Current Topics in Biochemical Research
Vol. 16, 2014

Review

Molecular chaperones: Ubiquitous proteins with great diversity of function in health and disease

Paul R. Johnson[1,4], Parag P. Shah[2,5], Michelle T. Barati[1,6], Evelyne Gozal[3,7] and Madhavi J. Rane[1,*]

[1]Department of Medicine, [2]James Graham Brown Cancer Center, [3]Department of Pediatrics, University of Louisville, Louisville, KY 40202, USA.

ABSTRACT

Heat shock proteins (Hsps) are highly conserved ubiquitous proteins and are present both in prokaryotes and eukaryotes. They are essential for fundamental cellular processes and are induced in response to a wide variety of physiological and environmental stresses, allowing cells to survive in most adverse conditions. However, their basal expression in the absence of any stress demonstrates the important role they play in maintaining cellular homeostasis. So far, various functions of Hsps have been described including their role as a scaffolding protein, as molecular chaperones involved in folding and unfolding proteins, regulating cytoskeletal stability, cell cycle control, hormone signaling, important modulators of muscle contraction, cell migration, cell survival. They also play a role in antigen presentation and serve as death signals to help the immune system to recognize dead or damaged cells. Hsps regulate the activity of multiple intracellular signaling intermediates, many of which are involved in the execution of the apoptotic signaling pathways. Recently, interest has emerged to study the role of Hsps in the pathogenesis of various diseases including diabetes, renal fibrosis, neurodegenerative disorders, cardiovascular disease and cancer. This review describes in detail, the different functions of Hsps in health and disease. Taking into consideration, the wide variety of functions of Hsps in health and disease, Hsps have the therapeutic potential to serve in numerous clinical implications.

KEYWORDS: Hsps, sHsps, disease, EMT, chaperones, Akt and MK2

INTRODUCTION

Molecular chaperones are ubiquitous proteins involved in multiple housekeeping cellular processes as well as stress response and are abundantly expressed across all species. Some of the house keeping functions of molecular chaperones include import of proteins into cellular compartments, protein folding in the cytosol, mitochondria and endoplasmic reticulum, prevention of protein aggregation, refolding of misfolded proteins, degradation of unstable proteins, dissolution of protein complexes and control of regulatory proteins. Heat shock proteins were first described as proteins that could be induced upon temperature increase (shock) in *Drosophila melanogaster* [1] and have been shown to regulate cellular apoptosis [2]. More recently, heat shock proteins (Hsp) complexed with immunogenic peptides released from necrotic cells have been linked to host defense. It has been proposed that Hsps-peptide complex released from necrotic cells initiate an innate or adaptive response to cellular injury [3]. Extracellular Hsps-peptide complex *via* binding to pattern recognition receptors stimulate antigen-presenting cells (APC) and initiate a response to cell death. These immunogenic peptides elicit potent CD8+ T cell responses only

*Corresponding author: mjrane01@louisville.edu
[4]paulrj3@hotmail.com
[5]ppshah04@louisville.edu
[6]michelle.barati@louisville.edu
[7]Evelyne.Gozal@louisville.edu

if these peptides are chaperoned with Hsps, thus, making Hsps the first mammalian adjuvant [3]. Hsps in general can be categorized into two groups; the first group consists of the well-conserved ATP-dependent chaperones Hsp60, 70 and 90, which are involved in folding of nascent proteins or refolding of misfolded proteins, protein trafficking and complex assembly, regulation of multiple kinases and transcriptional regulation, as well as removal of proteins by proteasomal-dependent and independent pathways [4-6]. In *E. coli*, these chaperones are classified by different names, specifically DnaK/Hsp70, DnaJ/Hsp40, and GroEL/GroES system (Hsp60/Hsp10). In the GroEL/GroES chaperonin system, GroEL forms two apposed rings of heptameric structure joined with a single heptamer of GroES. This complex is essential to *E. coli* survival, and facilitates the proper folding of proteins in this bacterium [7]. A pro-apoptotic role for Hsp60/Hsp10 has been described in Hela and Jurkat cells. Hsp60/Hsp10 was shown to associate with pro-caspase-3 and induce its activation [8, 9]. In contrast, a protective role for over-expressed Hsp60 and/or Hsp10 has been described in cardiac myocytes undergoing ischemia/reperfusion injury [10]. These differences can be attributed to different cell types as well as different stimuli used in both studies. This Hsp protein family is highly conserved in evolution, depends on an ATP-regulated switch between conformations for chaperone activity and generally work as part of a team of co-chaperones [6, 11-13]. ATP-dependent conformation changes regulate substrate binding and release and can be targeted to modulate chaperone functions [11-13]. The second group consists of the small molecular weight Hsps (sHsp), which serve to protect cells from a variety of stresses [14] and are critical to cytoskeletal integrity [15]. The sHsps do not possess ATPase activity. Both ATP-dependent and small molecular chaperones are molecularly defined as sharing a conserved C-terminal region containing a core α-crystallin domain [14]. This domain was found to be the most highly conserved portion of these proteins across species (archea, bacteria, fungi, metazoan, plants, and other eukaryotes) having an average length of 94 amino acids (aa) [16]. The variable N and C terminal regions, which contain an average of 56 aa and 10 aa, respectively, show the least degree of homology among species. Since the core α-crystallin domain lacks chaperone activity, the N and C terminal regions are believed to give sHsp their specific functions. Not only do the N and C termini differ among species, but the number of Hsps across species can vary widely. The human genome has been reported to encode 10 sHsp [14], whereas plants have been shown to encode more than 20 sHsp across species [16]. Buchner and colleagues in their recent genome-wide comparison of stress proteins compared species from all three domains of life (archaea, bacteria, and eukarya) reveals all forms of life contain sHsp, albeit in varying amounts [16]. The 10 human sHsps discovered thus far have been given the formal name HspB 1 through 10 according to the HUGO Gene Nomenclature Committee classification. Several of these have been classified according to more common names, for example Hsp27/HspB1, MKBP/HspB2, Hsp20/HspB6, Hsp22/HspB8, and ODF1/HspB10. All HSPs chaperone proteins functions are highly regulated by post-translational modifications such as phosphorylation, acetylation, glycosylation, methylation, nitrosylation or ubiquitination [11, 17-21]. These post-translational modifications may regulate co-chaperone and client protein binding, transport and localization, as well as ATPase activity, therefore could allow therapeutic intervention to modulate diseases associated with the various Hsps functions.

