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Research Article

Heat Shock Proteins

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ABSTRACT

Cells from virtually all organisms respond to a variety of stresses by the rapid synthesis of a highly conserved set of polypeptides termed heat shock proteins (HSPs). The precise functions of HSPs are unknown, but there is considerable evidence that these stress proteins are essential for survival at both normal and elevated temperatures. HSPs also appear to play a critical role in the development of thermotolerance and protection from cellular damage associated with stresses such as ischemia, cytokines, and energy depletion. These observations suggest that HSPs play an important role in both normal cellular homeostasis and the stress response. There is a widespread interest in the cellular mechanisms utilized by an organism to cope with a disruption in homeostasis. Current research is focused at severallevels, ranging from basic molecular biology approaches to therapeutic applications. One reason for this interest, and the complexity associated with thetopic, is evidence demonstrating that mammalian species have developed many different ways to deal with stress The intent of this mini-review is to summarize what is known about the various physiological factors that modulate HSP responses to stressors at cellular and systemic levels as well as to highlight studies suggesting that HSPs play a critical role in the development of thermotolerance and protection from stress-induced cellular damage.

Key Words: Heat shock proteins, stress protein; HSP70; heat stress; aging; gene expression; molecular chaperone, apoptosis.

INTRODUCTION

One of the "hottest" areas of current research involves a family of highly conserved stress proteins known as heat shock proteins (HSPs). These proteins are ubiquitous, occurring in all organisms from bacteria and yeast to humans. HSPs come in various forms and are categorized into families on the basis of their molecular weights There is substantial evidence that HSPs play important physiological roles in normal conditions and situations involving both systemic and cellular stress. Heat-shock proteins are produced in response to different types of stress conditions making cells resistant to stress induced cell damage. Under normal conditions, heat-shock proteins play numerous roles in cell function, including modulating protein activity by changing protein conformation, promoting multiprotein complex assembly/disassembly, regulating protein degradation within the proteasome pathway, facilitating protein translocation across organellar membranes, and ensuring proper folding of nascent polypeptide chains during protein translation. When cells are stressed, a common response is to undergo cell death by one of two pathways, either 'necrosis'or 'apoptosis'. Recently, both routes to cell death have been revealed to share similar mechanisms, with heat-shock proteins and their cofactors responsible for inhibiting both apoptotic and necrotic pathways. We therefore briefly summarize recent reports showing molecular evidence of cell death regulation by heat-shock proteins and their cochaperones.

History of HSP s

HSPs were first discovered in 1962[1] and described as a set of proteins whose expression was induced by heat shock and a variety of other stresses. Researchers have subsequently demonstrated that most HSPs have strong cytoprotective effects, are involved in many regulatory pathways, and behave as molecular chaperones for other cellular proteins[2,3].

Heat shock Protein Families

The HSPs have been extensively studied, especially with regard to their cellular localization, regulation, and functions. [4-9] HSPs are present in both prokaryotic and eukaryotic cells, and their high level of conservation suggests that they play an important role in fundamental cell processes.

HSP s in Prokaryotes

HSPs were initially discovered in *Drosophila melanogaster* larvae that were exposed to "heat shock"[10] and subsequent studies[11-13] identified several subsets of these proteins in the 70-kDa range. Over the past 30 years, a large number of additional proteins have been discovered within this family, and these are collectively referred to as "HSPs". The principal HSPs range in molecular mass from 15 to 110 kDa and are divided into groups based on both size and function[14]. They are present in the cytosol, mitochondria, endoplasmic reticulum, and nucleus, although these locations vary depending on the particular protein.

HSP s in Eukaryotes

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The most well-studied and understood HSPs in mammals are those with molecular masses of _60, 70, 90, and 110 kDa. These HSPs are expressed at euthermic body temperatures (_37°C) and in conditions of stress (e.g., heat shock) and have distinct locations and functional properties.

