Virginia Commonwealth University
VCU Scholars Compass

Theses and Dissertations | Graduate School

2011

HEAT SHOCK PROTEINS AS NOVEL CANCER THERAPEUTICS: TARGETING THE HALLMARKS OF CANCER

CHAO LI
Virginia Commonwealth University

Follow this and additional works at: https://scholarscompass.vcu.edu/etd

 Part of the Medical Genetics Commons

© The Author

Downloaded from
https://scholarscompass.vcu.edu/etd/2510

This Thesis is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.

www.manaraa.com

HEAT SHOCK PROTEINS AS NOVEL CANCER THERAPEUTICS:

TARGETING THE HALLMARKS OF CANCER

A dissertation submitted in partial fulfillment of the requirements for the degree of

Master of Science at Virginia Commonwealth University

By Chao Li

M.D. Hu Bei University of Medicine, 2001

M.S. Tong Ji Medical College, 2006

Mentor: Shawn E. Holt, Ph.D., Associate Professor

Department of Pathology, Department of Human and Molecular Genetics,

Department of Pharmacology & Toxicology, and The Massey Cancer Center

Virginia Commonwealth University
Richmond, Virginia
July, 2011

www.manaraa.com

Acknowledgements

First, I would like to thank my mentor, Dr. Shawn Holt, for his generous and strong support for my thesis preparation. He is always there when I am in need of suggestion and for someone to listen, as well as to encourage me to think broadly and deeply about scientific research, giving me suggestions about not just trouble-shooting for individual experiments, but more often about how to foster independent and critical thinking habits in science and in life. He is a good teacher, mentor, listener, and friend to me.

I am grateful to the members of my thesis defense committee, Dr. Alphonse Sirica and Dr. Rita Shiang for their time and effort spent reading my thesis, providing thoughtful suggestions, and for attending my defense.

I would also like to thank many other people for their support and advice during my years in graduate school. They are Drs. Joy Ware, David Gewirtz, Lawrence Povirk, and Lynne Elmore, for giving me ideas and suggestions to solve the many problems encountered in research and daily life.

I would also like to thank my dear wife, Jie Qian, for her unstoppable support and encouragement of my career and education. Her patience and wisdom have been really important for me to go through previous difficult times, and without her always by my side, this work could not have been done. I am deeply indebted to her constant love, numerous suggestions, and invaluable help during my graduate school years. Thanks to her for giving me a lovely son, David. He makes me truly believe life is not just made of difficulties but dreams and hopes and even miracles. God bless you all.

To my parents and my brother, your support and love cannot be expressed enough in words. Thank you all for these many years of support that has enabled me to chase my dreams.

Finally, I would also like to thank all the people in the Department of Pathology for their help to my family and my work here. Working in such a harmonious and friendly environment, to me, this is an honor that I am fortunate to have had.

If I have seen further it is only by standing on the shoulders of giants.

Sir Isaac Newton (1643 – 1727)

www.manaraa.com

TABLE OF CONTENTS

www.manaraa.com

www.manaraa.com

List of abbreviations

15-DSG	15-deoxyspergulain
17-AAG	17-allyamino, 17-demethoxy geldanamycin
17-DMAG	17-(2-dimethylaminoethyl) amino-17-demethoxygeldanamycin
ABD	ATPase-binding domain
AKT	Protein kinase B
AP-1	Activator protein
AR-JP	Autosomal Recessive Juvenile Parkinsonism
Apaf-1	Apoptosis protease activating factor-1
BAG-1	Bcl2-associated athanogene-1
BCR-ABL	Breakpoint cluster region-V-Abelson murine leukemia viral oncogene homolog
BIK/ NBK	Bcl-2 Interacting Killer/ Natural Born Killer
BiP	Immunoglobulin heavy chain binding protein
bFGF	Basic fibroblast growth factor
CDK	Cyclin-dependent kinase
CDK4	Cyclin-dependent kinase 4
C/EBP	CCAAT/enhancer-binding protein
CFTR	Cystic fibrosis (CF) transmembrane conductance regulator
c-RAF	C-Raf Proto-Oncogene Serine/Threonine Protein Kinase
CREB	cAMP-response-element-binding protein
DAF-16	Dauer formation abnormal 16
FGF	Fibroblast growth factor
FOXO3A	Forkhead box O3A gene

www.manaraa.com

GRP78	78-kDa glucose regulated protein
EC	Endothelial cell
EDEM	ER degradation-enhancing α-mannosidase-like protein
EGFR	Epidermal growth factor receptor
EphA2	Ephrin type-A receptor 2
ER	Estrogen receptor
ERAD	ER-associated degradation
ERK	Extracellular-signal-regulated kinase
FADD	Fas-associated death domain
FNACA	Fanconi anemia complementation group A
FGFR1	Fibroblast growth factor receptor 1
GSK	Glycogen synthase kinase
HER-2/ERBB2	Human epidermal growth factor receptor-2
HGF	Hepatocyte growth factor
HIF	Hypoxia inducible factor
HOP	Hsp70/Hsp90 organizing protein
HSF1	Heat shock transcription factor protein 1
Hsp90	Heat shock protein 90
Hsp70	Heat shock protein 70
IKK	IκB (inhibitor of nuclear factor κB) kinase
IGF-I and II	Insulin-like growth factor I and II
IGFR	Insulin-like growth factor receptor
IL	Interleukin

www.manaraa.com

IL-6	Interleukin-6
IL-8	Interleukin-8
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MAP2K	MAPK kinase
MAP3K	MAP2K kinase
MEKK	MAPK/ERK kinase kinase
MK	MAPK-activated protein kinase
MKP	MAPK phosphatase
MMP-2	Matrix metalloproteinases-2
NF-κB	Nuclear factor κB
Pael-R	Parkin-associated endothelin receptor-like receptor
PBD	Peptide-binding domain
PDGF	Platelet-derived growth factor
PD-ECGF	Platelet-derived endothelial cell growth factor
PGF	Placental growth factor
PHD	Prolyl hydroxylase
PKB	Protein kinase B
PR	Progesterone receptor
PTP	Protein tyrosine phosphatase
RACK1	Receptor for activated C kinase 1
RNAi	RNA interference
ROS	Reactive oxygen species

www.manaraa.com

R-Smad	Receptor-activated Smad
RTKs	Receptor tyrosine kinases
SDF-1	Stroma derived factor-1
STAT3	Signal Transducer and Activator of Transcription-3
TAFs	Tumor-associated fibroblasts
TGF-β	Transforming growth factor-β
TKIs	Tyrosine kinase inhibitors
TNF-α	Tumor necrosis factor-α
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor

www.manaraa.com

List of tables

www.manaraa.com

List of figures

www.manaraa.com

HEAT SHOCK PROTEINS AS NOVEL CANCER THERAPEUTICS: TARGETING THE HALLMARKS OF CANCER

Chao Li, M.S., M.D.

