

## ROLE OF HEAT SHOCK PROTEINS IN IMMUNE RESPONSE AND IMMUNOTHERAPY FOR HUMAN CANCER

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### ABSTRACT

Heat shock proteins (HSPs) are present in all living organisms, from bacteria to humans under normal conditions, immunological functions are conserved. They simply “monitor” the cell’s proteins i.e. they carry old proteins to the cell’s “recycling bin” or “garbage disposal” (proteasome) and they help newly synthesized proteins fold properly. They are expressed at high levels when exposed to a sudden temperature jump or other stress. This up-regulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor (HSF). Heat shock proteins act like ‘chaperones’, making sure that the cell’s proteins are in the right shape and in the right place at right time. HSPs are over-expressed in a wide range of human cancers and are implicated in tumor cell proliferation, differentiation, invasion, metastasis, death and recognition by the immune system. We review the current status of the role of the HSP expression in immune response and cancer with special emphasis on application of antigenic heat shock protein in immuno therapy for cancer.

**Keywords:** Heat Shock Factor, Chaperone, immune response, cancer.

### INTRODUCTION

FM Ritossa reported heat shock proteins for the first time in 1960. While working in lab he accidentally boosted the incubation temperature of *Drosophila* (fruit flies). On examining the chromosomes, he found a “puffing pattern” that indicated the elevated gene transcription of an unknown protein.<sup>1,2</sup> Increased synthesis of selected proteins in *Drosophila* cells following stresses such as heat shock was first reported in 1974.<sup>3</sup> This was later described as the “Heat Shock Response” and the proteins were termed as the “Heat Shock Proteins” (HSPs).

When a cell is exposed to increased temperature or other stresses, heat shock protein expression increases through enhancement of transcription. This is known as heat shock response and is thought to be caused by heat shock factor. Beginning in the mid-1980s, investigators recognized that many HSPs function as molecular chaperones and thus play a critical role in protein folding, intracellular

trafficking of proteins, and coping with proteins denatured by heat and other stresses. Most but not all, heat shock proteins are molecular chaperones. Molecular chaperones bind and stabilize proteins at intermediate stages of folding, assembly, translocation across membranes and degradation.

Heat shock proteins are named based upon their, molecular weight. For example the commonly studied HSP60, HSP70 and HSP90 refer to heat shock proteins of size 60, 70 and 90 kD. Heat shock proteins are among the most well-conserved proteins known. It account for 1-2% of total protein in unstressed cells which increases to 4-6% of cellular proteins when cells are heated.<sup>4</sup>

### Types of Heat shock proteins

The principal heat-shock proteins that have chaperone activity belong to five conserved classes: HSP60, HSP70, HSP90, HSP100 and the small heat-shock proteins (sHSPs).<sup>3</sup>

**Table -1:** Types of Heat Shock Proteins:

Approximate molecular weight (kD)	Prokaryotic proteins	Eukaryotic proteins	Function
10 kD	GroES	HSP10	-
20-30 kDa	GrpE	The HSPB group of HSP. Eleven members in mammals including HSP27 or HSPB1 <sup>5</sup>	-
40 kD	DnaJ	HSP40	Co-factor of HSP70
60 kD	GroEL, 60kDa antigen	HSP60	Involved in protein folding after its post-translational import to the mitochondrion/chloroplast
70 kD	DnaK	The HSPA group of HSP including HSP71, HSP70, HSP72, Grp78 (BiP), Hsx70 found only in primates	Protein folding and unfolding, provides thermo tolerance to cell on exposure to heat stress. Also prevents protein folding during post-translational import into the mitochondria/chloroplast.
90 kD	HtpG, C62.5	The HSPC group of HSP including HSP90, Grp94	Maintenance of steroid receptors and transcription factors
100 kD	ClpB, ClpA, ClpX	HSP104, HSP110	Tolerance of extreme temperature

