HEAT SHOCK PROTEINS – A MINI REVIEW


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ABSTRACT

This article provides an insight towards the various applications of heat shock proteins and also gives a brief introduction, discovery & functions of heat shock proteins. An overview of role of heat shock proteins in cardiovascular disease, infectious diseases & in cancer is discussed. Heat shock proteins, a family of stress inducible proteins are involved in the pathogenesis of atherosclerotic vascular diseases, various infectious disease & also in tumor cells.

KEY WORDS

Heat shock proteins, Chaperones, biomarker, atherosclerotic vascular disease, cancer and cytoprotective.

1. INTRODUCTION

Heat shock proteins (Hsps) are also known as stress proteins. They are practically found in all living organism from bacteria to humans. Heat and other types of stress stimuli can increase the cellular expression of Hsps. These proteins can be categorized into different families according to their molecular weight (Hsp110, 90, 70, 60, 40, 20-30, & 10). These are an evolutionary conserved family of proteins, whose expression increases in response to a variety of different metabolic results. Hsps are mostly regarded as intracellular molecules that mediate a range of essential housekeeping and cytoprotective functions. They function as intercellular signaling molecules; these molecules can be released and are present in the extracellular environment under physiological condition. They elicit cytokine production and adhesion.
molecule expression of a range of cell types and they can deliver maturation signals and peptides to antigen presenting cells through receptor mediated interactions. The induction of self Hsp immune reactivity can attenuate auto immunity and delay transplant rejection. Hsp derived from tumors & pathogens can induce specific protective immunity.

**Table1:** Brief classification and functions of Hsps and lists their functions

<table>
<thead>
<tr>
<th>Molecular Weight HSP in kDa</th>
<th>Prokaryotes</th>
<th>Eukaryotes</th>
<th>Function of HSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>GRoES</td>
<td>Hsp10</td>
<td>Chaperonin cofactor</td>
</tr>
<tr>
<td>20 – 30</td>
<td>GrpE</td>
<td>HspB group as well as Hsp27 or HspB1</td>
<td>Thermo-tolerance</td>
</tr>
<tr>
<td>40</td>
<td>DnaJ</td>
<td>Hsp40</td>
<td>Cofactor of Hsp70</td>
</tr>
<tr>
<td>60</td>
<td>GroEL</td>
<td>Hsp60</td>
<td>Involved in protein folding after its post-translational migration from mitochondrion/chloroplast</td>
</tr>
<tr>
<td>70</td>
<td>DnaK</td>
<td>HspA, Hsp70-72, Grp78, Hsx70</td>
<td>Protein folding/unfolding. Thermal tolerance to cells during heat stress. Prevents protein folding during post-translational migration from mitochondrion/chloroplast</td>
</tr>
<tr>
<td>90</td>
<td>HtpG, C62.5</td>
<td>HspC, Hsp90, Hsp94</td>
<td>Steroid receptor and transcription factor maintenance</td>
</tr>
<tr>
<td>100</td>
<td>ClpB, ClpA</td>
<td>Hsp104, Hsp110</td>
<td>Protection from extreme heat stress</td>
</tr>
</tbody>
</table>

1.1. **Functions:**

- Up regulators in stress
- Chaperones
- Housekeeping
- Cardiovascular
- Immunity
- Role in cancer

1.2. **Hsps in vascular disease**

Atherosclerosis vascular disease is classified into four main areas – carotid, coronary, aortic & peripheral
vascular disease. Each case the link between Hsp and atherosclerosis is outlined in systematic manner. Current evidence is that the components of immune system may be involved in pathogenesis of atherosclerosis. Heat shock proteins act as auto antigens in immune response. Heat shock protein levels may be increased in patients with vascular disease.¹

