CHARLES UNIVERSITY IN PRAGUE

THIRD FACULTY OF MEDICINE

Radek Rutkowski

Heat Shock Proteins - Focus on Therapeutic Approaches in Oncology

Diploma thesis

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Author of diploma thesis: Radek Rutkowski

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Advisor of the thesis: doc. RNDr. Ilona Hromadníková, Ph.D

Department of the advisor of the thesis: Oddělení molekulární biologie a patologie buňky, 3.LF UK

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Written Declaration

I declare that I completed the submitted work individually and only used the mentioned sources and literature. Concurrently, I give my permission for this diploma/bachelor thesis to be used for study purposes.

Prague, 21 March 2010 Radek Rutkowski

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1.0 Introduction

Since the discovery of heat shock proteins in 1960s and their immunogenic properties 20 years later in 1980s they have been under rigorous investigation with great hopes for advent of immunotherapy in oncology as well as other pathologies. So are the HSPs a breakthrough in therapy of cancers or will they be just another form of palliative treatment of these notorious and deadly diseases?

1.1 What are Heat Shock Proteins?

HSPs is a family of proteins expressed virtually by all living organisms. These proteins have a number of functions that are crucial to organism's intracellular and extracellular homeostasis. Due to the significance of HSPs, over the eons of the evolution, they have been highly conserved in our genetic code. The human HSPs shares almost 50 percent homology with simple unicellular prokaryotes to well over 95 percent homology with higher species of living organisms.[16]

1.2 The Discovery

The heat shock proteins, like many other significant scientific discoveries, were first identified accidentally in Italy during genetic research on Drosophila fruit flies which were accidentally exposed to higher than normal environmental

temperature. Since then the extensive research of these versatile moiety of proteins just began to shed some light on their significance in various homeostatic processes of living organism. So far among the others HSPs they were found to play role in: cellular stress response, proteosynthesis as chaperones, cardiovascular system, immune system, as well as proteodegradation.[16]

1.3 Classification

The number of newly identified HSPs is continuously growing so a coherent classification system is in a state of a flux. The HSPs originally have been classified according to their molecular weight where proteins of 10 kDa and 100 kDa could be named HSP10 or HSP104 respectively. However, due to the discovery of new molecules and their genetic fingerprints new more concise methods was needed. Currently the classification formulated by HUGO Gene Nomenclature Committee is being adopted by other institutions. [21]

According to the above mentioned nomenclature the HSPs have been divided into main families such as, Hsp70, Hsp110, Hsp40, small HSPs, human chaperone proteins, and chaperone like proteins. These families were further divided into subfamilies with number of different proteins belonging to these subfamilies. While the Family/Subfamily architecture of the nomenclature tries to group HSPs according to their biochemical structure, these proteins can also be grouped in another functional way where we can refer to HSPs as heat inducible or non inducible HSPs. Some of the examples of the heat inducible HSPs are;

HSPA1A, HSPA1B, HSPA6, or DNAJB1. On the other hand majority of HSPs belonging to the human chaperones proteins class were found to be non inducible by heat. Never the less the examples mentioned here are on the borders of the spectrum as many other heat shock proteins which can be induced to some extent by heat. [13] More complete list of the HSPs is listed in the Appendix, Tables 1-4.

2.0 Physiology of HSPs

2.1 Localization

Considering such broad scope of functionality of HSPs it is not surprising that these versatile proteins have been found in most of cellular compartments and well as extracellular space. So far HSP have been found in the; cytosol, endoplasmic reticulum, microsomes, mitochondria, and membrane bound.[13] HSP are also being investigated in the extracellular compartment; for example, Hsp70 are suspected to participate in the induction of tolerance to endotoxins such as lipopolysaccharides (LSP). [10][13]

In addition, expression of some HSPs was found to be tissue specific. For example heat shock protein HSP-90 was found to be elevated in the peripheral blood lymphocytes of some patient with systemic lupus erythematosus (SLE). [38]

2.2 Expression

The cellular expression of HSPs is very variable so they can constitute from 2 percent to 20 percent of all the soluble intracellular proteins. Their induction is primarily triggered by the cellular stress. This stress can come in many forms such as extracellular sources; temperature, cold, UV light, or an intracellular metabolic stress such as; hypoglycemia, acidosis, infection, inflammation, or toxin exposure.[20]

All the mechanisms of induction of HSP are still being investigated. Never the less, some of these pathways are becoming more clear. It has been found for example that transcription of heat shock proteins genes is modulated by heat shock factors. These HSFs are remaining dormant in the cytoplasm until the time when they come in contact with abnormal proteins created by various forms stress as described above. These abnormal proteins cause molecular and structural changes in these HSF via the process of phosphorylation and trimerisation. These, now active, HSFs can be transported to the nucleus and they can bind a heat shock binding protein 1 (HSBP1) and activate the expression HSPs. The expressed HSPs exert a negative feedback on the HSFs maintaining homeostasis. The process is depicted in a Figure 1.[35]