### Heat shock protein 27 (HSP 27)

HSP27 is a chaperone of the sHSP (small heat shock protein) and was identified as a protein with high homology to the eye lens  $\alpha$ -crystallin proteins<sup>6</sup>. For instance analysis of mouse homologue, HSP25, showed that this protein has a compact two-domain structure, composed mainly of  $\beta$ -sheets that are similar to  $\alpha$ - $\beta$  crystallin. HSP27 is constitutively expressed in several organs and tissues like eye, nervous system, heart, blood and blood vessels, lung, bladder, colon and stomach, as well as in estrogen responsive organs such as uterus, vagina, cervix and placenta. Lower levels were detected in other tissues including epithelial cells of the breast, testes and striated muscle. Elevated HSP27 levels have been detected in a range of different tumours including breast cancer, prostate cancer, gastric tumours, head and neck cancers, uterine and ovarian cancers as well as in cancers arising from urinary system (bladder and kidney) and the nervous system (meningiomas, astrocytomas and neuroblastomas).

Under stressful conditions such as following heat stress injury, HSP27 expression is increased at the transcriptional phosphorylation level and pre-existing and newly synthesized protein undergoes significant post-translational phosphorylation at specific amino acid residues, which alters its functions in these cells. Such modifications result in the dissociation of larger HSP27 complexes to form smaller complexes of the proteins (tetramers, dimers or monomers) which have distinct functions.

HSP27 has homologous and highly conserved amino acid sequence, the so-called  $\alpha$ -crystallin-domain at the C-terminus. These sequences consist of 80 to 100 residues with a homology between 20% and 60% and form  $\beta$ -sheets, which are important for the formation of stable dimers.<sup>7,8</sup> The N-terminus consists of a less conserved region, the so-called WD/EPF domain, followed by a short variable sequence with a rather conservative site near the C-terminus of this domain.

In vivo, HSP27 functions in thermotolerance. In vitro it acts as an ATP independent chaperone by inhibiting protein aggregation and by stabilizing partially denatured proteins. HSP27 is also involved in the apoptotic signaling pathway. HSP27 interacts with actin and intermediate filaments and protects actin filaments from fragmentation. It also preserves the focal contacts fixed at the cell membrane.<sup>9</sup> It is also involved in process of cell differentiation.<sup>10</sup>

### Heat Shock Protein 60 (HSP60)

Mammalian HSP60 was first reported as a mitochondrial P1 protein.<sup>11</sup> The amino acid sequence showed a strong homology to GroEL HSP60's bacteria homolog. HSP60 in eukaryotes is considered typically a mitochondrial chaperone (also called Cpn60) which also occurs in the cytosol, the cell surface, the extracellular space and in the peripheral blood under normal physiological conditions.<sup>12, 13</sup>

In order for HSP60 to act as a signal it must be present in the extracellular environment. In recent research it has

emerged that chaperonin 60 can be found on the surface of various prokaryotic and eukaryotic cells, and can even be released from cells.<sup>14</sup> HSP60 has the capability of activating monocytes, macrophages and dendritic cells and also of inducing secretion of a wide range of cytokines.<sup>15</sup> HSP60 constitutes 15-30% of cellular proteins.<sup>16</sup>

Under normal physiological conditions, HSP60 is a 60 kD oligomer composed of monomers that form a complex arranged as two stacked heptameric rings.<sup>17</sup> This double ring structure forms a large central cavity in which the unfolded protein binds via hydrophobic interactions.<sup>18</sup> This structure is typically in equilibrium with each of its individual components: monomers, heptamers, and tetradecamers.<sup>19</sup> Each subunit of HSP60 has three domains: the apical domain, the equatorial domain, and the intermediate domain. The equatorial domain contains the binding site for ATP and for the other heptameric ring. The intermediate domain binds the equatorial domain and the apical domain together.<sup>14</sup>

It catalyze the folding of proteins destined for the matrix and maintains protein in an unfolded state for transport across the inner membrane of the mitochondria.<sup>20</sup> HSP60 plays a key role in preventing apoptosis in the cytoplasm. The cytoplasmic HSP60 forms a complex with proteins responsible for apoptosis and regulates the activity of these proteins. Cytoplasmic HSP also plays role in a "danger signal cascade" immune response.<sup>13, 15</sup> Bacterial HSP60 play a role in autoimmunity causing the immune system to create anti-chaperonin antibodies. These new antibodies are then recognizing and attacking human HSP60 which causes an autoimmune disease.

