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Heat Shock Proteins: Heatingup skin cancer biology

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Abstract

Heat shock proteins (HSPs) are molecular chaperones with significant role in various physiological processes and pathological conditions including cancer. Certain cytotoxic insults or heat stress causes activation of the HSPs, which prevent cells from undergoing apoptosis and maintenance of cellular function. However, activation of HSPs also has detrimental effects particularly if the cells evading apoptosis possess oncogenic mutations. Over that last few decades there has been a steady rise in incidence of skin cancer globally and recent scientific and epidemiological evidence hypothesize that heat stress could also be a risk factor of skin carcinogenesis. More recently, HSP based vaccines have shown promise in treatment of early stage melanoma. Therefore, the aim of this article is to summarize the main concepts related to the expression and function of HSPs, from analysis of HSPs role in skin cancer and present final considerations related to HSP targeted therapy in this area.

Introduction

HSPs are polypeptides-proteins and famous in their role as molecular chaperones. HSPs are highly conserved molecular chaperones that are synthesized and expressed by the cell in response to stress conditions. The physiological body temperature of 37°C maintains expression and activity of proteins that are crucial for cellular homeostasis as well as for survival and cytoprotection [1-3]. However, an increment of 2°C can cause serious morphological damage to cells [4]. For instance, heat stress induces enlarged nuclei with distinct chromatin changes in heat-exposed keratinocytes, while heat induced enlarged cytoplasm can be observed in melanocytes [5,6]. In this context the HSPs play crucial roles and are responsible for many cytoprotective mechanisms. Recent studies have shown that HSPs are often highly expressed in a wide array of cancers [7,8]. HSPs could be stimulated by different stress signals and they intriguingly promote cell survival in various conditions. Furthermore, the active role of HSPs in tumor cell proliferation, differentiation, metastases and death, provides a strong rationale for intensive research and identifying molecular mechanisms involved. Several epidemiological studies have identified skin cancer to be closely associated with exposure to heat, such as in workers in bakery, mining and metallurgy industries [9]. These facts support the notion that heat may act an environmental stressor and an unidentified risk factor for skin cancer. In this review, we have summarized the roles of HSPs in physiology, cancer development and most importantly how HSPs serve as molecular targets for therapy in skin cancer.

Heat Shock Proteins (HSPs)

The folding of protein in the cells requires assistance of a number of cofactors called the molecular chaperones. Rittosa (1962) reported the expression of a set of proteins called heat shock proteins (HSP) associated with the puffed chromosomes of Drosophila when subjected to elevated temperature. These heat shock proteins were later found to be basically molecular chaperones assisting in the folding of unstructured proteins either newly synthesized or unfolded due to stress. The HSP identify non-native substrate proteins predominantly via their exposed hydrophobic residues. Since the demand of heat shock proteins depend primarily on the load of unfolded proteins present in the cell, HSPs need to be stringently regulated at the transcriptional and post translation levels to meet the demand of the cells [10,11]. Therefore at the cellular level meeting the chaperoning capacity includes temporary modifications in gene expression to survive changing environments, as well as altering cellular structure and function to deal with more permanent adverse conditions [12].

In the last couple of decades HSPs have emerged as a field of extensive research. Heat shock proteins are well conserved and have a ubiquitous presence occurring in all organisms from bacteria and yeast to humans. HSPs are best classified into families depending on their molecular weights. The first to be reported by Rittosa was the HSP 70 family of proteins. 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. HSPs vary in molecular mass from ~15 to 110 kDa and are classified into groups based on both size and function [13,14]. They are present in the cytosol, mitochondria, endoplasmic reticulum, and nucleus depending on the particular type of HSP and their isoforms. The most well-studied and understood HSPs in mammals are those with molecular masses of ~60, 70, 90, and 110 kDa. Small HSPs, with lower molecular weight exhibit tissue-specific expression pattern and include HSP32, HSP27, α/β-crystallin, and HSP20 chaperone.

Transcriptional Regulation of HSP

Heat shock proteins are stringently regulated at the transcriptional level by of the Heat shock transcription factors (HSF) binding to the promoter region Heat shock Element (HSE) lying upstream to most of the HSP genes [15]. As of now, four HSFs have been identified. HSF1,

