Review Article

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Heat Shock Proteins and Their Therapeutic Applications: An Overview

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Abstract

Heat shock proteins (HSPs) are a class of proteins that play important role in protein folding, maintaining homeostasis, and suppressing the aggregation of mis-folded proteins. The synthesis of these proteins in the cell is highly regulated, and is induced under various stress conditions that include the pH, temperature, and starvation, UV and chemical exposure and the oxidative stresses; however few Hsps also expresses constitutively. The major classes of HSPs include the HSP60, HSP70 and HSP90 and the small heat shock proteins that ranges from 12-40 kDa in size. The small heat shock proteins like Hsps 18 are well known to facilitate the refolding of substrate proteins and maintaining its biological activity, for which this protein has been explored as an efficient delivery system for the vaccines development. This review will discuss various HSPs and their close relatives involved in folding, assembly, regulation, and degradation of other proteins. The review will further highlight the various approaches by virtue of which the Hsps can be employed in therapeutic interventions.

Keywords: Molecular Chaperons; Folding; Therapeutics; Cancer; Stress; Heat Shock Protein.

Introduction

During stress conditions like high or low temperature, pH, osmotic stun, starvation, UV and chemical exposure, oxidative stress, almost all organisms expresses heat shock proteins that help these organisms to survive and perform their proper functions under these conditions [1-2]. These proteins function as molecular chaperons, due to their assisting role in proper folding of the partially or mis-folded proteins, it also suppress the aggregation of mis-folded proteins [3]. During this process they require ATP, and are assisted by their co-chaperons for efficient functioning. Synthesis of Heat shock proteins should be transitory even in sustained stress conditions, as their continuous synthesis would unfavorably affect the protein homeostasis as well as various cellular functions. A mechanism regulating the synthesis of Heat shock proteins involve binding of Hsp70 to transactivation domain of HSF1, thus repressing transcription of heat shock gene [4] Figure 1. Another mechanism leading to inhibition of heat shock protein synthesis involves binding of heat shock protein binding factor 1 (HSBP1) to HSF1 trimer and Hsp70, preventing HSF1 binding to DNA [5]. Heat shock response in prokaryotes is regulated by sigma factor, which is encoded by *rpoH* gene. This sigma factor binds to RNA polymerase and helps in transcription of the heat shock genes [6-7-8]. In *E. coli* various genes, whose transcription involve ó-32 factor, have been identified that include e.g. *htpY, dnaK, rpoD, grpE, groES, groEL, clpB* etc. Regulation of heat shock protein synthesis in prokaryotes is shut down by the feedback inhibition mechanism that is induced by *DnaK* chaperone machine. *DnaK* chaperone machine

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includes *DnaK, DnaJ* and *Grp E* proteins. These three proteins help in down regulation by repressing the translation of *rpoH* mRNA. Repression of mRNA translation lead to degradation of σ-32 and repression of its activity [9]. In eukaryotes, Heat Shock Factors (HSF), carry out the process of transcription [10]. Cells without stress have HSF bound to HSPs in the cytoplasm , whereas under stress conditions, HSF separates from the HSPs and form a trimer. HSF trimer enters into the nucleus and its phosphorylation occurs which are pre-requirements for binding of HSF to heat Shock Elements that carry the transcription process [11].

This review endeavors to offer an overview of heat shock proteins and their role in as autoimmunity, cancer and other vascular diseases. Various Hsps and their close relatives that are known to be involved in folding, assembly, regulation, and degradation of other proteins are also discussed in this article. It further highlights the mechanisms underlying these functions that provide insights into the approaches by which heat shock proteins can be used as therapeutic interventions. Primary objective of this review article is to glimpse the structure and functioning of various classes of HSPs and to further provide insights about their role in therapeutics.

Heat Shock Proteins as Molecular Chaperons

Molecular chaperones help in stabilizing unstable proteins by binding to them and assisting the processes like folding, assembly and regulation of denatured conformations of proteins [12]. Principal categories of chaperones that perform these functions are the small heat shock proteins, proteins of Hsp90 family [13], Hsp 100 family [14], Hsp 70 and Hsp 60 family [15]. These chaperones are sometime assisted by co-chaperonin that helps these chaperones in the folding process. For example, *Gro ES* is a co-chaperone that assist chaperone *Gro EL* in folding of proteins. Similarly, *Dna J* is a co-chaperone that assists *dnak*. During the folding process, chaperones require energy which it attain by hydrolyzing ATP into ADP. During ATP hydrolysis, conformational changes take place in the heat shock proteins [16]. The table below depicts members and functions of Heat shock proteins.