1.3. Hsps in tumor cells

Hsp such as Hsp70, 27, 90 inhibit apoptosis by direct physical interaction with apoptotic molecules and are also over expressed in tumor cells.² Hsp27 enhances the tumorigenicity of colon carcinoma cells. Hsp70 is highly expressed in human breast tumors. Hsp90 is expressed in prostate carcinomas. Hsps bind to procaspases and inhibit their activation. Depletion of Hsp70 from tumor cells by various methods induces their apoptosis. Inhibition of Hsp90 in tumor cells results in the dimerisation induced activation of death receptors, suggesting that Hsps keep death proteins in apoptosis resistant state by a direct association. Various diseases like neurodegenerative disease induce the accumulation of damage due to oxidative stress. Hsp induction and the degradation of damaged protein are all impaired under these conditions. In such cases the increased demand of chaperone capacity which results in the unbalancing of cellular homeostasis, also termed chaperone overload. Conventional antitumor therapies (chemotherapy, radiotherapy, hyperthermia etc.) all induce Hsp in surviving cells. The over expression of Hsps may help the accumulation of hidden mutation in tumors which can help their further progression to more aggressive types of metastatic cells. The induction of Hsp70 by hyperthermia and anticancer drugs was reviewed and was shown to be more effective in chemo resistant tumors (Fig 1). ¹³

![Fig. 01 Hsp proteins in tumor cells](image)

1.4. Biomarkers of spinal and brain ischemia

Tissue ischemia caused by decreased spinal cord or brain perfusion is a potent and powerful stressor that triggers many metabolic and inflammatory pathways.³ Cerebro Spinal Fluid (CSF) is produced continuously and the total CSF
Compartment volume is replaced three times a day under normal conditions. CSF bathes the neural tissues of the brain and spinal cord and should allow detection of the biochemical products of acute CNS ischemia more rapidly than in serum, particularly if the blood brain barrier is intact. Specific biochemical markers that have been examined to date include lactate, pCO₂, neuron-specific enolase (NSE) glucose, pH, S100β, and Hsps. These markers also markedly increase in serum at other times, including during surgical procedures unrelated to acute brain injury. They are elevated in marathon runners and may be released from a variety of tissues including brain injury during sepsis. Isoprostanes, the breakdown products of free-radical induced oxidation of arachidonic acid, have been examined in the urine and plasma of experimental animal models of spinal cord ischemia and have been correlated in human neurodegenerative diseases. Matrix metalloproteinase, which have a role in tissue remodeling and inflammation, have also been investigated.

1.5. Biomarkers of CNS ischemia in real time

Inducible Hsps, in particular Hsp70 and Hsp27, have also been used as markers for severely stressed cells and tissues in the CNS. Hsps are released during brain or spinal cord cellular stress. Induction and expression, or release of pre-formed Hsp70, is robust and extremely rapid and has been shown in numerous tissues and organs including brain and spinal cord. Hsp70 is induced by ischemia, surgical stress, hyper- and hypothermia, hypertension, and other near-lethal stress.⁴

1.6. As biomarkers of stress

Hsp70 and Hsp27 levels change with a variety of conditions, including age, vascular disease, diabetes, smoking, hypertension, sepsis, and renal insufficiency.⁵ Although it is not an acute maker, Hsp27 in serum was found to be a marker for diabetic neuropathy in a sub analysis of the EURODIAB study, independent of other risk factors. Hsp70 protein and mRNA are elevated in tumors and have been proposed as targets in solid cancers because Hsp70 over expression confers protection from chemotherapy or radiation. Elevated HSPs in serum or tissue appear to be nonspecific indicators of organ ischemia or dysfunction and in chronic diseases may suggest a lack of vascular delivery reserve or of microvascular disease. In humans, Hsp70 synthesis in cardiac tissue is significant within 2 h after cardioplegia exposure. Levels of Hsp70 are elevated in as little as 30 min after severe trauma. Although elevated Hsp70 in serum correlated with survival, it was not correlated with severity of injury or subsequent organ dysfunction, suggesting that a mechanism independent of release from necrotic cells was involved. Surgical stress induces endocrine responses including the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system.
Hsp70 and Hsp27 protein levels are less than 0.5 ng/ml in human CSF and serum, even after exercise to exhaustion. Although resting Hsp70 in CSF was higher than in resting serum, levels of Hsp70 in CSF did not increase with exercise, at a time when serum Hsp70 increased fivefold.  