Small-molecular-mass proteins, also termed small HSPs, exhibit tissue-specific expression and include heme oxygenase, Hsp32, Hsp27, _B-crystallin, and Hsp20 chaperone[15,16].

The HSP70 family

The primary focus of this minireview will be on the ubiquitous HSP70 family of proteins, which are the most temperature sensitive and highly conserved of the HSPs. The HSP70s are ATPbinding proteins and demonstrate a 60–80% base identity among eukaryotic cells (5, 18, 62). There are at least four distinct proteins in the HSP70 group (HSP72, HSP73, HSP75, and HSP78), and all of these proteins have several acronyms that can be redundant and confusing. Proteins in the HSP70 group share common protein sequences but are synthesized in response to different stimuli. For example, the 73-kDa protein (HSP73 or Hsc70) is constantly produced (hence, the term "constitutive"), whereas the 72-kDa protein (HSP72 or Hsp70) is highly inducible and its synthesis is increased in response to multiple stressors. The molecular structure of the HSP70 group of proteins and descriptions of HSP70 gene regulation will only be briefly covered in this mini-review, as there are several detailed reviews available on these topics[9,17,18].

The gene for Hsp70 is a 2,440-base pair gene containing a 212-base pair leader sequence and a 242-base pair downstream or 3-untranslated region[19]. There are at least two regulatory elements in the 5_-region that interact with heat shock transcription factors (HSFs). These HSFs bind to the promoter element during stress and are sufficient to induce Hsp70 transcription. In addition to hyperthermia, a number of stimuli are known to induce Hsp70 transcription, including energy depletion, hypoxia, acidosis, ischemia- reperfusion, reactive oxygen species (ROS), reactive nitrogen species such as nitric oxide, and viralinfection[20-22].

An important consideration regarding Hsp70 regulation involves the apparent discordance between transcription of message and Hsp70 translation. There is evidence suggesting that transcriptional activation of the Hsp70 gene is independent of protein synthesis. For instance, in cell culture experiments, Hsp70 mRNA can increase in response to a challenge, although there is little Hsp70 protein produced[23].

Functional Role of HSP s

The precise functions of proteins in the HSP70 family have not been completely delineated. However, the high degree of conservation of these proteins across species, coupled with their importance in cell survival in various conditions, suggests that these HSPs are critical for both normal cellular function and survival after a stress[46]. Therefore, one of the primary means to gain insight into HSP70 function in both in vitro and in vivo systems has been to assess their cellular responses after a stress-related induction.

Thermotolerance

One of the first physiological functions associated with the stress-induced accumulation of the inducible Hsp70 was acquired thermotolerance, which is defined as the ability of a cell or organism to become resistant to heat stress after a prior sublethal heat exposure[45].

Data from subsequent studies demonstrated that the induction of Hsp70 was associated with the development of tolerance to a variety of stresses, including hypoxia, ischemia, acidosis, energy depletion, cytokines such as tumor necrosis factor-_ (TNF-_), and ultraviolet radiation[47]. The phenomenon of acquired thermotolerance is transient in nature and depends primarily on the severity of the initial heat stress.

In general, the greater the initial heat dose, the greater the magnitude and duration of thermotolerance. The expression of thermotolerance following heating will occur within several hours and last 3–5 days in duration[48]. Additional supporting evidence includes observations that have linked the kinetics of thermotolerance induction and decay with parallel changes in HSP70 induction and degradation. However, these studies have generally been correlative in nature, with no causal link established between induction of HSP70 and acquired thermotolerance. The similar kinetics of thermotolerance demonstrated by cells, tissues, and animals suggest that the morbidity and mortality associated with whole body heating is due in part to the dysfunction of some critical target tissues[48]. It can be postulated that the development of thermotolerance results from the improved tolerance of the weakest organ and cell systems. Presumably, these tissues are both heat sensitive and vital to the animal.