Functions of ATP-dependent chaperones (Hsp60, Hsp70, and Hsp90)

In *E. coli*, Hsp70 (DnaK) complexes to Hsp40 (DnaJ) to facilitate the folding of nascent proteins [7]. In eukaryotes, on the other hand, Hsp70 and Hsp90 have long been recognized as chaperones implicated in protein folding but can also target proteins to proteasomal or non-proteasomal degradation [5, 22]. Hsps are very abundant in the cytosol but also localize in the nucleus and in various organelles [4, 6]. They bind, stabilize or target to degradation, and activate a multitude of protein substrates also referred to as client proteins. Over 200 HSP90 client proteins have been identified and more are being reported (see: http://www.picard.ch/downloads/Hsp90interactors.pdf). However no consensus binding sequence or motif has been identified among the client proteins.

By translocating to multiple subcellular locations, Hsp90 can regulate essential cellular functions involving protein complexes formation and stabilization such as RNA polymerase II assembly, teleomere complex assembly, proteasome assembly and RISC complex assembly [6]. Hsps mechanism of action involves homo- or hetero-dimerization and binding to co-chaperones and other proteins [4, 6]. Hsp90 is structurally composed of a highly conserved N-terminal domain that harbors a nucleotide-binding domain for ATP binding and several co-chaperone binding motives, connected by a highly conserved and charged linker domain to a middle domain for substrate binding. Finally, a C-terminal domain that harbors a MEEVD motif for a dimerization or binding of tetratricopeptide repeat-containing co-chaperones [6, 11, 13]. Hsp70 harbors the nucleotide binding domain, a linker region and a C terminal domain containing the substrate-binding domain [12]. Binding of a client protein and ATP changes the conformation of Hsp90 from an open conformation to a closed conformation with N-terminal domains from each monomer binding to each other, allowing ATP hydrolysis and re-opening the conformation to release the substrate [12, 13]. Therefore substrate binding and release are highly regulated by ATP hydrolysis, and by changes in dimer conformation as well as multiple post-translational modifications. A nascent protein coming off the ribosome during translation binds Hsp70, followed by Hsp90 binding [23]. Protein folding in mitochondria was first shown to be catalyzed by Hsp60 in an ATP-dependent manner. In addition, loss of Hsp60 in yeast led to deficiencies in mitochondrial protein assembly [24]. If a protein becomes misfolded, these chaperones are sometimes able to re-fold it into its correct conformation; otherwise, the protein can get degraded at the proteasome (loss of function) or form toxic aggregates through protein accumulation (gain of function) [23]. Regulation of these two opposite functions depends on the binding of co-chaperones such as CHIP and HOP to Hsp70 and Hsp90 C-termini. Phosphorylation of their C-termini by such kinases as CK1, CK2 and GSK3-β, prevents binding to CHIP, enhancing binding to HOP and protein folding rather than ubiquitination and degradation [22, 25, 26].

The classification and main functions of Hsps are summarized in Table 1. Both gain-of-function and loss-of-function misfolding have been described. Loss of function occurs when a necessary, but misfolded, protein is sent to the proteasome for degradation. Some diseases linked to this phenomenon are various cancers from loss of p53, nephrogenic diabetes insipidus from loss of vasopressin/aquaporin, and Fragile X Syndrome where the fragile X mental retardation protein is lost. Some well-known diseases from gain of function, which occurs when the misfolded protein forms aggregates, include Alzheimer's disease from β-amyloid and Tau proteins, Huntington's disease from aggregation of Huntingtin protein, and amyloidosis from Amyloid A protein (see Ref. [23] for a comprehensive list). Therefore, knowledge of the role of these molecular chaperones in diseases involving protein aggregation has led to the search for drugs that target Hsp70 and Hsp90 [23]. Several inducers of Hsp70 exist, some of which include the antioxidants α-lipoic acid, acetyl-L-carnitine, and resveratrol. However, this is not always the case with antioxidants, as quercetin has been demonstrated to inhibit Hsp70 in prostate cancer cell lines [23]. In addition, several prostaglandins have been reported to induce Hsp70. Despite the wide array of Hsp70 inducers, these compounds can act via direct stimulation of the Hsp70 machinery, or by first causing cellular stress that induces Hsp70. The principal inhibitors of Hsp90 are geldanamycin, novobiocin, and a more potent novobiocin derivative named "A-4". Interestingly, these same inhibitors of Hsp90 have an inducing effect on Hsp70, and this is believed to result from disruption of the cytosolic Hsp70/Hsp90/HSF-1 (heat shock factor-1) complex, allowing HSF-1 translocation to the nucleus. Activated HSF-1, a transcription factor, induces Hsps gene transcription [23]. Researchers investigating heat shock proteins in *D. melanogaster* decades ago at first concluded little association of Hsps with mitochondria [1]. However, as previously mentioned, Hsp60 was later recognized as playing a vital role for protein folding within mitochondria [24]. In addition, Hsp70 and Hsp90 have been found to play important roles in transporting proteins to the mitochondria [27]. For proteins to enter the mitochondria, it has long been

Table 1. ATP-dependent heat shock proteins and their functions.

Common name	Functions	References
Hsp110	Normal protein folding, thermotolerance,	[100]
	Ischemic tolerance	[101]
Hsp90	Protein folding and activation of assembly,	[102]
	Signal transduction and transcriptional regulation	
	Suppresses inflammation	[103]
	Alters expression of matrix-degrading enzymes	[104]
Hsp70	Normal protein folding and transport,	[105]
	Thermotolerance, cell protection	[106]
	Extracellular HSP70 suppresses cytokine secretion from FLS	[107]
	Involved in tolerance and autoimmune processes	[108]
Hsp60	Mitochondrial molecular chaperon, involved in protein folding after proteins have been dragged into the mitochondrion	[109]
Hsp40	Regulate ATP-dependent polypeptide binding	[110]
	by Hsp70 protein, regulate complex formation	[111]
	between Hsp70 and polypeptides	

recognized that the TOM (translocase of the mitochondrial outer membrane) complex facilitates pre-protein entry into outer mitochondrial membrane [28]. Earlier research demonstrated these Hsp70 and Hsp90 were important for bringing proteins to the TOM complex (specifically TOM70), and that the ATPase functions of Hsp70 and 90 were needed to release the transported protein to the TOM receptors [28]. Furthermore, it was believed that TOM70 alone transferred pre-proteins to the translocation pore (TOM40 complex). Recent work by Fan *et al.* has demonstrated that Hsp90 is important for both pre-protein targeting as well as translocation into the outer mitochondrial membrane [28]. Overall these studies uncovered a critical role for these Hsps in protein trafficking to the mitochondria. Using inhibitors of Hsp90, geldanamycin (GA) and novobiocin (NB), this group showed differential effects of the drugs on mitochondrial protein targeting mediated by Hsp90. Interestingly, novobiocin (which targets the C-terminal region of Hsp90) blocked pre-protein association with Hsp90 and blocked TOM70 association with Hsp90 to prevent pre-protein import. Geldanamycin (an inhibitor of N-terminal ATP-binding site of Hsp90), however, had no effect on pre-protein association with Hsp90, but prevented TOM70 import of the pre-protein by unknown mechanisms at the time [28].

