ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 03, pp.5670-5676, March, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

HEAT SHOCK PROTEINS IN PARASITE BIOLOGY: A REVIEW

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ARTICLE INFO

Article History:
Received 07th December, 2013
Received in revised form
20th January, 2014
Accepted 15th February, 2014
Published online 25th March, 2014

Key words:
Heat shock proteins,
HSP70,
Parasite biology.

ABSTRACT

The most abundant and highly conserved HSPs are the HSP70 family members. They are known to perform a range of vital cellular functions under stressed as well as in normal conditions to maintain the intracellular integrity and in adaptation and survival of the parasite within the hostile environment. In most of the parasitic infection, parasitein its life cycle undergoes an inevitable thermal shift during migration between vectors and hosts. This shift results in producing a significant heat shock response to which the parasite counteracts to protect itself from aberrant conditions. HSPs, as known to play an essential role as chaperon and its functions are also being explored in recent times for their role in immune response. This review is showing a detail study of HSPs in various parasitic infections.

INTRODUCTION

Many Organisms are exposed to a variety of stressful conditions, including sudden temperature increases that damage important cellular structures and interfere with essential functions. In response these stress conditions; organism develops variety of defense mechanism one of these mechanisms is activation of an ancient signaling pathway leading to the transient expression of heat shock or heat stress proteins (Hsps).

HSPs are the most abundant intracellular proteins expressed in stress conditions and are present in both prokaryotic and eukaryotic cells. Because of their highly conserved structure they plays important role in fundamental cellular processes like prevention of aggregation, folding to the native state and refolding of aggregated proteins. HSPs are defined as molecular chaperone that non-covalently binds with hydrophobic surfaces of nonnative proteins (Csermeley *et al*., 1998). Its synthesis is rapidly up-regulated by various stresses like temperature (high/low), glucose deprivation, infection and cancer. The first report on HSPs was found in 1962 (Ritossa, 1962) when Drosophila salivary gland cells were exposed to 37°C for 30 min and then returned to their normal temperature of 25°C for recovery, a puffing of the gene was found to have occurred in the chromosome in the recovering cells (Ritossa, 1962). These genes were subsequently found to lead for an increase in the expression of proteins of molecular weight of 27 - 70 kDa. HSPs are multi-gene families that range in molecular size from 10– 150 kDa and are found in all major cellular compartments. They are classified on the basis of their molecular weight and function. Five families of HSPs namely sHSPs, HSP60, HSP70, HSP90 and HSP100 have individual function to perform in various quantities in different cells.

The HSP70 is one of the highly conserved proteins of HSP family, its prokaryotic version, called DnaK, shares about 60% sequence identity with eukaryotic HSP70 proteins, which are found in the cytosol and in cellular organelles, such as the ER, mitochondria, and chloroplasts. Under normal physiological conditions, HSP70s undergoes in the de-novo folding of proteins. While they are under stress, they prevent the aggregation of unfolded proteins and refolding of aggregated proteins (Mayer and Bukau 2005). HSP70 have housekeeping functions in the cell. The HSP70 comprise one of the most highly conserved protein families across species, which plays a fundamental role in number of essential cellular processes that include the folding and assembly of newly synthesized proteins as well as refolding of misfolded and aggregated proteins. It also helps in membrane translocation of secretory proteins, control of regulatory proteins, and the proteolytic degradation of denatured or unstable proteins (Mayer and Bakau 2005; Kobiyama *et al*. 2010).

