Emerging Role of HSP70 in Human Diseases

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Abstract HSP70 are prominent stress proteins, which also act like molecular chaperones. The synthesis of HSP70 increases when the cell is exposed to any form of stress physical, biological or chemical. Under stress conditions, HSP70 recognize and bind to the unstable protein substrates and protect them from denaturation and aggregation. Besides, HSP70 are also essential during normal growth where they assist in folding of nascent proteins, degradation of misfolded and truncated proteins and, in subcellular localizations of proteins and vesicles. Since HSP70 are involved in a plethora of cellular activities, their role been implicated with several pathological diseases primarily related to apoptosis, carcinogenesis, amyloidogenesis. Here, we summarize the current knowledge on the HSP70 and their relevance in diseases such as cancer, diabetes, seizures and many more. Further, the relevance of HSP70 to serve as biomarkers and/or therapeutics in human diseases is also discussed.

Keywords Chaperone · Heat shock proteins · Human disease · Protein aggregation · Protein refolding · Stress

Abbreviations

AFLD	Alcoholic fatty liver diseases
AIF	Apoptosis-inducing factor
ATP	Adenosine triphosphate
DISC	Death inducing signaling complex
HSP	Heat shock protein
iNOS	Inducible nitric oxide synthase
MMP	Matrix metalloproteinase

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NAFLD	Nonalcoholic fatty liver diseases
NBD	Nucleotide binding domain
NEF	Nucleotide exchange factor
SBD	Substrate binding domain
TLR	Toll-like receptor

Introduction

Prokaryotes and eukaryotes are exposed to various environmental stresses. To counteract their effects, these have evolved a wide array of molecular and physiological processes. Stress proteins, also named as heat shock proteins, are one of the responses against the deleterious effects of many abiotic and biotic stresses including extreme temperatures, radiations, heavy metals, drought, hypoxia, ischemia and assaults of bacterial, viral and parasitic origin (Whitley *et al.* 1999). Heat shock proteins (HSP) are a class of evolutionarily conserved, functionally related cellular proteins which primarily act as chaperons (Verghese *et al.* 2012; Tóth *et al.* 2015). HSP are ubiquitous in nature and present in cytoplasm under normal conditions, but they are transferred to the nucleus and their expression is increased when cells are exposed to high temperature or shock (Xu *et al.* 2012). In 1962, Ferrucio Ritossa serendipitously discovered the response to heat shock in the form of heat-induced chromosomal puffing in salivary gland chromosomes of *Drosophila buscii* (Ritossa 1962). Later, Tissieres *et al.* (1974) observed that exposure to heat shock led to increased synthesis of a new kind of proteins in different tissues of *Drosophila melanogaster,* which were highly similar. However, it was observed that the concentrations of other proteins declined during heat shock (Tissieres *et al.* 1974). Based on initial studies in different organisms, HSP were considered to be upregulated in response to heat only, and were therefore named so. Later, it was found that in addition to temperature, several other stress factors are also responsible for higher expression of HSP (Kalmar and Greensmith 2009; Tóth *et al.* 2015). The most important function of HSP is to protect cells from stress by maintaining homeostasis and by assisting the folding of denatured proteins under stressed conditions (Hartl and Hayer-Hartl 2002). During heat-treatment, expression of cellular proteins is highly suppressed, while the expression of HSP mRNA is highly increased. HSP are essential to prevent the conformational changes in other proteins, prevent aggregation of misfolded proteins, refolding of misfolded proteins, support proteasomal removal of peptides that cannot be refolded and membrane protection. In addition, they are important for growth and development and have anti-apoptotic functions.

Types of Heat Shock Proteins

Initially, HSP were classified on the basis of their molecular weight into following groups:

(a) Small heat shock protein (sHSP) family
(b) HSP40 (J-proteins)
(c) Chaperonin (HSP60/GroEL) family
(d) 70-kDa heat shock protein (HSP70/DnaK) family
(e) HSP90 family
(f) HSP100/ClpB family.