Later evidence on protein trafficking revealed chimeric targeting signals exists on pre-proteins. It was originally thought that peptides contained unique signals that direct them to one of several different organelles (endoplasmic reticulum (ER), peroxisomes, and mitochondria). In the case of cytochrome P450s (CYP), Avadhani's group demonstrated that these proteins contain bimodal targeting sequences in which a cryptic (or hidden) mitochondrial targeting sequence is flanked by N-terminal ER targeting sequences [27]. Cytoplasmic endoproteases or phosphorylation of

certain serine residues exposed the hidden mitochondrial sequence. This group studied the role of Hsp70 and Hsp90 in allowing for pre-protein bypass of the peripheral TOM20 and TOM22 receptors. Using an *in vitro* protein translation system of ^{35}S-labeled CYPs imported into rat mitochondria, they showed CYP+33/1A1 (an N-terminal truncated form of CYP1A1) and CYP2E1 could bypass TOM20 and TOM22, sending the protein directly to the TOM40 translocation pore. However, Hsp70 alone brought these CYPs through the typical TOM20→ TOM22→ TOM 40 pathway. In case of CYP+5/1A1 (a different N-terminal truncation mutant) and CYP2E1, all three peripheral TOMs (TOM20, TOM22, and TOM70) were required for protein import in the presence of Hsp70 alone. However, when both Hsp70 and Hsp90 were present, these pre-proteins only required TOM70 for mitochondrial import. Similar to other models of Hsp70 and Hsp90 interaction [2] these authors found a requirement of Hsp70 first binding to the pre-protein before Hsp90 association [27]. One rationale for the observed differences in CYP import is the ability of the different Hsps (Hsp70 and Hsp90) to bind peripheral TOMs. For example, the proline-rich domain of TOM40 was shown to be the binding site for Hsp90, indicating how the presence of both Hsp70 and Hsp90 can facilitate bypass of certain TOMs (20 and 22 in this case).

In addition to their roles in protein trafficking, new evidence reveals an important role for Hsps as transcriptional co-factors. The vitamin D receptor (VDR) is one of several receptors belonging to the nuclear receptor (NR) superfamily. The NR superfamily consists of two distinct classes, class I and II. Among the class I NR are the steroid receptors: androgen, estrogen, progesterone, glycocorticoid, and mineralocorticoid receptors. VDR receptor belongs to the class II NR which, (similar to other nuclear receptors in this family, namely thyroid hormone, retinoids, and peroxisome proliferators), forms a heterodimer with retinoid X receptor (RXR) [29]. When class II NR dimerize with RXR, they influence gene transcription by binding to either direct repeat (DR) or inverted repeat (IR) elements within the promoters of target genes to influence gene transcription rates [30]. It has been known for some time that PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1α) functions as a co-activator with the class II nuclear receptors (NR) such as CAR, FXR, LXR, PPAR-α, PPAR-γ, PXR, and VDR in ligand and ligand-independent manners [31]. Recent evidence from the Wood Laboratory now implicates Hsp90 as a mediator of Vitamin D activation [32].

Using a Caco-2 human intestinal line that is known to be vitamin D responsive, the authors demonstrated that chemical inhibition of Hsp90 using geldanamycin (which blocks the ATPase domain of Hsp90) and siRNA to Hsp90β, impaired VDR responsiveness to $1\alpha,25(OH)_2D_3$ (the active form of vitamin D) stimulation. In addition, downstream activation of CYP24 (a known responsive gene to active vitamin D), was attenuated with geldanamycin (GA) treatment. Similar results were found when shRNA was used to knockdown Hsp90. The authors treated Caco-2 cells with or without active vitamin D and geldanamycin, and showed reduced DNA binding activity of VDR to the VDRE (Vitamin D response element) in cells treated with GA+Vit D versus no GA+Vit D. In summary, these studies demonstrate a vital role for Hsp90 in VDR activation [32]. However, separate studies suggest Hsp90 inhibition may not always be beneficial. In PC12 cells, geldanamycin has been demonstrated to facilitate Hsp90 complex disruption and production of reactive oxygen species (ROS). These effects were blocked by the antioxidant N-acetyl cysteine (NAC) suggesting that Hsp90 is directly involved in ROS production, glutathione depletion, and cytotoxicity, in these cells [5].

Thus, the myriad outcomes of Hsp90 disruption seem to have both beneficial and potentially deleterious effects in human health. Activation of VDR by Hsp90 is important as low vitamin D levels (and low VDR activation) have been strongly linked to various cancers, such as those of the breast, colon and prostate [33]. On the other hand, other data suggests activation of Hsp90 could lead to elevated levels of ROS [5], which has also been linked to inflammatory diseases and cancer pathogenesis. From these studies, one might conclude that a specific balance of these chaperones is important for maintaining optimum health.

Functions of small Hsps (sHsp)

The small Hsps are a group of proteins similar, but uniquely distinct from, the ATP-dependent chaperones (Hsp60, Hsp70, Hsp90). sHsps vary in size from 15 to 30 kDa and to date nine different members of this family have been identified: Hsp10, Hsp27, p20, HspB3, MKBP/HspB2, HspB8, HspB9, cvHsp, α-A crystallin and α-B crystallin [34]. Although members of this family share low amino acid homology, they are grouped together based on similar structural and functional properties, with all sHsps having a conserved core region that was first identified within the α crystallin proteins of the vertebrate eye [35]. As mentioned earlier, unlike Hsp70 or Hsp90, genome data from a variety of different species shows a wide array of functional variance of the sHsp [16]. The classification and main functions of small Hsps are summarized in Table 2.

Hsp27 has been reported to be virtually detected in all organisms from prokaryotes to mammals [36]. Like many other Hsps, Hsp27 has been shown to be highly inducible following exposure to variety of stressors, thereby enhancing cellular resistance to heat shock, oxidative stress and inflammatory mediators such as tumor necrosis factor (TNF)-α *in vitro* and *in vivo* [37, 38]. Moreover, in several *in vitro* models Hsp27 has been shown to inhibit apoptosis following oxidative stress [39]. Hsp27 is a substrate for phosphorylation by mitogen-activated protein kinase-activated protein kinase-2 (MAPKAPK-2) [40] and is believed to modulate the cytoskeletal arrangement of actin filaments in pulmonary endothelial cells, in a manner which is at least partially dependent on its phosphorylation state [41, 42]. Hsp27 regulates neutrophil apoptosis through the interaction of Hsp27 with protein kinase B (Akt) [19, 21]. Activation of Akt has been demonstrated to inhibit apoptosis in the variety of systems [43, 44]. Hsp27 has been reported to block the release of cytochrome c from mitochondria in the cells exposed to staurosporine, etoposide or cytochalasine D [45]. It also mediates inhibition of pocaspase-3 activation, through its ability to prevent initiator caspases like caspase-9 from gaining access to the residues whose cleavage is essential for

Table 2. Small heat shock proteins and their functions.