### Heat Shock Protein 70 (HSP70)

HSP70s are a family of ubiquitously expressed heat shock proteins. It is found in prokaryotes and eukaryotes.<sup>21, 22</sup> and is mainly localized in the cytosol, mitochondria and endoplasmic reticulum and exhibit constitutive and inducible regulation. It is not typically expressed in all kind of cells, but it is expressed at high levels in stress conditions. HSP70 is overexpressed in malignant melanoma<sup>23</sup> and underexpressed in renal cell cancer.<sup>24, 25</sup>

HSP 70 contains two distinct functional regions: a peptide binding domain (PBD) and the amino-terminal ATPase domain (ABD).

Peptide binding domain contains a groove with an affinity for neutral, hydrophobic amino acid residues. Amino terminal/C-terminal domain – rich in alpha helical structure acts as a 'lid' for the substrate binding domain. When an HSP70 protein is ATP bound, the lid is open and peptides bind and release relatively rapidly. When HSP70 proteins are ADP bound, the lid is closed, and peptides are tightly bound to the substrate binding domain.

Under normal conditions, HSP70 functions as ATP-dependent molecular chaperone that assist the folding of newly synthesized polypeptides, the assembly of multi protein complexes and the transport of proteins across cellular membranes. Under stressful conditions, elevated HSP70 levels allow cells to cope with increased concentrations of unfolded or denatured proteins<sup>26</sup>. It also inhibits apoptosis.<sup>27</sup>

### Heat Shock Protein 90 (HSP90)

HSP90 is a molecular chaperone and is one of the most abundant proteins expressed in cells.<sup>28</sup> It is highly conserved and expressed in a variety of different organisms from bacteria to mammals – including prokaryotic analogue htpG (high temperature protein G)<sup>29</sup> It has been identified in the cytosol, nucleus and endoplasmic reticulum, and is reported to exist in many tissues.<sup>30</sup> There are two isoform of HSP90 in mammalian cells – HSP90 $\alpha$  and HSP90 $\beta$ . Recently, a membrane associated variant of cytosolic HSP90, lacking an ATP-binding site, has been identified and was named as HSP90N.<sup>31</sup>

It consists of four structural domains.<sup>32, 33, 34</sup> A highly conserved N-terminal domain in which crystal structures are available.<sup>35, 36, 37</sup> A “charged linker” region, that connects the N-terminus with the middle domain. A middle domain is involved in client protein binding. It also increases the ATPase activity of HSP90. The C-terminal domain possesses an alternative ATP-binding site, which become accessible when N-terminal Bergerat pocket is occupied.<sup>38, 39</sup>

In unstressed cells, HSP90 plays a number of important roles, which include assisting in folding,<sup>40</sup> intracellular transport, maintenance and degradation of proteins as well as facilitating cell signalling. It acts as a general protective chaperone.<sup>41, 42, 43</sup> HSP 90 also participates in many key processes in oncogenesis such as self-sufficiency in growth signals, stabilization of mutant proteins, angiogenesis and metastasis.<sup>44-48</sup>

#### Release of heat shock proteins from mammalian cells

Increase in temperature was the first stimulus identified that promoted the induction of HSP, numerous other stimuli promote the induction of HSP. Skeletal muscle contraction is associated with the number of cellular stresses that may induce the heat shock response, e.g. increases in muscle temperature, changes in muscle pH, oxidative stress, mechanical stress and substrate depletion.

Exercise induces the expression of numerous HSP. Including HSP72, in the liver of exercised rodents<sup>49</sup> via a specific exocytotic pathway, as opposed to non-specific processes such as cell lysis.

It has been reported that specific cells within the brain can synthesis HSP72 in response to various cellular stresses, and, importantly, it has been shown that glial cells actively release HSP72 following stimulation<sup>50</sup>.

In addition to exercise stress, it has recently been demonstrated that psychological stress results in an increase in the systemic HSP72 concentration.