ABSTRACT

Molecular chaperones, commonly known as heat shock proteins (HSPs), are essential for mammalian cells to maintain homeostasis, and HSPs function by inducing an ATPase-coupled structural change, followed by interactions with diverse co-chaperones and over 200 client proteins implicated in many critical signaling networks. These highly expressed HSPs participate in the onset and progression of several human diseases including cancer, and their connection with tumorigenesis has facilitated research and clinical trials related to targeting HSPs as a novel anti-tumor therapy. The predominant mechanism of chaperone inhibition is through either disruption of the HSP association with client protein or an altered binding state that ultimately leads to proteasome-mediated degradation. Importantly, chaperone inhibition results in the degradation of several client proteins that play critical roles in many of the pathways known as the Hallmarks of Cancer, such as proliferation, angiogenesis, invasion, metastasis, and drug resistance. Here, we discuss: (1) the current knowledge of HSPs, particularly studies related to Hsp90-targeted cancer therapy, (2) the targeting of Hsp90-mediated signaling interactions to prevent emergence of core Hallmarks of Cancer, (3) the recent progression of Hsp90 inhibitors in clinical trials. Finally, we propose combinatorial therapy, additional inhibitor discovery, and location-specific inhibition of HSPs as necessary next steps in chaperone-targeted research relevant to cancer therapy.

www.manaraa.com

CHAPTER ONE

INTRODUCTION

The eukaryotic heat shock proteins (HSPs) are a set of the most highly conserved proteins in nature, collectively known as essential and ubiquitous molecular chaperones for their cytoprotective functions during the maintenance of organism homeostasis under both physiological and pathological conditions (Lindquist 1988; Hendrick *et al* 1993). Mammalian HSPs have also been recognized to play a series of critical roles in tumorigenesis, as well as their function in protein assembly and the prevention of protein misfolding and aggregation under stress conditions. Chaperones are divided into four major families, Hsp90, Hsp70, Hsp60, and Hsp20, according to their relative molecular mass (Calderwood *et al* 2006; Jego *et al* 2010), while additional novel families include Hsp110 and Hsp170 (Easton *et al* 2000). In order to survive under the harsh conditions within the tumor microenvironment, cancer cells typically become dependent on stress-inducible HSPs in order to become refractory to chemotherapy, tolerant to hypoxia, resistant to apoptosis, and to suppress antitumor immunity, all the while acquiring the properties of invasiveness and metastasis during cancer progression. To date, more than 200 HSP client proteins have been identified involving nearly all fundamental cellular activities and processes, including cell growth, proliferation, and cell survival (Jego *et al* 2010). Interestingly, many cancer-associated proteins have been reported as HSP clients, likely as a mechanism for promoting oncogenic transformation. Therefore, targeting HSPs would result in simultaneous inhibition of multiple signaling pathways responsible for modulation of various events

www.manaraa.com

involved in cancer progression for a broad range of tumor types, such as neoplastic growth, sustained angiogenesis, chemotherapeutic resistance, evasion of cell death, and ultimately, invasion and metastasis (Barginear *et al* 2008).

Although the exact molecular mechanism(s) of HSP inhibitors have not yet been fully determined, a significant number of client proteins are either part of mechanistic studies (bench) or under evaluation as part of clinical trials (bedside). For example, histone deacetylase (HDAC) inhibitors as novel anticancer agents are found to hyperacetylate Hsp90, causing an increase in its binding to an Hsp90 inhibitor now in phase II trials, ultimately showing anti-tumor activity in leukemia and prostate cancer (Barginear *et al* 2008). Glucose-regulated protein 78 kD (GRP78), also known as immunoglobulin heavy chain binding protein (BiP), is a member of the HSP family of molecular chaperones and serves as an unfolded protein response marker. GRP78 is involved in cellular adaptation and survival to facilitate tumorigenesis through active interaction with a variety of partners/ligands within tumor cells (Dudek *et al* 2009). On the basis of shared homology with other HSP family proteins, GRP78 is also a molecular target of HDAC inhibitors, resulting in the phosphorylation and activation of initiating factor 2α (p-IF2α) and an increase in ATF4 and C/EBP homologous protein synthesis (Kahali *et al* 2010), all of which are important for progression of certain cancer types.

Thus, overall, HSPs are implicated in cancer and have been shown to specifically interfere with current antitumor therapies that target phenotypic responses like apoptosis, necrosis, autophagy, and senescence. Not surprisingly, inhibition of Hsp90's ATPase activity and disruption of ongoing chaperone folding cycles results in dissociation, destabilization,

www.manaraa.com

and proteasomal degradation of a variety of client proteins, including the cancer-associated targets ErbB1, ErbB2, Bcl-2, Apaf-1, Akt, and MMP-2 (Jego *et al* 2010; Wang *et al* 2009). Even though clinical trials often show inherent toxicity of Hsp90 inhibitors and strong induction of cytoprotective function of Hsp70, a combination of Hsp90 and Hsp70 inhibitors with traditional chemo- and/or radio-therapy may provoke tumor regression in a synergistic manner.

Here, we will review studies describing the connection between molecular chaperones and tumorigenesis, especially focusing on the roles of the molecular chaperone families Hsp90 and Hsp70 (for example, GRP78). We start by reviewing our current understanding of the roles of individual chaperone family members in malignant progression and other types of diseases. We then focus on available strategies employing specific Hsp90 inhibitors in certain types of cancer to alter the Hallmarks of Cancer. Finally, we will evaluate potential novel approaches such as combinatorial therapy including inhibitors for Hsp90 and discuss future directions to improve the effects of anti-tumor treatment.

www.manaraa.com

CHAPTER TWO

CYTOPROTECTIVE FUNCTIONS OF HSPS

A. HSP Function in Protein Folding and Degradation

Molecular chaperones are essential for nascent polypeptides or proteins to fold correctly and prevent them from degradation and aggregation in an intracellular environment. Understanding of the molecular mechanisms of how different proteins synthesized in the cell use specific molecular chaperone machinery will provide fundamental implications for both translational research and the clinic practice.