Common name	Functions	References
Hsp27	Chaperon, anti-apoptotic,	[34]
	Regulates f-actin formation	[45]
	Involved in cell migration	[49]
MKBP	Myotonic dystrophy kinase binding protein, Enhances DMPK activity and protects it against heat inactivation	[112]
αA & αB-crystallin	Forms multimers with other sHsps	[113]
Hsp22, H11 kinase	Promotes cells survival, and increase resistance to oxidative stress	[114]
Hsp20	Regulates f-actin, cytoskeletal structures,	[115]
	Suppresses platelet aggregation	[116]
Hsp10	Chaperone in mitochondria, immunomodulation	[117]
	and cell proliferation and differentiation	[118]
		[119]

procaspase-3 activation. Moreover, Hsp27 has been reported to block DAXX-mediated apoptosis by preventing its translocation to the membrane and thus, inhibiting its interaction with Fas and ASK- [46]. Hsp27 also participates in smooth muscle contraction [47]. They showed bombesin-induced contraction of permeabilized intestinal smooth muscle cells was inhibited by anti Hsp27 antibody treatment [47]. Subsequently, several groups found that inhibiting the p38 MAPK/MK2/Hsp27 signaling pathway reduces contraction in variety of smooth muscles [48-50].

Cell proliferation and migration depends on actin filament remodeling which involves detachment and rounding of cells. Landry and coworkers previously showed that p38 MAPK pathway was necessary in migration of vascular endothelial cells [51]. Consistent with these results, Hedges *et al.* showed that activation of p38 Map kinase leading to Hsp27 phosphorylation is necessary for migration of tracheal smooth muscle cells [52]. Subsequently, several other groups have demonstrated that the p38 MAPK/MK2/Hsp27 pathway is necessary for migration of vascular smooth muscle cells [49], fibroblasts [53] and breast epithelial cells [54].

A separate group recently demonstrated the ability of human Hsp27 overexpression (OX) to protect against hepatic ischemia/reperfusion injury in mice [55]. In rats undergoing ischemia reperfusion (IR), overexpression of human Hsp27 in rat liver cells reduced apoptosis (as measured by active caspase 3) versus wild type Hsp27 levels. In addition, Hsp27 OX was able to attenuate vascular permeability of rat liver cells after ischemia and reperfusion. The findings from this study supports the earlier work of Tezel and Wax who postulated increased serum levels of Hsp27 autoantibodies in glaucoma patients as an underlying cause of retinal cell death in these patients. By treating human retinal cells with an exogenous Hsp27 antibody, the authors were able to demonstrate increased apoptosis in these cells supporting the idea that loss of Hsp27 function is associated with increased cell death [56]. In human neutrophils, our group showed that pre-treatment of neutrophils with Hsp27 antibody led to increased cell death as evidenced by Annexin V binding and assessed by electron microscopy [19, 21]. These findings were subsequently shown to be due to the requirement of Hsp27 dependent-Akt activation, as loss of Hsp27 prevented Akt activation, by MK2, which acts as PDK2 for Akt, promotes cellular survival [57]. Disruption of Akt-Hsp27 resulted in inhibition of Akt activity and key upstream positive regulators of Akt such as p38 MAPK, mitogen-activated protein kinase-activated protein kinase-2 (MAPKAPK2), and 3-phosphoinositide-dependent protein kinase-1 (PDK1), dissociated from the Akt signal complex. In contrast, Akt-Hsp27 disruption resulted in recruitment of PKC-δ, a negative regulator of Akt, within the Akt signaling complex. In addition, apoptosis-related proteins PARP-2, caspase-3, and caspase-8 also associated with the Akt signal complex after disruption of the Akt-Hsp27 interaction, suggesting that disruption of the Akt signaling complex may promote caspase-3 activation, leading to neutrophil apoptosis [58]. Thus, this strategy can have therapeutic potential to inhibit Akt activity and induce neutrophil apoptosis in neutrophil-mediated inflammatory pathologies.

Heat shock proteins and their clinical implications

Given their ubiquitous nature, it comes as little surprise that a wide array of diseases has been linked to aberrant Hsp function. Cells and tissues from a wide variety of tumors have been shown to express unusually high levels of Hsps including Hsp27 and/or Hsp70. Hsp27 and Hsp70 have been shown to increase the tumorigenic potential of rodent cells transplanted into syngeneic hosts [59-60]. Hsp27 over expression has been also reported to increase the metastatic potential of human breast cancer cells inoculated into nude mice [61]. Antisense constructs of Hsp70 have been shown to sensitize cancer cells to apoptosis and to eradicate tumors (breast and colon carcinomas) in various models [62, 63] indicating the potential role of Hsp27 and Hsp70 in tumorigenesis. Increased expression of Hsp70 has been correlated with high-grade malignant tumors including endometrial and renal cell tumors [64]. In human renal cell carcinoma, diversity in expression of Hsp27 isoforms represents an useful tumor marker [65]. In prostate cancer, Hsp27 is an independent predictor of clinical outcome. A low

level of expression of Hsp27 is associated with a delay in prostrate tumor progression [66]. Expression of Hsp27 in primary breast cancer has been shown to be associated with short survival for node-negative patients [67]. Moreover, Hsp27 staining in case of ovarian carcinoma has been shown to be associated with decreased survival [68]. Feng and coworkers have demonstrated that evaluation of the soluble level of Hsp27 in human serum may be a useful biomarker for breast cancer or hepatocellular carcinoma [69]. By deletion mutagenesis previous studies from our laboratory has identified acidic linker region (amino acids 117-128) on Akt as an Hsp27 binding region [21]. By immunoprecipitation and glutathione *S*-transferase pulldown studies, we showed that deletion of amino acids 117-128 on Akt resulted in loss of its interaction with Hsp27 and MK2 but not with Hsp90 as Hs90 binding site on Akt is distinct from Hsp27 [21]. Our studies demonstrated that Hsp27 is positive regulator of Akt. Moreover, Hsp27 is expressed at high levels in clinical breast tumors and possibly promotes tumor progression by activating Akt [61]. We believe targeted delivery of excess amount of recombinant TAT-$Akt_{117-128}$ protein at tumor sites will competitively inhibit endogenous Akt-Hsp27 interaction. The consequence of this disruption will inhibit Akt activation and will induce apoptosis of tumor cells. Thus, TAT-$Akt_{117-128}$ protein may serve as a therapeutic target to control cancer.