HSF2 and HSF4 have been identified in mammals, whereas HSF1, HSF2 and HSF3 are identified in avian [16]. Two HSFs redundant in human cells, HSF 1 and HSF 2 binds to the same HSEs (Heat Shock Element) and have 38% sequence identity [17]. These factors are activated by distinct stimuli; HSF 1 is responsive to classical stress signals such as heat and oxidative reagents, whereas hemin-mediated differentiation of human erythroleukemia cells activates HSF2. HSP70 is regulated by the transcription factor, heat shock factor 1 (HSF1). Under cellular stress HSF1, which primarily resides in the cytosol trimerizes and migrates to the nucleus. In the trimeric state, HSF1 has a high affinity for cis-acting heat shock elements (HSEs) in the promoter region of heat shock protein genes [18]. The bound trimer forms a complex with the potential to activate transcription of the gene. HSP90 remains in complex with HSF1 in the cytosol under condition of "no stress" and prevents its subsequent binding to HSE and induction of HSPs (HSP70 and HSP90). However inhibiting HSP90 destabilizes the HSP90-HSF1 complex and the free HSF1 can induce HSP70 synthesis in the cell [19]. One of the important pre-requisites of the Heat shock Factor activation or deactivation is its phosphorylation at multiple phosphorylation sites. Each of these sites is acted upon by different signaling kinases which make HSF1 an important molecular switch to regulate stress response and decide the fate of the cell [20] [21].

Physiological Processes Regulated by HSPs

Physiological role of heat shock proteins ranges from embryonic development to apoptotic cell death. Functionally, heat shock proteins act as cytoprotective agent by maintaining proteostatis through (1) interacting with client proteins to ensure proper folding and function (2) translocation and transport of certain proteins to specialized organelles like mitochondria and (3) rapid degradation of damaged proteins [2]. In addition to these chaperoning functions of protein quality control, HSPs can act independently to be a critical player in triggering immune responses [22] and apoptotic signaling pathways [1].

Apoptosis: Phosphorylation and oligomerization of HSP27 as an immediate aftermath of stress response is thought to stabilize the cytoskeletal components such as actin microfilaments to promote survival. HSP27 can act more directly in resisting apoptosis is by virtue of blocking the mitochondrial translocation of Bid and suppression of cytochrome C release [23]. In melanoma cells HSP27 is reported to inactivate Smac (second mitochondria-derived activator of caspases) [24,25]. The effect HSP72 (inducible form) in modulating apoptotic pathways depend partially on its activity as a chaperone and partly by its direct interaction with proteins involved in the apoptotic pathway as evident from its role in influencing TNF mediated activation of tBid and NO meditated translocation of Bax in the mitochondria. HSPs might also modify apoptotic signaling downstream of mitochondrial release of Cytochrome c and upstream of activation of executioner caspases. Current research has thrown ample light on the role of HSP70 in disrupting the apoptosome complex, recruitment and activation of procaspase-9. It appears that HSP70 takes up a multilevel target approach to ensure efficient blocking of apoptosis in the stressed cells. [26]. HSP90 and HSP27 are also reported to prevent Apaf-1 oligomerization by directly associating with Apaf-1 and cytochrome c, respectively. The role of co chaperones in assisting these HSPs is still to be elucidated, but HSP40 seems to play a prominent role. So the above discussion suggests that the role of HSPs in preventing apoptosis is very similar to that of IAPs (inhibitor of apoptosis proteins such as livin, survivin etc).

Immune response: Extracellular HSPs can trigger innate immune response by interacting with antigen and stimulating the antigen presenting cells and leading to the activation of anti-tumor CD8+ and CD4+ T cells. These can have physiological benefits as providing a quick response to chemotherapeutic agents (discussed in the following section) or it can trigger pathological conditions like atherosclerosis and other inflammatory disorders. HSP60 activates human PBMC and monocyte-derived macrophages through CD14 signaling and p38 mitogen-activated protein kinase [27]. The immunogenic properties of heat shock proteins (HSPs) have been exploited to develop immuno-modulatory agents. HSPs have been used as potent adjuvants in immunotherapy of cancer and infectious diseases. The HSP-based vaccines can induce specific and non-specific cellular immune responses all of which are important to induce tumor rejection. Clinical application of such vaccine is currently underway for melanoma treatment. The most promising results have been observed in patients with melanoma and renal clear cell cancer without advanced disease [28].

Heat and Skin Cancer

Heat stress significantly impacts cellular physiology and can influence the activity of signal transduction pathways, particularly cell proliferation, survival and cell death in keratinocyte and melanocyte. A direct evidence of heat induced cancer can be identified in a small population of remote Himalayan region where the practice to carry burning coal in a willow basket tucked (locally called Kangri) near the lower abdomen still persists among the low income group people to stay warm during the freezing winter months. A 5-year study was conducted during which 17 patients who were documented as Kangri cancer treated. Sixteen patients had cancer on a thigh and 1 had cancer on the abdominal wall. These tumors have an aggressive biological behaviour with a substantial risk of loco-regional metastasis in 30-50% cases. Because of unique geographical distribution of Kangri cancer, there is dearth of literature regarding the natural history, loco-regional and distant metastatic pattern and treatment recommendations in these tumors. There exists an increased incidence of skin cancers among workers constantly exposed to intense temperatures, in mining and metallurgical industries. Further research is warranted to determine the role of heat in skin cancer formation, alone or in synergism with UV radiation [29-35].