Most heat-shock proteins (HSPs) and their constitutively-expressed relatives are ubiquitously expressed under normal conditions to protect cells from the dangerous consequences of protein misfolding and aggregation. Highly expressed HSPs are considered key components in the cellular protective response to various cytotoxic exposures including temperature, hypoxia, inflammation, starvation, radiation, infection, heavy metals, and acidic conditions. The induction of HSP expression is at least partially controlled by the specific transcription factor heat shock factor 1 (HSF1) and also by other oncogenic signaling proteins such as heregulin-HER2-PI3K (Calderwood *et al* 2006; Workman *et al* 2007). HSF1 monomers are complexed with the Hsp90 chaperone in the cytoplasm of cells, rendering both proteins functionally inactive. Only under stress-inducing conditions the interaction is disrupted, likely through phosphorylation events, and the functional HSF1 trimer induces transcriptional upregulation of HSPs (Voellmy *et al* 2004; de Billy et al 2009; Pirkkala *et al* 2001). HSP stimulation allows for an increase in the critical pathways responsible for

www.manaraa.com

protecting cells from destruction (e.g. cell death pathways). Importantly, each member of HSP family plays a unique role in cytoprotection. Many of these key proteins will be discussed in more details in the subsequent sections.

A1. Hsp90: Hsp90α and Hsp90β

Hsp90 is unique among the chaperones because it is not necessary for the biogenesis of most polypeptides; rather, it seems to control aspects of protein activity, stabilization, and complex assembly. There are over 200 Hsp90 identified "client proteins" or cellular substrates reported to interact with Hsp90, compromising a variety of functional pathways for cell growth, proliferation, and survival (Figure 1). Many of these clients are *bona fide* oncoproteins involved in oncogenic signal transduction implicated in tumor progression (for an updated list, refer to: http://www.picard.ch/downloads/HSP90interactors.pdf). Hsp90 is composed of three functional domains: an amino-terminal ATP binding domain, a charged middle linker domain with high affinity for client protein binding, and a carboxy-terminal dimerization domain. Hsp90 undergoes modulated nucleotide-dependent cycling of ATP binding and hydrolysis, with ultimate release of the modified client protein. Different post-translational modifications including phosphorylation, acetylation and S-nitrosylation may affect the function of Hsp90. The most prevalent members of the Hsp90 family are Hsp90α and Hsp90β isoforms (also called HSPC1 and HSPC3, respectively), which are expressed by two distinct genes whose protein products are mainly cytoplasmic. So far only Hsp90 α has been reported to stabilize MMP-2 and prevents it from degradation in cancer cells through the interaction between the Hsp90α middle domain and the MMP-2 C-terminal

www.manaraa.com

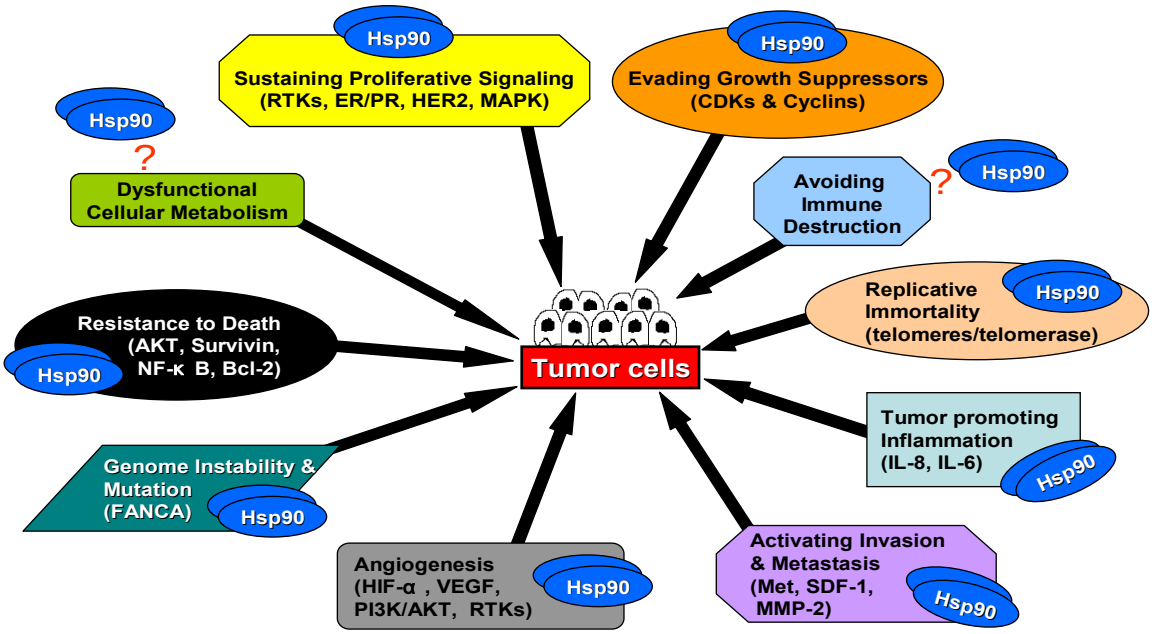


FIGURE 1. Current therapeutic targeting of the hallmarks of cancer using Hsp90 inhibitors. Drugs targeting Hsp90 in certain forms of cancer either through competitive binding to N-terminal or C-terminal of Hsp90 thereby prevention of ATP-dependent Hsp90 cycle eventually leading to client proteins degradation. The genes listed are illustrative examples implicated in the development of the hallmarks of cancer. There is a vast amount of literature reports with different molecular therapeutic targets *in vitro* and *in vivo* alone or in combination to modulate most of the hallmarks of cancer. So far two hallmarks of cancer regarding to Hsp90 inhibitors for the cancer therapy are still under development, which hold a promise for future research as potential cancer therapeutics. FNACA, Fanconi anemia complementation group A. Akt, protein kinase B. VEGF, vascular endothelial growth factor. SDF-1, stroma derived factor-1. IL-8, interleukin-8. IL-6, interleukin-6. ER, estrogen receptor. PR, progesterone receptor. HER-2, human epidermal growth factor receptor-2. RTKs, receptor tyrosine kinases. MMP-2, matrix metalloproteinases-2. MAPK, mitogen-activated protein kinase.

www.manaraa.com

hemopexin domain (Song *et al* 2010).

Hsp90 inhibition has attracted considerable attention in the past two decades as a promising approach for cancer therapy, leading to degradation of multiple oncogenic client proteins. A number of compounds and their derivatives have been shown to bind the ATP binding pocket of Hsp90, which prevents ATP hydrolysis and blocks protein folding and assembly. Instead, Hsp90 inhibition results in the targeting of its client proteins to a proteosomal degradation pathway. In addition, molecular mechanisms underlying Hsp90 translocation to the extracellular matrix and nucleus still remain obscure, even though secretion can be observed after stimulation by stressful conditions, growth factors, and influenced by phosphorylation and acetylation of the chaperone. Tapia and Morano recently discovered a novel targeting event of Hsp90 into the nucleus from the cytoplasm upon glucose exhaustion in the yeast. They employed an Hsp90-Green Fluorescent Protein (GFP) fusion protein to show nuclear accumulation of Hsp90 was a specific response to transition through the shift into quiescence (Tapia *et al* 2010). In another study, Diehl et al found elevated nuclear Hsp90 expression during breast cancer progression, which suggested that nuclear Hsp90 could be an indicator of malignancy and a viable target for cancer therapy (Diehl *et al* 2009). Previous studies also indicated both Hsp90 and Hsp23 were involved in specific nuclear events. In one study, Hsp90 and Hsp23 were demonstrated to promote telomerase activity by enhancing telomerase binding to DNA as well as nucleotide processivity (Forsythe *et al* 2001). In higher eukaryotes, the Hsp90/Hsp70 chaperone complex is required for proper delivering p53 and steroid receptors such as glucocorticoid, androgen and oestrogen receptors into nucleus thus promoting their DNA binding activities.