Hsp90 is an abundant chaperone protein which is expressed by all eukaryotic cells. In addition, Hsp90 expression and/or ATPase activity has been reported to be increased in cancer cells including breast. Hsp90 over expression is associated with the expression of estrogen receptor and HER2/erbB2, as has been described as an independent marker of bad prognosis associated with decreased survival [70]. Increased Hsp90 activity has also been shown to be associated with lung cancer in which the epidermal growth factor receptor is mutated [71]. Several inhibitors of Hsp90 are currently undergoing the PhaseI/II trials to find the better therapeutic regimen for cancer therapy. It has been shown that Hsp70 expression is induced following treatment with Hsp90 inhibitors which has been well demonstrated *in vitro*, in leukemic cells and peripheral blood mononuclear cells [72], and also *in vivo*, in tumors of treated patients [73]. Moreover, depletion of Hsp70 by siRNA strongly increases cells sensitivity to 7-AAG [74], indicating that Hsp70 inhibitors can be use as local chemo-sensitization agents in Hsp70-expressing human tumors. Furthermore, over expression of the small heat shock protein α B crystalline has been observed in glial tumors such as astrocytoma, glioblastoma and oligodendroglioma [75], and in renal carcinoma tumors [76]. Xu *et al.* recently reported that Hsp70 may be served as therapeutic target towards laryngeal carcinoma radiotherapy by inhibiting degradation of nucleolin which is known to be involved in cell proliferation [77]. Epithelial mesenchymal transition (EMT) has been shown to be a key step during tumor progression and metastasis [78]. More recently Slotta-Huspenina *et al.* [79] in their study involving adenocarcinoma patients showed that drugs against Hsp90, which have been recently introduced in cancer treatment, may be used to treat adenocarcinoma tumors by targeting Hsp90 alone or in combination with Her2. Extracellular Hsp90 (eHsp90) has been shown to promote cell motility and invasion in cancer cells and metastasis in preclinical models [80]. Recently, Liu *et al.* [81] have further provided evidence that Hsp70 attenuates epithelial-to-mesenchymal transition of peritoneal mesothelial cells by inhibiting high glucose-induced Smad3 activation. Therefore, the family of molecular chaperones (Hsp90) can be considered as possible therapeutic targets in the treatment of a variety of human cancers.

Serum Hsp27 concentrations were found to be lower in patients with type-1 diabetes and this correlated with large nerve fiber dysfunction and higher levels of serum Hsp27 was associated with better nerve function and fewer neuropathic signs in type 2 diabetes [82, 83]. Moreover, the EURODIAB prospective complications study identified Hsp27 as a novel circulating marker for diabetic neuropathy but not a marker for vascular complications in type 1 diabetes [84, 85]. Hsps including Hsp25, Hsp60, Hsp70, and Hsp90 expression/phosphorylation is modulated in the glomeruli and in the medulla of streptozotocin (STZ)-induced diabetes rats [86]. Over-expression of Hsp27 transgene in the pancreatic beta cells

attenuated STZ-induced diabetic nephropathy *in vivo* while *in vitro* Hsp27 over-expression inhibited cytokine-induced islet apoptosis [87]. In an additional study with MK2 (-/-) mice, it was demonstrated that loss of MK2 and loss of its downstream signaling including Hsp27 phosphorylation had no protective effects on STZ-induced early diabetic nephropathy [88]. Djamali and his group has demonstrated a role for Hsp27 in regulating kidney tubulointerstitial fibrosis and they have demonstrated that over-expression of Hsp27 in renal tubules inhibits fibrogenesis in obstructive nephropathy [89-91].

Plasma levels of soluble Hsp27 have been shown to be lower in patients with atherosclerosis compared to healthy controls [92]. Serum Hsp27 levels may serve as a therapeutic target for atherosclerosis [93]. Wang *et al.* showed that resveratrol enhances Hsp27 expression in cultured human aortic smooth muscle and reduces proliferation [94]. Hsp27 has been shown to be phosphorylated in Gemcitabine-resistant pancreatic cancer cells and phosphorylated form of Hsp27 may be involved in providing the resistance to Gemcitabine in pancreatic cancer cells [95]. Thus, Hsp expression and secretion may prove to be beneficial in a wide range of clinical applications.

Severity of cardiovascular disease is characterized by existence of heat shock proteins in the peripheral circulation, presence of which has been reported by many investigators [96-98]. Zhu and coworkers measured serum Hsp70 concentrations in individuals evaluated for CAD (coronary artery disease) by coronary angiography, and found that concentrations are significantly higher in patients without evidence of CAD, indicating that heat shock proteins influence the progression of cardiovascular disease [97]. Moreover, Hsp70 has been shown to involve in ischemic preconditioning. Overexpression of the major 70-Kda heat shock protein (Hsp70) in the transgenic mice has been shown to improve myocardial function [99]. Thus, it is clear that Hsps expression is altered in a great variety of diseases and therefore modulation of Hsp signaling may allow development of novel therapeutic strategies to control disease phenotype.

CONCLUSIONS

In conclusion, Hsps are rapidly and ubiquitously engaged in response to physical, chemical, and environmental stresses and have been shown to functionally integrate several important signaling events including proliferative, migratory, survival, and cell death. Taking into consideration the wide variety of their functions, these Hsps have significant therapeutic potential in diagnosis and treatment of a variety of diseases including cancer.

ACKNOWLEDGEMENTS

This work was supported by a grant from NIH R01AI075212 (MJR).

AUTHOR'S CONTRIBUTION

PRJ, PPS, MTB, EG were responsible for collection of information and writing the draft of the manuscript. MJR has provided important input in writing the manuscript and critically revising the manuscript. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

None declared.

ABBREVIATIONS

Hsp27, heat shock protein 27; Hsp70, heat shock protein 70; Hsp90, heat shock protein 90; Hsp60, heat shock protein 60; Hsp10, heat shock protein 10; sHsps, small molecular weight heat shock proteins; PDK1, phosphoinositide-dependent kinase-1; PDK2, phosphoinositide-dependent kinase-2; MAPK, mitogen-activated protein kinase; MK2, MAPK-activated protein kinase-2; TOM, translocase of the mitochondrial outer membrane; CYP, cytochrome P450s; EMT, epithelial to mesenchymal transition.

REFERENCES

1. Velazques, J. M., DiDomenico, B. J. and Lindquist, S. 1980, Cell, 20, 679.
2. Garrido, C., Gurbuxani, S., Ravagnan, L. and Kroemer, G. 2001, Biochem. Biophys. Res. Commun., 286(3), 433-42.
3. Anderson, K. M. and Srivastava, P. K. 2000, Immunol. Lett., 74(1), 35-9.
4. Pratt, W. B. and Toft, D. O. 2003, Exp. Biol. Med., 228, 111-133.