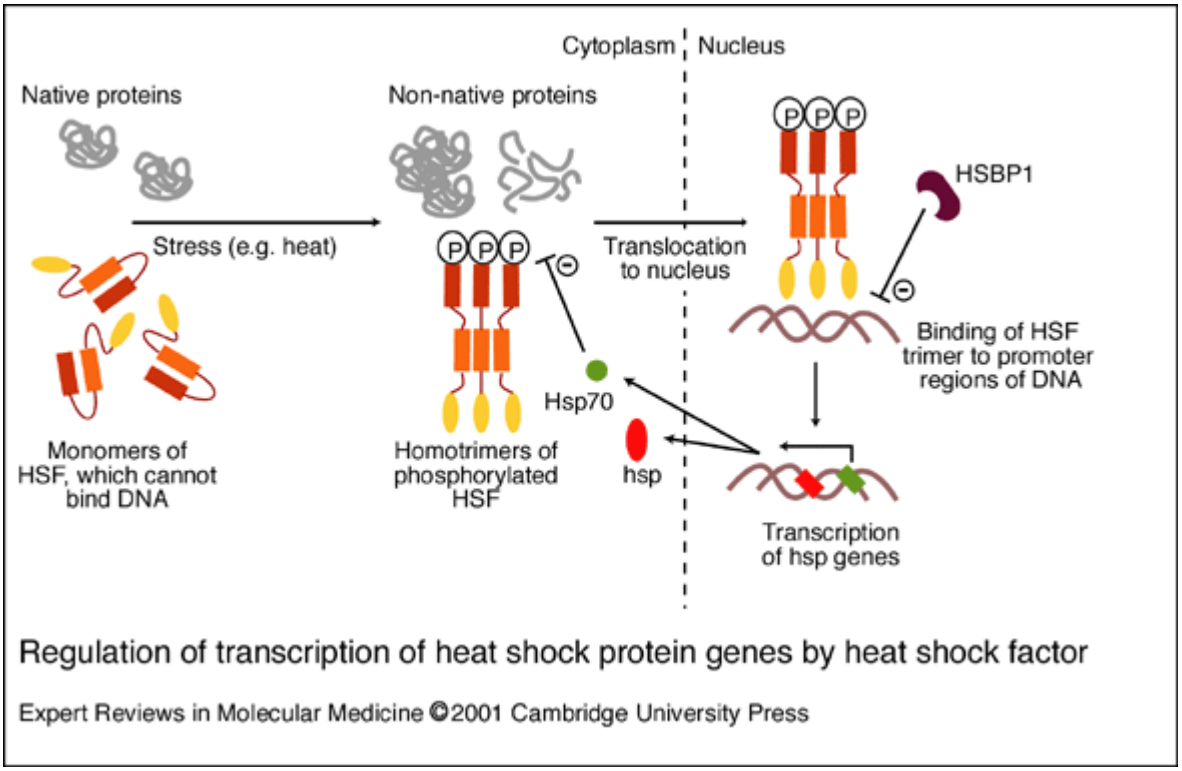

Figure 1. Regulation of HSP expression diagram from the reference [35]

2.3 Apoptosis Modulation

The apoptosis is form of programmed cellular death. It is very complex process driven by the activation of caspase cascades and modulated by myriad of proteins and cellular pathways. However, from a general perspective we can divide the trigger of caspases cascades into direct signal transduction and mitochondrial pathways. In the direct signal transduction pathways the process is mediated by tumor necrosis factor (TNF) or Fas-Fas ligand interaction. Both of these mechanism result in an activation of the caspases and induction of apoptosis.[11]

In the mitochondrial pathways of apoptosis the damage induced increase in mitochondrial permeability leads to release of SMACs (second mitochondria-derived activator of caspases). These proteins bind an inhibitor of apoptosis proteins (IAPs) deactivating it, thus allowing apoptosis to proceed. An additional pathway of apoptosis triggered by mitochondria is formation of MAC channel in the mitochondrial membrane that results in release of cytochrome c into a cytosol. That cytochrome then interacts with other cytosolic proteins such as Apaf-1 which together with other caspases forms so called apoptosome which in turn activated the executing caspases of the entire apoptotic processes. [11]