Major Families of Heat Shock Proteins

Hsp90 Family

HSP90 is an ATP-dependent molecular chaperone, which is known to stabilize the substrate proteins during the formation of steroid receptor complexes [31]. Hsp90 maintain the active or inactive conformation of client proteins and inhibit the aggregation process [32]. Structurally, It is a dimeric protein comprises of four domains that include the N-domain, charged linker, middle domain and C-domain. Middle domain consists of ATPase site, and is responsible for ATP hydrolysis and binding of substrate proteins [33]. Interestingly, Hsp90's, ATP-binding region has a lid that generally exists in an open conformation during ADP-bound structure, but changes to a closed conformation when ATP bound [34]. C- Terminal domain comprises of three stranded β-sheet and α-helix coil [35]. The middle domain of Hsp90 has three regions- two α–β–α domain (large and small) and a helical coil [36]. Charged linker region comprises of repeats of charged amino acids which are highly conserved and are connected with N terminal domain of *Hsp90.* ATP bound conformation of *Hsp90* was depicted along with *p*[NH] *ppA* (adenosine 5_-[β,γ - imido] triphosphate; 'AMP-PNP') and p23/Sba1 co- chaperone [37]. *Hop/ Sti1* (*Hsp70-Hsp90* organizing protein) is a co-chaperone which associates *Hsp70* chaperone system with Hsp90 chaperone machinery and also inhibits ATPase activity of *Hsp90* [38-39]. Notably, inhibition of ATPase activity is associated with another co-chaperone *Cdc37/p50* [40].

Genes of *Hsp90* family from various dissimilar organisms like fruit flies, yeasts, chickens, Mammals, trypanosomes, and bacteria has shown very much identity in these sequences. All the proteins of *Hsp90* family in eukaryotes and bacteria *E. coli* contains a region of high negative-charge at the carboxyl end, which is usually observed to be different, except four amino acid residues, *glu-glu-val-asp,* that is observed to be similar among *Hsp90* of eukaryotes. Earlier studies have demonstrated that proteins of the *Hsp90* family are present abundantly even under normal temperatures conditions, and are induced by heat. In *Drosophila melanogaster*, only one gene of this family i.e. *Hsp83* is known. In yeast *S. cerevisiae*, two genes of this family are known – *Hsc83* and *Hsp83*, while *Hsc83* is reported to be constitutively expressed and induced moderately by heat, in contrary, the *Hsp83 i*s constitutively expressed at a minor level and is strongly induced by heat [41]. In *Arabidopsis thaliana,* truncated cDNA of *Hsp83* was isolated, sequenced and cloned to full length by primer extension methods. The study revealed that the level of homologous transcripts of this cDNA increases upon induction at high temperature [42]. The protein of the endoplasmic reticulum also contains the sequence *glu-glu-val-asp* at the same position in the protein, but not at the C-terminal position. The ER

protein *GRP94* is glucose regulated protein as it is induced by glucose starvation and the cytosolic one is induced by glucose restoration [43].

Hsp70 Family

The 70 kDa family of proteins is the most abundant family of heat shock proteins. Hsp70 play a role in folding of the denatured proteins into their native state and further holds the unfolded polypeptides [15]. The structure of Hsp70 consists of an ATPase domain, a β- sandwich sub domain at the Carboxy end which is its substrate binding domain and an α-helical subdomain. The activity of Hsp70 takes place when hydrophobic peptides of the proteins interact with substrate binding domain of Hsp70 in an ATP-dependent manner. Hsp40 is a co-chaperone that helps Hsp70 in folding process, and is essential to activate the ATPase activity of Hsp70 that results in Hsp70-ADP complex. The release of ADP is aided by a nucleotide-exchange factor for the opening of nucleotide binding cleft. When ATP binds the ATPase domain of Hsp70, a conformational change in the substrate binding domain takes place and substrates that are bound gets released which completes the ATPase cycle [44]. All Hsp40 comprises of a domain known as J-domain which necessarily help in cellular activity via interacting with it. The J-domain has a conserved sequence of tripeptides - Histidine-Proline-Aspartic acid (HPD) [45]. Hsp40s are classified into three classes on the basis of the functional domains contained in them. Type I Hsp40s are highly conserved and consist of a glycine-phenylalanine (G/F) and cysteine rich region that consists of four motifs of CXXCXGXG which is a glycine/methionine rich region. A carboxy terminal peptide binding domain and a dimerization domain. Type II Hsp40s consist of the J domain and the G/F rich region, along with the peptide-binding region at the C-terminus. Type III Hsp40s possess only the conserved J-domain that can exist anywhere on its sequence [46]. Apart from folding, members of 70 kDa heat shock proteins appear to play necessary roles in Clathrin-dissociation activity from vesicles coated with bovine and depict high ATPase activity too [47]. Hsp70kDa family is also required for the synthesis of protein, its translocation as well as storage [48]. Various cell organelles like cytoplasm, nucleus, mitochondria and endoplasmic reticulum found to have hsp70 and other members of its family [49]. Various proteins associated with Hsp70 family are inducible only under stressful conditions, whereas, few are constitutive in nature and expresses under normal conditions too [49-50].