It is pertinent to mention here that a few reports have also included ubiquitin (8.5 kDa) as one of the HSP class in eukaryotes (Vierling 1997). There is a distinct pattern of ATP usage in HSP. For example, high molecular weight HSP (hHSP; 27–110 kDa), i.e., HSP60, HSP70, HSP90 and HSP100 are ATP dependent while smaller HSP (sHSP; 15–42 kDa) are ATP independent. Expression of hHSP at euthermic or stress temperatures show distinct set of functions such as protein folding and translocation, cytoprotection, regulation of nuclear hormone receptors as well as regulation of apoptosis, whereas sHSP are mostly tissue specific, and play an important role as chaperone for protein folding as well as strong anti-apoptotic effectors. In 2009, Kampinga *et al.* proposed a new classification system for human HSP families and categorized them into (Kampinga *et al.* 2009):

(a) Small heat shock protein (HSPB)
(b) DNAJ (HSP40)
(c) HSPA (HSP70)
(d) HSPC (HSP90)
(e) HSPH (HSP110)
(f) Chaperonin family HSPD/E (HSP60/HSP10)
(g) CCT (TRiC)

In addition to the above HSP classes, human exclusively contain HSP33 (De Maio 1999). The detailed comparison between the different families of HSP is presented in the Table 1.

In the present chapter, we would be focusing primarily on the HSP of the HSP70 family, which are well characterized, ubiquitous and highly conserved ATP-dependent chaperones. In comparison to other HSP, HSP70 are the most prominent response to the heat stress, toxic chemicals and heavy metals. In stressed cells, HSP70 are mostly localized in the nucleus and nucleolus. Under stress, HSP70 are over-expressed, refolds denatured proteins and induces tolerance. In addition to helping the cell to survive in stress, HSP70 have several functions in unstressed conditions also, e.g., folding of nascent peptides, intracellular protein transport and apoptosis. It has also been shown that expression level profile of HSP70 vary for healthy and diseased conditions (Radons 2016).

Table 1 The heat shock protein families (Kaminga *et al.* 2009)

Characters	Family						
	Small heat shock protein (HSPB)	DNAJ (HSP40)	HSPA (HSP70)	HSPC (HSP90)	HSPH (HSP110)	HSPD/E (HSP60/HSP10)	CCT (TRiC)
Number of member in human	11 (mammals)	~50	13	5	4	–	–
Characteristics	HSPB have conserved α-crystalline C-terminal domain of 100 amino acids (de Jong *et al.* 1998)	HSP40 contains a conserved N-terminal J-domain that stimulates ATPase activity (Qiu *et al.* 2006)	HSPA contains N-terminal regulatory ATPase domain and C-terminal substrate binding domain (Gragerov *et al.* 1994; Zhu *et al.* 1996)	HSPC contain three regions: the N-terminal region, central region and C-terminal (Csermely *et al.* 1998)	The HSPH are homologous to HSPA. HSPH have longer linker region between N- and C-terminal domains (Kampinga *et al.* 2009)	HSPD has three domains i.e. apical domain, equatorial domain and intermediate domain these play important role in binding of substrate and co-chaperone, ATP binding and act as hinge prompting conformational change respectively (Fink 1999) and it is heat inducible protein	CCT is not upregulated during heat shock

Functions		Folding, refolding, translocation, responsible for stimulation of HSPA ATPase activity	Folding of newly synthesized proteins, protein transport across intracellular membrane, DNA repair	Suppress aggregation of unfolded proteins, disaggregates loose protein aggregates, and enhances refolding of partially denatured proteins and cellular signaling	Folding, nucleotides exchange factor and removal of ADP after ATP hydrolysis	Folding of nascent and misfolded proteins in ATP-dependent manner	Folding of newly synthesized cytosolic proteins, preventing protein aggregation
	HSPB proteins bind to unfolded, partially denatured and damaged proteins, and inhibiting their irreversible aggregation, retaining them in a refolding competent state, it provides protection aganist both apoptosis and oxidative stress						
Subcellular location	Cytoplasm, cytoskeleton and nucleus	Cytosol, nucleus, endoplasmic reticulum, mitochondria, endosomes and ribosomes	Mitochondria (HSPA9), endoplasmic reticulum (HSPA5)	Cytosol, endoplasmic reticulum and mitochondria	Cytosol and endoplasmic reticulum	Mitochondria and chloroplast	Cytosol
Comments	Present in both prokaryotes and eukaryotes that expressed under stress conditions	–	–	–	–	Represented by GroEL in prokaryotes and HSP60 in mitochondria	Present in eukaryotes