www.manaraa.com

In summary, these reports shed light on the roles of Hsp90 involved in translocation of client proteins into nucleus as part of its chaperone functional cycles. How can nuclear Hsp90 and its client proteins be targeted? Searching for specific approach to deliver Hsp90 inhibitors into the nucleus of cancer cells becomes an interesting question to be addressed for cancer therapeutics. For example, the use of silicon-based drug delivery vectors would be a good choice for cancer treatment and imaging, which will facilitate the delivery of multiple nano-components to particular cell compartments to achieve site-directed delivery of drugs. An *in vitro* study showed that the internalization of porous silicon microparticles by endothelial cells and macrophages is compatible with all the cellular physiological process including cell morphology, intracellular transportation, cell cycle and mitosis, cytokine secretion, and cell viability (Serda *et al* 2011).

A2. Hsp70 Family

Hsp70 family members are not only responsible for protein conformational assembly, but also preventing protein misfolding and aggregation during a variety of post-translational processes including protein targeting and degradation, membrane translocation, and apoptosis. The HspA group of HSPs includes Hsp71, Hsp70, Hsp72, and GRP78 (BiP). The members of this Hsp70 family represent the most highly conserved molecular chaperones. They have two major functional domains: an N-terminal ATPase-binding domain (ABD) responsible for substrate binding and refolding, and a C-terminal peptide-binding domain (PBD) to facilitate the release of client protein after ATP hydrolysis.

The 78-kDa glucose regulated protein (GRP78), also well known as immunoglobulin

www.manaraa.com

heavy chain binding protein (BiP), was originally found as a major protein for maintaining intracellular homeostasis in endoplasmic reticulum (ER) called the unfolded protein response (UPR). High level of GRP78 confers multiple survival advantages to facilitate the proliferation of cancer cells through harsh conditions and to acquire chemotherapeutic resistance (Gonzalez-Gronow *et al* 2009; Dong *et al* 2008; Lee, 2007; Lee, 2001; Li *et al* 2006). While it is difficult to detect GRP78 expression in normal cells, over-expression of GRP78 can be detected in many tumor cell lines and primary tumors, such as breast and prostate cancer cells. *In vivo* studies demonstrate a critical role of GRP78 in tumor growth, metastasis, and angiogenesis in xenograft models and in the Grp78 heterozegous mice with partial reduction of GRP78 (Lee, 2007). It has been shown that GRP78 interacts with BH-3 only proapoptotic protein, Bcl-2 Interacting Killer or Natural Born Killer (BIK, or NBK) and specific caspases, such as caspase-7, on the ER membrane, thereby regulating the balance between cell survival and apoptosis (Rao *et al* 2001; Fu *et al* 2007; Rauschert *et al* 2008; Reddy *et al* 2003). GRP78 is commonly found inside the ER lumen because of a presumed N-terminal ER localization signal. GRP78 can be detected as a cell surface protein in a broad variety of tumor cells by global profiling of the cell surface proteins, suggesting cancer cells may have evolved a specific mechanism for presenting GRP78 epitopes on the cell surface (Gonzalez-Gronow *et al* 2009; Misra *et al* 2006; Misra *et al* 2010).

Hsp72 is another major heat shock-induced protein capable of protecting cells from stressful conditions. Hsp72 can be present at elevated levels in various forms of tumors and in many transformed cell lines. It has been shown that the oncogenic potential of Hsp72 is confined in its peptide binding domain since the expression of this domain alone was

www.manaraa.com

sufficient for tumorigenic transformation of Rat-1 cells (Volloch *et al* 1999). Based on more novel findings of roles of Hsp70 from clinical and basic research, Hsp70 targeted therapy is clearly an attractive approach for anti-tumor treatment. Considerable progress has been made in targeting Hsp70 using small molecule inhibitors in cancer as well as in other protein folding diseases. In this review, we will focus on small molecule inhibitors targeting Hsp90.

A3. Hsp60

In eukaryotes, the chaperonin proteins Hsp60 and Hsp10 are structurally and functionally nearly identical to the bacterial GroEL and GroES proteins, respectively. Hsp60/GroEL belongs to the molecular chaperones in the alkalai family. GroEL requires the lid-like cochaperonin protein complex GroES to function normally to help proteins fold correctly. Hsp60 is involved in protein folding after its post-translational import to the mitochondria (or chloroplasts in plants). These chaperonins aid the folding process by assembling into large complexes to provide specific folding spaces where clients can undergo the appropriate intramolecular interactions to obtain the correct three dimensional structure. Accumulating data showed alteration of Hsp60 expression in tumor development. It has been demonstrated Hsp60 as an intramitochondrial protein directly interacts with cyclophilin D (CypD), a component of the mitochondrial permeability transition pore, thereby preventing CypD-dependent cell death through formation of a multiple complex with Hsp90, and tumor necrosis factor receptor-associated protein-1 in tumors (Ghosh *et al* 2010). However, Hsp60 has also been found in the cytosol, cell membrane, vesicles, extracellular space as well as serum (Cappello *et al* 2011). The elucidation of molecular mechanism of Hsp60 distribution

www.manaraa.com

in different cellular compartment may provide a novel target for cancer therapy. For example, Chun et al demonstrated the cytosolic Hsp60 promoted the TNF-α-mediated activation of the IKK/NF-κB survival pathway via direct interaction with IKKα/β in the cytoplasm. Mechanism for IKK activation by the cytosolic Hsp60 was showed to work via up-regulating the activation-dependent serine phosphorylation in a chaperone-independent manner, which indicated the role of Hsp60 for tumor cell survival may work through NF-κB pathway (Chun *et al* 2010).