Hsp60 Family

Hsp60 (GroEL) is a chaperonin that belongs to molecular chaperone family and assist protein folding of denatured proteins [18-51]. The structure of GroEL is in the form of a cage consisting of two seven-membered rings of 57kDa each within the central cavity [25]. The detail investigation of the structure GroEL structure has revealed the presence of three domains – Equatorial domain, Apical domain and a middle Hinge domain. The equatorial domain is an ATP-binding region and the apical domain is a substrate-binding region consisting of hydrophobic residues. The third domain middle hinge is a connecting domain between the other two domains [52-53]. Co-chaperonin named as GroES (Hsp10) assists GroEL in the protein folding process which binds upon the open-cavity of GroEL as a lid (Figure3). Binding of GroES causes rotation of the subunits of Hsp60 in a way that protein is released from the hydrophobic site into hydrophilic part which facilitates its folding. Interestingly, energy in the form of ATP is required for the same. Denatured protein binds to the hydrophobic region of apical domain on the inner side of GroEL. Consequently, ATP binding leads to a conformational change which facilitates release of substrate protein and binding of GroES. Furthermore, hydrolysis of ATP causes release of GroES lid and liberates the substrate protein. Complete folding of the client protein is achieved after manifold of cycles [53-54].

Contrary to GroEL-GroES chaperone system, TRiC is member of Hsp60 Family present in the eukaryotic cytosol which is constitutively expressed [51]. Large sized proteins that could not undergo folding via GroES-GroEL chaperone machinery undergo folding *via* binding to TRiC. TRiC binds to emerging polypeptides from the ribosomes which are brought to TRiC through a chaperone GimC or through DnaK-DnaJ chaperone machinery [26-54]. Hsp60 activity is regulated by several post-translational modifications that include acetylation [55], glycosylation [56] and ubiquitination [57].

Small Heat Shock Protein Family

Small Heat Shock Protein Family comprises of members of molecular size ranging from 12kDa to 40kDa. These are unique in having an 'alpha-crystallin domain' which remains conserved throughout. Besides this, sHsps also consists of a C-domain and N-domain, as revealed by X-ray crystallography studies [58-59]. Small heat shock proteins display chaperone activity. sHSP 18 act as molecular chaperone same as that of alpha-crystallin. Both sHSP 18 and alpha-crystallin are effective in preventing inactivation of restriction enzyme from

heat [45]. Hsp18 play a role in maintaining the biological activity of the proteins as depicted in one study, where authors have showed the role of sHsp18.1 preventing the denaturation of enzymes NdeI and SmaI from thermal inactivation [60]. Another study also confirm that Hsp18 bound to a heat denatured luciferase enzyme could re activate it in the presence of wheat germ extracts or rabbit reticulocyte and thus shows that this small heat shock protein can help in refolding of substrate proteins [61-62]. Due to its chaperone activity in maintaining the protein stability, sHsp18 is also used as an efficient delivery system for the vaccines [60].

HSP20/α-crystallin, another small heat shock proteins categorized as a molecular chaperone Members of the sHsp 20 family have a common structure that consists of α -crystallin core structure that is found in the C-terminal position . Any alteration in these chaperones are reported to be associated with different diseases such as prion disease, cystic fibrosis, cataracts, or neurodegenerative diseasesincluding Huntington's disease, Parkinson's disease and Alzheimer's diseasedue to the aggregation of a protein because of partial unfolding and exposure of hydrophobic surfaces of proteins [63].

Hsp27(HspB1), a Class1 Heat shock protein is known to repress senescence [64] and block apoptosis in cancer by inhibiting the mitochondrial release of apoptotic proteins. Hsp27 facilitates apoptosis by binding to cytochrome c, inhibiting the formation of apoptosome and thus preventing the activation of Caspase-3 [30]. Another study related to sHsp30 proposed that induction of this protein is known to down regulate the stress stimulation of H^+-ATPase activity. It provides various tolerances to several stresses like heat shock, osmotic stress, glucose starvation, organic acid stress etc. These stress tolerances are not affected by the loss of hsp30 in cells rather the time required for adaptation to these stress conditions is extended [65].