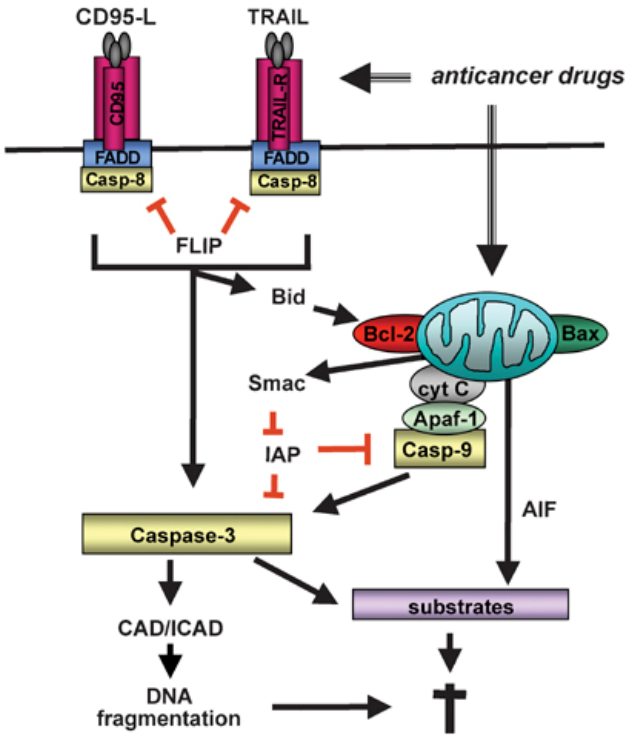

Figure 3. Apoptotic pathways [11]

According to various research it has been found that HSPs play important role in the apoptosis process. For example, Hsp70 has been found to stop the apoptosis by binding to Apaf-1 protein thus stopping activation of the caspase cascade. In addition, Hsp70 was found also able to block the non caspase depended apoptosis mechanism via apoptosis inducing factor (AIF) as depicted in the Figure 3 above. [15]

Furthermore, it has also been found that HSPs also affect the direct signaling pathways of inducing apoptosis. For example, reduced levels of Hsp90 were found to be protective against apoptosis triggered by the TNF-alpha receptors. [14][25]

2.4 Effects on Immunity

The original discovery of the linkage of HSPs to immunity was done independently by a graduate student in India (Pramod Srivastava) in 1980 and by another group of researchers in Germany in 1984. As that research showed that HSPs interaction with immunity altered ability of experimental animals to resist tumors it resulted in very active exploration in that field. To this days the research in immunological applications of HSPs includes tumor vaccines, viral infections (such as HIV, HSV-2), bacterial infections such as Tuberculosis, and autoimmune diseases such as Multiple Sclerosis or Diabetes Mellitus type I.[16][17] So far, HSPs can were found to be capable of activating both humoral and cellular immune response. [13]

In all the living cells the intracellular HSPs bind the various cytosolic proteins. During normal cell cycle and apoptosis these proteins are sequestered and never seen extracellularly. However, virus infected cells and tumors cells generate various anomalous proteins that are captured by the cytosolic HSPs . Some of these HSPs such as membrane bound molecules like Hsp70 are capable of presenting antigens to the immune system. Whereas other HSPs, while bound to the cytosolic proteins both normal and abnormal, are released into extracellular space when the affected cells die by necrosis. The released HSPs can interact with the immune cells via the CD91 that allows dendritic cells, platelets and macrophages to respond to these proteins. In this process the activated immune cells up regulate B7 co-stimulator that promotes better T cell response. [16]

What is interesting with HSPs that even though in general humoral antigen should stimulate the humoral immunity. In the case of APCs activated by HSPs complexed with immunogenic peptides the cytotoxic and NK cellular response is invoked instead. The full mechanism of that hasn't been yet deciphered. [16]

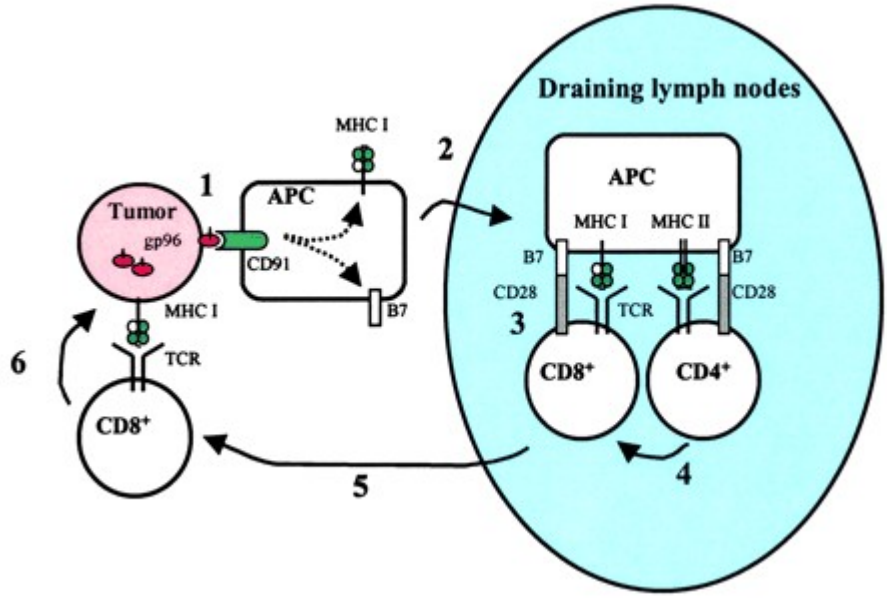

Figure 2. HSP in tumor immunity interactions [31]