A4. Hsp27 and the small HSPs

Protein folding also involves the Hsp27 family, known as the small HSPs (sHSPs, 15-30 kDa). They assemble into large aggregates that mediate holding and folding in an ATP-independent manner, which differs from the larger ATP-dependent molecular chaperones (e.g. Hsp90). The common functions of sHSPs are chaperone activity, thermotolerance, inhibition of apoptosis, regulation of cell development, and differentiation (Garrido *et al* 2006). Hsp27 belongs to a family of sHSPs that includes ubiquitin, α-crystallins, Hsp20, and others. sHSPs have significant sequence homology and biochemical properties in common including phosphorylation and oliogmerization. Hsp27 contains a central domain called α-crystallin, which is universally conserved among all the sHSPs family members. The N-terminus is made of a less conserved region, called WDPF motif representing amino acid (16-19 residues) tryptophan, aspartate, proline, and phenylalanine, which is necessary for chaperone-like activity of sHSP, since substrate binding occurs through hydrophobic interactions (Takeuchi 2006). The WDPF motif affects the Hsp27's

www.manaraa.com

oligomerization, which is regulated by the phosphorylation of the protein. Hsp27 may serve as a differentiation marker since it is induced in the early stages of differentiation, decreasing when a cell divides.

Hsp27 enhances the activation of the nuclear factor-κB (NF-κB) pathway, which controls many pathophysiological processes, such as tumor cell proliferation, inflammation, drug resistance, and stress response (Romani *et al* 2007; Andrieu *et al* 2010). The cytoprotective properties of Hsp27 result from its ability to modulate reactive oxygen species (ROS) and to raise glutathione levels in its reduced (non-oxidized) form.

A5. Hsp40 family

Hsp90 and Hsp70 are relatively thoroughly investigated for their roles in molecular mechanisms underlying tumor pathogenesis. However, the role of DnaJ/Hsp40 family in physiological and pathological conditions is still to be further investigated. Because hydrolysis of ATP is essential for Hsp70s activities, DnaJ/Hsp40 proteins stabilize Hsp70s interaction with client proteins through binding to the ATPase domain of Hsp70 particularly with its conserved tripeptide of Histidine-Proline-Aspartic acid (HPD) motif in the J domain (Tsai J *et al* 1996; Qiu *et al* 2006). The J domain is usually located at the N-terminal region of the DnaJ/Hsp40 proteins and composed of a 70-amino acid sequence forming four helices and a loop region containing the HPD motif between helices II and III. Depending on the presence of the Gly/Phe-rich region (G/F) with/without the cysteine repeats, DnaJ/Hsp40 proteins can be categorized into type I, II and III.

Several studies have shown that several Hsp40 family members including hTid I (class

www.manaraa.com

DNAJA3) and HLJ 1 (class DNAJB4) are associated with the regulation of tumor progression (Kim *et al* 2004; Wang *et al* 2005). In addition, some DnaJ family members have unique domains which display the functional diversity of these proteins. For example, the mammalian DnaJ protein ERdj5, as a novel endoplasmic reticulum (ER) chaperone, has an unique combination of domains including a danj, a protein disulfide isomerase-like and a thioredoxin domain (Cunnea *et al* 2003; Hosoda *et al* 2003). Recently, Ushioda et al reported that ERdj5 has a reductase activity; it can cleave disulfide bonds of misfolded proteins and accelerates ER-associated degradation (ERAD) through its interactions with ER degradation-enhancing α-mannosidase-like protein (EDEM) and BiP/GRP78 (Ushioda *et al* 2008 & 2011). Thomas *et al* reported that ERdj5 enhanced apoptosis in neuroblastoma cells utilizing its dnaj domain to block the ER stress-induced phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) and the subsequent translational suppression (Thomas *et al* 2009). The compromised UPR was observed in ERdj5-overexpressing ER-stressed cells. This was due to inhibition of eIF2α phosphorylation which impaired neuroblastoma cell survival under ER stress conditions. These findings indicated that ERdj5 reduced neuroblastoma cell survival through negative regulation of the UPR, providing the possibility that this chaperone protein could be a novel target for cancer therapy.

A 6. HOP

Hsp70/Hsp90 organizing protein (HOP) is one of the most extensively studied co-chaperones, which is able to directly associate with both Hsp70 and Hsp90. The current dogma proposes that HOP functions primarily as an adaptor that directs Hsp90 to

www.manaraa.com

Hsp70-client protein complexes in the cytoplasm (Odunuga *et al* 2004). However, mounting studies indicate that HOP can also associate with a number of Hsp90-independent complexes including the intriguing role as a receptor for prion proteins (Martins *et al* 1997; Romano *et al* 2009).

The diverse location of HOP inside of the cell, the versatility of tetratricopeptide (TPR) domain, coupled with the association with various other cellular Hsp90-independent proteins uncovered novel roles of HOP. For example, Marozkina *et al* recently showed HOP is a critical target of S-nitrosoglutathione (GSNO), and its S-nitrosylation by GSNO inhibited the association of HOP with DeltaF508 cystic fibrosis (CF) transmembrane conductance regulator (CFTR) in the ER. This effect was necessary and sufficient to mediate GSNO-induced cell-surface expression of DeltaF508 CFTR. HOP siRNA recapitulated the effect of GSNO on DeltaF508 CFTR maturation and expression. In summary this study demonstrated that GSNO corrects DeltaF508 CFTR trafficking by inhibiting HOP expression, and the combinational therapies targeting different mechanisms of action may have additive treatment beneficial for CF (Marozkina *et al* 2010).

B. Functional Role of HSPs in Cancer Development

Molecular chaperones are essentially housekeeping proteins as they function to: 1) assist nascent protein folding, 2) prevent misfolding, and 3) reduce aggregation of unfolded or misfolded proteins during stressful conditions. In transformed cells, mechanisms for controlling protein aggregation are critical for preventing cell death that can be induced by the increase in cell stress and the ultimate loss of cellular homeostasis. Interestingly, these

www.manaraa.com

pathways are interrelated with an underlying theme: cancer initiation and progression. A list of major key cancer-associated mechanisms is discussed in this section.

B1. Diversified roles of HSPs in cell death pathways

As transformed cells develop, their microenvironment plays an important role in tumor cells ability to progress to a more aggressive phenotype. During this time of adaptation to their surrounding environment, tumor cells develop survival capabilities through several known and unknown mechanisms in order to acclimate to stressful conditions that accompany tumor progression, including inflammation, hypoxia, radiation, and chemotherapy. HSPs can protect tumor cells not only from apoptosis induced by a variety of diverse stimuli but also from type II programmed cell death (i.e. autophagy), replicative senescence, and mitotic catastrophe.