Heat Shock Proteins As Therapeutic Targets

Heat Shock proteins are important in maintaining the homeostasis in cells. One of their main functions includes transport of peptides among the components of the cell. Due to this approach, stress proteins are targeted for the regulation of immune system. Foreign antigenic peptides induce the expression of heat shock proteins and generate immune response in the body. These proteins also help in presenting the foreign peptides to the immune cells. HSPs can also be used in the anti-cancer vaccines, as these help in presenting the foreign peptides from the cytosol to the MHC-1 complex in the endoplasmic reticulum. MHC-1 and antigen complex after binding with the CD4 receptors of the T-cytotoxic cells induces an immune response against the antigenic peptide. MHC-1 complex becomes active by the cytokines released by T-helper cells which are associated with MHC-2 complex to generate an immune response. This strategy helps in designing an approach for the vaccination in which antigenic peptides and chaperone complex can be introduced into the tumor cells to generate an immune response [66].

HSP Based Anticancer Vaccines

Heat shock proteins are important due to their functional role in preventing the accumulation of degraded or misfolded proteins. Hence, *Hsps* play a major role in preventing body tissue degradation and aging process [67]. Moreover, the level of expression of these chaperone elevate in cancer [68-69]. *HspC2* was found to have high expression level in breast cancer cells [70], while *HspB1* increased expression is associated with prostate cancer, liver cancer, pancreatic cancer as well as gastric cancer [71-72]. Overexpression of heat shock proteins has been known to be problematic in various anticancer therapies, and can cause resistance to these therapies *via* refolding of proteins and preventing the apoptosis [71-73]. Hence, down regulation of over-expressed *Hsps* can be an effective way to overcome this problem. Hsp90, one of the most abundant proteins among Heat shock proteins have been targeted in a variety of cancers, such as Breast cancer, Colon cancer, Solid Neoplasm, Gastric Carcinoma. Targeting and inhibiting Hsp90 resulted in degradation of oncogenic proteins [74]. Various clinical trials are recruiting related to Hsp90 inhibitors as mentioned in table below. The first inhibitor of Hsp90 as 17-AAG (17 Allylamino-17-Demethoxygeldanamycin) [75]. Nonetheless, more research is essential to state a clear and thorough mechanisms of Hsps in allograft rejection. Immunogenic properties of the Hsps is well known [76]. Mechanisms lying behind Hsps mediated immunity involve the binding of these Hsps to antigenic peptides and their presentation to Antigen Presenting Cells (APCs) and cross priming of Cytotoxic T-lymphocytes [77-78]. Thus, immunotherapy can be used to deploy Hsp vaccines to the patient. An example illustrating the use of Hsps as vaccines involves *Gp96* vaccine in the treatment of melanoma and carcinoma is in clinical trials [79-80]. Hsps belonging to large molecular weight chaperone families including Hsp110 and Grp170

are important targets in the development of anticancer vaccines as they are known to bind to the antigens Trp2 and Gp100 in case melanoma. In addition to this, studies revealed that both Hsp110 and Grp170 increased the immunogenic peptide effects greatly [81-82]. Some Hsps are known to be expressed on surface tumor cells but are absent on the surface of normal cells. Thus, natural killer cells recognize these tumor cells due to the responsiveness of Hsps expressed on their surface [83-84]. Hsp based vaccines have the potential to treat various kinds of cancer as mentioned in the table depicting interventions used in clinical trials.

Heat Shock Proteins in Neurodegenerative Diseases

Neurodegenerative diseases occur due to generation of neurotoxicity through misfolding and aggregation of proteins. These include Huntington's Disease, Parkinson's Disease, Sclerosis, Muscular Atrophy, Ataxias etc. [85]. Among neurodegenerative diseases, Alzheimer's is a type of dementia which is characterized by the formation of amyloid plaques leading to brain cell death. In the diseased state, a protein named Amyloid Precursor Protein (APP) is cleaved by β and γ secretase to generate $A\beta_{42}$ which is a neurotoxic fragment. The aggregation and oligomerization of this neurotoxic fragment leads to the plaque formation; hence causing neuronal cell death [86- 87]. Heat shock proteins have been observed to be linked with the protein aggregates. HspB proteins have been found in plaques and amyloids [88] and HspA1 proteins have been associated with Aβpeptides [89].

Parkinson's disease is another neurodegenerative disease which is characterized by the presence of mutations in α-synucleic gene. Various studies suggest that several members of Hsps have been found to be associated with the treatment of Parkinson's disease. An example includes small heat shock protein Hsp27 which inhibits the toxic effects caused due to mutations in α-synuclein [90]. Moreover, Hsp27 and α-B crystallins have been found successful in the treatment of neurodegenerative diseases by preventing the fibril formation through their binding to Aβ peptides [91].These studies suggest that Heat shock proteins have been implicated in the treatment of neurodegenerative diseases.