2.5 Cardiovascular System

HSPs also play a significant role in a cardiovascular system where they participate in relaxation of smooth muscles as well as smooth muscle contraction. In addition they also play role in platelet anti aggregation pathways.[13]

For example, it has been found that a small Hsp20 is an active participant in many important homeostatic processes such as vasodilatation, and platelet aggregation. It has been shown that the increase of these proteins in the ischemic myocyte improves its contractibility and prevents beta agonist induced apoptosis of overstressed heart cells thus improving cardiac functions and preventing

reperfusion injury. An additional study has also shown that Hsp70 induced in ischemic myocard also exerts cardio protective effect on the ischemic cells. [31]

Yet another Hsp90 was found capable of modulating activity of endothelial nitric oxide synthase (eNOS) thus affecting vasodilatation thus having possible role in prevention of ischemic injury in hypoperfused tissues. [22]

2.6 Housekeeping

Another role where HSPs actively participate is a protein folding and protein housekeeping. The HSPs are key chaperones that assist in the end stages of the proteosynthesis by folding the newly made proteins, they can also assist in re-folding or miss folded proteins as well as in delegation of proteodegradation by proteosomes.[13]

3.0 HSP in Clinical Oncology

3.1 General Therapeutic Approaches

Due to the complexities of the HSPs function and their significance in cellular homeostasis and immunity there have been various attempts to exploit their function for therapeutic purposes. The two independent approaches include

HSP ability to modulate the immunity thus making it possible to develop a form of cancer vaccine. [13]

The other approaches focus more on the function of HSP in the intracellular processes and cell cycle control. So far there have been some attempts to affect cancers by modulating activity of HSPs and their ability to perform their normal function. Discussed further in hematologic cancers section.[13]

3.2 HSPs in Cancer Immunotherapy

Currently there are few approaches in use the HSPs in immunotherapy. One approach was developed by Antigenics and it called the Oncophage vaccine. The protocol used to develop this vaccine consists of: preservation of the resected tumor from the patient, extraction of all the HSPs from the tumor cells, including HSPs bound with normal cellular proteins, then re-injection of that extract back into the patient promoting immunity. The advantage of this approach is that it works virtually on any tumor as long as adequate number of tumor cells are obtainable, and the disadvantage is that this treatment is a form of a tailored treatment specific for a patient as various tumors constantly mutate possibly altering its immunogenic properties.[30]

The alternative to the tailored therapy has been tried as well where the tumor specific antigens are injected. The advantage is that this therapy could mass produce the pharmaceuticals via cellular methods however the disadvantage is the constant flux of the gen expression in mutating living tumor cells.[13]

As Antigenics holds the patents for all immunotherapy of human HSPs there have been alternative approaches tried by the competition using bacterial heat shock proteins. The disadvantage of the last approach is that the non human HSPs could be themselves immunogenic causing formation of immunoglobulins rendering them useless.[13]

Another domain of possible application of HSP in immunotherapy are the autoimmune disease such as MS or DM I. The principle behind this believe is in a fact that it has been found that the larger doses of HSPs were found to down regulate the immune response thus providing yet another way of modulating the immunity that may play role in development of these diseases. [13]

4.0 Clinical Trials

As described so far HSP have rich portfolio of functions, both intracellular and extracellular. Since these functions include modulation of immunity as well as interaction with cellular mechanisms controlling apoptosis one could assume that these proteins could also play role in developments of tumors and thus they could possibly lead to new forms of treatments. [31]

So far HSPs immunotherapy has been tried for various solid and hematological cancers such as; kidney cancer, melanoma, pancreatic cancer, colon cancer, gastric cancer, non-Hodgkin lymphoma, chronic myelogenous leukemia and others with promising result.[13]

In general assessment of new drugs happens during series of clinical phases 1 to 4. Each phase has a specific objectives and is performed on a adequate group of patients to come up with statistically significant results. The description of the main clinical trials as defined as follow:

"*In Phase 1 clinical trials, researchers test a new drug or treatment in a small group of people (20-80) for the first time to evaluate its safety, determine a safe dosage range, and identify side effects.*

In Phase 2 clinical trials, the study drug or treatment is given to a larger group of people (40-100) to see if it is effective and to further evaluate its safety.

In Phase 3 studies, the study drug or treatment is given to large groups of people (more than 200) to further determine its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the drug or treatment to be used safely.

Phase 4 studies are done after the drug or treatment has been marketed. These studies continue testing the study drug or treatment to collect information about their effect in various populations and any side effects associated with long-term use."

