses activation of the NF-κB-mediated monocyte response and LPS-induced secretion of TNF-α [102]. Induction of Hsp70 synthesis by oral administration of geranyl-geranyl-acetone decreased the LPS-induced levels of NO, TNF-α, and IL-6 and reduced the mortality rate in experimental animals [115]. In a study of a mixture of the inducible and constitutive Hsp70 forms isolated from bovine muscle tissue, its single intravenous administration prior to injection of *E. coli* and *Salmonella typhimurium* LPSs efficiently decreased the rate of deaths due to endotoxin shock in Wistar rats [116]. Preventive administration of exogenous Hsp70/Hsc70 normalized several physiological parameters whose abnormalities lead to fatal outcome in septic shock, including a decrease in blood pressure and an increase in blood clotting rate [116]. In addition, Hsp70 administration promoted the recovery of several blood chemistry parameters (bilirubin, creatinine, serum albumin, total protein, etc.). Preventive recombinant Hsp70 administration prior to injecting LPS prevented heart failure and hepatic insufficiency [104]. Hsp70 administration after LPS injection normalized several physiological and chemistry parameters, but failed to improve the survival in animals. Earlier studies showed that exogenous human recombinant Hsp70 (HSPA1A) decreases the production of ROS, NO, and TNF-α in neutrophils, downregulates expression of the CD11b/CD18 receptors, and restores the normal level of neutrophil apoptosis and several biochemical parameters of the blood after exposure to LPS or lipoteichoic acid (a main endotoxin of Gram-positive bacteria) [101, 117]. The results received independent support from another study [88], which demonstrated that the secreted Hsp70 level undergoes a more than tenfold increase in rats with endotoxin shock induced experimentally and that the survival in rats with higher blood Hsp70 levels is higher than in rats with lower Hsp70 levels.

The kinetics of the inhibitory effect of Hsp70 on LPS-induced ROS production in neutrophils showed that the effect of Hsp70 administration develops within 4–5 min [101, 118, 119]. The interaction of LPS with the CD14/TLR4 receptors is similarly characterized by a rapid kinetics (approximately several minutes), which corresponds to the time it takes for exogenous Hsp70 to inhibit the LPS effect [120]. Thus, a hypothetic mechanism whereby Hsp70 suppresses endotoxin shock is based on competing with LPS for TLR4 and blocking the LPS interactions with neutrophils and other cells of the immune system (Fig. 1b).

There are data that TLR4 is capable of mediating eHsp70 endocytosis [13, 94]. The data are supported by the fining that Hsp70 added to the culture medium is more intensely internalized in cells that express surface TLR4 than in TLR4-lacking cell lines [107]. Thus, phagocytosis of the TLR4/Hsp70 complex may contribute to the block of TLR4–LPS interaction. The hypothesis agrees with the fact that Hsp70 acts efficiently only when administered preventively, 10 min before a LPS injection. The Hsp70 synthesis inducer geranyl-geranyl-acetone is similarly more effective when administered preventively (16 h before a LPS injection) [115].

The protective effect of eHsp70 in endotoxin shock possibly arises not only because eHsp70 competes with LPS for TLRs, but also because synthesis of endogenous Hsps is induced in target cells in response to eHsp70 administration [97]. The effect is similarly mediated by the interaction of eHsp70 with TLR4. However, the interaction does not activate NF-κB in contrast to LPS, but triggers the alternative TLR4-dependent signaling cascade that involves PI3K and Akt. In turn, Akt inhibits glycogen synthase kinase 3β (GSK-3β), which acts as a negative regulator of HSF1, the main transcription factor responsible for transcription of the Hsp genes [97]. It is of interest that intracellular Hsp70 mediates TLR4 ubiquitination, acts as an inhibitor of the NF-κB cascade triggered by TLR4 activation, and consequently decreases the LPS-induced overproduction of cytokines, like eHsp70 [121]. Thus, recombinant Hsp70 preparations or Hps70 synthesis inducers, such as geranyl-geranyl-acetone and low-dose interferon γ, are possible to recommend for clinical testing as drugs to prevent septic complications.

Large amounts of eHsp70 are secreted by various tumors. A high level of membrane-associated Hsp70 exposed on the cell surface is a feature of many cancer cells [122–124]. An artificial increase in surface Hsp70 associated with the plasma membrane in tumor cells may lead to activation of natural killers and certain types of effector T cells with subsequent lysis of the tumor. The effect is possible to induce by administering low doses of interferon γ or exogenous Hsp70 [123, 125–127].

Discrepant data are available on the role that is played by Hsp70 secreted from tumor cells in exosomes. On the one hand, the exosomes are capable of activating dendrite cells, improving the efficiency of tumor antigen recognition, and increasing secretion of several cytokines [127]. On the other hand, secretion of exosomes exposing Hsp70 on their surfaces is often considered to be a mechanism whereby tumor cells suppress the immune response. There are data that the exosomes are recognized by TLR2 of myeloid suppressor cells, which block activation of tumor-specific T cells [71, 128, 129].

Finally, a method was proposed to stimulate the antitumor adaptive immune response with the use of Hsp70 isolated directly from tumor tissue. It was observed that Hsp70 and Hsp90 isolated from tumor cells and then administered to the patient induce the immune response by activating antigen-presenting and cytotoxic T cells [94]. The effect is explain by the fact that Hsp70 and Hsp90 are isolated from tumors in complex with oligopeptides, which can act as antigens to trigger the immune response [47, 94, 130]. Then the Hsp complexes with tumor peptides are phagocytized by antigen-presenting macrophages as a result of Hsp recognition by the CD91 receptor. Tumor-specific antigens are consequently presented with both MHCI and MHCII molecules via the TAP1-dependent and endosomal pathways [47, 94]. The events lead to activation of effector T cells, which recognize and attack tumor cells. Therefore, Hsp70 and Hsp90 in complex with antigenic peptides purified from a patient's tumor are possible to consider as an individualized vaccine and to use together with standard therapies to treat cancer patients [131].

Hsp70 AS A NEUROPROTECTIVE FACTOR

Neurodegenerative diseases and primarily Alzheimer's disease (AD) have become a serious medical and social problem. Senile (age-related) AD continuously increases in incidence because of the increasing life span, especially in developed countries, presenting a substantial burden on the economy [132, 133]. AD is thought to be due to a pathological aggregation and accumulation of the amyloid β (Aβ) peptide (an amyloid precursor protein (APP) processing product), followed by a tau protein aggregation and neuroinflammation [134–136]. Main AD symptoms include long-term memory loss, speech impairment, and other cognitive disorders. The functions of the central nervous systems (CNS) are gradually lost because of mass neuronal death, inevitably leading to fatal outcome [134, 137, 138]. There is still no adequate means to prevent and to treat AD. Attempts were made to treat AD symptomatically with several drugs, the majority of which have an unproven or extremely low efficacy [139–142].

Because neurodegenerative diseases are directly associated with a distorted folding of proteins, such as the tau protein in AD, it is only natural to assume that Hsps play a role in neuropathology. A decrease in intracellular Hsp70 was postulated for neurodegeneration. There are data that the aggregation of the tau protein in AD is associated to a substantial extent with a decrease in chaperone activities of Hsp70 and other Hsps [143]. Lower Hsp90 concentrations in the blood serum and the frontal cortex of the brain were observed in AD. On the other hand, Hsp70 was found to increase in early AD, colocalizing with tau protein aggregates [144]. Hsp70 plays a role in degradation of hyperphosphorylated tau by facilitating its ubiquitination with CHIP ubiquitin ligase [144, 145]. The Hsp110/Hsp70/Hsp40 complex is capable of disrupting α-synuclein fibrils to produce α-synuclein monomers in vitro [66, 67]. Recent studies with the specific Hsp70 synthesis inducer geranyl-geranyl-acetone showed that Hsp70 induction suppresses many cognitive disorders in a valid transgenic mouse model of AD [146].