Other Studies Related to Hsp Vaccines

Several investigations have associated heat shock proteins to autoimmune diseases. Evidences suggested that Hsp60 is implicated in rheumatoid arthritis [92] and Type 1 diabetes [93]. Hsp60 also facilitates the secretion of various cytokines, thus can be a potential target for inflammatory diseases [94]. A study has demonstrated the use of therapeutic agents which are competitive inhibitors of Hsp70 against Gaucher disease, lysosomal storage diseases and β-galactosidase deficiency disorders in order to stabilize the damaged proteins [95]. Another therapeutic agent, Salvianolate has shown to treat ischemia in a rat model [96]. Small Heat shock proteins like Hsp27, Hsp22 and HspB5 are characterized by the presence of α-crystallin domain that is correlated with inflammation, apoptosis and tumorogenesis [97-98-99]. Heat shock proteins find

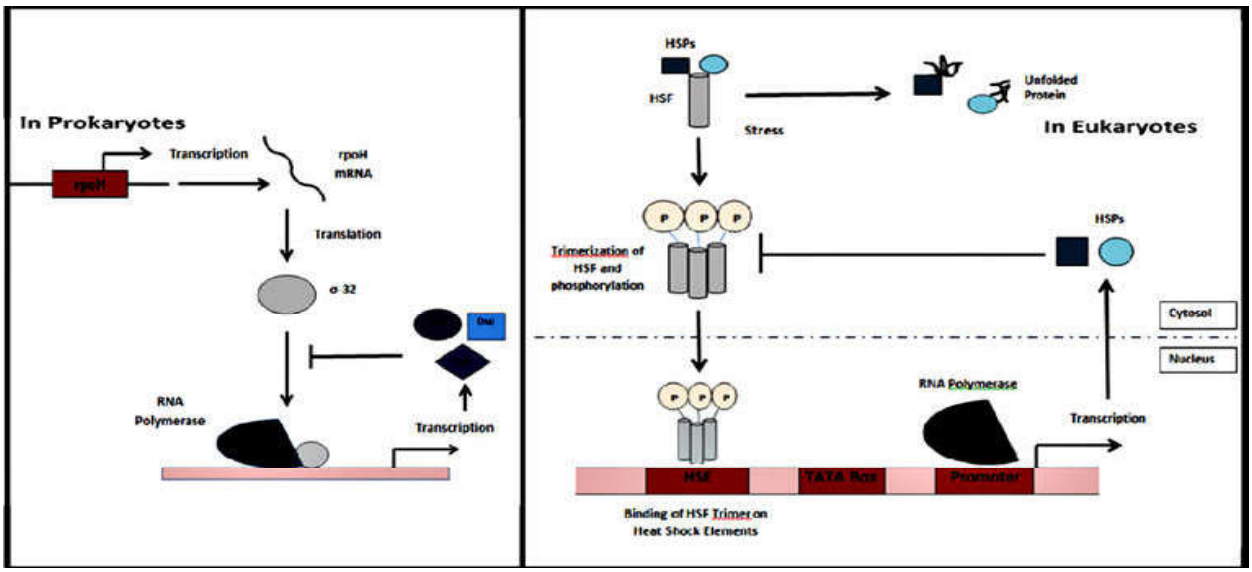

Fig. 1: Regulation of Heat Shock Proteins in **(a)**Prokaryotes **(b)**Eukatyotes