1. Heat shock proteins in various parasites

There is renewed interest in the HSP70 protein family in a number of medically and economically significant parasites such as *Trypanosomal* species; they are the cause of fatal disease in both man and animals. *Trypanosoma brucei* and *Trypanosoma cruzi* infections cause African sleeping sickness

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and chagas disease, respectively, while *Leishmania major* infection results in debilitation and death from Leishmaniasis, *Brugia malayi* and *Wuchereriabancrofti* (Simpson et. al., 2005). The parasitic protozoa, *Trypanosoma brucei* and *Leishmania major* are transmitted by insect vectors to their mammalian hosts. There is a temperature shift (25°C and 37°C) between the hosts and parasite may induce a heat shock response in the parasite. The heat shock genes (homologous to HSP70 and HSP83) were 25 to 100 times more abundant in *Trypanosoma brucei* bloodstream forms (trypomastigotes) than in insect(procyclic) stages. The patterns of heat shock gene expression in *Leishmania*, *promastigotes* (insect-adapted) and *amastigotes* (mammal-adapted) were different. The temperature shift induced differentiation of *Leishmania major* from promastigotes to amastigotes. Therefore, heat shock gene may be responsible for differentiation of these vector-borne parasites.

1.1 In protozoa

HSP70 has been previously characterized in *Entamoeba histolytica* (Ortner *et al*. 1992). Interestingly, the expression of HSP70 is induced during encystations of the reptile parasite *Entamoebainvadens* (Field *et al*. 2000). The expression of HSP100 could also contribute to the resistance of the parasite. By screening the *Entamoeba histolytica* genome databank, Ankri (2002) has identified a gene that encodes a protein (Ehserp) with strong homologies to members of the serpin super family. Ankri (2002) proposed that Ehserp, HSP100 and arginase are involved in the parasite evasion mechanism. The major HSPs such as HSP70 and HSP90 are highly abundant in *Leishmania promastigotes* under all culture conditions which play stage-specific roles during infection (Brandau S, 1995). The protein-coding region is highly conserved when it is compared with its *Leishmania major*homologue, while 5'- and 3'-flanking DNA sequences display considerable divergence. Under heat stress, translation of encoded mRNA has an unusually long 5-leader sequence typical for RNAs. The gene product of HSP100 is abundant only during sustained heat stress, not under common chemical stresses (Huble *et al*. 1995). HSP100 associates into trimeric complexes and mostly found in a cytoplasm, which is possibly membrane-associated. Its localization was determined by immune electron microscopy and it shows immediate early expression kinetics during axenic amastigotes development. In its absence, expression of at least one amastigotes stage-specific protein family is impaired (Krobitsch *et al*., 1991). The replacement of the corresponding clpB gene in *Leishmania major* had a moderate effect on the thermo tolerance of this species reducing viability at 35°C (Hubel *et al*. 1997). The gene replacement also greatly affected the virulence of this parasite in laboratory animals. Krobitsch and clos (1999) has investigated that diverged structure and function of HSP100 may be responsible for the different permissive temperature ranges of *Leishmania major* and *Leishmania donovani*.

1.2 In helminthes

Thompson and coworkers has suggested that the expression of sHSPs (small HSPs) cDNA controlled at the level of RNA synthesis (Thompson *et al*. (1996). It is highly stage specific and temperature dependent in microfilaria of *Brugiaphangi*. Small heat shock protein gene of microfilariae of *Brugiaphangi* identified and cloned by Thompson *et al*. (1996). The development of microfilariae of *Brugiaphangi* in the mammalian host is blocked until uptake by a mosquito vector when the development cycle is re-initialed. Devaney *et al*. (1992) have compared the profile of polypeptide labeled in microfilariae in mammalian temperature (37°C) and mosquito temperature (28°C) which revealed a complex of low molecular weight protein (18kDa and 22-24 kDa) synthesized only in microfilariae at 37°C.

During heat shock condition, the synthesis of these proteins was induced which suggested that these are heat shock proteins. The sSHPs play a role in development of several other nematode species such as *Ancylostoma caninum*, *Dictyocaulus viviparous* and *Toxocar acanis* (Hartman *et al*. 2003). Salen *et al*. (2001) found the expression of HSPs and their immunogenic role in the host parasite relationship in *Trichinella spiralis*. The proteins of 71kDa and 81 kDa from heat shock larvae are suggesting that the HSPs can render the host more refractory to re-infection. The HSPs and other thermoregulatory protein play an important role in maintenance of the developmental block. Differential gene expression in microfilariae is not restricted. HSPs, with subsequent study identified a number of cDNAs up regulated in mammalian or mosquito (Emes *et al*. 2002).