Proteomic analysis of murine skin has shown that a variety of heat shock proteins (HSPs) are constitutively expressed in the skin. HSP27 is present in the epidermis, and HSP70 can be found in both the epidermis and dermis [36]. Role of HSPs in initiating immune responses has already been discussed. Research work using murine allergic contact hypersensitivity as a model established that HSPs play an important role to mount a severe allergic response to carcinogenic agents and act as a first line of defense to subsequently protect the skin from developing cancer. Inhibition of HSP27 and HSP70 produced a reduction to a hapten induced contact hypersensitivity response and further resulted in the induction of antigen-specific unresponsiveness [37]. Hapten induced immune response is triggered through increased activity of HSPs, their interaction with TLR4, and, in turn, increased production of cytokines that are known to enhance antigen presentation by T cells providing a crucial link between adaptive and innate immunity during the early stages of contact hypersensitivity. When mice were pretreated with anti-HSP27 and anti-HSP70

antibodies in vivo prior to subjecting them to a standard two-stage cutaneous carcinogenesis protocol, the percentage of mice with tumors was much greater ($p < 0.05$) in anti-HSP27- and HSP70-pretreated animals compared with mice pretreated with control antibodies arguing strongly in favor of a protective role of HSPs in prevention of skin cancer. The following section will discuss how a HSP based approach can be taken to formulate a targeted therapy for skin cancer [37].

Skin Cancer and Current HSP based Therapy

Melanoma is a form of skin cancer which if left untreated can become deadly. Until recently, dacarbazine (DTIC) was the standard therapy for metastatic melanoma patients, with IL-2 used in the US and fotemustine in some European countries [38]. Nevertheless, the few objective responses (ORs) obtained with these drugs were not associated with an improved overall survival (OS). Combined regimens of chemotherapy and immunotherapy increased ORs and toxicity without improving OS.

In recent years, this scenario has changed completely due to the introduction of two distinct therapeutic approaches, immunomodulation and mutation-driven therapies, which have shown survival benefits in randomized clinical trials for metastatic melanoma patients [39,40].

Nevertheless, both these therapeutic approaches have some limitations and need to be further evaluated in the long-term period. Immunomodulation resulted in adverse events in up to 40% of patients and only genetically defined subgroups of patients benefitted from mutation based therapies with short-lived responses and development of clinical resistance 6 – 9 months after the initiation of therapy [41].

In this complex scenario, several HSPs have been applied in the development of vaccines for cancer therapy. HSPs form macromolecular complexes (HSP peptide complexes [HSPPCs]) by non-covalently binding to low-molecular-weight peptides, and could be isolated and purified from cells. In preclinical and clinical models, HSPPCs isolated from tumor cells induced immunity against the entire and unique repertoire of antigenic peptides expressed by the cells they were derived from. Melanoma, renal clear cell carcinoma, and glioblastoma are among suitable targets for this treatment as demonstrated by clinical benefit obtained in subsets of patients, mainly those with early stage of disease and limited tumor burden [42,43]. Mechanistically, vaccination with heat-shock protein peptide complexes (HSPPCs) purified from metastatic lesions when injected intradermally or subcutaneously in melanoma patients induced both innate and adaptive immune responses. Interaction of HSPPCs with HSP receptors on antigen presenting cells such as the macrophages triggered signaling cascades along with endocytotic pathway. In a parallel response, interaction of the HSPs with CD91, CD14, TLRs resulted in secretion of inflammatory cytokines and chemokines. Following receptor-mediated endocytosis via CD91, Lox-1, chaperoned peptides are processed and presented in complex with MHC class I and class II molecules on the cell surface, leading to activation of CD8+ and CD4+ T cells [44,45].

Merkel Cell Carcinoma (MCC) is another rare and highly aggressive neuroendocrine skin cancer for which no effective treatment is available. HSP70 inhibitor has been recently found to be effective to limit proliferation and survival of various MCC cell lines [46]. Further research is warranted to identify more HSP-targeted based therapy for successful outcomes in skin cancer.

Conclusion

The incidences of people diagnosed with various forms of skin cancer annually are increasing and this skin cancer is emerging as one of the major health concerns across the globe. As currently treatment options for metastatic forms of melanoma are sparse, so critical understanding of HSPs' regulation and function can be utilized to develop targeted therapy for the disease.

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