ANTIGENICS INC. "*About Clinical Trials*". http://www.antigenics.com/trials/about/>, 20 March, 2010.

4.1 Kidney cancer

4.1.1 Epidemiology

Renal cell carcinoma constitutes 3 percent of all adult cancers and it is a ninth most common cancer cause of death in U.S. RCC is inherently resistant to chemotherapy and it is usually in advanced stages at the time of diagnosis in adults. The mean 5 year survival rate ranges from 67 percent (stage I) to 11 percent in stage IV of the disease. Currently main stream therapy for this cancer is nephrectomy followed by interferon alpha and IL2 treatment.[36]

4.1.2 Studies performed

In the scope of immunotherapy, the renal cell carcinoma has been found to be one of the most vulnerable cancers to the HSPs vaccination. [40] Following studies with Antigenics' Oncophage were preformed: [37]

- Phase 1/2, metastatic kidney cancer
- Phase 2, metastatic kidney cancer
- Phase 3, non-metastatic kidney cancer

4.1.2.1 Phase 1 - Clinical Studies (metastatic renal cell carcinoma)

During this clinical study Dr. P. Srivastava first demonstrated application of heat shock protein gp96-peptide complexes (HSPPC-96) in the clinical setting. In this study 29 patients have completed 4 weekly intradermal injections with a four week follow up. The vaccination shown to be safe and it did not demonstrate any significant side effects. In addition the promising biologic activity in the 59 percent of the patients prompted need for further studies. [1]

4.1.2.2 Phase 2 - Clinical Studies (metastatic renal cell carcinoma)

The results of phase 2 clinical trials for metastatic renal cell carcinoma using Antigenics' heat shock protein gp96-peptide complexes have shown some promise as well. After intradermal injection of patients with autologous vaccine derived of patients own tumor cells, combined with IL-2 treatment, increased median progression free survival (PFS) for the whole study group from 18 to 25 weeks, compared to IL-2 treatment alone. Two years after initiation of the vaccine, 30% of all pts remain alive compared to standard 15% overall survival rate. As in the previous study the treatment was without significant or unexpected toxicity. [13][33]

4.1.2.3 Phase 3 - Clinical Studies (non-metastatic renal cell carcinoma)

The results of phase 3 clinical trials for non-metastatic renal cell carcinoma using Antigenics' HSPPC-96 have shown even greater promise than the phase 2 studies for metastatic cases. It has been found that the adjuvant vaccination, administered via weekly intradermal injections of autologous HSPs from patients own tumor cells, in combination with nephrectomy, significantly increased the progression free survival (PFS) in the affected patients.[18] According Antigenics' reports almost 50 percent of the phase I patients were still alive 2 years after autologous vaccination, this is compared to standard 2 year survival rate of 15 percent. [3]

The side effects associated with vaccine were minimal and much less severe than other modalities of treatment. The most commonly encountered side effect was erythema and indurations at the site of injection experienced by approximately 50% of the patients. [13]

4.2 Melanoma

4.2.1 Epidemiology

Melanoma accounts for only approximately 5 percent of skin cancers with the lifetime risk of developing that disease of 1 per 75. The incidence of

melanoma increases by 5-7% annually, that acceleration is rate is very high and second only to increase of lung cancer in women. The geographic distribution of this disease varies geographically where countries like Australia experience the highest number of incidents. This disease is in a way unique that it typically affects young and middle-aged people unlike other solid tumors that are more common in older adults [26]

4.2.2 Clinical Studies

- Phase 1, metastatic melanoma
- Phase 2, metastatic melanoma (combination)
- Phase 3, metastatic melanoma

4.2.2.1 Phase 1, Metastatic Melanoma

Autologous tumor derived HSPPC-96 was used to induce a T-cell responses and protective immunity against melanoma in 36 patients with advanced disease, stage III and IV. The focus of this clinical trial was mainly to prove safety of the HSPPC-96 vaccination. The study was completed successfully completed with positive results as no serious toxicity was encountered. [5]

4.2.2.2 Phase 2, Metastatic Melanoma

This clinical trial was performed in the Istituto Nazionale Tumori in Milan, Italy. It involved vaccination of 45 metastatic (stage IV) melanoma patients with HSPPC-96 using both subcutaneous and intradermal injections. The vaccination was found to invoke a T cell mediated immunity reaction in over 50 percent of the patients without serious side effects. [8]