1.3 Heat shock proteins in higher organism

HSP70s were extensively studied in livestock species as well as in human also. It was purified from different breeds of bovine (Guerriero and Raynes 1990; Kamwanja *et al*. 1994; Lacetera *et al*. 2006). The HSP70 gene has also been identified in the microalgae *Chlorococcum littorale* (Beuf *et al*. 1999) and *Volvox carteri* (Cheng *et al*., 2006). The conservation of HSP70 sequence reflected the conserved functional properties across the species. In the case of, Drosophila HSP70 expressed in mammalian cells efficiently protects them against heat stress (Pelham, 1984) and rodent HSP70 can be functionally complimented by human HSP70 to grant cellular protection against various in vitro stresses (Li, 1991).

It was also expressed in transgenic animals (Plumier 1995; Radford 1996). The most studied HSPs in higher organism are HSP70 and HSP90. Hightower and White (1981) reported the cell also contains a constitutively expressed protein which is similar to the nature of HSP70 except being slightly high in molecular weight and with a low isoelectric point. In bovine, endometrial tissue (Putney *et al*. 1988) and conceptuses (Putney *et al*. 1988) are responded to heat stress by an increased synthesis of HSP70 and HSP90. Heat stress affects productivity of livestock, but little is known about how animals respond to heat at the cellular level. Lymphocytes isolated from various animals were heat stressed and responded by synthesizing heat stress proteins. This cellular response may be an important mechanism by which these animals respond to heat stress. All species examined showed increased synthesis of both HSP70 and HSP90, which implies a common response to heat stress. However, there were differences in the less dominant proteins, which indicate species uniqueness.

2. ROLE OF HSP70

2.1 Protective role of heat shock protein 70

2.1.1 *In Vivo* studies

There are many evidences which indicate that heat shock protein is capable to protect the cells organs and animal from normal lethal heating as well as different types of noxious condition and ischemia. It has been reported that heat shock protein protects human gastric cells from sepsis-induced injury (Villar *et al*. 1994), transplanted pig kidneys from warm ischemic injury (Perdrizet *et al*. 1990), rabbit heart from ischemic reperfusion injury (Currie *et al*. 1993) and rat small intestine from ischemic-reperfusion injury (Stojadinovic *et al*. 1995). In rat, over expression of HSP70 displays the protective role to the lungs from sepsis–induced injury and reduced the hepatocytes apoptosis, which is induced by TNFa (Takano *et al*. 1998).

2.1.2 In Vitro Studies

The evidence has been derived from the study of cultured cells after heat shock, HSP70 gene promote over-expression of HSP70. It is reported that, the protection of human monocytes from hydrogen peroxide induced toxicity (Polla *et al*. 1987) and apoptosis (Samali and Cotter 1996), guinea pig gastric mucosal cells from ethanol damage (Nakamura *et al*. 1991) by the induced expression of HSP70. Protection of the human A-431 cells from NaCN toxicity (Kiang *et al*. 1997), rat FRTL-5 thyroid cells from hypoxia/deoxygenating injury (Kiang *et al*. 1996b), and human breast cancer T47D cells and MCF-7 cells from lethal temperature (Kiang *et al*. 1998) has also been reported. Kiang and coworker studied on the protective effect of heat shock is possibly mediated by over-expressed HSP70 because there is a lag between heat shock and the development of protection correlated with the production of HSP70 after 2hr of heat shock, and protection is affected when HSP70 production is inhibited by treatment with inhibitors (Kiang *et al*. 1996b).