For instance, the small intestine is capable of generating thermotolerance and is also reported to be the tissue most sensitive to heat damage. Both the small intestine and whole animal are sensitive to in vivo temperatures ranging from 41°C to 42°C, whereas gastrointestinal disorders are frequently observed after whole body heating (42°C for 120 min) and during heat stroke in humans[49]. In support of this concept, demonstrated that the gut and liver are the first organs to accumulate HSP70 following whole body hyperthermia.

Advances in molecular biology techniques have provided researchers with tools to address the issue of a causal link between HSP induction and thermotolerance more directly[50]. Cellular manipulations that either block HSP70 accumulation or overexpress certain HSPs have been shown to either increase or decrease heat sensitivity. For example[51-54] transfection of a plasmid containing the *Drosophila* HSP70 gene into a monkey fibroblast cell line produced large increases in HSP70 accumulation in these cells and improved tolerance to a heat shock paradigm.Elevations in cellular HSP27 levels via plasmid transfection have also yielded a state of thermotolerance without the need for a conditioning thermal stress..

Conversely, microinjections of monoclonal antibodies specific for HSP70 inhibited the synthesis of these proteins, resulting in a reduction in thermotolerance. As

noted, HSPs appear to play a role in protecting cells from damage generated by a variety of stressors. Their synthesis is associated with protection against light-induced damage to the retina and ischemiareperfusion injury to the heart, liver, and kidney .In addition, studies of cardiac shock followed by resuscitation have revealed that hepatocytes synthesize members of the HSP70 family early in the course of recovery[55]. The fact that HSP70 message is preferentially translated by a cell under stress to the exclusion of other messages may result in the inability of the cell to produce some proteins or respond to additional signals.

In this model, the cell may "choose" self-preservation over tissue preservation to the detriment of the organ. This model may be particularly relevant in a situation where HSP70 accumulation could be utilized as a biomarker of cellular injury.

In this scenario, cells of tissues most at risk would also be the cells most likely to accumulate HSP70 during stress, and this HSP70 accumulation could mark a tissue for potential failure[56,57]. Although the precise mechanisms for the improvement in cellular thermotolerance in association with an increase in HSP levels have not been delineated, it is tenable to postulate that proteins in the HSP70 family are involved in preventing protein denaturation and/or processing denatured proteins and protein fragments that are produced by stressors such as hyperthermia. Supporting evidence for this scenario comes from a set of in vitro experiments by Mizzen and Welch[58], who demonstrated that heat stress results in translational arrest within a cell, and this arrest is proportional to both the intensity and duration of the applied heat stress. Subsequent resumption of translation resulted in HSP mRNA being translated into HSPs before the synthesis of other proteins took place within the cell. Interestingly, the period of translational arrest in response to heat stress could be shortened in these experiments if cells were first made thermotolerant.

One interpretation of these results is that a primary function of HSPs during cellular stress is to maintain translation and protein integrity. Cells that were made thermotolerant also produced less HSP during a second challenge compared with previously unheated cells, suggesting there is a regulation of HSP synthesis that is dependent on the levels of these proteins existing within the cell[59]. Although a majority of data in this area has been derived from in vitro methodologies, a unique set of experiments in humans by Moseley and colleagues generated data supportive of this concept. Healthy men performed a challenging exercise protocol in either hot (46°C) or more moderate (30°C) ambient conditions[60,61]. Leukocytes obtained from subjects after the protocol were then incubated at 41°C. The increase in Hsp70 synthesis in heat-stressed leukocytes was inversely proportional to the length of the initial "conditioning" exercise stress, suggesting that cells regulate the amount of these stress proteins inresponse to repeated challenges[60].

An additional issue related to the development of thermotolerance deals with the possibility that HSPs,through their interaction with cellular proteins during translational arrest, play a role in preventing protein denaturation and processing denatured proteins that are generated in response to stressors such as heat[62]. For example, data suggest that the injection of denatured proteins into cells or the generation of abnormal proteins can induce HSP activity. Although these different sets of data clearly demonstrate a broad range of physiological processes that involve the HSPs, the evidence that the HSPs are responsible for cellular thermotolerance is circumstantial rather than conclusive[63].