B1.1 Apoptosis

HSPs regulate the intrinsic apoptotic signaling pathway (mitochondrial). Hsp90 and its co-chaperones also modulate and mediate tumor cell apoptosis through interacting with the Akt kinase, tumor necrosis factor (TNF) receptor, HER2/ErbB2 receptor, and transcription factors like NF-κB. However, Hsp90 plays a more significant role during oncogenic transformation than simple inhibition of apoptosis. Using genetic and pharmacological techniques, Hsp90 has been shown to facilitate numerous transient low-affinity protein-protein interactions that were previously unknown, partly because these client proteins become actively involved in oncogenic transformation (Basso *et al* 2002; Vanden *et*

www.manaraa.com

al 2003; Chen *et al* 2002; Xu *et al* 1999; Zhang *et al* 2008). For instance, wild type SRC tyrosine kinase needs only limited assistance from the Hsp90 chaperone machinery for assembly into its mature and functionally active protein; yet v-SRC mutants demonstrated an uncommonly stable interaction with Hsp90 (Oppermann *et al* 1981; Brugge *et al* 1983). This aberrant interaction between chaperone and mutated client protein was later shown to be essential for acquiring and maintaining the transforming activity of v-SRC in tumor cells (Xu et al 1993; Whitesell et al 1994). Because of the accumulation of such mutant proteins in tumor cells, novel phenotypes may emerge as a result of alterations in the levels of unbound or uncomplexed Hsp90, which can be virtually eliminated by environmental stress and/or tumorigenic transformation. Numerous *in vitro* studies suggested an important role for HSPs in the regulation of caspase activation because they were able to block cell death at different stages by interaction with a number of apoptosis-related client proteins.

Hsp27 is actively involved in the apoptotic pathway. Hsp27 interacts with the outer mitochondrial membrane and sequesters cytochrome C, which was released into the cytosol from the mitochondria, thereby preventing the formation of the apoptosome and the activation of procaspase-9. In addition, knockdown of Hsp27 by siRNA induces apoptosis through activation of caspase-3.

Hsp70 is a negative regulator of the intrinsic apoptotic pathway and can inhibit cell death at different stages. At a premitochondrial stage, Hsp70 suppresses stress inducing signals through binding to c-Jun N-terminal Kinase (JNK1) via an ATPase domain independent manner (Park *et al* 2001; Mosser 2000), as well as binding to nonphosphorylated protein kinase C (PKC) and AKT (Gao *et al* 2002), resulting in their stabilization, at the

www.manaraa.com

mitochondrial stage, Hsp70 prevents Bax translocation to inhibit mitochondrial membrane permeabilization (Stankiewicz et al 2005). Finally, Hsp70 functions at the post-mitochondrial level by interacting with the apoptosis-inducing factor (AIF) and the apoptosis protease activating factor-1 (Apaf-1) or by protecting essential nuclear proteins from caspase-3 cleavage (Li et al 2000).

B1.2 Autophagy

sHSPs are reported to act at different steps in protein quality control with differential potential to prevent aggregation of insoluble mutant proteins. Aggregation of polyglutamine proteins results in the development of neurological disorders such as Huntington disease and spinocerebellar ataxias. One of the HSP family members, HSP7 can prevent toxicity of polyglutamine-containing proteins in cells by assisting the loading of misfolded proteins or small protein aggregates into autophagosome. However, the mechanism of heat shock 27kDa protein family, member 7 (HSPB7) involvement with the autophagic machinery has yet to be investigated. Recently, Jiang *et al* reported Hsp90-mediated inactivation of NF-κB signaling pathway turned protective autophagy into apoptosis in human leukemia NB4 cells when treated with sodium selenite, which was an essential dietary component for animals and humans considered as a protective agent against cancer. In their study, reduction of Hsp90 in selenite-treated NB4 cells attenuated IKK/NF-κB signaling pathway and led to inhibition of autophagy-related gene (Atg) 6 expression (also known as Beclin 1) and vesicular accumulation of microtubule-associated proteins 1 light chain 3 (LC3), both of which are the markers of autophagic activity, thereby activating apoptotic pathway in these cancer cells.

www.manaraa.com

These findings suggested that the potential role of overexpression of Hsp90 may be served as a protective factor for drug resistance in some types of cancer (Jiang *et al* 2011).

B1.3 Necrosis, Telomerase and ROS

Small HSPs, especially Hsp27 and α-crystallin, can protect cardiomyocytes from ischemia-induced necrosis both *in vitro* and *in vivo*, while the larger Hsp70 protein protects human β-cells from nitric oxide-induced necrosis via rescue of mitochondria functions. The potential mechanisms of this phenomenon may be associated with inhibition of the stress kinases JNK and p38 by Hsp70, indicating that these kinases are targets of the anti-necrotic effect of Hsp70 in the myocardium. Although the molecular mechanisms of HSPs in protection of cancer cells from ischemia-induced necrosis has not yet been reported, it may be interesting to investigate the effect of Hsp70 on JNK and p38 regarding to higher levels of expression of Hsp27 and/or Hsp70 in tumor versus normal tissue. Akalin *et al* showed Hsp90 chaperone-mediated enhancement of telomerase assembly contributed to tumorigenic conversion. In the same study, telomerase activity was dramatically increased as prostate cancer cells progressed to tumorigenic state *in vitro* using an appropriate prostate cancer model system within a similar genetic background (Akalin *et al* 2001). The interactive relationship among stress, telomeres, telomerase and molecular chaperones needs to be further examined. Therefore, telomerase inhibition as a potential adjuvant therapy to anti-tumor approaches becomes appealing if Hsp90 inhibition can affect telomerase activity at low doses without detrimental effects. In addition, it has shown that chronic inhibition of Hsp90 using an established antibiotic inhibitor, radicicol, resulted in telomere shortening and

www.manaraa.com

subsequent cell death through the generation of reactive oxygen species (ROS) through the deregulation of the NOS pathway with a significant increase in NOS-dependent O_2^- radicals (Compton *et al* 2006).

B2. Angiogenesis

High levels of HSP expression are important for cancer cells to survive in a hypoxic tumor microenvironment, which is generally attributed to their effects on the transcription factor HIF-1α by mediating its stabilization and/or aggregation. HIF-1 is a heterodimer that is composed of both HIF-1α (120 kD) and HIF-1β (91-94 kD). HIF-1α is stabilized by Hsp90 in hypoxic conditions and normally degraded by prolyl hydroxylase (PHD), the von Hipple-Lindau (VHL)/Elongin-C/Elongin-B E3 ubiquitin ligase complex through proteasomes dependent manner (Liu *et al* 2007; Isaacs *et al* 2003;).