HSP70: Structure and Mechanism

There are three distinct regions of HSP70: (a) Conserved N-terminal ATPase domain or nucleotide binding domain (NBD) of ~40 kDa; composed of four subdomains (IA, IB, IIA, IIB) surrounding the ATP-binding pocket, (b) Substrate binding domain (SBD) of ~18-kDa and (c) Variable C-terminal of ~10-kDa. Each of the three domains has different functions. The SBD binds to the substrate proteins and their association is regulated by NBD. The variable C-terminal acts as a "lid" of HSP70 and helps to hold the substrates at SBD (Zhu *et al.* 1996). Human HSP70 (HSPA) is a dimer of N-terminal ATPase domain (45 kDa) (Flaherty *et al.* 1990) and a C-terminal peptide binding domain (25 kDa) (Zhu *et al.* 1996). A small linker domain separates the N-terminal domain and the C-terminal domain. In order to help in protein folding, HSPA repeatedly binds and release the unfolded protein. The binding occurs at hydrophobic regions, since they are exposed in unfolded proteins. This cyclic process is dependent on the ATPase activity of HSP70, which is assisted by co-chaperones J-proteins and nucleotide exchange factor (Miot *et al.* 2011). J-proteins induce the hydrolysis of ATP that is required for binding of solvent-exposed hydrophobic amino acids of substrate proteins whereas nucleotide exchange factor is associated with ATP–ADP exchange which release ADP from HSP70 and ultimately the substrate.

Functions of HSP70

The HSP70 family of proteins is housekeeping proteins and is highly conserved across all living domains. The major responsibilities of HSP70 are folding of nascent proteins in normal cells and refolding of denatured proteins under shock condition. Apart from this, HSP70 are also involved in multiple biological functions including import and translocation of proteins and vesicles into organelles across membranes, growth, apoptosis, proteolytic degradation of unstable proteins by targeting the proteins to lysosomes or proteasomes and the degradation of unwanted proteins. The functions of HSP70 family members highly depend on their cellular localization, and on the basis of their localization they are broadly classified in two types, intracellular and extracellular HSP70. The intracellular residing HSP70 protect cells against lethal damage induced by stress, and support folding and transport of newly synthesized polypeptides and aberrant proteins as well as assembly of multi-protein complexes. Further, extracellular HSP70 are considered as molecules with immunomodulatory functions, which act either as cross-presenters of immunogenic peptides via MHC antigen or in a peptide-free version as chaperokines or stimulators of innate immune responses. The major cellular functions of HSP and their molecular mechanism are as follows:

1. *Unfolding/refolding*: HSP70 family members under normal physiological conditions act as molecular chaperones. In response to the stress-induced damages,

intracellular HSP70 bind to the exposed hydrophobic amino acids of non-native conformation of proteins, thus protecting them against denaturation or aggregation until the cell attain the favorable condition (for reviews, see (Boston *et al.* 1996; Hartl 1996)). In conjunction with other chaperones i.e., dimeric HSP40 and co-chaperones i.e., nucleotide exchange factor (NEF), HSP70 recognizes stable misfolded polypeptides and convert them into native proteins by repeated cycle of binding, ATP-dependent unfolding, and spontaneous refolding. Improper unfolding/refolding phenomenon might lead to attachment of substrate to "holdases", including small HSP and HSP90, which maintain the substrate in a non-aggregated folding-component state and pass it to the HSP70 unfoldase machinery for refolding. Additionally, HSP70/HSP110 heterodimer converts protein aggregates into natively unfolded substrates and form NEFs by acting reciprocally on each other and, cooperatively, they efficiently disassemble stable protein aggregates.