5. Clark, C. B., Rane, M. J., El Mehdi, D., Miller, C. J., Sachleben Jr., L. R. and Gozal, E. 2009, Free Radic. Biol. Med., 47(10), 1440-1449.
6. Makhnevych, T. and Houry, W. A. 2012, Biochim. Biophys. Acta, 1823, 674-682.
7. Voet, D. and Voet, J. 2004, Biochemistry, 3rd Ed., 291-294.
8. Samali, A., Cai, J., Zhivotovsky, B., Jone, D. P. and Orrenius, S. 1991, EMBO J., 18, 2024-2048.
9. Xanthoudakis, S., Roy, S., Rasper, D., Hennessey, T., Aubin, Y., Cassady, R., Tawa, P., Ruel, R., Rosen, A. and Nicholson, D. W. 1999, EMBO J., 18, 2049-2056.
10. Lin, K. M., Lin, B., Lian, I. Y., Mestril, R., Scheffler, I. E. and Dillmann, W. H. 2001, Circulation, 103, 1787-1792.
11. Li, J., Soroka, J. and Buchner, J. 2012, Biochim. Biophys. Acta, 1823, 624-635.
12. Mayer, M. P. 2010, Mol. Cell, 39(3), 321-31.
13. Mayer, M. P. 2013, Trends Biochem. Sci., 38(10), 507-14.
14. Kappe, G., Franck, E., Verschuure, P., Boelens, W. C., Leunissen, J. A. M. and de Jong, W. W. 2009, Cell Stress & Chaperones, 8, 53.
15. Guay, J., Lambert, H., Gingras-Breton, G., Lavoie, J. N., Huot, J. and Landry, J. 1997, J. Cell Sci., 110(Pt 3), 357-68.
16. Kriehuber, T., Rattei, T., Weinmaier, T., Bepperling, A., Haslbeck, M. and Buchner, J. 2010, FASEB J., 156992.
17. Barati, M. T., Rane, M. J., Klein, J. B. and McLeish, K. R. J. 2006, Prot. Res., 5, 1636-1646.
18. Cloutier, P. and Coulombe, B. 2013, Biochim. Biophys. Acta, 1829, 443-454.
19. Rane, M. J., Pan, Y., Singh, S., Powell, D. W., Wu, R., Cummins, T., Chen, Q., McLeish, K. R. and Klein, J. B. 2003, J. Biol. Chem., 30, 27828-27835.
20. Jog, N. R., Jala, V. R., Ward, R. A., Rane, M. J., Haribabu, B. and McLeish, K. R. 2007, J. Immunol., 178(4), 2421-8.
21. Wu, R., Kausar, H., Johnson, P., Montoya-Durango, D. E., Merchant, M. and Rane, M. J. 2007, J. Bio. Chem., 282(30), 21598-21608.
22. Muller, P., Ruckova, E., Halada, P., Coates, P. J., Hrstka, R., Lane, D. P. and Voijtesek, B. 2013, Oncogene, 32(25), 3101-3110.
23. Sloan, L., Fillmore, M. and Churcher, I. 2009, Curr. Opin. Drug Discov. Devel., 12, 666.
24. Ostermann, J., Horwich, A. L., Neupert, W. and Hartl, F. U. 1989, Nature, 341, 125.
25. Johnson, B. D., Schumacher, R. J., Ross, E. D. and Toft, D. O. 1998, J. Biol. Chem., 273, 3679-3686.
26. Connell, P., Ballinger, C. A., Jiang, J., Wu, Y., Thompson, L. J., Hohfeld, J. and Patterson, C. 2001, Nat. Cell Biol., 3, 93-96.
27. Anandatheerthavarada, H. K., Sepuri, N. B. V. and Avadhani, N. G. 2009, J. Biol. Chem., 284, 17352.
28. Fan, A. C. Y., Bhangoo, M. K. and Young, J. C. 2006, J. Biol. Chem., 281, 33313.
29. Olefsky, J. M. 2001, J. Biol. Chem., 276, 36863.
30. Hermanson, O., Glass, C. K. and Rosenfeld, M. G. 2002, Trends in Endocrinology and Metabolism, 13, 55.
31. Finck, B. N. and Kelly, D. P. 2006, J. Clin. Invest., 116, 615.
32. Angelo, G., Lamon-Fava, S., Sonna, L. A., Lindauer, M. L. and Wood, R. J. 2008, Biochem. Biophys. Res. Commun., 367, 578.
33. Slattery, M. L. 2007, Nutrition Reviews, 65, 102.
34. Concannon, C. G., Gorman, A. M. and Samali, A. 2003, Apoptosis, 8, 61.
35. De Jong, W. W., Leunissen, J. A. and Voorter, C. E. 1993, Molecular Biology and Evolution, 10, 103.
36. Parsell, D. A. and Lindquist, S. 1993, Annu. Rev. Genet., 27, 437.
37. Hastie, A. T., Everts, K. B., Zangrilli, J., Shaver, J. R., Pollice, M. B., Fish, J. E. and Peters, S. B. 1997, Am. J. Physiol., 273, L401.
38. Meredino, A. M., Paul, C., Vignola, A. M., Costa, M. A., Melis, M., Chiappara, G., Izzo, V., Bousquet, J. and Arrigo, A. P. 2002, Cell Stress Chaperones, 7, 269.
39. Arrigo, A. P. 2001, IUBMB Life, 52, 303.
40. Knauf, U., Jakob, U., Engel, K., Buchner, J. and Gaestel, M. 1994, EMBO J., 13, 54.