Inflammatory processes in the CNS received substantial attention in the two past decades because they are involved in the pathogenesis of AD and other proteinopathies, as was independently demonstrated by many teams [137, 147–149]. It was observed that AD is accompanied by a chronic inflammatory response that arises in brain tissues and involves activation of

microglia and astrocytes and secretion of several proinflammatory cytokines, NO, and ROS, which exert a cytotoxic effect on neurons. The brain levels of receptors involved in the inflammatory response increase in AD, and the production of TNF-α, IL-1, IL-6, and complement components increases both in the brain and at the periphery (in the blood plasma) in AD patients. In addition, the TLR4 level and NF-κB activity increase in the brain [147, 150–153]. Similar data were obtained in transgenic mouse models of AD that express mutant APP forms and presenilins and are prone to accumulating Aβ aggregates and developing cognitive disorders [154]. Risk of developing sporadic AD is known to correlate with certain allelic variants of the genes for TLR4, CD14 (a coreceptor of TLR4), CD33, TREM2 (Triggering receptor expressed on myeloid cells 2), CR1 (a complement receptor), and IL-6, which are involved in the immune response and inflammation [152, 155, 156]. The oligomeric Aβ form acts as a TLR4 ligand, and its interaction with TLR4 can oppositely affect microglial cells and astrocytes. On the one hand, the TLR4–Aβ interaction facilitates Aβ phagocytosis and degradation [157]. On the other hand, activation of TLR4 by Aβ leads to overproduction of proinflammatory cytokines and ROS with subsequent damage to neurons in AD [158, 159].

As described in the previous section, eHsp70 efficiently inhibits the TLR4 activation-induced overproduction of proinflammatory factors. Therefore, eHsp70 is of interest to investigate as a potential neuroprotector in AD and a nonspecific adaptogen in other neurodegenerative diseases as well. The neuroprotective role of Hsp70 has been demonstrated to date with several models in vitro and a few cases in vivo [15, 17, 19, 160, 161]. Overexpressio of Hsp70 in a primary culture of cortical nervous tissue decreases the rate of neuronal apoptosis induced at a high Aβ concentration. Endogenous Hsp70 prevents oxidative stress associated with inflammatory responses [162, 163]. Both eHsp70 secreted by astrocytes and other glial cells and purified recombinant Hsp70 added to the culture medium increase the survival of motor neurons deprived of growth factors in culture and the resistance of neuroblastoma cells to HS and staurosporine [75]. It was demonstrated that Hsps secreted by glial cells are internalized in axons in synaptic regions. This mechanism may compensate for Hsp deficiency that arises in the axoplasm because of a substantial distance from the neuron body [13]. Recombinant Hsp70 and Hsp90 added to the culture medium activate microglia and facilitate Aβ phagocytosis in AD models in vitro [15].

In addition to its role in regulating the folding of tau, Hsp70 promotes Aβ phagocytosis and cleavage in glial cells and blocks oligomerization of Aβ peptides penetrating into cells [145, 164–166].

Transgenic mice and *Drosophila* were engineered to secrete Hsp70 into the intercellular medium in recent years. Extracellular Hsp70 was found to exert a marked neuroprotective effect in strains obtained by crossing the extracellular Hsp70 producers with transgenic strains that express the Aβ peptide and are prone to developing neurodegenerative processes [19].

Our team performed a series of works to study the possibility of using eHsp70 in AD therapy. Recombinant human Hsp70 (the major stress-inducible protein HSPA1A) was synthesized in various expression systems and was free from LPS contamination. To study the neuroprotective effect of recombinant Hsp70, we used two AD models, bulbectomized mice of the NMRI strain (wild type) and mice of the 5XFAD transgenic strain (which expresses two mutant human proteins, APP and PS1).

Removal of the olfactory bulb (bulbectomy) in mice causes symptoms characteristic of AD, including an increase in Aβ; mass neuronal death in the temporal cortical and hippocampal regions; serotonin-, acetylcholine-, and glutamatergic system deficiency; and various cognitive disorders [167, 168]. Transgenic 5XFAD mice display a rapid Aβ accumulation, development of amyloid plaques in brain tissues (thus providing the most adequate model to study human AD), mass neuronal death, and progressive loss of cognitive functions [169].

Delivery of the therapeutic agent into the brain is a problem to be solved in in vivo studies, especially when using a neurotropic drug (recombinant Hsp70 in our case). The blood–brain barrier substantially limits the set of conventional drug delivery methods, especially in the case of macromolecular drugs [170]. The absorption kinetics and tissue distribution of iodine-labeled Hsp70 after its intravenous administration were determined as early as 2005 [91]. The experiments showed that the CD91 receptors plays a role in Hsp70 internalization and that Hsp70 is rapidly eliminated from the blood and accumulates in the liver. Similar results were obtained when the protective role of Hsp70 was studied using the rat model of LPS-induced sepsis [116]. More recent studies showed that many agents, including high-molecular-weight proteins purified chromatographically, are efficiently transported into the brain through the olfactory and trigeminal nerves after intranasal administration [171]. The efficiency of protein delivery into the brain is 5–8% of the dose upon intranasal administration, while the oral and intravenous routes are far less efficient. Experiments with the classical transport protein albumin labeled with radioactive iodine showed that albumin is rapidly (within 5 min) transferred into all regions of the brain and appears in the blood of laboratory animals after intranasal administration [172].

Fluorescently labeled recombinant Hsp70 was intranasally administered to mice, and sections of various brain regions were examined by confocal microscopy. As early as 3 h after administration, the fluorescent label was detected in various brain regions,

including the olfactory bulb, neocortex, hippocampus, dorsal raphe nucleus, locus coeruleus, and cerebellum. The label showed an extracellular localization in the majority of cases, occurring in the perinuclear region. Thus, eHsp70 efficiently penetrates the blood–brain barrier upon intranasal administration. To understand whether eHsp70 remains intact or undergoes proteolysis after penetrating into the brain, proteins of the cortex and hippocampus were isolated from NMRI mice at several times points after intranasal administration of ^{125}I-labeled native Hsp70 and resolved electrophoretically. The full-size eHsp70 was observed in the brain 30 min after administration and became undetectable within 1 h. The same pattern was observed after intranasal administration for ^{125}I-labeled bovine serum albumin, which is similar in molecular weight (66 kDa) to Hsp70. Thus, the two proteins entered the brain soon (within a few minutes) after administration and then rapidly underwent proteolysis [17, 107].

Further studies showed that that intranasal administration of recombinant Hsp70 decreased both the soluble Aβ concentration in bulbectomized and transgenic mice and the amount and size of amyloid plaques in 5XFAD transgenic mice as compared with mice receiving saline or heat-inactivated Hsp70 in place of native Hsp70. Cognitive functions (learning in the Morris maze) improved in the mice treated with native Hsp70. Histology showed a substantial increase in the absolute and relative (%) amounts of intact neurons and their higher density in mice treated with intranasal Hsp70 for 3 weeks as compared with control mice. Thus, eHsp70 prevented neuronal death and sustained the normal function of neurons in the DA models [17].

Hippocampal transcriptome sequencing in transgenic 5XFAD mice showed that the expression profiles of several genes in brain tissues substantially changed after intranasal administration of Hsp70. In particular, upregulated transcription was observed for genes responsible for dopamine metabolism, amine transport, neuropeptide production, and other processes that increase nerve impulse conduction. Significant downregulation was established for several genes whose products are involved in the inflammatory response; the finding confirmed that recombinant eHsp70 acts as a nonspecific anti-inflammatory agent [18].