2. *Anti-apoptotic Activity*: HSP70 are potent anti-apoptotic proteins and block apoptosis at many different levels. HSP70 block the mitochondrial translocation and activation of Bax, inhibiting mitochondrial membrane permeabilization and release of pro-apoptotic factors, and also inhibit assembly of death inducing signaling complex (DISC) (Gurbuxani *et al.* 2003; Lanneau *et al.* 2008).
3. *Repairing*: HSP70–1 in nucleus, assist the repairing machines of ssDNA by binding to poly (ADP-ribose) polymerase 1 (PARP-1), thus mediating their assembly and initiating their functions.
4. *Tumorigenic*: In cancer cells, a constitutive high-level expression of cytosolic HSP70 is observed frequently. Here, they provide resistance to stress-induced apoptosis, assist in suppressing default senescence, and are correlated with the development of metastasis and drug resistance. HSP70 stabilize the lysosomal membranes and affect autophagy, leading to the survival of cancerous cells. Cell senescence is initiated when HSP70-1 undergoes down-regulation via p53-dependent and p53-independent pathways (Yaglom *et al.* 2007). Another role of extracellular HSP70-1 in tumor invasion and metastasis comes from its ability to increase the MMP-9 expression by activating NF-κB and activating protein-1 (AP1) (Lee *et al.* 2006). However, cytosolic HSP70 have negative impact on cancer patients. Extracellular HSP70 are associated with cancer immunity and thus can be used as drug.
5. *Immunomodulation*: HSP70 act as stimulators of the adaptive immune response through their ability to bind antigenic peptide during intracellular antigen processing. The extracellular HSP70 may act as a danger signal to the innate immune system and is also relevant for the establishment of cancerous and autoimmune diseases. HSP70 exert anti-inflammatory properties, by modulation of cytokine production of dendritic cells that provide a link between innate and adaptive immune response.

HSP70 Superfamily in Mammals

In mammalian cells, there are four major isoforms of HSP70 localized in different organelles: the constitutively expressed heat shock cognate 70 (HSC70/HSPA8/HSP73) in the cytoplasm and nucleus, the stress-induced HSP70 (or HSP72/HSPA1A) in cytoplasm, the glucose-regulated BiP (or Grp78/HSPA5) in endoplasmic reticulum (ER) and mtHSP70 (Grp75/mortalin/HSPA9/mito-HSP70) in mitochondrion. Despite the difference in expression pattern of HSP70 and HSC70, their major functions are same i.e., to avoid protein aggregation; folding and assembly of nascent polypeptides, to refold misfolded or aggregated proteins, to enhance the ubiquitination and the degradation of misfolded protein. These proteins are also involved in translocation of protein through intracellular membrane and show interaction with signal transduction proteins. BiP is a major regulator of ER stress, which binds to the proteins transported to the ER and assist in the formation of quaternary structure. The mtHSP70 is mainly involved in protein transportation from mitochondria.

In humans, HSP70 has 13 members, which share several structural and functional features. For example, HSPA1 (HSP70) (reviewed in (Kampinga *et al.* 2009)) is induced by high temperature, has subfamilies called HSPA1A (HSP70-1) and HSPA1B (HSP70-2), which differ by only two amino acids. The gene sequence of another member, HSPA6 (HSP70B') is 77% similar to the HSPA1 gene. The expression of these proteins is transcriptionally controlled by Heat Shock Factors (HSF) which includes four members: HSF1, HSF2, HSF3 and HSF4. HSF have distinctive and overlapping functions and have tissue-specific patterns of expression. Among all HSF, HSF1 is the prime transcriptional regulator and is required for transactivation of HSP genes and maintenance of thermo-tolerance. During stress, HSF1 is induced and binds to the promoter of HSP70 to enhance its transcription.