41. Guay, J., Lambert, H., Gingras-Breton, G., Lavoie, J. N., Huot, J. and Landry, J. J. 1997, Cell Sci., 110, 357.
42. Hirano, S., Rees, R. S., Yancy, S. L., Welsh, M. J., Remick, D. G., Yamada, T., Hata, J. and Gilmont, R. R. 2004, Cell Biol. Toxicol., 20, 1.
43. Ahmed, N. N., Grimes, H. L., Bellacosa, A., Chan, T. O. and Tsichlis, P. N. 1997, Proc. Natl. Acad. Sci. USA, 94, 3627.
44. Kennedy, S. G., Wagner, A. J. and Conzen, S. D. 1997, Genes & Development, 11, 701.
45. Paul, C., Manero, F., Gonin, S., Kretz-Remy, C., Virot, S. and Arrigo, A. P. 2002, Mol. Cell. Biol., 22, 816.
46. Charette, S. J., Lavoie, J. N., Lambert, H. and Landry, J. 2000, Mol. Cell. Biol., 20, 7602.
47. Bitar, K. N., Kaminski, M. S., Hailat, N., Cease, K. B. and Strahler, J. R. 1991, Biochem. Biophys. Res. Commun., 181, 1192.
48. Meier, M., King, G. L., Clermont, A., Perez, A., Hayashi, M. and Feener, E. P. 2001, Hypertension, 38, 1260.
49. Lee, H. M., Won, K. J., Kim, J., Park, H. J., Kim, H. J. and Roh, H. Y. 2007, J. Pharmacol. Sci., 103, 427.
50. Macintyre, D. A., Tyson, E. K., Read, M., Smith, R., Yeo, G. and Kwek, K. 2008, Endocrinology, 149, 245.
51. Rousseau, S., Houle, F., Landry, J. and Huot, J. 1997, Oncogene, 15, 2169.
52. Hedges, J. C., Dechert, M. A., Yamboliev, I. A., Martin, J. L., Hickey, E. and Weber, L. A. 1999, J. Biol. Chem., 274, 24211.
53. Hirano, S., Rees, R. S., Yancy, S. L., Welsh, M. J., Remick, D. G. and Yamada, T. 2004, Cell Biol. Toxicol., 20, 1-14.
54. Kim, H. H. and Kim, K. 2003, FEBS Lett., 535, 190.
55. Chen, S. W. C., Park, S. W., Kim, M., Brown, K. M., D'Agati, V. D. and Lee, H. T. 2009, Transplantation, 87, 1478-1487.
56. Tezel, G. and Wax, M. B. 2000, J. Neurosci., 20, 3552-3562.
57. Rane, M. J., Coxon, P. Y. and Powell, D. W. 2001, J. Biol. Chem., 276, 3517.
58. Luerman, G. C., Uriarte, S. M., Rane, M. J. and McLeish, K. R. 2010, J. Proteomics, 73(3), 552-61.
59. Jaattela, M. 1995, Int. J. Cancer, 60, 689.
60. Garrido, C., Fromentin, A., Bonnotte, B., Favre, N., Moutet, M., Arrigo, A. P., Mehlen, P. and Solary, E. 1998, Cancer Res., 58, 5495.
61. Lemieux, P., Oesterreich, S., Lawrence, J. A., Steeg, P. S., Hilsenbeck, S. G., Harvey, J. M. and Fuqua, S. A. 1997, Invasion Metastasis, 17, 113.
62. Gurbuxani, S., Schmitt, E., Cande, C., Parcellier, A., Hammann, A., Daugas, E., Kouranti, I., Spahr, C., Pance, A., Kroemer, G. and Garrido, C. 2003, Oncogene, 22, 6669.
63. Nylandsted, J., Rohde, M., Brand, K., Bastholm, L., Elling, F. and Jaattela, M. 2000, Proc. Natl. Acad. Sci. USA, 97, 7871.
64. Nanbu, K., Konishi, I., Mandai, M., Kuroda, H., Hamid, A. A., Komatsu, T. and Mori, T. 1998, Cancer Detect. Prev., 22, 549.
65. Sarto, C., Valsecchi, C., Magni, F., Tremolada, L., Arizzi, C., Cordani, N., Casellato, S., Doro, G., Favini, P., Perego, R. A., Raimondo, F., Ferrero, S., Mocarelli, P. and Galli-Kienle, M. 2004, Proteomics, 4, 2252.
66. Rocchi, P., So, A., Kojima, S., Signaevsky, M., Beraldi, E., Fazli, L., Hurtado-Coll, A., Yamanaka, K. and Gleave, M. 2004, Cancer Res., 64, 6595.
67. Thanner, F., Sutterlin, M. W., Kapp, M., Rieger, L., Morr, A. K., Kristen, P., Dietl, J., Gassel, A. M. and Muller, T. 2005, Anticancer Res., 25, 1649.
68. Geisler, J. P., Tammela, J. E., Manahan, K. J., Geisler, H. E., Miller, G. A., Zhou, Z. and Wiemann, M. C. 2004, Eur. J. Gynaecol. Oncol., 25, 165.
69. Feng, J. T., Liu, Y. K., Song, H. Y., Dai, Z., Qin, L. X., Almofti, M. R., Fang, C. Y., Lu, H. J., Yang, P. Y. and Tang, Z. Y. 2005, Proteomics, 5, 4581.
70. Pick, E., Kluger, Y., Giltnane, J. M., Moeder, C., Camp, R. L., Rimm, D. L. and Kluger, H. M. 2007, Cancer Res., 67, 2932.
71. Shimamura, T. and Shapiro, G. I. 2008, J. Thorac. Oncol., 3, S152.
72. Guo, F., Rocha, K., Bali, P., Pranpat, M., Fiskus, W., Boyapalle, S., Kumaraswamy, S., Balasis, M., Greedy, B., Armitage, E. S., Lawrence, N. and Bhalla, K. 2005, Cancer Res., 65, 10536.