4.2.2.3 Phase 3, Metastatic Melanoma

The phase 3 of the clinical studies using Oncophage vaccination with autologous tumor-derived head shock protein complexes was performed in 76 centers word wide, including countries such as Europe, Russia, Australia, US. The study consisted of 322 patients with stage IV disease, including M1a (Distant skin metastasis, normal LDH) and M1b (lung metastasis, with normal LDH), and M1c (Other Distant Metastasis OR Any Distant Metastasis with Elevated LDH) patients. Approximately 2/3 of the patients were treated with the vaccine where as another control group was treated by the physician choice (PC) therapy. The vaccinated patients were injected subcutaneously the vaccine once a week for 4 weeks following biweekly regiment until vaccine was exhausted. The physician choice treatment mainly consisted various combinations of IL-2, dacarbazine/temozolamide, and surgery. The overall results were compared by assessing the overall survival statistics of the patients. [6][37]

It has been found that that Oncophage vaccine was most effective in the M1a group of patients where the overall survival was increased over the PC group by approximately 60 percent. The M1b vaccination group also showed some increased in overall survival rate of approximately 7 percent. Never the less the group of physician choice had 30 percent longer survival in the M1c group over the Oncophage vaccination. [13]

4.3 Other Solid Cancers

4.3.1 Clinical Trials

Overall, the scope of the studies and possible therapies using HSPs, whether immunotherapy or other modalities, is still actively researched with multitude of clinical trials underway.

The HSP immunotherapy has been assessed in various other clinical with focus on some solid cancers such as colon, pancreatic cancer, gastric cancer, and glioma.[13]

Also other therapeutic modalities pretaining to HSPs, such as the HSP-90 inhibitors, have also been tried agains HRE2 positive breast cancer, gastrointestinal cancer, gliomas, cervical cancer and prostate cancers and others [32]

4.3.1.1 Pancreatic Cancer

This particular cancer is often diagnosed in more advanced stages when the radical curative treatment is often not possible. There has been phase I (Antigenics, 1997), and II clinical trials for a treatment of this pathology, unfortunately due to difficulties with pancreatic enzymes digesting the HSPs after surgery there have been some modification to the process making further studies possible. [13]

4.3.1.2 Cervical Cancer

The HPSs have been also used to create a cancer vaccine for a cervical cancer. The competitor of Antigenics, due to patent constrains, have attempted to use bacterial HSP to create a vaccine with some success. Although the usage of non human HPS molecules may have its own complications such autoimmunization and autoanibodies to the treatment. [13]

4.4 Hematologic Malignancies

4.4.1 Pathophysiology of HSP-90 in tumor genesis

The HSPs are also being assessed in the treatment of hematologic malignancies. One of the key pathophysiological processes that is characteristic of the cancers, among others, is over expression of various oncogenic proteins regulating proliferation, survival and apoptosis. It has been found that one of the heat shock proteins, HSP-90, is significantly elevated in various tumors, including number of solid tumors as well as lymphomas. That finding brought in question possibility of affecting tumor by controlling that particular family of heat shock proteins via drugs. Unlike the HSPs approach for solid tumors the extraction of tumor cells in hematologic malignancies is not as simple warranting a different approach to the potential therapies. [4]

4.4.2 Geldanamycins

These chemicals were originally isolated as antibiotics. Even though they turned out to have relatively weak antibiotics properties, they were shown to have antitumor activity. Their mode of action (MOA) was found to be mediated via inhibition of HSP90. Combined with the knowledge of the role of HSP-90 in carcinogenesis they become a focus of research as an antitumor pharmaceutical. [12]

The geldanamycin itself has proven to be relatively hepatotoxic, thus forcing researches to look for better alternatives. So far two such chemicals have been used clinicaly and these are geldanamycin analogues: 17-AAG (17-allylamino - 17 - demethoxygeldanamycin) and 17-DMAG (17-dimethylaminoethylamino - 17 - demethoxygeldanamycin). [12]

4.4.3 Preclinical trials with 17-AAG

In this study, the geldanamycin analog 17-AAG showed ability to binds, very specifically, to HSP-90 inhibiting its function. Studies using various lymphoma cell lines (9 in total), including; Hodgkin Lymphoma, Anaplastic Large Cell Lymphoma, Mantle Cell Lymphoma have shown that 17-AAG induced cell deaths in all the lines. The pharmaceutical was able to arrest the cell cycle in either G0/G1 or G2/M phase and induce apoptosis via caspase pathways. [34]

4.4.4 Phase I clinical trials of 17-AAG in lymphomas

The purpose of this study performed in 2004 was to assess the pharmacokinetics and pharmacodynamics of the 17-AAG in human patients. The chemical under turned out to be relatively well tolerated although some dose related toxicities were reported. These were; elevation of liver enzymes, fatigue, anorexia, diarrhea, vomiting, pancreatitis. The effect on levels of heat shock

protein in mononuclear cells of the peripheral blood was found relatively unchanged.[32]

4.4.5 Phase II clinical trials of 17-AAG in lymphomas

The phase II of the clinical study of 17-AAG was conducted on 22 patients with mantle cell or Hodgkin lymphoma (in more advanced stages). The patients were given 17-AAG intravenously weekly over a period of a month. The purpose of the study was to qualitatively assess the clinical response to the drug.[9]