The variety of stressors used to condition cells will likely induce other important cellular defense proteins in addition to HSPs, such as antioxidant enzymes. It should also be noted that thermotolerance can be generated in the absence of HSPs[64]. In these studies, thermotolerance was manifested under conditions of protein synthesis inhibition (i.e., no HSP accumulation) as well as a chronic exposure to a lower temperature than is required for HSP accumulation. Other studies have demonstrated that inhibition of transcription during the conditioning heat stress also allows the maintenance of thermotolerance.

In addition, oxidative stresses, which can confer thermotolerance, may not increase the levels of HSPs[64]. In other stresses, such as ischemia,where HSPs are thought to play a role, HSP overexpression has also not been found to confer tolerance. Therefore, generating a scenario in which the development of stress tolerance in a cellular system is causally linked to an increase in Hsp70 expression is difficult because organisms and cells respond to stress in a variety of complex ways[65-68]. The mechanisms contributing to thermal injury vs. thermotolerance are even less clear in the intact organism. One obvious explanation for thermal injury at the cellular level is direct heat damage. However, this cellular damage is likely due in part to functional impairment of a tissue or organ (e.g., reductions in blood flow) and the possible impact of systemic factors such as endotoxin-mediated cytokine production. Moreover, much of the research attempting to gain an understanding of the intact organism's adaptive response to heat has focused on heat acclimatization processes. Because the factors involved in heat injury at the whole organism level are complex and the mechanisms contributing to the protective role of HSPs are not well defined, issues such as these remain a central challenge in this field of research[69].

HSP70 functions associated with stress tolerance

Although the evidence linking stress-induced HSP70 accumulation with tolerance to heat and other stressors is compelling, the mechanisms by which HSPs confer stress tolerance are not well understood[72]. Attention has primarily been focused on the role of HSP70 as a chaperone and its potential ability to contribute to cellular repair processes in response to interventions such as heat, oxidative stress, activation of proteases, release of lysosomal and proteolytic enzymes, and alterations of the cytoskeleton[70,71,73-75].

Several important cytoprotective functions have been attributed to HSPs and, in particular, the HSP70family. These include[76]

- the folding of proteins in various intracellular compartments,
- the maintenanceof structural proteins
- the refolding of misfolded proteins,
- translocation of proteins across membranes and into various cellular compartments,
- the prevention of protein aggregation, and
- the degradation of unstable proteins

Interestingly, it has also been noted that HSPs can play a role in apoptosis. HSP27, HSP70, and HSP90 proteins are predominantly antiapoptotic, whereas HSP60 is proapoptotic[77]. Moreover, it appears that these HSPs function at multiple points in the apoptotic signaling pathway to elicit this response. Although there are numerous studies available demonstrating the broad range of physiological processes that involve HSPs, including protein translocation, receptor regulation, cytoskeleton stabilization, and management of protein folding and repair, evidence directly demonstrating that the HSPs are responsible for stress tolerance is not conclusive. In addition, the complexity of the integrated response to a physiological challenge in vivo makes it difficult to ascertain what "stressor" is responsible for stimulating an increase in HSP synthesis. In a situation such as an aerobic exercise of moderate intensity and duration, additional signals besides an elevation in core temperature(Tc) are present that could potentially activate HSP expression, including acidosis, energy depletion, reductions in blood flow to visceral organs and an associated tissue hypoxia, and generation of ROS[78-81].

Furthermore, in addition to HSPs, cells will express other important stress proteins such as antioxidant enzymes, providing an organism with multiple cytoprotective options. It is also important to note that there are numerous studies demonstrating that thermotolerance can be generated in the absence of intracellular HSP accumulation. Therefore, it is problematic, especially at the whole organism level, to definitively link an increase in HSP70 expression directly to the acquisition of stress tolerance, partly because mammalian species respond to stress in a multitude of complex, integrated ways[82].