Thus, the neuroprotective properties of eHsp70 were described with two AD models in vivo and confirmed at the molecular level. The mechanism of action of Hsp70 in this case is probably the same as in experiments with endotoxin shock models; i.e., Hsp70 interacts as a ligand with certain receptors and thereby triggers the signaling cascades that lead to the biochemical and physiological effects observed. The assumption is based on the fact that Hsp70 has an extremely low effective dose (4 μg daily) and occurs as a full-sized protein in the brain for a short period of time after administration. The N-methyl-D-aspartate (NMDA) receptor was identified as a possible target of Hsp70 [161]. Hyperactivation of the NMDA receptor contributes to nervous cell death during the inflammatory response and facilitates the accumulation of prion-like aggregates. Antagonists of the NMDA receptor promote cell survival during accumulation of PrP aggregates [173]. CD91, which acts as an apolipoprotein E receptor and is capable of recognizing many ligands varying in nature, is another candidate receptor of Hsp70 [174, 175]. It was demonstrated that eHsp70 is efficiently recognized by macrophage CD91 [90], which is similar to TLR4 in mediating phagocytosis of Hsp70 and other exogenous Hsps [13, 91, 94]. CD91 is exposed on certain neurons and astrocytes and is involved in AD pathogenesis [176]. Finally, TLR4, which recognizes eHsp70, plays an important role in the inflammatory response in AD [13]. Our and other teams showed that eHsp70 suppresses secretion of proinflammatory cytokines in immune cells exposed to LPS in vitro [101–106, 116, 117]. In addition, eHsp70 activates microglia, which facilitates dissolution of protein aggregates and thus facilitates Aβ phagocytosis in vitro [15, 135]. It is possible that eHsp70 switches TLR4 from cytokine production to Aβ phagocytosis and subsequent degradation in the bulbectomized and 5XFAD mouse models because a decrease in Aβ was observed in both of the models.

A negative correlation was observed between the level of soluble Aβ peptides and the endogenous Hsp70 content in the brain in mice with neurodegeneration due to bulbectomy. The Aβ level significantly increased 1.5 months after bulbectomy, the increase coinciding with cognitive impairment. A decrease in Aβ was observed 6 months after bulbectomy, and the capabilities of memorizing and learningbeing were temporarily restored. This remission period coincided with a substantial increase in intracellular Hsp70 after surgery. Then the Hsp70 level decreased again, the Aβ concentration reached its maximum, and cognitive functions were impaired irreversibly. The findings make it possible to assume that an increase in endogenous Hsp70 acts as a compensatory mechanism in neurodegeneration or during recovery after brain injury [177].

The results indicate that both recombinant Hsp70 (or its mimetics with similar effects) and inducers of endogenous Hsp70 synthesis are promising as potential drugs to treat AD and certain other proteinopathies.

CONCLUSIONS

Based on the data obtained independently by several teams, the intracellular and secreted Hsp70 forms act as potent nonspecific adaptogens that improve the resistance to various stress factors and possess marked neuroprotective and immunoregulatory activities. However, it is clear that different mechanisms mediate the protective effects of endogenous and exogenous,

including recombinant, Hsp70 proteins. Induction of intracellular Hsp70 synthesis may promote the survival of cells and the total body on exposure to various stress factors by preventing the accumulation of denatured and damaged proteins and blocking the signaling cascades that lead to apoptosis. On the other hand, even when used in trace amounts, exogenous Hsp70 may act as an alarmin, or an alarm signal, to trigger various signaling pathways involved in regulating the nonspecific immune response and inducing the inflammatory reaction. This effect is probably responsible for neuroprotective activity observed for recombinant Hsp70 in several animal models of AD. Recombinant Hsp70 therefore attracts particular attention as a promising therapeutic agent; the more so, because it is simple to obtain in conventional expression systems and to deliver into the brain via intranasal administration.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project nos. 18-04-00895 and 18-04-00865), the Russian Science Foundation (project no. 17-74-30030), and the Program of Basic Research at the State Academies of Sciences for the Period from 2013 to 2020 (project no. 01201363817).

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

1. Laskey R.A., Honda B.M., Mills A.D., Finch J.T. 1978. Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA. *Nature*. **275**, 416–420.
2. Mayer M.P. 2010. Gymnastics of molecular chaperones. *Mol. Cell*. **39**, 321–331.
3. Craig E.A., Jacobsen K. 1984. Mutations of the heat inducible 70 kilodalton genes of yeast confer temperature sensitive growth. *Cell*. **38**, 841–849.
4. Feder M.E., Hofmann G.E. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annu. Rev. Physiol*. **61**, 243–282.
5. Barua D., Heckathorn S.A. 2004. Acclimation of the temperature set-points of the heat-shock response. *J. Therm. Biol*. **29**, 185–193.
6. Gong W.J., Golic K.G. 2006. Loss of Hsp70 in *Drosophila* is pleiotropic, with effects on thermotolerance, recovery from heat shock and neurodegeneration. *Genetics*. **172**, 275–286.
7. Evgen'ev M.B., Garbuz D.G., Zatsepina O.G. 2014. *Heat Shock Proteins and Whole Body Adaptation to Extreme Environments*. Dordrecht, Netherlands: Springer.
8. Kampinga H.H., Hageman J., Vos M.J., Kubota H., anguay 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.
9. Evgen'ev M.B., Garbuz D.G., Zatsepina O.G. 2005. Heat shock proteins: Functions and role in adaptation to hyperthermia. *Ontogenez*. **36**, 265–273.
10. Hartl F.U., Bracher A., Hayer-Hartl M. 2011. Molecular chaperones in protein folding and proteostasis. *Nature*. **475**, 324–332.
11. Zatsepina O.G., Przhiboro A.A., Yushenova I.A., Shilova V., Zelentsova E.S., Shostak N.G., Evgen'ev M.B., Garbuz D.G. 2016. A *Drosophila* heat shock response represents an exception rather than a rule among Diptera species. *Insect. Mol. Biol*. **25**, 431–449.
12. Asea A., Rehli M., Kabingu E., Boch 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. *J. Biol. Chem*. **277**, 15028–15034.
13. Calderwood S.K., Mambula S.S., Gray P.J., Jr., Theriault J.R. 2007. Extracellular heat shock proteins in cell signaling. *FEBS Lett*. **581**, 3689–3694.
14. Ghosh A.K., Sinha D., Mukherjee S., Biswas R., Biswas T. 2015. LPS stimulates and Hsp70 down-regulates TLR4 to orchestrate differential cytokine response of culture-differentiated innate memory CD8+ T cells. *Cytokine*. **73**, 44–52.
15. Kakimura J., Kitamura Y., Takata K., Umeki M., Suzuki S., Shibagaki K., Taniguchi T., Nomura Y., Gebicke-Haerter P.J., Smith M.A., Perry G., Shimohama S. 2002. Microglial activation and amyloid-beta clearance induced by exogenous heat-shock proteins. *FASEB J*. **16**, 601–603.
16. 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 stresstolerance. *Brain Res*. **914**, 66–73.
17. Bobkova N.V., Garbuz D.G., Nesterova I., Medvinskaya N., Samokhin A., Alexandrova I., Yashin V., Karpov V., Kukharsky M.S., Ninkina N.N., Smirnov A.A., Nudler E., Evgen'ev M. 2014. Therapeutic effect of exogenous Hsp70 in mouse models of Alzheimer's disease. *J. Alzheimers Dis*. **38**, 425–435.
18. Evgen'ev M.B., Krasnov G.S., Nesterova I.V., Garbuz D.G., Karpov V.L., Morozov A.V., Snezhkina A.V., Samokhin A.N., Sergeev A., Kulikov A.M., Bobkova N.V. 2017. Molecular mechanisms underlying neuroprotective effect of intranasal administration of human Hsp70 in mouse model of Alzheimer's disease. *J. Alzheimers Dis*. **59**, 1415–1426.
19. De Mena L., Chhangani D., Fernandez-Funez P., Rincon-Limas D.E. 2017. secHsp70 as a tool to approach amyloid-β42 and other extracellular amyloids. *Fly*. **11**, 179–184.
20. Milner C.M., Campbell R.D. 1990. Structure and expression of the three MHC-linked *HSP70* genes. *Immunogenetics*. **32**, 242–251.