The HSP70 also provides protection when induced by methods other than heat shock, such as treatment of human breast cancer T47-D cells with estrogen (Kiang *et al*. 1997), rat vascular smooth muscle cells with nitric oxide generating agents (Xu *et al*. 1997), and rat lung cells with arsenate or glutamine (Wischmeyer *et al*., 1997). Cells transfected with the HSP70 gene are protected from many harmful agents (Li *et al*. 1991; Kiang *et al*. 1998; Samali and Cotter 1996; Uney *et al*. 1993). It is reported that induction of HSP70 enhances the hydrogen peroxide cytotoxicity in Drosophila (Love *et al*. 1986) and the TCR/CD3- and Fas/APO-1/CD95-mediated apoptotic cell death in Jurkat cells (Liossis *et al*. 1997). Inhibition of HSP70 expression diminishes cell survival (Riabowol *et al*. 1988). These results further support the view that HSP70 is important for the cell survival. Evidence presented above suggests that harnessing endogenous protective systems such as HSPs can possibly provide therapeutic benefit. Harmless manipulation that increases the synthesis of cytoprotective HSP70 may prove to be of clinical use in organ transplantation and functional recovery of organs that require reperfusion as a result of lost blood supply.

2.2. Clinical importance of HSP70

2.2.1 Immunology and Infectious Diseases

Several studies have raised the possibility that HSP70 may be involved in various aspects of the immune system (Kaufmann and Schoel 1994). Genes encoding two members of the HSP70 family are found to reside in the major histocompatibility complex (MHC) and a protein binding motif of HSP70 is very similar to the peptide binding cleft of the MHC class-I proteins. A peptide-binding protein related to the HSP70 named as PBP74 family is involved in peptide loading of MHC Class II molecules. Deoxyspergulain, an immunosuppressant, is found to bind to HSP73 specifically (Nadler *et al*. 1992). Another immunosuppressant, FK506, appears to bind to HSP56 (Yem *et al*. 1992). It is likely that these two self-peptides are involved in immune processes, such as antigen presentation and cytotoxic cell killing of immune targets, which may lead to autoimmunity. In many bacterial infections in animals, the immunodominent antigen is *E. coli* chaperone 60kDaproteins (GroEL). Antibodies raised against GroEL from one bacterial species tend to recognize the protein from all other species of the same genus, but not those of a different genus. Several studies have shown that immunization with HSPs purified from pathogens protects against diseases such as blinding trachoma (Zhang and Brunham 1992), Legionnaires disease (Blander and Horwitz 1993), and malaria (Dubois *et al*. 1984). In some cases, the immunity against pathogenic HSPs tends to exacerbate diseases such as Lyme disease (Morrison *et al*. 1989; Shanafelt *et al*. 1991). Generally, HSPs are present only intra cellular part of the cell. It is suggested that bacterial GroEL is secreted or present on the surface membrane and allows T-cells access to the GroEL antigen. The other possibility is cytolysis of infected cells release pathogenic HSPs to the extracellular space, where they are detected by host immune cells. Macrophages in the host respond to infection by releasing cytokines, oxygen free radicals, and nitric oxide, which are involved in killing the infecting cells (Snyder *et al*. 1992). The cytokines, oxygen free radicals, and nitric oxide have been shown to increase HSPs synthesis in macrophages (Manthey and Voge 1992; Vogel and Hogan 1990). However the increased level of HSPs in macrophages may act to inhibit the release of cytokine, oxygen free radicals and nitric oxide (Van Dissel *et al*. 1987; Peetermans *et al*. 1993; Shigetada *et al*. 1996; Fincato *et al*. 1991). In this way, the HSP increase in macrophages may not benefit the host.