Two major pathways for apoptosis induction have been identified: intrinsic and extrinsic. The hallmarks of the intrinsic pathway are mitochondrial involvement and the formation of the 'apoptosome'. In the intrinsic pathway, cell death signals induce the release of cytochrome c (Cyt c) from the mitochondria, which then binds to the apoptosis protease activating factor-1 (Apaf-1), inducing oligomerization and eventual recruitment of procaspase-9[24-26]. Apoptosome formation results in the processing and activation of caspase-9, which triggers the caspase pathway by activating the downstream caspase-3 Overexpression of Hsp27 increases the resistance of cells to various apoptotic stimuli. One mechanism by which Hsp27 could interfere with apoptosis is by directly binding to cytosolic Cyt c and sequestering it from Apaf-[27]. The antiapoptotic activity of Hsp27 appears to be highly dependent on its oligomeric status. Hsp27 is able to shift between different oligomeric states in a phosphorylation-dependent manner and only the high molecular weight Hsp27 appears competent to inhibit apoptosome formation. Recently, modification of Hsp27 by methylglyoxal (a glycolysis by-product that modifies proteins at arginine and lysine residues) was reported to repress the formation of large Hsp27 oligomers and inhibit Cyt c-dependent apoptosis. These results support a role for Hsp27 inapoptosis by inhibition of apoptosome formation[28,29,30].

Two groups reported a direct interaction between Apaf-1 and Hsp70 that prevents apoptosome formation. The precise mechanism by which this interaction affords apoptosis inhibition is unclear, but it appears to be mediated by the caspase recruitment domain of Apaf-1 and to require the presence of ATP, implying that the 'foldase' activity of Hsp70 is involved[31].

Recently, it was shown that Hsp70 protects serum depletion-induced cell death in cells lacking Apaf-1 indicating that Hsp70 also plays a role in diminishing Apaf-1-independent apoptosis. One of the targets in this situation may be apoptosis-inducing factor (AIF), which normally resides in the intermembrane space of the mitochondria, but upon apoptosis induction translocates to the cytosol and nucleus, where it participates in caspase-independent apoptotic pathways. Hsp70 directly binds to AIF, inhibiting AIF-dependent apoptosis[32-34]. This binding is mediated by the peptide domain of Hsp70, but the ATPase domain is dispensable for cell death inhibition, suggesting that this mechanism is not chaperone-activity dependent.

Hsp60 is a heat-shock protein that primarily localizes to the matrix of the mitochondria. Recently, two independent groups reported a role for Hsp60 in caspase-3 maturation In Jurkat T cells, a subpopulation of caspase-3 is found in the mitochondria in complex with Hsp60[35]. Upon induction of apoptosis with staurosporine, mitochondrial procaspase-3 is activated and dissociates from Hsp60 prior to the release of both proteins into the cytosol In vitro, recombinant Hsp60 accelerated the rate at which procaspase-3 was activated by Cyt c and dATP in an ATP-dependent manner, suggesting that the chaperone function of Hsp60 is involved in this process .Cytosolic Hsp60 has also been shown to be complexed with the antiapoptotic protein Bax .Under hypoxic conditions, Hsp60 and Bax dissociate, whereupon Bax translocates to the mitochondria to participate in apoptosis. The above situations suggest a role for heat-shock proteins upstream of caspase activation[36,37].

However, Hsp70 overexpression can also inhibit caspase-dependent events that occur much later in apoptosis, such as activation of cytosolic phospholipase A2 and changes in nuclear morphology. Hsp70 could also protect cells from forced expression of caspase-3[38]. Thus, heat-shock proteins also inhibit events occurring downstream of caspase activation. During the final phases of apoptosis, chromosomal DNA is digested by the DNase CAD (caspase activated DNase, also known as DFF 40, DNA fragmentation factor 40), following activation by caspase-3. Recently, the enzymatic activity and proper folding of CAD/ DFF40 was reported to be regulated by Hsp70, Hsp40, and ICAD (inhibitor of CAD)[39-42]. ICAD appears