21. Milner C.M., Campbell R.D. 1992. Polymorphic analysis of three MHC-linked *HSP70* genes. *Immunogenetics*. **36**, 357–362.
22. Walter L., Rauh F., Gunther E. 1994. Comparative analysis of the three major histocompatibility complex-linked heat shock protein 70 (*hsp70*) genes of the rat. *Immunogenetics*. **40**, 325–330.
23. Garbuz D.G., Astakhova L.N., Zatsepina O.G., Arkhipova I.R., Nudler E., Evgen'ev M.B. 2011. Functional organization of hsp70 cluster in camel (*Camelus dromedarius*) and other mammals. *PLoS One*. **6**, e27205.
24. Hess K., Oliverio R., Nguyen P., Le D., Ellis J., Kdeiss B., Ord S., Chalkia D., Nikolaidis N. 2018. Concurrent action of purifying selection and gene conversion results in extreme conservation of the major stress-inducible *Hsp70* genes in mammals. *Sci. Rep*. **8**, 5082.
25. Flaherty K.M., DeLuca-Flaherty C., McKay D.B. 1990. Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. *Nature*. **346**, 623–628.
26. Flajnik M.F., Canel C., Kramer J., Kasahara M. 1991. Which came first, MHC class I or class II? *Immunogenetics*. **33**, 295–300.
27. Welch W.J., Feramisco J.R. 1985. Rapid purification of mammalian 70000-dalton stress proteins: Affinity of the proteins for nucleotides. *Mol. Cell. Biol*. **5**, 1229–1237.
28. Nollen E.A., Morimoto R.I. 2002. Chaperoning signaling pathways: Molecular chaperones as stress-sensing 'heat shock' proteins. *J. Cell Sci*. 2002. **115**, 2809–2816.
29. Guzhova I., Margulis B. 2006. Hsp70 chaperone as a survival factor in cell pathology. *Int. Rev. Cytol*. **254**, 101–149.
30. Frydman J. 2001. Folding of newly translated proteins in vivo: The role of molecular chaperones. *Annu. Rev. Biochem*. **70**, 603–647.
31. Goloubinoff P., Sassi A.S., Fauvet B., Barducci A., De Los Rios P. 2018. Chaperones convert the energy from ATP into the nonequilibrium stabilization of native proteins. *Nature Chem. Biol*. **14**, 388–395.
32. Chakraborty K., Chatila M., Sinha J., Shi Q., Poschner B.C., Sikor M., Jiang G., Lamb D.C., Hartl F.U., Hayer-Hartl M. 2010. Chaperonin-catalyzed rescue of kinetically trapped states in protein folding. *Cell*. **142**, 112–122.
33. Reeg S., Jung T., Castro J.P., Davies K.J.A., Henze A., Grune T. 2016. The molecular chaperone Hsp70 promotes the proteolytic removal of oxidatively damaged proteins by the proteasome. *Free Radic. Biol. Med*. **99**, 153–166.
34. Bercovich B., Stancovski I., Mayer A., Blumenfeld N., Laszlo A., Schwartz A.L., Ciechanover A. 1997. Ubiquitin-dependent degradation of certain protein substrates in vitro requires the molecular chaperone Hsc70. *J. Biol. Chem*. **272**, 9002–9010.
35. Nelson R.J., Ziegelhoffer T., Nicolet C., Werner-Washburne M., Craig E.A. 1992. The translation machinery and 70 kD heat shock protein cooperate in protein synthesis. *Cell*. **71**, 97–105.
36. Ku Z., Yang J., Menon V., Thomason D.B. 1995. Decreased polysomal HSP70 may slow polypeptide elongation during skeletal muscle atrophy. *Am. J. Physiol*. **268**, 1369–1374.
37. Arias E., Cuervo A.M. 2011. Chaperone-mediated autophagy in protein quality control. *Curr. Opin. Cell Biol*. **23**, 184–189.
38. Fan A.C., Young J.C. 2011. Function of cytosolic chaperones in Tom70-mediated mitochondrial import. *Protein Pept. Lett*. **18**, 122–131.
39. Sousa R., Lafer E.M. 2006. Keep the traffic moving: mechanism of the Hsp70 motor. *Traffic*. **7**, 1596–1603.
40. Kutik S., Guiard B., Meyer H.E., Wiedemann N., Pfanner N. 2007. Cooperation of translocase complexes in mitochondrial protein import. *J. Cell Biol*. **179**, 585–591.
41. van der Laan M., Hutu D.P., Rehling P. 2010. On the mechanism of preprotein import by the mitochondrial presequence translocase. *Biochim. Biophys. Acta*. **1803**, 732–739.
42. Hamman B.D., Hendershot L.M., Johnson A.E. 1998. BiP maintains the permeability barrier of the ER membrane by sealing the lumenal end of the translocon pore before and early in translocation. *Cell*. **92**, 747–758.
43. Dudek J., Pfeffer S., Lee P.H., Jung M., Cavalié A., Helms V., Förster F., Zimmermann R. 2015. Protein transport into the human endoplasmic reticulum. *J. Mol. Biol*. **427**, 1159–1175.
44. Melnick J., Argon Y. 1995. Molecular chaperones and the biosynthesis of antigen receptors. *Immunol. Today*. **16**, 243–250.
45. Sawa T., Imamura T., Haruta T., Sasaoka T., Ishiki M., Takata Y., Takada Y., Morioka H., Ishihara H., Usui I., Kobayashi M. 1996. Hsp70 family molecular chaperones and mutant insulin receptor: Differential binding specificities of BiP and Hsp70/Hsc70 determines accumulation or degradation of insulin receptor. *Biochem. Biophys. Res. Commun*. **218**, 449–453.
46. Plemper R.K., Böhmler S., Bordallo J., Sommer T., Wolf D.H. 1997. Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. *Nature*. **388**, 891–895.
47. Nishikawa S., Brodsky J.L., Nakatsukasa K. 2005. Roles of molecular chaperones in endoplasmic reticulum (ER. quality control and ER-associated degradation (ERAD). *J. Biochem*. **137**, 551–555.
48. Lasunskaia E.B., Fridlianskaia I.I., Guzhova I.V., Bozhkov V.M., Margulis B.A. 1997. Accumulation of major stress protein 70 kDa protects myeloid and lymphoid cells from death by apoptosis. *Apoptosis*. **2**, 156–163.
49. Takano M., Arai T., Mokuno Y., Nishimura H., Nimura Y., Yoshikai Y. 1998. Dibutyryl cyclic adenosine monophosphate protects mice against tumor necrosis factor-alpha-induced hepatocyte apoptosis accompanied by increased heat shock protein 70 expression. *Cell Stress Chaperones*. **3**, 109–117.
50. Ahn J.H., Ko Y.G., Park W.Y., Kang Y.S., Chung H.Y., Seo J.S. 1999. Suppression of ceramide-mediated apoptosis by HSP70. *Mol. Cells*. **9**, 200–206.

51. Brar B.K., Stephanou A., Wagstaff M.J., Coffin R.S., Marber M.S., Engelmann G., Latchman D.S. 1999. Heat shock proteins delivered with a virus vector can protect cardiac cells against apoptotic as well as against thermal or hypoxic stress. *J. Mol. Cell. Cardiol.* **31**, 135–146.