1.7. Hsp in anticancer therapy
Hsp inhibitor could stop tumor formation and significantly extend survival of mice. Hsp70 is an intracellular quality control officer, refolding misfolded proteins and preventing protein aggregation, which among other disorders, is associated with neurodegenerative diseases. Hsp70 also ferries proteins to their proper intracellular locations. Tumor cells, which face an abundance of cellular stresses, typically over express Hsp70, are making it a potentially interesting anticancer target.

The cancer microenvironment exposes malignant cells to a variety of stressful conditions that promote protein misfolding. Hsp70 helps cancer cells deal with this stress. Unlike normal cells, which typically express little, if any, of Hsp70, cancer cells contain high levels of this protein all of the time. Indeed, Hsp70 has been termed a cancer-critical survival factor, since cancer cells probably require the actions of this protein to survive the protein-altering adverse conditions. The inhibitor, called PES, interferes with the Hsp70 activities that the cancer cell needs to survive. So by targeting Hsp70, one can target the cancer cell. PES interacts with Hsp70 by blocking its stress-relieving functions. It also induces Hsp70-dependent cell death by disrupting the cell’s ability to remove damaged components. PES specifically targets Hsp70, a protein that is differentially expressed in normal versus cancerous cells, and one which is essential for the survival of the cancer cell. This pathway and protein target is important for cancer cells. Given the extreme heterogeneity of cancer cells, simultaneously disabling networks of signaling pathways may be important. Because PES targets a protein that is essential for a multiple of pathways, it could be effective against a variety of cancers.

Like many proteins, Hsp70 it functions through a cadre of interacting proteins, which augment its activity. It was found out that several known Hsp70-interacting proteins were no longer interacting properly when the cells were exposed to PES. Among those were proteins that help Hsp70 refold misfolded proteins and proteins that aids in its protein trafficking functions. PES blocks the cell’s ability to get rid of the proteins damaged by cellular stress in a process called autophagy, a process in which cells were basically eating themselves to death. PES will thus be vital to those scientists studying Hsp70 in depth. Other Hsp70 inhibitors exist but they are neither generally available, nor sufficiently specific. PES, thus provides a new basis for anticancer therapeutics, either directly as a treatment, or as a starting point for further development.

1.8. Plasma Hsp60 and cardiovascular disease risk:
The recent study at Whitehall has identified psychological distress as a major risk factor for coronary heart disease. The levels of circulating Hsp60 in 860 participants from the Whitehall cohort and 761 individuals diagnosed with diabetes have been measured and related to psychological, biological, and genetic factors. In the participants' serums, the concentrations of Hsp60 ranged from undetectable to mg/mL levels. Circulating Hsp60 correlated with total and low-density lipoprotein (LDL) cholesterol and was positively associated with cortisol decline over the day. Levels of this stress protein also correlated with measures of psychological stress including psychological distress, job demand, and low emotional support. Mass spectrometric analysis of circulating immunoreactive Hsp60 reveal that it is predominantly the intact protein with no mitochondrial import peptide, suggesting that this circulating protein emanates from mitochondria. The Hsp60 is stable when added to plasma and the levels in the circulation of individuals are remarkably constant over a 4-year period, suggesting plasma levels are partly genetically controlled. Sequence analysis of the Hsp60-Hsp110 intergenic promoter region identified a common variant 3175 C>G where the G allele had a frequency of 0.30 and was associated with higher Hsp60 levels in 761 type 2 diabetic patients. The extended range of plasma Hsp60 concentrations in the general population is genuine and is likely to be related to genetic, biological, and psychosocial risk factors for coronary artery disease.