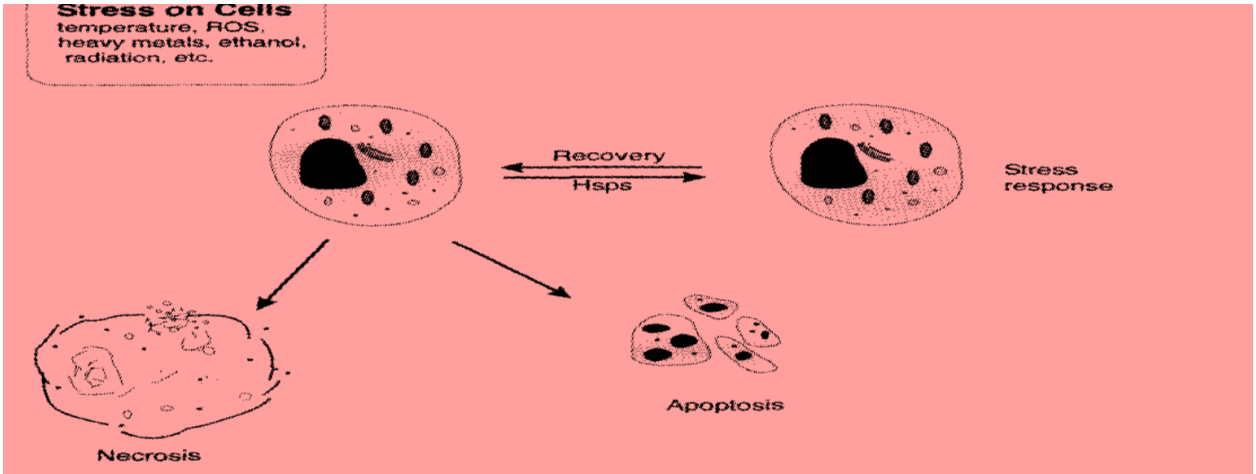

Fig. 1 The effects of stress-inducing agents on cells. When a cell experiences low level stress, it activates the stress response which involves rapid induction of Hsps. Accumulation of Hsps accompanies thermotolerance and resistance to cell death. When the stress element is removed, the cells return to their normal activities. As the stress level increases, the injured cells activate their own demise and undergo apoptosis. However, under extreme conditions, when the stress level eliminates the capacity for regulated activation of apoptotic cascade, then the cells undergo necrosis.

Heat-shock proteins regulate the mitochondrial pathway of apoptosis

to recognize an intermediate folding state conferred by Hsp70–Hsp40, suggesting that these heat-shock proteins may promote the formation of the CAD–ICAD complex during protein translation[43-45].

Role of heat-shock proteins in ischemic and degenerative disorders

During focal ischemia, the cells surrounding the core infarct rapidly upregulate Hsp27, 70, and 90 mRNA levels, implying that increases in heat-shock proteins represent a stress response to ischemia–reperfusion injury. The overexpression of Hsp70 in the neurons of transgenic mice or mice injected with Hsp70-expressing viral vectors results in cytoprotection in several different models of nervous system injury, including ischemia[83].

In myocardial infarction models, heart-specific transgenic mice or in vivo gene transfer of Hsp70-expressing vectors have increased resistance to cell death and better functional recovery .In muscle cell death, caspase-mediated cleavage of the intermediate filament desmin is associated with muscle cell death .An important role for caspase-8and caspase-8antagoni sts in heart development has also been revealed through gene knockout studies in mice, providing additional evidence for a link between caspases and muscle physiology[84,85]. Overall, however, the mechanisms regulating caspase activation and apoptosis in muscle are largely unknown.