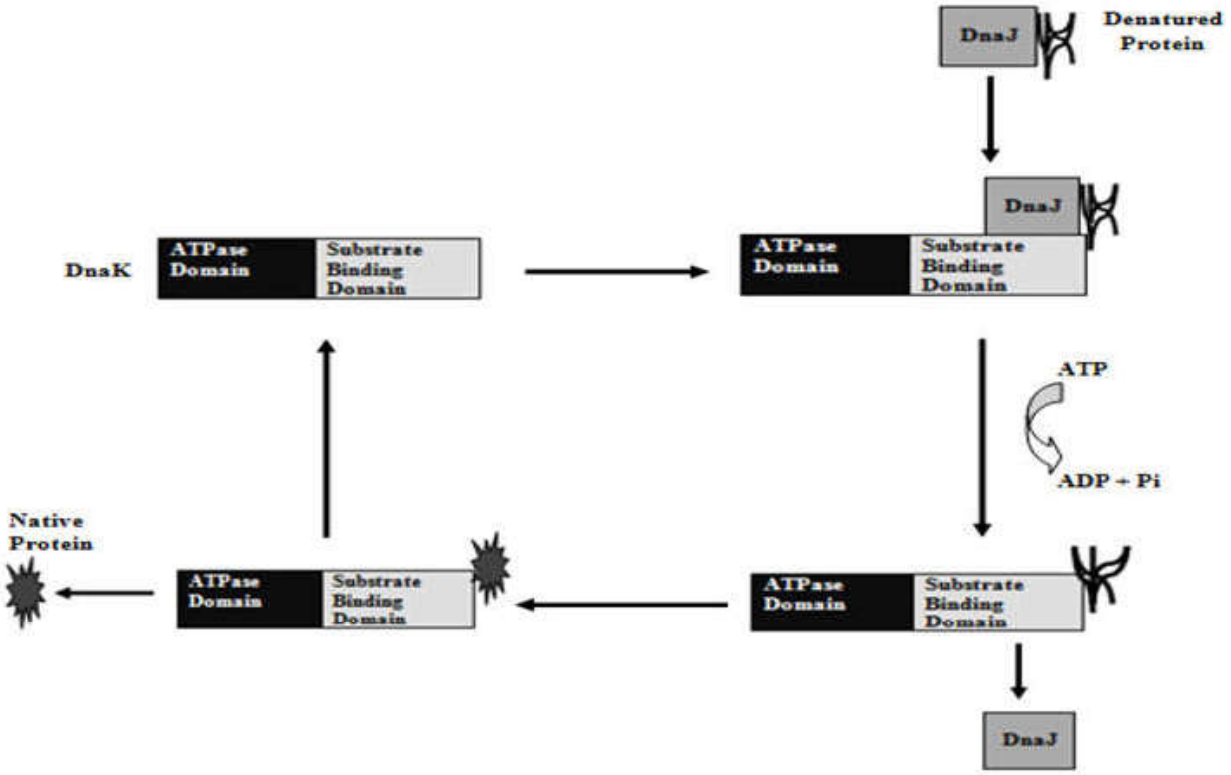

Fig. 2: Schematic Representation of DnaK-DnaJ Chaperone Machinery

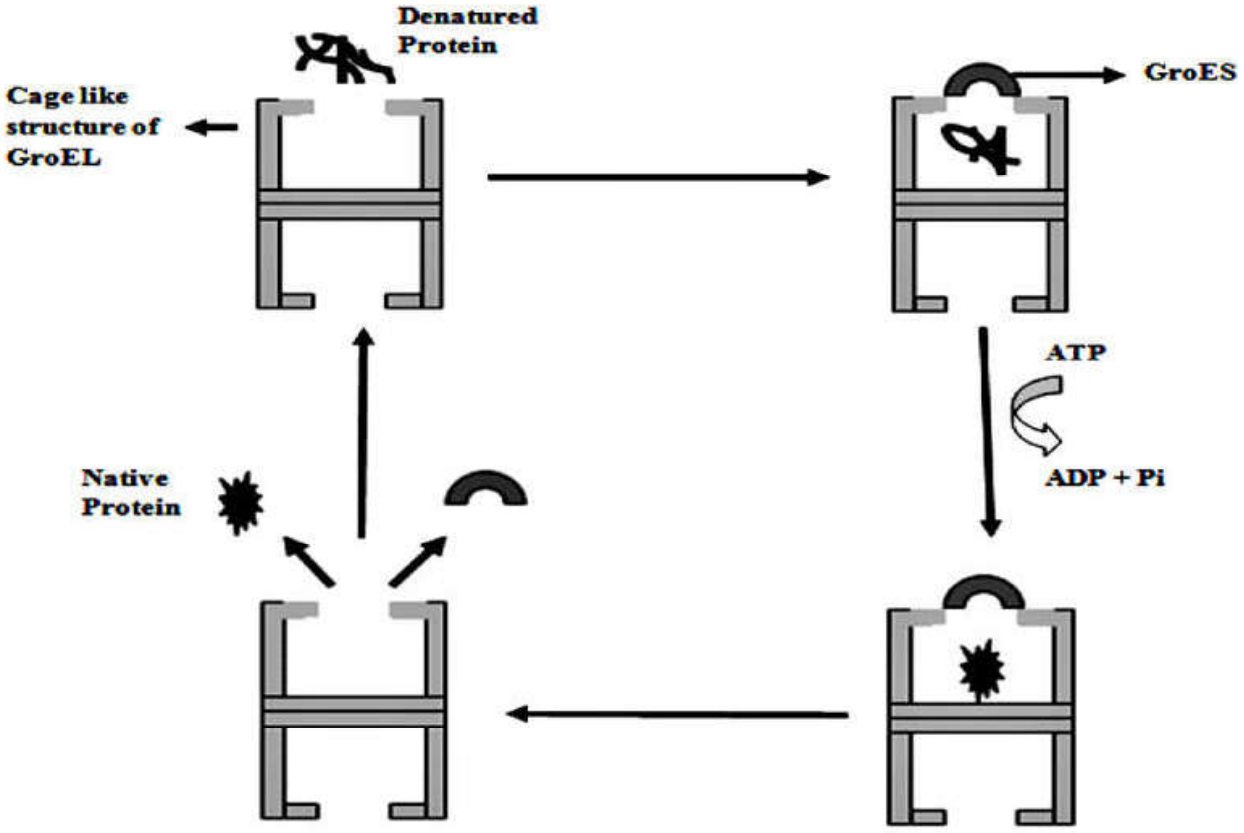

Fig. 3: Protein Folding *via* GroEL-GroES chaperone system

Table 1: The table below depicts members and functions of Heat shock proteins.