1.9. Hsp in infectious diseases

Microbial pathogens expressed increased levels of Hsp as a defense mechanism to survive in their hosts during infection. Hsp act as major antigens and very strong inducers of humoral and cellular immune response to various infectious diseases. The evidence for the Hsp as major antigens during infectious diseases have been confirmed by experiments revealing increased levels of anti Hsp antibodies and activation of specific cellular immune response by activation of γ ST cells. Hsp 60 specific antibodies have been detected in patients suffering from TB, leprosy, typhoid. Increased antibody levels to hsp70 have been demonstrated in sera of patients with malaria, leishmaniasis, schistosomiasis and candidiasis. Hsp60 acts as major antigen in diseases like pertussis-gastritis caused by helicobacter pylori, histoplasmosis, toxoplasmosis, and Lyme disease. Table 2 shows presence of specific Hsps in relation to various pathogenic diseases.
Table 2. List of heat shock proteins associated with various pathogens

<table>
<thead>
<tr>
<th>PATHOGEN</th>
<th>DISEASE</th>
<th>HSP FAMILY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Tuberculosis</td>
<td>Hsp60, Hsp70</td>
</tr>
<tr>
<td>Mycobacterium leprae</td>
<td>Leprosy</td>
<td>Hsp10, Hsp60, Hsp70</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Gastritis</td>
<td>Hsp60</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>Pertussis</td>
<td>Hsp60</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>Typhoid</td>
<td>Hsp60, Hsp70</td>
</tr>
<tr>
<td><strong>PROTOZOA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmodium falciparum</td>
<td>Malaria</td>
<td>Hsp60, Hsp70</td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td>Chaga's disease</td>
<td>Hsp70, Hsp90</td>
</tr>
<tr>
<td>Leishmania donovani</td>
<td>Leishmaniasis</td>
<td>Hsp70</td>
</tr>
<tr>
<td>** FUNGI**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Candidiasis</td>
<td>Hsp90</td>
</tr>
<tr>
<td>Histoplasma capsulatum</td>
<td>Histoplasmosis</td>
<td>Hsp60, Hsp70</td>
</tr>
</tbody>
</table>

2. MATERIALS & METHODS

Detailed literature search.

3. DISCUSSION & CONCLUSION

There is a strong link between Hsp expression and atherosclerotic vascular disease. The study illuminates the role of Hsp in different infectious and autoimmune diseases. Hsps are expressed everywhere and act as dominant antigens of microbes during infection. The unique ability of HSP to activate or suppress both innate and adaptive immune responses to its associated antigens under different circumstances made them to be exploited as therapeutic antigens as well as therapeutic targets in many infections and cancers. The involvement of HSP in a multitude of intracellular actions places them as central coordinators in deciding the fate of cell. The level of various HSP, the amount of HSP, which are not occupied by damaged, misfolded proteins can be critical in cytoprotection and cell survival. Hsp 70
and Hsp27 are important markers in the detection of severe cellular stress and primarily ischemia. The role of these HSPs as a biomarker for ischemia is intimately related to but separate from the role of the HSPs in intracellular protection. It appears that HSPs can be secreted and do not reflect solely release from necrotic or damaged cells and organs. Secretion and circulation of extracellular Hsp70 (and Hsp27) is involved in antigen presentation, immune response, and patterns of “danger” signaling. The secretion or release of intracellular and extracellular Hsp70 during or immediately after organ ischemia has local and systemic physiological responses that are only now beginning to be understood. The protective properties of the HSPs have been described in a wide variety of species, organs, and tissues. However, most reports of the protective roles of the intracellular Hsps are from the cardiac and CNS tissues.

4. ACKNOWLEDGEMENT

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5. CONFLICTS OF INTEREST/ COMPETING INTERESTS

All authors of the manuscript do not have any conflicts of interest with publication of the manuscript or an institution or in the manuscript and/or is important to the outcome of the study presented.

6. REFERENCES