Hsp90 plays a critical role in structural modulation of oncoproteins including Akt, HER-2/ERBB2, RAF1, eNOS, BCR-ABL and mutated p53 (Kim *et al* 2008; Bohonowych *et al* 2010). Hypoxia and other stressful stimuli induce HIF expression as well as subsequent cellular response, resulting in a cascade of signaling events that induce VEGF expression and angiogenesis. Importantly, several critical mediators in this angiogenic signaling pathway, including HIF, VEGF-receptor and IL-8/NF-κB are dependent upon Hsp90 for their function. Receptor Tyrosine Kinase (RTK) activation also potentially induces HIF expression via Akt/mTOR -mediated translation pathway. RTKs additionally transactivate Ephrin type-A receptor 2 (EphA2), a novel Hsp90 client protein known to be involved in tumor angiogenesis. In addition, HIF also promotes the expression of several RTK ligands, for

www.manaraa.com

example, hepatocyte growth factor (HGF) and TGF-α, as well as RTK receptors including endothelia growth factor receptor (EGFR) and insulin-like growth factor receptor (IGFR), thereby reinforcing these signaling interactions. Moreover, Hsp90 plays a role in NF-κB-induced VEGF expression and regulates downstream effectors, including Akt-mediated eNOS phosphorylation. Given that Hsp90 is required for activation of VEGFR, Akt, eNOS, and NFκB, Hsp90 inhibitors can be employed to target multiple signaling molecules of angiogenesis pathway, as demonstrated by the potent suppression of VEGF and NO release both *in vitro* and *in vivo* with the overall outcome of inhibiting tumor angiogenesis (Bohonowych *et al* 2010).

Lang et al showed that Hsp90 inhibitors can also disrupt HIF-1α/Signal Transducer and Activator of Transcription-3 (STAT3) mediated autocrine loop for IL-6 and IGF-I in pancreatic adenocarcinoma cells and the highly metastatic L3.6p1 pancreatic cells by direct disturbance with the functions of HIF-1α and STAT3. In the same study, ELISA data also demonstrated a marked reduction of VEGF-A expression after treatment with 17-allylamino-geldanamycin (17-AAG), a Hsp90 inhibitor (Lang *et al* 2007). In tumor cells, STAT3 is commonly activated and blocks apoptosis as wells promotes cell transformation. Much of the known roles of Hsp90 in angiogenesis is shown schematically in Figure 2.

www.manaraa.com

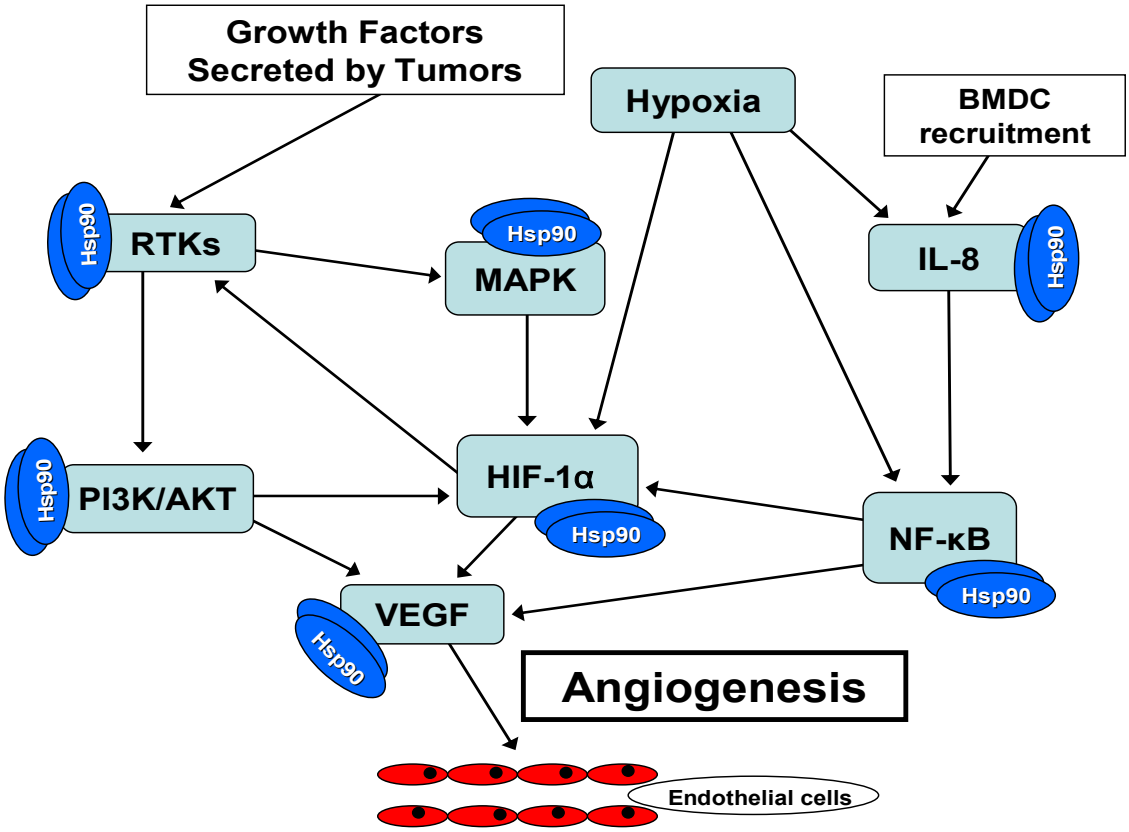


FIGURE 2. Hsp90 inhibitors targeting angiogenic signaling network in cancer. Hsp90 mediates multiple channels of angiogenesis signaling in cancer cells through its interaction with multiple client proteins including HIF-alpha, RTKs, AKT, and NF-κB. Traditional oncogenic angiogenesis pathway works through HIF-dependent manner, which can be activated either by direct stimulant, hypoxia or through phosphorylation of several tyrosine kinase receptors (RTKs) thereby activating downstream HIF-1 α-associated pathways including RTKs-PI3K/Akt-HIF-alpha-VEGF and RTKs-MAPK-HIF-alpha-VEGF. In addition, recruitment of bone marrow derived cells (BMDC) stimulates a proangiogenic factor IL-8 secretion to activate NF-κB-dependent VEGF expression. Recently, several studies showed NF-κB induced HIF-alpha expression in transformed cells and canonical NF-κB pathway is required for inflammatory gene expression when exposed to hypoxia (Qiao *et al* 2010; Fitzpatrick *et al* 2011). Over all these findings indicate that a complicated signaling network intertwined for collaboration of angiogenesis in cancer, which provides a strong evidence for Hsp90 inhibitors to be considered as multi-targets-inhibition strategy for clinical trial.

www.manaraa.com

B3. Invasion and Metastasis

Over-expression of heat shock factor protein 1 (HSF1) and HSPs in tumor cells displayed an increasing trend to invade tumor microenvironment and metastasize to distant sites (Ciocca *et al* 2005; Hoang *et al* 2000), though molecular mechanisms have not yet fully understood. In addition to transcriptional regulation of HSPs expression, recent data also showed that HSF1 is an important facilitator for tumor progression. Accumulating research suggests that highly expressed downstream factors of HSF1, including Hsp27 and Hsp70, in tumor cells are at least partially responsible for the invasive and/or metastatic properties of tumors.

