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MULTIDIMENSIONAL APPLICATIONS OF HEAT SHOCK PROTEINS: A REVIEW OF THE PERSPECTIVES FOR ENVIRONMENT AND HEALTH

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ABSTRACT

The heat shock response is a rapid transient reprogramming of all cellular activities leading to a fast and complete recovery of the cell. Heat shock proteins (Hsps) form the most ancient cytoprotective system in all living organisms on earth and generally interact with a number of cellular systems. Both *in vivo* and *in vitro* studies proved that various environmental stressors over express Hsps to protect the cells. These proteins usually help to refold the misfolded proteins in an organism and also help to eliminate them if they become irreversibly damaged. Experiments are gradually revealing the promise of Hsps as pharmacological targets in health researches. Hsps are found to be over expressed in human diseases like cancer, arthritis, diabetes, cardiac and autoimmune diseases. Based on a direct correlation between the level of stress and the expression of heat shock proteins, an idea in research that has emerged is the use of stress genes or proteins expression as an indicator to evaluate the level of toxicity. Heat shock proteins can be used extensively as molecular biomarkers in the biomonitoring and toxicological researches. Study of the expressions of heat shock proteins in common soil organisms such as microbes, rotifers, earthworms and in the higher vertebrates as well can reveal the extent of contamination and possible health hazards. However, it is very difficult to identify a specific environmental problem which is solely responsible for heat shock response in a particular organism. Further investigations are required to get deeper insights in the multidimensional appilcations of Hsps in the medical diagnostics and environmental monitoring.

INTRODUCTION

Stress can be considered as a physiological disturbance that can be correlated with various abnormalities.[1] In natural world, organisms are constantly being exposed to acute and chronic stresses, which are able to cause deleterious effect on cellular infrastructure and can disturb cellular homeostasis. In response to stresses, the conformation of structural proteins can be disrupted. Organisms have to respond to the different stresses by altering their cellular metabolism and by activating their defense mechanisms.[2] When a cell experiences environmental stress, it stops, or at least slows down most of its original functions, such as transport processes, DNA, RNA and protein synthesis.[2] The cellular stress response pathways are highly conserved in evolutionary history and play a central role in responding to environmental stresses.[2] Paradoxically, damage to cells can engage one of two opposing responses: apoptosis or programmed cell death that removes damaged cells to prevent inflammation and the heat shock or stress response that prevents damage or facilitates recovery to maintain cell survival.[3] Interactions between these two pathways determine the fate of a cell. One of the responses of a cell to the unfavourable alteration in its cellular environment is the sudden changes in genotypic expression of a group of stress genes which synthesize a group of proteins known as heat shock proteins (Hsps).

In 1962, Ritossa and co-workers first noticed that specific genes were activated by temperature induction in the salivary glands of *Drosophila melanogaster*.[4] In 1974, the products of the genes were identified and the 'heat shock proteins' term was introduced.[5] The heat shock response is a rapid transient reprogramming of all cellular activities leading to a fast and complete recovery of the cell. Hsps generally interact with a number of cellular systems and form efficient cytoprotective mechanisms. The heat shock or stress response is one of the most highly conserved adaptive responses in nature. For example, 50% of its sequence conserved between E. coli and human, and some domains are 96% similar.[6]

The term 'heat shock proteins' is a misnomer, because they are not solely induced by heat shock. These proteins are induced by heat and many other stresses such as infection, inflammation, exercise, exposure of toxins (like ethanol, arsenic, trace metals, heavy metals, solvents, effluents, pesticides and ultraviolet light), starvation, oxygen deprivation, nitrogen deficiency (in plants), water stress, salinity, osmotic stress, cold, etc.[7,8,9] Both in vivo and in vitro studies proved that various environmental stressors over express Hsps to protect the cells.[7]

Hsps are categorized into several families that are named on the basis of their approximate molecular mass. For example, the 70-kd protein is referred to as Hsp70. In mammalian cells, the stress response involves the induction of five major classes of Hsp families: Hsp27 (the small Hsps), Hsp60, Hsp70, Hsp90, and Hsp104.[2] Different types and amounts of Hsps are expressed by plants in natural conditions.[10] Higher plants are characterized by the presence of at least 20 types of small Hsps, which play an important role in membrane quality control and can potentially contribute to the maintenance of membrane integrity under stress conditions.[10]

Hsps are mainly localized in the cytosol, mitochondria and endoplasmic reticulum and exhibit constitutive and inducible regulation.[2] Hsp60, Hsp70, or Hsp90 are constitutively expressed in mammalian cells while others, Hsp27 and Hsp 70 are strongly induced by different stresses, such as heat, oxidative stress or anticancer drugs.[7] The Hsp70 family represents the most highly conserved among the Hsps, with almost identical functional counterparts can be found in the hierarchies of the entire living world.[2]

Heat Shock Proteins as Molecular Chaperones:

Apoptosis or Programmed cell death (PCD) is a common phenomenon in developmental processes or in normal physiological conditions, where the old or damaged cells are eliminated.[11] During apoptosis, distinct morphological and physiological changes occur in the cells (like nuclear condensation, cell shrinkage, fragmentation of nuclear DNA etc.).[11] Apoptosis is induced by an array of extra- or intracellular stimuli. The genetic machinery of an organism can decide whether to prevent or induce cell death depending upon the severity of the stress. Here the role of Hsps comes to play. These proteins usually help to refold the misfolded proteins in an organism and also help to eliminate them if they become irreversibly damaged. They have been referred to as "molecular chaperones" because of this function.[7] The majority of chaperones prevent the aggregation of "sticky" protein folding intermediates. By binding to their targets, chaperones are acting as "collectors" of damaged proteins.[2] However, in some cases, wherein it is better if the cell dies, there is no reason for any further defense. Hsps also occur under non-stressful conditions, simply to monitor the normal proteins of the cells.[2]

Heat Shock Proteins in Cancer and other diseases:

Hsps are generally found to be overexpressed in the tumor cells. The reasons behind the elevated levels of Hsps in tumor cells are still not clear. Probably an elevation in the expression of misfolded proteins increase the demand for Hsp mediated refolding capacity. Alternatively,

tumor cells experience a lot of stresses (hypoxia, nutrient deprivation, etc.), which can induce heat shock protein synthesis. The microenvironment of the tumor cells often experience hypoxic and glucose restricted conditions, which in turn can elevate the Hsp levels.[12] The major inducible chaperones Hsp70 and Hsp27 are present at elevated levels in various human tumors. These Hsps are expressed at high levels in a large fraction (up to 50%) of breast, endometrial, lung, prostate and other types of tumor biopsies.[13,14,15] Heat shock proteins regulate the biological mechanisms of cancer by promoting cancer development by suppression of various anti-cancer mechanisms, like apoptosis and senescence, as well as by helping in the expression of metastatic genes. They can also facilitate tumor rejection by the immune system.[2] On another side, Hsp27, the major member of the small heat shock proteins family, is overexpressed in a variety of cancers, and can play a major role in tumor development. The reason behind it is the anti-apoptotic activity of Hsp27, which upon over-expression blocks apoptosis caused by a variety of stimuli.[16] Cancer therapies could use the Hsps for treatment of the affected people. HSP gp96 could possibly be used as a vaccine against formation of certain tumor types in humans as it has worked properly in mice. This would be beneficial to patients with risks of developing a specific type of cancer.[17] Another possible use in the clinical research could be the over expression of Hsp 27 in normal cells which has also been shown to protect against necrosis that is induced by anti-cancer or chemotherapy drugs and radiation stresses.[18]

Hsps are also over expressed in some common human diseases like arthritis, diabetes, cardiac and autoimmune diseases. Reactivity to Hsp70 has also been implicated in the induction of disease in toxin induced interstitial nephritis.[19,20] The induction of molecular chaperones, most notably Hsp70, may prevent damage to cardiac muscle by both ischemia and reperfusion. So, in modern researches, molecular chaperones are actively being investigated as possible tools in the treatment of heart attack or stroke.[21] Induction of Hsps is also beneficial in transplanted organs, where moderate heat treatment reduces the chances of damage in transplanted organs and the risk of organ rejection.[22] They are cytoprotective molecules and their induction during and after the transplantation periods are likely to be a protective response for the maintenance of cell and tissue integrity. Recently, a single mutation in the small Hsp core domain human αB-crystallin was linked to abnormal intracellular aggregates of intermediate filaments in human muscle. The evidence can show that the overexpressions of stress proteins can contribute to an autoimmune response and can protect proteins that contribute to disease processes.[23]

Heat shock proteins and Infections:

When a parasite or bacterium enters the host organism, it usually finds the environment highly stressful. Temperature, pH, ionic strength and nutritional composition are all abruptly changed, and also the highly hostile reception by the immune system. Thus, over expression of a large panel of various Hsps occur to protect the organism. Many of these proteins are also expressed on the surface of the parasites or bacteria, providing an easy target for immune recognition. Since the structure of the Hsps has been highly conserved during evolution, the "stress-epitope repertoire" found on the surface of a wide variety of infecting agents is rather similar. Therefore, a very strong and generalized immune response develops against these proteins at an early stage and acts as a "first line of defense" during later infections.[24,25] Early stages of viral infections and intracellularly growing bacteria are stressful not only for the infecting organism, but also for the infected cells. This is reflected by an increased expression of Hsps, including Hsp90.[24]

Heat Shock Proteins and Aging:

Increasing age is associated with a reduced capacity to maintain homeostasis in all physiological systems. There is a progressive decline in the ability to produce Hsps in the cells with aging. Moreover, individual Hsps can become less able to mitigate the effects of stress on proteins due to aging. So, an attenuated heat shock protein response could contribute to the increased susceptibility to environmental challenges and the more prevalent morbidity and mortality seen in aged individuals.[26] However, if this hypothesis is correct, then treatments that both reduce damage to protein and increase Hsp expression should prolong life.