Role of HSP70 in Human Diseases

As discussed earlier, the HSP70 chaperones are mainly involved in folding of translated proteins, intracellular localization and prevention of aggregation. Therefore, improper functioning of HSP lead to several diseases related to defects in protein folding or trafficking. The implications of malfunctioning of HSP70 in some of the major human diseases are discussed below:

HSP70 in Cancer

HSP70 act as an important factor in development of different types of cancers and can be used as potential tumor biomarker. Usually HSP70 is overexpressed on the cell surface of tumors. Because of their chaperonin activity as well as cell signaling

regulation activity, HSP70 are involved in tumor cell proliferation, differentiation, invasion, metastasis and death. However, in some cancers (renal and cervix), survival is not correlated with the Hsp70 levels. The sequential increase in the level of HSP70 has a potential prognostic value in patients with chronic hepatitis, liver cirrhosis and liver carcinomas intrahepatic cholangiocarcinoma (IH-ChCa) and metastatic tumors (Yang *et al.* 2010). Hence, change in the expression level of HSP70 could be used as a biomarker and prognosis in cancers like colon cancer, breast cancer, melanoma, bladder cancer, cholangiocarcinoma and squamous cell carcinoma of the head and neck (SCCHN). The clinical outcome of radiotherapy can also be monitored by ascertaining the levels of HSP70 in SCCHN patients. HSP70 are considered as favorable target for treating several cancers. The association of HSP70 and Bag3 (nucleotide exchange factor of HSP70) changes the activity of certain transcription factors (NF-κB, FoxM1, Hif1α), the translation regulator (HuR) and the cell-cycle regulators (p21, survivin) (Colvin *et al.* 2014). One of the ways to check the proliferation of tumors is to induce senescence; in some cases it is done by down-regulating HSP72 via p53-dependentand p53-independent pathways. HSP70 are also associated to base pair excision system, therefore inhibition of HSP70-based DNA repair in cancer cell might be important in chemotherapeutic regimens. Furthermore, the combination of the two chaperones HSP70 and HSP90 along with conventional anti-cancer drugs is a favorable therapeutic selection for patients suffering with advanced bladder cancer.

HSP70 in Apoptosis

Besides their role as molecular chaperones, HSP70 are also anti-apoptotic proteins. HSP70 inhibit the apoptosis at multiple points in intrinsic as well as extrinsic pathways. HSP70 interact with stress-induced kinases and inhibit their functions in apoptosis. In the intrinsic pathway, HSP70 inhibit the disruption of the mitochondrial membrane potential and help to prevent the release of pro-apoptotic factors such as cytochrome c and apoptosis-inducing factor (AIF) (Gurbuxani *et al.* 2003). In extrinsic pathway, it responds to the apoptotic stimulus by inhibiting the assembly of DISC (Lanneau *et al.* 2008). HSP70 also provide protection against hypoxia/reoxygenation-induced apoptosis and maintaining intestinal epithelial cells, with the increase in expression level of BCL-2.

HSP70 in Diabetes Mellitus

The expression of HSP70 is reported to high in type I diabetes mellitus (TIDM) and type II diabetes mellitus (TIIDM) (Nakhjavani *et al.* 2010). Generally, the pancreas regulates the levels of HSP70 where they protect the susceptible beta cells from exocrine pancreatic damage and from the stress associated with insulin hypersecretion. Recently, it has been shown that if the expression of HSP decreases in TIIDM patients, their wound healing process is impaired (Singh *et al.* 2015). It has also

been proposed that one of the most effective and feasible strategy to improve the glucose tolerance in hyperglycemic (i.e. high blood sugar) condition is to increase the HSP70 level, potentially by targeting hyperglycemia-related deficits in HSF1 (Kavanagh *et al.* 2011). HSP70 have a direct correlation with several molecules and can be used as an indicator for variety of diseases. For example, (i) increased serum HSP70 and hemoglobin A1c (HbA1c) levels in women indicates gestational diabetes mellitus, (ii) In patients with high C-reactive protein (CRP) and in case of hunger inhibiting hormone such as leptin, higher levels of HSP70 and asymmetric dimethyl arginine (ADMA) were reported. Furthermore, a relationship between chronic inflammation with diabetes mellitus and diabetes mellitus-associated albuminuria can be postulated from the higher levels of HSP70 observed in diabetic patients with albuminuria (i.e. presence of albumin in the urine).