52. Wagstaff M.J., Collaço-Moraes Y., Smith J., de Belleroche J.S., Coffin R.S., Latchman D.S. 1999. Protection of neuronal cells from apoptosis by HSP27 delivered with a herpes simplex virus-based vector. *J. Biol. Chem*. **274**, 5061–5069.

53. Kumar S., Stokes J. 3rd, Singh U.P., Scissum Gunn K., Acharya A., Manne U., Mishra M. 2016. Targeting Hsp70: A possible therapy for cancer. *Cancer Lett.* **374**, 156–166.

54. Pandey M.K., Prasad S., Tyagi A.K., Deb L., Huang J., Karelia D.N., Amin S.G., Aggarwal B.B. 2016. Targeting cell survival proteins for cancer cell death. *Pharmaceuticals.* **9**, e11.

55. Sharp F.R., Zhan X., Liu D.Z. 2013. Heat shock proteins in the brain: Role of Hsp70, Hsp27, and HO-1 (Hsp32) and their therapeutic potential. *Transl. Stroke Res.* **4**, 685–692.

56. Schett G., Steiner C.W., Gröger M., Winkler S., Graninger W., Smolen J., Xu Q., Steiner G. 1999. Activation of Fas inhibits heat-induced activation of HSF1 and up-regulation of HSP70. *FASEB J.* **13**, 833–842.

57. Mosser D.D., Caron A.W., Bourged L., Denis-Larose C., Massie B. 1997. Role of the human heat shock protein HSP70 in protection against stress-induced apoptosis. *Mol. Cell. Biol.* **17**, 5317–5327.

58. Gabai V.L., Meriin A.B., Yaglom J.A., Volloch V., Sherman M.Y. 1998. Role of HSP70 in regulation of stress-kinase JNK: Implications in apoptosis and aging. *FEBS Lett*. **438**, 1–4.

59. Kumar Y., Tatu U. 2003. Stress protein flux during recovery from simulated ischemia: Induced heat shock protein 70 confers cytoprotection by suppressing JNK activation and inhibiting apoptotic cell death. *Proteomics*. **3**, 513–526.

60. Stankiewicz A.R., Lachapelle G., Foo C.P., Radicioni S.M., Mosser D.D. 2005. Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation. *J. Biol. Chem*. **280,** 38729–38739.

61. Garrido C., Galluzzi L., Brunet M., Puig P.E., Didelot C., Kroemer G. 2006. Mechanisms of cytochrome *c* release from mitochondria. *Cell Death Differ*. **13**, 1423–1433.

62. Mosser D.D., Caron A.W., Bourget L., Meriin A.B., Sherman M.Y., Morimoto R.I., Massie B. 2000. The chaperone function of Hsp70 is required for protection against stress-induced apoptosis. *Mol. Cell. Biol*. **20**, 7146–7159.

63. Jaattela M., Wissing D., Kokholm K., Kallunki T., Egeblad M. 1998. HSP70 exerts its anti-apoptosic function downstream of caspase-3-like proteases. *EMBO J.* **17**, 6124–6134.

64. Guzhova I.V., Margulis B.A. 2000. Induction and accumulation of HSP70 leads to formation of its complexes with other cell proteins. *Tsitologiya*. **42**, 647–652.

65. Hargitai J., Lewis H., Boros I., Rácz T., Fiser A., Kurucz I., Benjamin I., Vígh L., Pénzes Z., Csermely P., Latchman D.S. 2003. Bimoclomol, a heat shock protein co-inducer, acts by the prolonged activation of heat shock factor-1. *Biochem. Biophys. Res. Commun.* **307**, 689–695.

66. Finka A., Sharma S.K., Goloubinoff P. 2015. Multilayered molecular mechanisms of polypeptide holding, unfolding and disaggregation by HSP70/HSP110 chaperones. *Front. Mol. Biosci.* **2**, article 29.

67. Gao X., Carroni M., Nussbaum-Krammer C., Mogk A., Nillegoda N.B., Szlachcic A., Guilbride D.L., Saibil H.R., Mayer M.P., Bukau B. 2015. Human Hsp70 disaggregase reverses Parkinson's-linked α-synuclein amyloid fibrils. *Mol. Cell.* **59**, 781–793.

68. Jaattela M. 1999. Escaping cell death: Survival proteins in cancer. *Exp. Cell Res*. **248**, 30–43.

69. Multhoff G., Hightower L.E. 2011. Distinguishing integral and receptor-bound heat shock protein 70 (Hsp70) on the cell surface by Hsp70-specific antibodies. *Cell Stress Chaperones*. **16**, 251–255.

70. Zhai L.L., Xie Q., Zhou C.H., Huang D.W., Tang Z.G., Ju T.F. 2017. Overexpressed HSPA2 correlates with tumor angiogenesis and unfavorable prognosis in pancreatic carcinoma. *Pancreatology*. **17**, 457–463.

71. Boudesco C., Cause S., Jego G., Garrido C. 2018. Hsp70: A cancer target inside and outside the cell. In: *Chaperones: Methods and Protocols.* Eds. Calderwood S.K., Prince T.L. *Method in Molecular Biol*. **1709**, 371–396.

72. 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. *J. Cell. Physiol*. **138**, 257–266.

73. 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. *Immunol. Invest*. **27**, 367–377.

74. Clayton A., Turkes A., Navabi H., Mason M.D., Tabi Z. 2005. Induction of heat shock proteins in B-cell exosomes. *J. Cell. Sci*. **118**, 3631–3638.

75. 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. *J. Neurosci*. **25**, 9735–9745.

76. Davies E.L., Bacelar M.M., Marshall M.J., Johnson E., Wardle T.D., Andrew S.M., Williams J.H. 2006. Heat shock proteins form part of a danger signal cascade in response to lipopolysaccharide and GroEL. *Clin. Exp. Immunol*. **145**, 183–189.

77. 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. *Biochem. Biophys. Res. Commun.* **387**, 229–233.

78. Beckett K., Monier S., Palmer L., Alexandre C., Green H., Bonneil E., Raposo G., Thibault P., Le Borgne R., Vincent J.P. 2013. *Drosophila* S2 cells secrete wingless on exosome-like vesicles but the

wingless gradient forms independently of exosomes. *Traffic*. **14**, 82–96.