S. no.	Hsp Family	Members	Functions	Reference
1.	Hsp100	Class I proteins - Hsp104, bacterial ClpB and their distant relatives ClpA, ClpC); Class II - ClpX and HslU.	Protein disaggregation in association with Hsp70 chaperone system; Proteolytic degradation of proteins; Remodeling of client proteins	[14, 17, 18]
2.	Hsp90	HtpG in the bacterial cytosol; Grp94/gp96 in the endoplasmic reticulum of eukaryotes; Hsp75/TRAP1 in the mitochondrial matrix and; Hsp90 in eukaryotic cytosol (Hsp83 in *Drosophila* Hsc82 and Hsp82 in yeast, Hsp90α and Hsp90β in humans, Hsp86 and Hsp84 in mice).	Protein folding; Regulation of the stability and active state of substrate proteins; Contribution in a ide range of cellular processes like signal transduction, intracellular transport, and protein degradation.	[19, 20, 21]
3.	Hsp70	DnaK, HscA or Hsc66 and HscC or Hsc62 in Prokaryotes; Hsc70 in Cytosol, Hsp70 and its paralogs HSPA1A, HSPA1B, and HSPA1L in Eukaryotes ; Binding immunoglobulin protein (BiP or Grp78) in endoplasmic reticulum and; mtHsp70 or Grp75 in mitochondria.	Folding and refolding of client proteins; Proteolytic degradation of unfolded proteins; Transmembrane transport of proteins	[22, 23]
4.	Hsp60	Group I proteins - GroEL in bacterial cytosol, Hsp60 in mitochondria and Rubisco binding protein (RuBisCoBP) in chloroplasts; Group II- Thermosome/TF55 in archaea and TRiC/CCT in the eukaryotic cytosol.	Binding to the substrate protein and enabling its folding	[24, 25, 26, 27]
5.	Small Heat Shock Proteins	Class I sHsps - Hsp27 (HspB1), αB-crystallin (HspB5), Hsp20 (HspB6) and Hsp22 (HspB8); Class II sHsps - HspB2, HspB3,HspB4 (αA-crystallin), HspB7, HspB9 and HspB10.	Prevents aggregation of denatured proteins; Increase the stability of microfilaments, intermediate filaments and microtubules; Blockage of apoptosis (e.g., Hsp27)	[28, 29, 30]

Table 2: Table below shows Interventions involving Heat Shock Proteins in Clinical Trials

S. No.	Heat Shock Protein involved	Intervention	Disease	Phase of trial	Source
1.	Heat Shock Protein Peptide Complex-96 (HSPPC-96)	Biological: **Heat Shock Protein Peptide Complex-96 (HSPPC-96)**; Procedure: Tumor Resection; Radiation: Radiation	Glioblastoma Multiforme; Astrocytoma, Grade III; Anaplastic Ependymoma; Clear Cell Ependymoma; Ependymoma	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02722512?term=heat+shock+proteins&rank=1
2.	Hsp70	Biological: **Heat Shock Protein 70-peptide complexes (HSP70)**	Breast Neoplasms	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT00027131?term=heat+shock+proteins&rank=2
3.	Heat Shock Protein gp96	Biological: **HSPPC-96**; Procedure: conventional surgery	Brain and Central Nervous System Tumors	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT00293423?term=heat+shock+proteins&rank=4
4.	Heat Shock Protein gp96	Biological: **gp96**	Glioma	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02122822?term=heat+shock+proteins&rank=6
5.	Hsp70	Biological: **Heat Shock Protein 70 HSP70**	Leukemia,Myeloid, Chronic; Leukemia,Myeloid,Philadelphia-Positive	Phase 1	https://clinicaltrials.gov/ct2/show/NCT00027144?term=heat+shock+proteins&rank=7