Like all other pharmaceuticals, 17-AAG treatments come with some side effects, ranging from diarrhea to thrombocytopenia, pleural effusions, diarrhea, fatigue, and nausea. Three of the enrolled patients died during the treatment due to cardiac and pulmonary complications.[9]

There results of that study, done on a small group of 22 patients, showed promising clinical results. Almost 40 percent of cases in that study showed reduction in the tumor load and 11 percent responded with partial remission. [9]

5.0 Other Applications of HSP

5.1 HSPs and HIV

Considering the fact that HSPs can present any intracellular peptides to APSs invoking the immune response prompted investigation of possibility of vaccination for non-oncological pathologies. One of such investigation involved the HIV infected cells where a selected HIV peptide, HIV Gag p24 peptide EV32, was used stimulate CD8+/CD4+ immune response. The results of that studies showed promise a give rise to possibilities of vaccination against viral epitopes. [24]

5.2 HSPs and HSV-2

Antigenics is also developing a vaccine using its HSPs technology agains HSV-2 virus. The vaccine, called AG-707, is a polyvalent vaccine consisting of 32 synthetic peptides complexed with HSPs. This vaccine has an advantage over other types of HSPs immunotherapy that it is not specific to a given patient unlike other patient specific HSPs immunotherapies developed for cancer vaccines.[2]

The goal of the vaccine is to induce stimulate both 'helper' and killer' T-cell which would lead to reduction in outbreaks and severity of recurrent attacks.

Currently the vaccine is entering the phase 1 clinical trails stage where its safety and qualitative response will be evaluated.[13]

5.3 HSPs and Neurodegenerative disease

Considering that many neurodegenerative diseases such Alzheimer's, Parkinson's, ALS (amyotrophic lateral sclerosis) are linked to protein misfolding it is probable that HSPs, that among other functions act as a molecular chaperones and protein antiagregates, could play role in pathogenesis of these diseases. The studies are ongoing an the HSP-90 have been found to be involved opening a doorway to clinical investigation of drugs modifying HSPs behavior as for example previously mentioned HSP90 inhibitors. [23]

5.4 Miscallenous applications of HSP

In addition, being already quite versatile, HSPs are also being assessed for the genetic manipulations in GMO crops. As researchers believe that hybridize plants may perform better in certain difficult environment, such as hot dry climate. [13]

6.0 Conclusion

Despite of the fact that HSPs have been knows for approximately 50 years and despite of the intensive resears in the field, currently Russia is the only place where HSP immunotherapy has been approved as of 2008 for treatment in renal cell carcinoma. [29]

The HSPs immunotherapy has proven benefitial as an adjuvant treatment in some stages of a selected cancers. The advantage of HPSs immunotherapy is that it has less side effects compared to standart chemotherapy although. Neverthe less, the HPSs immunotherapy doesn't offer a radical cure.

There are other attempts to use assess HSPs in infectious diseases as well as in some degenerative and autoimune diseases. Although the research in the last two is still far from being conclusive.

7.0 Appendix

Family	Protein Name	Older Names	Localization	Function
				stabilizer of ZNF198 protein which is involved in a chromosome rearrangement with the FGFR1 gene in an atypical myeloproliferative disease [10]
Hsp70 (HSPA)	HSPA1A	HSP70-1; HSP72; HSPA1		
	HSPA1B	HSP70-2		
	HSPA1L	hum70t; hum70t; Hsp-hom		
	HSPA2	Heat-shock 70kD protein-2		
	HSPA5	BIP; GRP78; MIF2	Endoplasmatic Reituculum	chaperone
	HSPA6	Heat shock 70kD protein 6 (HSP70B')		chaperone
	HSPA7	Heat shock 70kD protein 7		
	HSPA8	HSC70; HSC71; HSP71; HSP73	cytosolic, membranebound	folding and translocation of proteins across intracellular membranes
	HSPA9	GRP75; HSPA9B; MOT; MOT2; PBP74; mot-2		mitochondrial housekeeping
	HSPA12A	FLJ13874; KIAA0417		
	HSPA12B	RP23-32L15.1; 2700081N06Rik		
	HSPA13	Stch	microsomes	
	HSPA14	HSP70-4; HSP70L1; MGC131990		

Table 1 ***Modified from [13]. In red are the most heat inducibled HSPs***

Family	Subfamily	Protein Name	Older Names	Localization	Function
Hsp110 (HSPH)		HSPH1	HSP105	cytosolic	
		HSPH2	HSPA4; APG-2; HSP110	cytosolic	
		HSPH3	HSPA4L; APG-1	cytosolic	
		HSPH4	HYOU1/Grp170; ORP150; HSP12A	microsomes	