2.2.2 In the autoimmune diseases

It has been reported that T-cell receptor, ab-positive T-cells can recognize an epitope of HSP65, and this recognition causes autoimmune diseases, such as adjuvant arthritis (Van den Broek *et al*. 1989) and non-adjuvant arthritis in mice (Ito *et al*. 1991, Anderton *et al*. 1994; Van Eden *et al*. 1989). However, T-cell receptor ab-positive T-cells also recognize a different epitope of HSP65 and this recognition enables the modulation of autoimmune diseases in rats (Anderton *et al*. 1995). The HSP70 may also serve as an antigen which is recognized by a subset of T lymphocytes, which expresses g and d chains in place of a and b chains (Tamura *et al*. 1993; Hisaeda *et al*. 1997). This T-cell subset is disproportionally increased in patients with the systemic autoimmune disease lupus

erythematosus and an important role of HSP70 in autoimmune disease was documented (Rajagopalan *et al*., 1990). Numerous reports have claimed the presence of anti-HSP (e.g., 60 or 70 kDa) antibodies in sera from patients with rheumatic diseases, Graves` disease and Hashimoto`s thyroiditis. Until evidence is produced that unequivocally proves a definitive role of HSPs in the origin and pathogenesis of human rheumatic diseases, we should consider that the anti-HSP antibodies probably represent an effort by the body to protect itself from the HSP-like proteins released by stressed cells and infectious pathogens (Schultz and Arnold 1993).

2.2.3 In Cancer

It has been found that cancer cells exposed simultaneously to heat and chemotherapeutic agents die at a higher rate than cells treated with chemotherapeutic agents alone (Hahn and Li 1990). However, cells exposed to heat before treatment with chemotherapeutic agents exhibit resistance to drug treatment. For example, cultured breast cancer MCF-7 and MDA-MB-231 cells express high levels of HSP70 and HSP27 which exhibit resistance for treatment with doxorubicin, actinomycin D, and amphotericin (Ciocca *et al*. 1982; Shen *et al*. 1987; Rice and Hahn 1987; Hahn and Li 1990). It is clear that the increased expression of the multidrug resistance protein mediates the development of resistance to many cancer chemotherapeutic agents (Gottesman and Pastan 1988). Interestingly, the gene that encodes multidrug resistance protein contains an appropriate HSE. Therefore, modulation of the HSP70 content may enhance the therapeutic efficacy of chemotherapy and perhaps allow the use of lower doses of chemotherapeutic agents. Two studies have claimed that certain tumor cell lines express HSP70 on the cell surfaces (Chant *et al*. 1995; Multhoff *et al*. 1995). However, these studies have not shown in a definitive manner that HSP70 proteins are indeed membrane-anchored proteins and not just loosely associated with the outer surface. On the basis of reports that natural killer cells are involved in eliminating cellular targets that express HSP70 (Blachere *et al*. 1993; Botzler *et al*. 1996), it is tempting to speculate that HSP70 vaccination may have powerful anti-neoplastic activity (Nakagawa *et al*. 1991; Rajagopalan *et al*. 1990; Udono and Srivastava 1993).

2.2.4 In reproduction

HSP are also involved in several processes essential for cellular function under physiological conditions. Its production enhanced during the in-vitro embryo culture and they are among the first proteins produced during mammalian embryo development (Neuer, 2000). The spontaneous expression of HSP as an essential part of embryo development is well documented and presence or absence of HSP influences various aspects of reproduction in many species. HSPs are immunodominent antigen of numerous microbial pathogens e.g. *Chlamudio trachomatis*, which have been recognized as the main cause of tubal infertility. The fertility problems have a previous genital tract infection which becomes sensitized to microbial HSP. A prolonged and asymptomatic infection may trigger immunity to microbial HSP epitope that are also expressed in man (Neuer, 2000).

Conclusion

HSPs play a crucial role in various parasitic infections in various aspects. In earlier study Srivastava et. al., 2010 has reported in immunodiagnostic role in filarial infection. Here is an urgent need to study the role of HSPs in parasitic infection. HSPs play a protective role for parasite under stress condition during host-parasite interaction. These ideas may helpful to explore the role of HSPs as new chemotherapeutic agents / diagnostic tools for parasitic infection.

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