79. Takeuchi T., Suzuki M., Fujikake N., Popiel H.A., Kikuchi H., Futaki S., Wada K., Nagai Y. 2015. Intercellular chaperone transmission via exosomes contributes to maintenance of protein homeostasis at the organismal level. *Proc. Natl. Acad. Sci. U. S. A.* **112**, E2497–E2506.
80. Mambula S.S., Calderwood S.K. 2006. Heat shock protein 70 is secreted from tumor cells by nonclassical pathway involving lysosomal endosomes. *J. Immunol.* **177**, 7849–7857.
81. Mambula S.S., Stevenson M.A., Ogawa K., Calderwood S.K. 2007. Mechanisms for Hsp70 secretion: Crossing membranes without a leader. *Methods*. **43**, 168–175.
82. Prudovsky I., Mandinova A., Soldi R., Bagala C., Graziani I., Landriscina M., Tarantini F., Duarte M., Bellum S., Doherty H., Maciag T. 2003. The nonclassical export routes: FGF1 and IL-1alpha point the way. *J. Cell. Sci*. **116**, 4871–4881.
83. Ferrari D., Pizzirani C., Adinolfi E., Lemoli R.M., Curti A., Idzko M., Panther E., Di Virgilio F. 2006. The P2X7 receptor: A key player in IL-1 processing and release. *J. Immunol*. **176**, 3877–3883.
84. Arispe N., Doh M., Simakova O., Kurganov B., De Maio A. 2004. Hsc70 and Hsp70 interact with phosphatidylserine on the surface of PC12 cells resulting in a decrease of viability. *FASEB J.* **18**, 1636–1645.
85. Schilling D., Gehrmann M., Steinem C., De Maio A., Pockley A.G., Abend M., Molls M., Multhoff G. 2009. Binding of heat shock protein 70 to extracellular phosphatidylserine promotes killing of normoxic and hypoxic tumor cells. *FASEB J.* **23**, 2467–2477.
86. Asea A. 2007. Mechanisms of HSP72 release. *J. Biosci*. **32**, 579–584.
87. Vega V.L., Rodríguez-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. *J. Immunol*. **180**, 4299–4307.
88. Tsai T.N., Lee T.Y., Liu M.S., Chuang I.C., Lu M.C., Dong H.P., Lue S.I., Yang R.C. 2015. Release of endogenous heat shock protein 72 on the survival of sepsis in rats. *J. Surg. Res*. **198**, 165–174.
89. Macleod C., Bryant C.E. 2017. Visualising pattern recognition receptor signalling. *Biochem. Soc. Trans*. **45**, 1077–1085.
90. 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.
91. Takemoto S., Nishikawa M., Takakura Y. 2005. Pharmacokinetic and tissue distribution mechanism of mouse recombinant heat shock protein 70 in mice. *Pharm. Res.* **22**, 419–426.
92. 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.
93. Asea A. 2008. Hsp70: A chaperokine. *Novartis Found. Symp.* **291**, 173–179.
94. Srivastava P. 2002. Interaction of heat shock proteins with peptides and antigen presenting cells: Chaperoning of the innate and adaptive immune responses. *Annu. Rev. Immunol*. **20**, 395–425.
95. 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. *Nat. Med.* **6**, 435–442.
96. Fleshner M., Johnson J.D. 2005. Endogenous extracellular heat shock protein 72: Releasing signal(s) and function. *Int. J. Hyperthermia*. **21**, 457–471.
97. Lee K.H., Jeong J., Yoo C.G. 2013. Positive feedback regulation of heat shock protein 70 (Hsp70) is mediated through Toll-like receptor 4-PI3K/Akt-glycogen synthase kinase-3β pathway. *Exp. Cell Res.* **319**, 88–95.
98. Ko R., Lee S.Y. 2016. Glycogen synthase kinase 3β in Toll-like receptor signaling. *BMB Rep.* **49**, 305–310.
99. Bausinger H., Lipsker D., Ziylan U., Manié S., Briand J.P., Cazenave J.P., Muller S., Haeuw J.F., Ravanat C., de la Salle H., Hanau D. 2002. Endotoxin-free heat-shock protein 70 fails to induce APC activation. *Eur. J. Immunol*. **32**, 3708–3713.
100. Gao B., Tsan M. F. 2003. Endotoxin contamination in recombinant human heat shock protein 70 (Hsp70) preparation is responsible for the induction of tumor necrosis factor alpha release by murine macrophages. *J. Biol. Chem*. **278**, 174–179.
101. Rozhkova E., Yurinskaya M., Zatsepina O., Garbuz D., Karpov V., Surkov S., Murashev A., Ostrov V., Margulis B., Evgen'ev M., Vinokurov M. 2010. Exogenous mammalian extracellular HSP70 reduces endotoxin manifestations at the cellular and organism levels. *Ann. N.Y. Acad. Sci*. **1197**, 94–107.
102. 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. *J. Immunol.* **177**, 7184–7192.
103. Borges T.J., Lopes R.L., Pinho N.G., Machado F.D., Souza A.P., Bonorino C. 2013. Extracellular Hsp70 inhibits pro-inflammatory cytokine production by IL-10 driven down-regulation of C/EBPβ and C/EBPδ. *Int. J. Hyperthermia*. **29**, 455–463.
104. Hsu J.H., Yang R.C., Lin S.J., Liou S.F., Dai Z.K., Yeh J.L., Wu J.R. 2014. Exogenous heat shock cognate protein 70 pretreatment attenuates cardiac and hepatic dysfunction with associated anti-inflammatory responses in experimental septic shock. *Shock*. **42**, 540–547.
105. Troyanova N.I., Shevchenko M.A., Boiko A.A., Mirzoev R.R., Pertseva M.A., Kovalenko E.I., Sapozhnikov A.M. 2015. Modulating effect of extracellular HSP70 on generation of reactive oxigen speciesin populations of phagocytes. *Russ. J. Bioorg. Chem*. **41** (3), 271–279.
106. 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* (Moscow). **81** (11), 1384–1395.