Intriguingly, in vivo evidence does indeed suggest that in response to various physiological stimuli, HSP can be actively released and subsequently accumulate in the systemic circulation. Several years ago, it has been demonstrated that the circulating concentration of a specific member of the HSP family, HSP72 – the stress-inducible member of the HSP70 family, was significantly increased following a bout of moderate-intensity exercise<sup>51</sup>. Importantly, this increase was observed in the absence of

any overt tissue damage and suggested that exercise stress may stimulate the active release of certain HSP from intracellular locales, to the extracellular environment.

The discovery that mammalian cells have the capacity to actively release specific HSP was originally made over 15 years ago. In 1989, it was demonstrated that mammalian cells possessed the capacity to release selective HSP in both the basal and stress-induced state.<sup>52</sup> In this study, culture medium from rat embryo cells, incubated at either 37 or 45°C for 10 minutes followed by a 2h recovery period, was collected and subjected to two-dimensional polyacrylamide gel electrophoresis. It was shown that cultured cells released a selective panel of proteins in the basal state, and in response to heat shock HSP72 was readily detectable in the cell culture medium. To address the cellular mechanism by which these proteins were released cells were treated with pharmacological inhibitors of the common secretory pathway. Intriguingly, neither monensin (a Na<sup>2+</sup> ionophore that disrupts the structure of the Golgi apparatus and inhibits vesicular transport) nor colchicines (an inhibitor of microtubule assembly) had any effect on basal, or stress-induced, HSP release. Importantly, further experiments provided strong evidence that the observed HSP release is indeed an actively regulated process as opposed to a non-specific release mechanism such as cell lysis<sup>52</sup>. While these data convincingly demonstrate that mammalian cells are indeed capable of releasing stress proteins, the cellular mechanism/s facilitating this transport remained, until recently, unknown.

#### Applications of Antigenic heat shock proteins in immuno therapy for cancer

HSP trigger immune response through activities that occurs both inside the cell (intracellular) and outside the cell (extracellular). HSPs are normally found inside cells. Due to cell necrosis the cell contents spills out. The abnormal peptides so spilled out gives the ‘danger signal’ to the immune system to generate a response in order to prevent any infection or disease.

Antigenic heat shock protein mechanism works by mimicking these ‘danger signal’ naturally triggered by extracellular HSPs. Depending on the abnormal peptides that have spilled out of the cell, the immune system can be activated to target different cancers and certain infectious agents.<sup>53</sup> The abnormal peptides found within diseased cells are different from cancer to cancer and from person to person. Therefore library of abnormal peptides is unique to each individual’s disease and can be thought of as the cancer’s ‘fingerprint’.<sup>54</sup> Antigenic patient specific vaccine consists of HSP-peptide complexes that have been isolated from that patient’s cancer cells. When the vaccine is injected into the body, the fingerprint of HSP-peptide complexes can directly encounter the immune system’s cells, which is designed to stimulate the immune cells to target cancer cells bearing the fingerprint.

#### Intracellular activities

HSP end up binding virtually to every protein because of their normal functions of inside the cell (such as helping proteins fold, preparing proteins for disposal, etc.). This

means that at any given time, HSPs can be found inside the cell bound to a wide array of peptides that represent a 'library' of all the proteins inside the cell. This library contains normal peptides that are found in all cells as well as abnormal peptides that are only found in sick cells.<sup>55</sup>

Research suggests that inside the cell, the heat shock proteins take the peptides and hand them over to another group of molecules. These other molecules take the abnormal peptides that are found only in sick cells and move them from inside the cell to outside on the cell's surface. When these abnormal peptides called antigens are displayed in this way, they act as red flags, warning the immune system that the cell has become sick.

### Extracellular activities

Heat shock proteins are normally found inside cells. When they are found outside the cell, it indicates that a cell has become so sick that it has died and spilled out all of its contents. This kind of messy, unplanned death is called necrosis, and only occurs when something is very wrong with the cell. Extracellular HSPs are one of the most powerful ways of sending a 'danger signal' to the immune system in order to generate a response that can help to get rid of an infection or disease

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