Hsp70 gene expression declines during normal aging in human retina, and heat shock-induced Hsp70 expression is decreased in senescent and late-passage cells, both of which suggest that the process of aging itself might be associated with reduced Hsp70 production.[27,28]

The accumulation of misfolded proteins is the major cause of neurodegeneration conditions like Alzheimer's, Parkinson's, Huntington's or prion disease. Overexpressions of Hsp40 and Hsp70 have beneficial effects in these conditions.[29,30,31]

Heat Shock Proteins as Biomarkers:

Due to industrial and vehicular pollution, pesticide contamination and other unsustainable anthropogenic activities, several thousand chemicals are affecting the health of our ecosystems significantly. However, gene expression analysis can indicate the extent of damage in biological systems if the target molecule is part of the defense, repair or detoxification machinery of the

cell. The stress proteins provide information on the biological impact of the toxic chemicals to organisms and predict adverse consequences of that exposure.

Based on a direct correlation between the level of stress and the expression of Hsps, an idea in research that has emerged is the use of stress genes or proteins expression as an indicator to evaluate the level of toxicity. Hsps can be used extensively as biomarkers in the biomonitoring and toxicological researches. In many cases, Hsps are especially useful biomarkers because their induction is much more sensitive to stress than traditional indices such as growth inhibition. Hsps are the potential indicators of pollutants or toxins in the environment, mainly in the aquatic environment. In the terrestrial environment, heavy metal contamination and pesticide accumulation affect the ecosystems severely. Study of the expressions of heat shock proteins in plants, common soil organisms such as microbes, rotifers, earthworms etc. can reveal the extent of contamination and possible health hazards.[32,33,34,35] Increased levels of Hsp70 have been measured in tissues of fish exposed to industrial effluents and polycyclic aromatic hydrocarbons; heavy metals such as copper, zinc and mercury; pesticides and arsenite.[36] Juvenile rainbow trouts exposed to metals in water or feed showed significantly higher Hsp 70 levels in the gill tissues.[37] Potentially, combinations of heavy metals can over express Hsps in soil nematodes in such patterns that these can become diagnostic fingerprints for specific toxicants in soil.[38] It was shown that environmental contaminants like naphthoflavone, cadmium chloride, and bleached kraft paper mill effluent (BKME) can increase the levels of Hsps at concentrations that were several fold lower than their LC50 values.[39] Heat shock proteins can act as biomarkers and can reveal the overall health status of an ecosystem. However, Organisms in the field often undergo multiple stresses simultaneously, interaction of which can yield significant Hsp overexpression even when no single monitored toxicant is at harmful levels. So, often it is very difficult to identify a specific environmental problem which is solely responsible for heat shock response in a particular organism. According to some authors, Hsps levels will not be regarded as an authentic biomarker because of adaptation to fluctuations in natural environmental conditions.[40]

CONCLUSION

Molecular chaperones were necessary for the establishment of life on Earth, for the bursts of activity during evolution, and are crucial for the protection of our own life against various stresses. Chaperones are one of the most abundant proteins in our cells, and may help to maintain and remodel the structure of the cytoplasm in eukaryotes. Hsps represent a remarkable example

of molecular "descent with modification" at the levels of gene sequence, genomic organization, regulation of gene expression, and protein structure and function. The patterns are so clear that they can be used to establish the evolutionary origins and the phylogenetic affinities of the major groups of organisms.

Although Hsp70s, Hsp60s, and Hsp90s have been shown to interact with a variety of polypeptides with physiologically important consequences, the chemistry of the interactions has been understood superficially. Among the molecular chaperones, Hsps have a dominant role in apoptotic inhibition. There are clearly many aspects of heat shock protein biology that remain puzzling. The proteins so far have executed both pro-apoptotic and anti-apoptotic activities. The synthesis and degradation of Hsps could also consume a large fraction of a cell's or organism's nutrient and energy stores, and can also occupy a huge portion of the metabolic system of an organism, that can create problems in processing of other essential biomolecules.[41] Experiments are gradually revealing the promise of Hsps as pharmacological targets both for inducing and inhibiting apoptosis. Whether Hsps over expressions are associated directly with the process of carcinogenesis still remains to be elucidated. Multiple cellular signaling pathways may converge to alter the expression of same gene products, which, further complicates the understanding of mechanisms of action of toxicants based on stress gene expression. Further investigations are also required on the role of co- chaperones in the regulation of apoptosis as they can also be interesting pharmacological targets. Knowledge about various kinds of small Hsps are still lacking. Recently, compounds which down regulate Hsp levels in cancer cells have been tested to study their anti-cancerous properties.[42] In near future, it is hoped that the identification of crucial points in the apoptotic pathway controlled by Hsps will provide rational targets for the development of a new generation of therapeutics.

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