107. Yurinskaya M., Zatsepina O.G., Vinokurov M.G., Bobkova N.V., Garbuz D.G., Morozov A.V., Kulikova D.A., Mitkevich V.A., Makarov A.A., Funikov S.Y., Evgen'ev M.B. 2015. The fate of exogenous human HSP70 introduced into animal cells by different means. *Curr. Drug Deliv*. **12**, 524–532.
108. Peri F., Calabrese V. 2014. Toll-like receptor 4 (TLR4) modulation by synthetic and natural compounds: an update. *J. Med. Chem*. **57**, 3612–3622.
109. Ofengeim D., Yuan J. 2013. Regulation of RIP1 kinase signalling at the crossroads of inflammation and cell death. *Nat. Rev. Mol. Cell Biol*. **14**, 727–736.
110. Angus D.C., Wax R.S. 2001. Epidemiology of sepsis: An update. *Crit. Care Med*. *29*, S109–S116.
111. van Zanten A.R., Brinkman S., Arbous M.S., Abu-Hanna A., Levy M.M., de Keizer N.F.; Netherlands Patient Safety Agency Sepsis Expert Group. 2014. Guideline bundles adherence and mortality in severe sepsis and septic shock. *Crit. Care Med*. **42**, 1890–1898.
112. Zhang Y.H., Takahashi K., Jiang G.Z., Zhang X.M., Kawai M., Fukada M., Yokochi T. 1994. In vivo production of heat shock protein in mouse peritoneal macrophages by administration of lipopolysaccharide. *Infect. Immun*. **62**, 4140–4144.
113. Gupta A., Cooper Z.A., Tulapurkar M.E., Potla R., Maity T., Hasday J.D., Singh I.S. 2013. Toll-like receptor agonists and febrile range hyperthermia synergize to induce heat shock protein 70 expression and extracellular release. *J. Biol. Chem*. **288**, 2756–2766.
114. Wheeler D.S., Fisher L.E., Jr., Catravas J.D., Jacobs B.R., Carcillo J.A., Wong H.R. 2005. Extracellular hsp70 levels in children with septic shock. *Pediatr. Crit. Care Med*. **6**, 308–311.
115. Nakada J., Matsura T., Okazaki N., Nishida T., Togawa A., Minami Y., Inagaki Y., Ito H., Yamada K., Ishibe Y. 2005. Oral administration of geranylgeranylacetone improves survival rate in a rat endotoxin shock model: Administration timing and heat shock protein 70 induction. *Shock*. **24**, 482–487.
116. Kustanova G., Murashev A., Karpov V.L., Margulis B.A., Guzhova I.V., Prokhorenko I.R., Grachev S.V., Evgen'ev M.B. 2006. Exogenous heat shock protein 70 mediates sepsis manifestations and decreases the mortality rate in rats. *Cell Stress Chaperones*. **11**, 276–286.
117. Vinokurov M., Ostrov V., Yurinskaya M., Garbuz D., Murashev A., Antonova O., Evgen'ev M. 2012. Recombinant human Hsp70 protects against lipoteichoic acid-induced inflammation manifestations at the cellular and organismal levels. *Cell Stress Chaperones*. **17**, 89–101.
118. Yurinskaya M.M., Vinokurov M.G., Zatsepina O.G., Garbuz D.G., Guzhova I.V., Rozhkova E.A., Suslikov A.V., Karpov V.L., Evgen'ev M.B. 2009. Exogenous heat shock proteins HSP70 suppress endotoxin-induced activation of human neutrophils. *Dokl. Akad. Nauk*. **426**, 406–409.
119. Ostrov V.F., Slashcheva G.A., Zharmukhamedova T.Yu., Garbuz D.G., Evgen'ev M.B., Murashev A.N. 2010. The Influence of the recombinant human heat shock protein Hsp70 in the biochemical properties of blood during endotoxic shock simulation in rats. *Russ. J. Bioorg. Chem*. **36** (3), 310–314.
120. Shin H.J., Lee H., Park J.D., Hyun H.C., Sohn H.O., Lee D.W., Kim Y.S. 2007. Kinetics of binding of LPS to recombinant CD14, TLR4, and MD-2 proteins. *Mol. Cells*. **24**, 119–124.
121. Afrazi A., Sodhi C.P., Good M., Jia H., Siggers R., Yazji I., Ma C., Neal M.D., Prindle T., Grant Z.S., Branca M.F., Ozolek J., Chang E.B., Hackam D.J. 2012. Intracellular heat shock protein-70 negatively regulates TLR4 signaling in the newborn intestinal epithelium. *J. Immunol*. **188**, 4543–4557.
122. Multhoff G., Botzler C., Wiesnet M., Müller E., Meier T., Wilmanns W., Issels R.D. 1995. A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells. *Int. J. Cancer*. **61**, 272–279.
123. Multhoff G., Botzler C., Jennen L., Schmidt J., Ellwart J., Issels R. 1997. Heat shock protein 72 on tumor cells: A recognition structure for natural killer cells. *J. Immunol*. **158**, 4341–4350.
124. Jun Ho Jang, Hanash S. 2003. Profiling of the cell surface proteome. *Proteomics*. **3**, 1947–1954.
125. Roigas J., Wallen E.S., Loening S.A., Moseley P.L. 1998. Heat shock protein (HSP72) surface expression enhances the lysis of a human renal cell carcinoma by IL-2 stimulated NK cells. *Adv. Exp. Med. Biol*. **451**, 225–229.
126. Ponomarev E.D., Tarasenko T.N., Sapozhnikov A.M. 2000. Splenic cytotoxic cells recognize surface HSP70 on culture-adapted EL-4 mouse lymphoma cells. *Immunol. Lett*. **74**, 133–139.
127. 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. *J. Immunol*. **175**, 2900–2912.
128. Chalmin F., Ladoire S., Mignot G., Vincent J., Bruchard M., Remy-Martin J.P., Boireau W., Rouleau A., Simon B., Lanneau D., De Thonel A., Multhoff G., Hamman A., Martin F., Chauffert B., et al. 2010. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *J. Clin. Invest*. **120**, 457–471.
129. Rérole A.L., Gobbo J., De Thonel A., Schmitt E., Pais de Barros J.P., Hammann A., Lanneau D., Fourmaux E., Demidov O.N., Micheau O., Lagrost L., Colas P., Kroemer G., Garrido C. 2011. Peptides and aptamers targeting HSP70: A novel approach for anticancer chemotherapy. *Cancer Res*. **71**, 484–495.
130. Zihai Li. 2003. Role of heat shock protein in chaperoning tumor antigens and modulating anti-tumor immunity. In*: Tumor Antigens Recognized by T Cells and Antibodies*. Eds. Hans J. Stauss, Kawakami Y., Parmiani G. New York: Taylor and Francis, pp. 20–33
131. Shevtsov M., Multhoff G. 2016. Heat shock protein-peptide and HSP-based immunotherapies for the treatment of cancer. *Front. Immunol*. **7**, article 171.
132. Galvin J.E., Howard D.H., Denny S.S., Dickinson S., Tatton N. 2017. The social and economic burden of frontotemporal degeneration. *Neurology*. **89**, 2049–2056.

133. Marešová P., Dolejš J., Kuca K. 2018. Call for a uniform strategy of collecting Alzheimer's disease costs: A review and meta-analysis. *J. Alzheimers Dis.* **63**, 227–238.

134. Reisberg B., Saeed M.U. 2004. Alzheimer's disease. In: *Comprehensive Textbook of Geriatric Psychiatry,* 3rd ed. Eds. Sadavoy J., Jarvik L.F., Grossberg G.T., Meyers B.S. New York: W.W. Norton, pp. 449–509

135. Clayton K.A., Van Enoo A.A., Ikezu T. 2017. Alzheimer's disease: The role of microglia in brain homeostasis and proteopathy. *Front. Neurosci.* **11**, article 680.

136. Schwarzman A.L., Sarantseva S.V. 2017. Transmission of pathogenic protein aggregates in Alzheimer's disease. *Mol. Biol.* (Moscow). **51** (3), 368–371.

137. Grimm A., Friedland K., Eckert A. 2016. Mitochondrial dysfunction: The missing link between aging and sporadic Alzheimer's disease. *Biogerontology*. **17**, 281–296.

138. Ahmad K., Baig M.H., Mushtaq G., Kamal M.A., Greig N.H., Choi I. 2017. Commonalities in biological pathways, genetics, and cellular mechanism between Alzheimer disease and other neurodegenerative diseases: An in silico-updated overview. *Curr. Alzheimer Res.* **14**, 1190–1197.

139. Yang G., Wang Y., Tian J., Liu J.P. 2013. Huperzine A for Alzheimer's disease: A systematic review and meta-analysis of randomized clinical trials. *PLoS One*. **8**, e74916.

140. Ehret M.J., Chamberlin K.W. 2015. Current practices in the treatment of Alzheimer disease: Where is the evidence after the Phase III trials? *Clin. Ther.* **37**, 1604–1616.

141. Tatarnikova O.G., Orlov M.A., Bobkova N.V. 2015. Beta-amyloid and Tau protein: Structure, properties, and prion-like properties. *Usp. Biol. Khim.* **55**, 351–390.

142. Kumar D., Ganeshpurkar A., Kumar D., Modi G., Gupta S.K., Singh S.K. 2018. Secretase inhibitors for the treatment of Alzheimer's disease: Long road ahead. *Eur. J. Med. Chem.* **148**, 436–452.

143. Franklin T.B., Krueger-Naug A.M., Clarke D.B., Arrigo A.P., Currie R.W. 2005. The role of heat shock proteins Hsp70 and Hsp27 in cellular protection of the central nervous system. *Int. J. Hyperthermia*. **21**, 379–392.

144. Leak R.K. 2014. Heat shock proteins in neurodegenerative disorders and aging. *J. Cell Commun. Signal.* **8**, 293–310.

145. Sulistio Y.A., Heese K. 2016. The ubiquitin-proteasome system and molecular chaperone deregulation in Alzheimer's disease. *Mol. Neurobiol.* **53**, 905–931.

146. Sun Y., Zhang J.R., Chen S. 2017. Suppression of Alzheimer's disease-related phenotypes by the heat shock protein 70 inducer, geranylgeranylacetone, in APP/PS1 transgenic mice via the ERK/p38 MAPK signaling pathway. *Exp. Ther. Med.* **14**, 5267–5274.

147. Dursun E., Gezen-Ak D., Hanağası H., Bilgiç B., Lohmann E., Ertan S., Atasoy İ.L., Alaylıoğlu M., Araz Ö.S., Önal B., Gündüz A., Apaydın H., Kızıltan G., Ulutin T., Gürvit H., Yılmazer S. 2015. The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. *J. Neuroimmunol.* **283**, 50–57.