Table 2 ***Modified from reference [13].***

Family	Subfamily	Protein Name	Older Names	Localization	Function
Chaperonins	HSPD	HSPD1	HSP60; GroEL	mitochondria	folding newly synthesized cytosolic proteins and preventing protein aggregation
	HSPE	HSPE1	HSP10; chaperonin 10; GroES	mitochondria	
	CCT			cytosol	
		CCT1	TCP1; CCTA; CCT-alpha; TCP-1-alpha		
		CCT2	CCTB; CCT-beta; TCP-1-beta		
		CCT3	CCTG; CCT-gamma; TCP-1-gamma; TRiC-P5		
		CCT4	CCTD; CCT-delta,;TCP-1-delta; SRB		
		CCT5	CCTE; CCT-epsilon; TCP-1-epsilon		
		CCT6A	CCT6; CCTZ; CCT-zeta; CCT-zeta1; TCP-1-zeta; HTR3; TCP20		
		CCT6B	CCTZ2; CCT-zeta2; TSA303		
		CCT7	CCTH; CCT-eta; TCP-1-eta		
		CCT8	CCTQ; CCT-theta; TCP-1-theta; KIAA002		
	Other chaperonin-like				
		MKKS	McKusick–Kaufman syndrome; MKS; Bardet–Biedl syndrome 6; BBS6		
		BBS10	Bardet–Biedl syndrome 10		

Table 3 ***Modified from reference [13].***

Family	Subfamily	Protein Name	Older Names	Function
Hsp40	DnaJA	DNAJA1	DJ-2; DjA1; HDJ2; HSDJ; HSJ2; HSPF4; hDJ-2	
		DNAJA2	DNJ3; mDj3; Dnaj3; HIRIP4	
		DNAJA3	Tid-1; Tid1l	
		DNAJA4	Dj4; Hsj4	
	DnaJB	DNAJB1	HSPF1; HSP40	HSPA recruitment and stimulation of the HSPA ATPase activity?
		DNAJB2	HSJ1; HSPF3; Dnajb10; MDJ8	
		DNAJB3	Hsj3; Msj1; MSJ-1; Hcg3a	
		DNAJB4	Hsc40	
		DNAJB5	Hsc40; HSP40-3	
		DNAJB6	Mrj; mDj4	
		DNAJB7	Dj5; mDj5	
		DNAJB8	mDj6	
		DNAJB9	Mdg1; mDj7; ERdj4	
		DNAJB11	Dj9; ABBP-2; Erdj3	
		DNAJB12	Dj10; mDj10	
		DNAJB13	Tsarg6; Tsarg 3 protein	
		DNAJB14	EGNR9427; FLJ14281	
	DnaJC	DNAJC1	MTJ1; ERdj1; ERj1p; Dnajl1	This family plays a role in possible recruitment of HSPA members to specific subcompartments and/or functions
		DNAJC2	Zrf1; Zrf2; MIDA1;M-phase phosphatase protein 11; MPP11; zuotin; ZUO1	
		DNAJC3	p58; mp58; Prkri; Dnajc3; p58IPK; Dnajc3b	
		DNAJC4	HSPf2; Mcg18	
		DNAJC5	Csp	
		DNAJC5B	CSP-beta	
		DNAJC5G	MGC107182; gamma-CSP	
		DNAJC6	mKIAA0473; auxilin	
		DNAJC7	Ttc2; mDj11; mTpr2	
		DNAJC8	AL024084; AU019262; splicing protein (spf31)	
		DNAJC9	AU020082; RcDNAJ9	
		DNAJC10	JPDI; ERdj5; macrothioredoxin	
		DNAJC11	FLJ10737; dJ126A5.1	
		DNAJC12	Jdp1; mJDP1	
		DNAJC13	Rme8; RME-8; Gm1124	
		DNAJC14	HDJ3; LIP6; DRIP78	
		DNAJC15	Dnajd1; MCJ; Cell growth-inhibiting 22 protein	
		DNAJC16	mKIAA0962	
		DNAJC17	C87112	
		DNAJC18	MGC29463	
		DNAJC19	TIM14; TIMM14	
		DNAJC20	JAC1; HSC20; HscB	
		DNAJC21	GS3; JJJ1; DNAJA5	
		DNAJC22	FLJ13236; Wurst	
		DNAJC23	Sec63; AI649014	
		DNAJC24	DPH4; zinc finger, CSL-type containing 3	
		DNAJC25	bA16L21.2.1; DnaJ-like protein; AAH48318; LOC552891; G-protein gamma 10	
		DNAJC26	GAK; cyclin G associated kinase; auxilin-2	
		DNAJC27	RBJ; RabJ	
		DNAJC28	Orf28 open reading frame 28; C21orf55, oculomedin	
			Sacsin; SACS	
		DNAJC30	WBSCR18; Williams–Beuren syndrome	

Table 4 ***Modified from reference [13]. In red are the most heat inducibled HSPs***

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