73. Lanneau, D., Brunet, M., Frisan, E., Solary, E., Fontenay, M. and Garrido, C. J. 2008, J. Cell. Mol. Med., 12, 743.
74. Powers, M. V., Clarke, P. A. and Workman, P. 2008, Cancer Cell, 14, 250.
75. Aoyama, A., Steiger, R. H., Frohli, E., Pockley, A. G., Georgiades, A. and Thulin, T. 2003, Hypertension, 42, 235.
76. Pinder, S. E., Balsitis, M., Ellis, I. O., Landon, M., Mayer, R. J. and Lowe, J. J. 1994, Pathol., 174, 209.
77. Xu, J., Wang, K., Zhang, X., Qiu, Y., Huang, D., Li, W., Xiao, X. and Tian, Y. 2010, J. Exp. Clin. Cancer Res., 29, 106.
78. Thiery, J. P., Acloque, H., Huang, R. Y. and Nieto, M. A. 2009, Cell, 139, 871.
79. Slotta-Huspenina, J., Becker, K. F., Feith, M., Walch, A. and Langer, R. 2014, Cancers (Basel), 6, 1382.
80. Hance, M. W., Nolan, K. D. and Isaacs, J. S. 2014, Cancers (Basel), 6, 1065.
81. Liu, J., Bao, J., Hao, J., Peng, Y. and Hong, F. 2014, Mol. Med. Rep., 10, 1089.
82. Pourhamidi, K., Skärstrand, H., Dahlin, L. B. and Rolandsson, O. 2014, Diabetes Care, 37(3), e49-50.
83. Pourhamidi, K., Dahlin, L. B., Boman, K. and Rolandsson, O. 2011, Diabetologia, 54(12), 3143-9.
84. Gruden, G., Bruno, G., Chaturvedi, N., Burt, D., Schalkwijk, C., Pinach, S., Stehouwer, C. D., Witte, D. R., Fuller, J. H. and Perin, P. C. 2008, Diabetes, 57(7), 1966-70.
85. Burt, D., Bruno, G., Chaturvedi, N., Schalkwijk, C., Stehouwer, C. D., Witte, D. R., Fuller, J. H., Pinach, S., Perin, P. C. and Gruden, G. 2009, Diabetes Care, 32(7), 1269-71.
86. Barutta, F., Pinach, S., Giunti, S., Vittone, F., Forbes, J. M., Chiarle, R., Arnstein, M., Perin, P. C., Camussi, G., Cooper, M. E. and Gruden, G. 2008, Am. J. Physiol. Renal Physiol., 295(6), F1817-24.
87. Dai, T., Patel-Chamberlin, M., Natarajan, R., Todorov, I., Ma, J., LaPage, J., Phillips, L., Nast, C. C., Becerra, D., Chuang, P., Tong, L., de Belleroche, J., Wells, D. J., Wang, Y. and Adler, S. G. 2009, Endocrinology, 150(7), 3031-9.
88. Park, J. K., Ronkina, N., Höft, A., Prohl, C., Menne, J., Gaestel, M., Haller, H. and Meier, M. 2008, Nephrol. Dial Transplant., 23(6), 1844-53.
89. Vidyasagar, A., Reese, S. R., Hafez, O., Huang, L. J., Swain, W. F., Jacobson, L. M., Torrealba, J. R., Chammas, P. E., Wilson, N. A. and Djamali, A. 2013, Kidney Int., 83(1), 84-92.
90. Vidyasagar, A., Wilson, N. A. and Djamali, A. 2012, Fibrogenesis Tissue Repair, 5(1), 7.
91. Vidyasagar, A., Reese, S., Acun, Z., Hullett, D. and Djamali, A. 2008, Am. J. Physiol. Renal Physiol., 295(3), F707-16
92. Martin-Ventura, J. L., Duran, M. C., Blanco-Colio, L., M., Meilhac, O., Leclercq, A. and Michel, J. B. 2004, Circulation, 110, 2216-2219.
93. Seibert, T. A., Hibbert, B., Chen, Y, X., Rayner, K., Simard, T., Hu, T., Cuerrier, C. M., Zhao, X., de Belleroche, J., Chow, B. J., Hawken, S., Wilson, K. R. and O'Brien, E. R. 2013, J. Am. Coll. Cardiol., 62(16), 1446-54.
94. Wang, Z., Chen, Y., Labinskyy, N., Hsieh, T. C., Ungvari, Z. and Wu, J. M. 2006, Biochem. Biophys. Res. Commun., 346, 367-376.
95. Taba, K., Kuramitsu, Y., Ryozawa, S., Yoshida, K., Tanaka, T., Maehara, S., Maehara, Y., Sakaida, I. and Nakamura, K. 2010, Anticancer Res., 30(7), 2539-2543.
96. Pockley, A. G., Georgiades, A. and Thulin, T. 2003, Hypertension, 42, 235.
97. Zhu, J., Quyyumi, A. A. and Wu, H. 2003, Arterioscler. Thromb. Vasc. Biol., 23, 1055.
98. Hunter-Lavin, C., Hudson, P. R. and Mukherjee, S. 2004, Cell Stress Chaperones, 9, 344.
99. Plumier, J. C., Ross, B. M., Currie, R. W., Angelidis, C. E., Kazlaris, H., Kollias, G. and Pagoulatos, G. N. 1995, Journal of Clinical Investigation, 95, 1854.
100. Ren, F., Xu, Y., Mao, L., Ou, R., Ding, Z., Zhang, X., Tang, J., Li, B., Jia, Z., Tian, Z., Ni, B. and Wu, Y. 2010, Cancer Biol. Ther., 9(2), 134.

101. Xue, J. H., Fukuyama, H., Nonoguchi, K., Kaneko, Y., Kido, T., Fukumoto, M., Fujibayashi, Y., Itoh, K. and Fujita, J. 1998, Biochem. Biophys. Res. Commun., 247(3), 796.
102. Pratt, W. B. and Toft, D. O. 2003, Exp. Biol. Med., 228, 111.
103. Rice, J. W., Veal, J. M., Fadden, R. P., Barabasz, A. F., Partridge, J. M., Barta, T. E., Dubois, L. G., Huang, K. H., Mabbett, S. R., Silinski, M. A., Steed, P. M. and Hall, S. E. 2008, Arthritis Rheum., 58(12), 3765.
104. Kimura, H., Yukitake, H., Tajima, Y., Suzuki, H., Chikatsu, T., Morimoto, S., Funabashi, Y., Omae, H., Ito, T., Yoneda, Y. and Takizawa, M. 2010, Chem. Biol., 17(1), 18.
105. Wong, H. R., Mannix, R. J., Rusnak, J. M., Boota, A., Zar, H., Watkins, S. C., Lazo, J. S. and Pitt, B. R. 1996, Am. J. Respir. Cell Mol. Biol., 15(6), 745.
106. Wang, Y. R., Xiao, X. Z., Huang, S. N., Luo, F. J., You, J. L., Luo, H. and Luo, Z. Y. 1996, Shock, 6(2), 134.
107. Luo, X., Zuo, X., Zhou, Y., Zhang, B., Shi, Y., Liu, M., Wang, K., McMillian, D. R. and Xiao, X. 2008, Arthritis Res. Ther., 10(2), R41.
108. Borges, T. J., Wieten, L., van Herwijnen, M. J., Broere, F., vander Zee, R., Bonorino, C. and van Eden, W. 2012, Front. Immunol., 3, 95.
109. Wick, G., Knoflach, M. and Xu, Q. 2004, Annu. Rev. Immunol., 22, 361.
110. Szabo, A., Langer, T., Schröder, H., Flanagan, J., Bukau, B. and Hartl, F. U. 1994, Proc. Natl. Acad. Sci. USA, 91(22), 10345.
111. Cyr, D. M., Langer, T. and Douglas, M. G. 1994, Trends Biochem. Sci., 19(4), 176.
112. Shama, K. M., Suzuki, A., Harada, K., Fujitani, N., Kimura, H., Ohno, S. and Yoshida, K. 1999, Cell Struct. Funct., 24(1), 1.
113. Gruvberger-Saal, S. K. and Parsons, R. J. 2006, Clin. Invest., 116(1), 30.
114. Morrow, G., Samson, M., Michaud, S. and Tanguay, R. M. 2004, FASEB J., 18(3), 598.
115. Tessier, D. J., Komalavilas, P., Panitch, A., Joshi, L. and Brophy, C. M. 2003, J. Surg. Res., 111, 152.
116. Kozawa, O., Matsuno, H., Niwa, M., Hatakeyama, D., Oiso, Y., Kato, K. and Uematsu, T. 2002, Life Sci., 72(2), 113.
117. Macario, A. J. and Conway de Macario, E. 2005, N. Engl. J. Med., 353, 1489.
118. Johnson, B. J., Le, T. T., Dobbin, C. A., Banovic, T., Howard, C. B., Flores Fde, M., Vanags, D., Naylor, D. J., Hill, G. R. and Suhrbier, A. 2005, J. Biol. Chem., 280, 4037.
119. Corrao, S., Campanella, C., Anzalone, R., Farina, F., Zummo, G., Conway de Macario, E., Macario, A. J., Cappello, F. and La Rocca, G. 2010, Life Sci., 86, 145.