148. Taipa R., Sousa A.L., Melo Pires M., Sousa N. 2016. Does the interplay between aging and neuroinflammation modulate Alzheimer's disease clinical phenotypes? A clinico-pathological perspective. *J. Alzheimers Dis.* **53**, 403–417.

149. Pugazhenthi S., Qin L., Reddy P.H. 2017. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim. Biophys. Acta*. **1863**, 1037–1045.

150. Heppner F.L., Ransohoff R.M., Becher B. 2015. Immune attack: The role of inflammation in Alzheimer disease. *Nat. Rev. Neurosci*. **16**, 358–372.

151. Zhang F., Jiang L. 2015. Neuroinflammation in Alzheimer's disease. *Neuropsychiatr. Dis. Treat*. **11**, 243–256.

152. Heneka M.T., Carson M.J., Khoury J.E., Landreth G.E., Brosseron F., Feinstein D.L., Jacobs A.H., Wyss-Coray T., Vitorica J., Ransohoff R.M., Herrup K., Frautschy S.A., Finsen B, Brown G.C., Verkhratsky A., et al. 2015. Neuroinflammation in Alzheimer's disease. *Lancet Neurol*. **14**, 388–405.

153. Bolós M., Perea J.R., Avila J. 2017. Alzheimer's disease as an inflammatory disease. *Biomol. Concepts*. **8**, 37–43.

154. Nazem A., Sankowski R., Bacher M., Al-Abed Y. 2015. Rodent models of neuroinflammation for Alzheimer's disease. *J. Neuroinflammation*. **12**, 74.

155. Balistreri C.R., Grimaldi M.P., Chiappelli M., Licastro F., Castiglia L., Listì F., Vasto S., Lio D., Caruso C., Candore G. 2008. Association between the polymorphisms of *TLR4* and *CD14* genes and Alzheimer's disease. *Curr. Pharm. Des*. **14**, 2672–2677.

156. Chen Y., Yip P., Huang Y., Sun Y., Wen L.L., Chu Y.M., Chen T.F. 2012. Sequence variants of Toll Like receptor 4 and late-onset Alzheimer's disease. *PLoS One*. **7**, e50771.

157. Tahara K., Kim H.D., Jin J.J., Maxwell J.A., Li L., Fukuchi K. 2006. Role of Toll-like receptor signalling in Aβ uptake and clearance. *Brain*. **129**, 3006–3019.

158. Jin J.J., Kim H.D., Maxwell J.A., Li L., Fukuchi K. 2008. Toll-like receptor 4-dependent upregulation of cytokines in a transgenic mouse model of Alzheimer's disease. *J. Neuroinflammation*. **5**, 23.

159. Tang S.C., Lathia J.D., Selvaraj P.K., Jo D.G., Mughala M.R., Cheng A., Siler D.A., Markesbery W.R., Arumugam T.V., Mattson M.P. 2008. Toll-Like receptor-4 mediates neuronal apoptosis induced by amyloid β-peptide and the membrane lipid peroxidation product 4-hydroxynonenal. *Exp. Neurol*. **213**, 114–121.

160. Meriin A.B., Sherman M.Y. 2005. Role of molecular chaperones in neurodegenerative disorders. *Int. J. Hyperthermia*. **21**, 403–419.

161. Ekimova I.V., Nitsinskaya L.E., Romanova I.V., Pastukhov Y.F., Margulis B.A., Guzhova I.V. 2010. Exogenous protein Hsp70/Hsc70 can penetrate into brain structures and attenuate the severity of chemically-induced seizures. *J. Neurochem*. **115**, 1035–1044.

162. Magrané J., Smith R.C., Walsh K., Querfurth H.W. 2004. Heat shock protein 70 participates in the neuro-

protective response to intracellularly expressed beta-amyloid in neurons. *J. Neurosci*. **24**, 1700–1706.

163. Calabrese V., Stella A.M., Butterfield D.A., Scapagnini G. 2004. Redox regulation in neurodegeneration and longevity: Role of the heme oxygenase and HSP70 systems in brain stress tolerance. *Antioxid. Redox Signal*. **6**, 895–913.
164. Lu R., Tan M., Wang H., Xie A.M., Yu J.T., Tan L. 2014. Heat Shock Protein 70 in Alzheimer's disease. *Biomed. Res. Int.* **2014**, 435203.
165. Lazarev V.F., Mikhaylova E.R., Guzhova I.V., Margulis B.A. 2017. Possible function of molecular chaperones in diseases caused by propagating amyloid aggregates. *Front. Neurosci*. **11**, 277.
166. Rivera I., Capone R., Cauvi D.M., Arispe N., De Maio A. 2018. Modulation of Alzheimer's amyloid β peptide oligomerization and toxicity by extracellular Hsp70. *Cell Stress Chaperones*. **23**, 269–279.
167. Bobkova N.V., Nesterova I.V., Medvinskaya N.I., Aleksandrova I.Y., Samokhin A.N., Gershovich Y.G., Gershovich P.M., Yashin V.A. 2005. Possible role of olfactory system in Alzheimer's disease genesis. In: *New Trends in Alzheimer and Parkinson Related Disorders: ADPD 2005.* Eds. Fisher A., Hanin L., Memo M., F. Stocchi. Medimond, pp. 91–95.
168. Holland D., Brewer J.B., Hagler D.J., Fennema-Notestine C., Dale A.D., and the Alzheimer's Disease Neuroimaging Initiative. 2009. Subregional neuroanatomical change as a biomarker for Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A*. **106**, 20954–20959.
169. Oakley H., Cole S.L., Logan S., Maus E., Shao P., Craft J., Guillozet-Bongaarts A., Ohno M., Disterhoft J., Van Eldik L., Berry R., Vassar R. 2006. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. *J. Neurosci*. **26**, 10129–10140.
170. Jogani V., Jinturkar K., Vyas T., Misra A. 2008. Recent patents review on intranasal administration for CNS drug delivery. *Recent Pat. Drug. Deliv. Formul*. **2**, 25–40.
171. Ying W. 2008. The nose may help the brain: Intranasal drug delivery for treating neurological diseases. *Future Neurol*. **3**, 1–4.
172. Falcone J.A., Salameh T.S., Yi X., Cordy B.J., Mortell W.G., Kabanov A.V., Banks W.A. 2014. Intranasal administration as a route for drug delivery to the brain: Evidence for a unique pathway for albumin. *J. Pharmacol. Exp. Ther.* **351**, 54–60.
173. Black S.A., Stys P.K., Zamponi G.W., Tsutsui S. 2014. Cellular prion protein and NMDA receptor modulation: Protecting against excitotoxicity. *Front. Cell. Dev. Biol*. **2**, 45.
174. Rebeck G.W., Reiter J.S., Strickland D.K., Hyman B.T. 1993. Apolipoprotein E in sporadic Alzheimer's disease: Allelic variation and receptor interactions. *Neuron*. **11**, 575–580.
175. Xiao H., Gao Y., Liu L., Li Y. 2017. Association between polymorphisms in the promoter region of the apolipoprotein E (APOE) gene and Alzheimer's disease: A meta-analysis. *EXCLI J*. **16**, 921–938.
176. Strickland D.K., Kounnas M.Z., Argraves W.S. 1995. LDL receptor-related protein: A multiligand receptor for lipoprotein and proteinase catabolism. *FASEB J*. **9**, 890–898.
177. Bobkova N., Guzhova I., Margulis B., Nesterova I., Medvinskaya N., Samokhin A., Alexandrova I., Garbuz D., Nudler E., Evgen'ev M. 2013. Dynamics of endogenous Hsp70 synthesis in the brain of olfactory bulbectomized mice. *Cell Stress Chaperones*. **18**, 109–118.

Translated by T. Tkacheva