Differentiation-induced apoptosis of myogenic cells is regulated by the small heat-shock protein aB-crystallin, which is closely related to Hsp27. aB-crystallin inhibits the proteolytic activation of caspase-3 during myoblast differentiation. This protective effect is blocked when aB-crystallin carries a phosphorylation- deficient point mutation at Arg120, which is the same mutation responsible for Desmin-like myopathy (myofibrillar myopathy). The overexpression of Arg120Gly aB-crystallin causes aberrant desmin function and aggregation of aB-crystallin with early death and myopathy. In muscle atrophy, phosphorylated small heat-shock proteins are recruited to aggreosomes for quality control of proteins, suggesting a role for Hsp27 in proteasome regulation[86]. Recently, the ubiquitin protein ligase (MAFbx/Atrogin-1) and ring finger protein (MuRF1) have been cloned as inducible genes of muscular atrophy.It would be interesting to explore the possible functional interactions between small heat-shock proteins and atrophy-specific proteasome machinery[87,88].

Role of HSP s in neuro degenerative disorders

Neurodegenerative disorders, such as Parkinson's and Huntington's disease, are caused by the deposition of misfolded proteins, which in turn cause neuronal cell death. Hsp27 is capable of protecting motor neurons from apoptosis following mechanical injury. This protective mechanism requires the proper phosphorylation state of Hsp27 and appears to act somewhere between Cyt c release and caspase-3 activation[89]. The phosphorylation requirement may be important for Hsp27 dissociation to the oligomeric form that would then be competent to interact with newly released Cyt c[90].

Immune surveillance and antigen presentation.

Although the primary focus of research on HSPs has been directed toward their functions and accumulation inside the cell in response to a physiological stress, there is emerging recognition that HSPs serve as modulating signals for immune and inflammatory responses. This concept was recently detailed in a concise review by Moseley[91].

One area of investigation pertinent to the topic of stress tolerance has dealt with the potential role of HSPs in cytokine production. Elevations in intracellular HSP levels have been shown to improve cell tolerance to

inflammatory cytokines such as TNF- and interleukin-1. HSP accumulation within a cell produces both transcriptional inhibition and a decrease in TNF-_ and interleukin-1 secretion demonstrated that heat conditioning and the resultant increase in intracellular Hsp70 levels protected animals from an endotoxin dose that was lethal in unconditioned rats. Moreover, this paradigm was associated with a decrease in serum TNF-_ levels after administration of endotoxin in the heat-conditioned animals. These results suggest that intracellular HSP accumulation may contribute to a reduction in inflammatory cytokine production with cellular challenge[92-94].

Conversely, when HSPs are present on the surface of cells or released into the local extracellular environment during conditions such as necrotic cell death or viral infection, these proteins have an immune-stimulating response. The situation involving cell necrosis is quite relevant to conditions of physiological challenge, such as heat stress, where widespread cellular injury and necrotic cell death have been noted[95]. Hsp70 is also known to facilitate antigen presentation in cells such as macrophages and dendrites[90,96]. When Hsp70 is applied to the environment external to cells, macrophages and lymphocytes produce inflammatory cytokines. Finally, studies have demonstrated the presence of Hsp70 on the surface of tumor cells[97,99], potentially functioning as recognition molecules for natural killer (NK) cells. Together, these observations demonstrate that HSPs are important modulators of antigen presentation, T-lymphocyte activation, cytokine production, and NK cell killing, placing them in a unique position of contributing to both intracellular and extracellular responses to a physiological stress[100].

CONCLUSION

In this mini-review, an attempt was made to summarize the physiological factors that modulate HSP responses to stressors at cellular and systemic levels. From the literature presented, it should be evident that the HSP70 family of proteins is essential for cellular survival from heat stress and other types of physiological challenge. It is clear that the heat shock proteins [HSP s]are ubiquitously present in cells under both normal and stressful conditions and that their structure is well conserved among species[101]. In addition, there is a large body of evidence to support the role of HSPs for improving cell survival to otherwise lethal challenges. Strategic alliances among research teams could forge new directions and accelerate progress in this promising area, which, ultimately, could succeed in exploiting endogenous pathways to enhance physiological health and to reduce physiological attrition associated with cardiovascular diseases.

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