HSP70 in Obesity, Non-alcoholic Fatty Liver Disease, Alcoholic Fatty Liver Disease and Hepatic Steatosis

The nonalcoholic fatty liver diseases (NAFLD) and hepatic steatosis (HS) induces the risk of type 2 diabetes (T2D) and cardio-cerebrovascular diseases (Qu *et al.* 2015a); moreover, obesity, NAFLD, alcoholic fatty liver disease (AFLD) and HS increase the inflammation. In case of NAFLD, there is a decrease in the expression level of HSF-1 of liver and adipose tissue, which affects the HSP70-dependent anti-inflammation. The HSP70 inhibition in NAFLD patients occurs in kupffer cells. Obese patients with NAFLD also have a lower HSP70 serum concentration (Di Naso *et al.* 2015). Additionally, in comparison to patients with mild alcoholic fatty liver disease (AFLD) or alcohol consuming individuals without AFLD, the lower level of HSP70 was found in AFLD patients (Qu *et al.* 2015b). In case of hepatocellular injury in AFLD patients, HSP70 shows increased positive immunoreactivity and could be used as a sensitive marker.

HSP70 in Chronic Glomerulonephritis

The expression of HSP70 in urine is also higher in case of higher chronic glomerunephritis (CGN) activity and transient creatinine as compared to inactive nephritis, active CGN and preserved renal function, and persistent proteinuria and chronic renal failure (Chebotareva *et al.* 2014).

HSP70 in Stroke and Seizure-Related Pathological Events

One of the vital functions of HSP70 is to prevent the occurrence of apoptosis in brain. The neuro-protective effect is achieved via anti-apoptotic mechanism in association with the overexpression of HSP70 (Zhao *et al.* 2014). Extracellular HSP70 facilitates the production of cytotoxic levels of tumor necrosis factor alpha via

TLR4/MyD88 signaling cascade, which results in increased neuronal death (Dvoriantchikova *et al.* 2014). In case of seizure related pathologic events also, HSP70 has a potential value as a sensitive and specific biomarker.

HSP70 in Helicobacter pylori Infection

Helicobacter pylori (*H. pylori*) are important causative agents of gastritis, peptic ulcer diseases, and mucosa associated lymphoid tissue (MALT) lymphoma and gastric cancer. It has been reported that HSP70 level changes significantly during *H. pylori* infection, viz. H. pylori-associated chronic gastritis, ulcerative colitis, and glutamine-treated patients (Leri *et al.* 1996). Studies also suggested that during *H. pylori* infection, HSP70 expression level decreases. This might involve the initiation of HSP70 expression for cytoprotection against *H. pylori* infection to prevent the expression of inducible nitric oxide synthase (iNOS) (Yeo *et al.* 2004). Pierzchalski *et al.* suggested that HSP70 protects cytoplasmic and nuclear proteins from the damaging effects of bacterial products by delaying the apoptosis of monocytes (Pierzchalski *et al.* 2014).

HSP70 in Atherosclerosis

Atherosclerosis is an inflammatory disease that affects a large human population. HSP70 concentration changes during the progression of atherosclerosis, and thus it is an effective biomarker to monitor atherosclerosis. However, there are contradictory examples over the correlation of HSP70 in atherosclerosis disease: Dulin *et al.* measured a significantly lower concentration of extracellular HSP70 in atherosclerosis patients (Dulin *et al.* 2010) while other group has reported that in patients suffering from carotid artery disease and chronic lower limb ischemia, the concentration of serum HSP70 changed depending on the severity of atherosclerosis (Krepuska *et al.* 2011).

Conclusions

HSP70 are expressed in normal cellular conditions where they regulate protein homeostasis facilitating protein folding and degradation. However, their expression is increased manifold when the cell is exposed to stress. HSP70 protect the cell from stress-induced protein unfolding and other adversities. Thus, their expression has important implications on progression of several human diseases. Therefore, HSP70 have promising role as bio-molecular marker in diagnosis of several diseases and as potential drug targets.

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