6.	Heat Shock Protein Peptide Complex-96	Drug: **HSPPC-96 or Oncophage**	Malignant Melanoma	Phase 3	https://clinicaltrials.gov/ct2/show/NCT00039000?term=heat+shock+proteins&rank=8
7.	Heat Shock Protein gp96	Biological: **autologous gp96 vaccination**	Liver Cancer; Pancreatic Adenocarcinoma	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT02133079?term=heat+shock+proteins&rank=12
8.	Heat Shock Protein gp96	Biological: **autologous gp96 vaccination**; Drug: Oxaliplatin+S-1	Gastric Carcinoma	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT02317471?term=heat+shock+proteins&rank=13
9.	Heat Shock Protein Peptide Complex-96	Drug: ipilimumab; Drug: **HSPPC-96**	Melanoma	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT02452281?term=heat+shock+proteins&rank=14
10.	Hsp90	Drug: CDKI AT7519; Drug: **Hsp90 Inhibitor AT13387**	Adult Solid Neoplasm	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02503709?term=heat+shock+proteins&rank=19
11.	Heat Shock Protein Peptide Complex-96	Drug: **autologous human tumor-derived HSPPC-96**	Lymphoma, Follicular; Lymphoma, Small Lymphocytic	Phase 2	https://clinicaltrials.gov/ct2/show/NCT00081809?term=heat+shock+proteins&rank=21
12.	Heat Shock Protein Peptide Complex-96	Biological: **HSPPC-96**; Drug: bevacizumab	Recurrent Glioblastoma; Recurrent Adult Brain Tumor; Gliosarcoma	Phase 2	https://clinicaltrials.gov/ct2/show/NCT01814813?term=heat+shock+proteins&rank=22
13.	Heat Shock Protein Peptide Complex-96	Biological: **HSPPC-96**	Brain and Central Nervous System Tumors	Phase 2	https://clinicaltrials.gov/ct2/show/NCT00905060?term=heat+shock+proteins&rank=27
14.	Hsp70	Biological: **recombinant 70-kD heat-shock protein**	Leukemia	Phase 1	https://clinicaltrials.gov/ct2/show/NCT00030303?term=heat+shock+proteins&rank=28
15.	Hsp70	Biological: OVA BiP peptide; Biological: gp209-2M antigen; Biological**: recombinant 70-kD heat-shock protein**; Biological: tyrosinase peptide	Melanoma (Skin)	Phase 1	https://clinicaltrials.gov/ct2/show/NCT00005633?term=heat+shock+proteins&rank=29
16.	Heat Shock Protein Peptide Complex-96	Biological: **HSPPC-96**	Renal Cell Carcinoma	Phase 2	https://clinicaltrials.gov/ct2/show/NCT01147536?term=heat+shock+proteins&rank=38
17.	Hsp 90	Drug: Erlotinib Hydrochloride; Drug: **Hsp90 Inhibitor AT13387**; Other: Laboratory Biomarker Analysis; Other: Pharmacological Study	Recurrent Non-Small Cell Lung Carcinoma; Stage IV Non-Small Cell Lung Cancer	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT02535338?term=heat+shock+proteins&rank=55
18.	Hsp 90	Drug: Dabrafenib; Drug**: Hsp90 Inhibitor AT13387**; Other: Laboratory Biomarker Analysis; Other: Pharmacological Study; Drug: Trametinib	Recurrent Melanoma; Solid Neoplasm; Stage IIIA Skin Melanoma; Stage IIIB Skin Melanoma; Stage IIIC Skin Melanoma; Stage IV Skin Melanoma	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02097225?term=heat+shock+proteins&rank=57
19.	Hsp 90	Drug: **Hsp90 Inhibitor AT13387**;	Estrogen Receptor Negative; HER2/Neu Negative;	Phase 1	https://clinicaltrials.gov/ct2/show/NCT0247417

		Other: Laboratory Biomarker Analysis; Drug: Paclitaxel; Other: Pharmacological Study	Progesterone Receptor Negative; Recurrent Breast Carcinoma; Stage IIIA Breast Cancer; Stage IIIB Breast Cancer; Stage IIIC Breast Cancer; Stage IV Breast Cancer; Triple-Negative Breast Carcinoma		3?term=heat+shock+proteins&rank=61
20.	Hsp90	Drug: **MPC-3100 (an Hsp90 inhibitor)**	Cancer	Phase 1	https://clinicaltrials.gov/ct2/show/NCT00920205?term=heat+shock+proteins&rank=67
21.	Hsp110-gp100	Biological: Recombinant Human **Hsp110-gp100 Chaperone** Complex Vaccine; Other: Laboratory Biomarker Analysis	Recurrent Melanoma; Stage IIIB Skin Melanoma; Stage IIIC Skin Melanoma; Stage IV Skin Melanoma	Phase 1	https://clinicaltrials.gov/ct2/show/NCT01744171?term=molecular+chaperone&rank=1
22.	Hsp70	Biological: **CN54gp140 glycoprotein-hsp70 conjugate vaccine**	HIV Infections	Phase 1	https://clinicaltrials.gov/ct2/show/NCT01285141?term=hsp&rank=16

their immense applications in the transplantation processes, various Hsps like Hsp60 has been correlated with allograft rejection and autoimmunity [100].Clinical and experimental studies with Heat shock proteins have provided various opportunities to the researchers for treating the fatal diseases that remain uncured previously. Table 2 below present information resources towards the Heat Shock Proteins in Clinical Trials.

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