Hsp90 was detected on the cell surface and in conditioned medium of tumor cells, where it acted as a molecular chaperone to assist in the activation of matrix metalloproteinase-2 (MMP-2), working with a complex of co-chaperone proteins including Hsp90 organizing protein (HOP) and p23, leading to elevated tumor invasiveness. Accumulating evidences indicate that Hsp90 especially Hsp90α can be expressed and function in the extracellular space acting as a molecular chaperone that assist in the maturation of pro-MMP2 to its active form by stimulating propeptide cleavage. Activated MMP2 protease digests many of the major extracellular matrix (ECM) components surrounding tumor tissue including fibronectin, laminins, collagens, *etc* thereby facilitating tumor invasion process. As an ubiquitously expressed cytosolic protein in higher eukaryotes, Hsp90α also works in other cellular compartments in different cell types and specific disease conditions playing distinctive biological functions.

Several cochaperones including p23 and HOP were found in the conditioned medium of

www.manaraa.com

HT-1080 fibrosarcoma cells acting in concert with Hsp90α to activate MMP2. In addition, p23 was also shown to promote tumor progression and poor prognosis in breast cancer by increasing metastatic potential through the upregulation of several genes involved in metastasis and chemotherapeutic resistance by enhancing Akt signaling pathway, which plays a critical in tumorigenesis. These findings opened a new door to target extracellular Hsp90 and related chaperone complex for cancer therapy. However, there are still several important questions that need to be addressed before we become excited about these novel findings. For example, neutralizing antibody against extracellular Hsp90 only affects the regulation of cell migration and metastasis but did not affect tumor cells proliferation both *in vitro* and *in vivo*. Two possible explanations can be considered. First, Akt/PI3K signaling pathways are involved in cell survival and metastasis. Both of them are Hsp90 client proteins. Hence blockage of Hsp90 activity seems to activate apoptosis through attenuation of Akt/PI3K oncogenic pathways. Second, heregulin-induced HER2 phosphorylation in tumor cells can be ameliorated by neutralizing antibody against extracellular Hsp90 which inactivates downstream kinase signaling pathways, rearranges cytoskeleton and subsequent cell migration. These data provided molecular mechanistic evidence for the multifaceted Hsp90 as a metastasis promoter during the cancer progression and suggested targeting Hsp90 for the prevention of cancer metastasis.

B4. Chemotherapeutic Resistance

Since HSPs' cytoprotective effect is essential for cancer cell survival, it is not surprising that HSPs targeted therapy is considered an interesting pharmacological intervention stategy

www.manaraa.com

for cancer treatment. To that end, there are currently 13 Hsp90 inhibitors entered into clinical evaluation in cancer patients (Trepel et al 2010, Table 1). Most studies to identify Hsp90 inhibitors have concentrated on ligands binding to the N-terminal ATP-binding site, which disrupts Hsp90's ATPase activity and the ongoing ATP-dependent folding cycle. Because this cyclic event requires multiple co-chaperone proteins, inhibition results in the destabilization, ubiquitination, and ultimately proteasomal degradation of the client proteins, causing the disruption of multiple oncogenic signaling pathways simultaneously.

It has recently been confirmed there is an association of the GRP78 chaperone with client proteins of the Hsp70-Hsp90 complex, namely HER2, HER3, Akt and androgen receptor (AR) were observed in prostate cancer. The association of AR and GRP78 expression in untreated prostate cancer is novel and highlights the potential implication of targeting GRP78 as a novel molecular therapy in prostate cancer in both the hormone-naïve and castrate-resistant states. However, one of the critical problems with Hsp90 inhibition is the accumulation of Hsp70 after treatment, which can reduce cell death induced by Hsp90 inhibitors and therefore buffer their anti-tumor efficacy in the clinic. Those disappointing results may be related to inherent Hsp90 inhibitors toxicity and induction of Hsp70 by calcium mobilization and activation of TGF-β signaling pathway. Thus, Hsp70 inhibition combined with anti-Hsp90 compounds could be an interesting strategy to eliminate the toxic side-effects of Hsp90 inhibitors and enhance synergistic efficacy for the treatment of cancer. Currently compounds designed for inhibition of Hsp70 function are targeting different domains by interfering at specific steps within the chaperone dynamic cycle. For example, 15-deoxyspergulain (15-DSG) possibly interacts with the C-terminal EEVD motif

www.manaraa.com

Table 1. Hsp90 Inhibitors

Hsp90 Inhibitor	Interaction Site	References	Source
Geldanamycin	N-terminal ATP-binding	Supko 1995; Park 2003;Kim 2009; Samuni 2010	benzoquinone ansamycin antibiotic
17-AAG (Tanespimycin)	N-terminal ATP-binding	Janin 2005; Grem 2007; Erlichman 2009	Geldanamycin analog
17-DMAG	N-terminal ATP-binding	Palacios 2010	Geldanamycin analog
Retaspimycin hydrochloride	N-terminal ATP-binding	Hanson 2009; Dewaele 2008	IPI-504
IPI-493	N-terminal ATP-binding	Porter 2009; Croasdell 2010	Ansamycin class
Radicicol	N-terminal ATP-binding	Delmotte 1953; Soga 2003	Macrocylic antifungal
Purine-scaffold inhibitors	N-terminal ATP-binding	Zhang 2010	CNF-2024/BIIB021
Shepherin	N-terminal ATP-binding	Plescia 2005	Peptidomimetic antagonist
Pyrazoles (CCT-018159)	N-terminal ATP-binding	Rowlands 2004; Cheung 2005; Barril 2006; Sharp 2007	Antifungal antibiotic
PU24FCI	N-terminal ATP-binding	Vilenchik 2004	Synthetic inhibitor
SNX-5422(mesylate)	N-terminal ATP-binding	Huang et al 2009; Fadden 2010	Synthetic inhibitor
STA-9090	N-terminal ATP-binding	Lin 2008;Wang 2010	Resorcinol triazole
NVP-AUY922	N-terminal ATP-binding	Eccles 2008;Tauchi 2011;Gaspar 2010	Synthetic inhibitor
Oxime derivative KF58333	N-terminal ATP-binding	Soga et al 2001; Kurebayashi 2001	Synthetic inhibitor
Novobiocin	C-terminal ATP-binding*	Donnelly 2008; Yang 2003; Nordenberg 1992	From streptomyces
Coumermycin	C-terminal ATP-binding	Marcu 2000	From streptomyces

*The C-terminal ATP-binding site is putative

www.manaraa.com

to stimulate the steady-state ATPase activity of the constitutively expressed Hsc70 without affecting Hsc70 functions that require DnaJ (Brodsky et al 1999). In addition, 15-DSG was also found to bind Hsp90. Hsc70s are the constitutive cellular analogues of the stress-inducible Hsp70 molecular chaperones, 70-kDa heat shock proteins that bind and release polypeptide substrates concomitant with ATP-dependent dynamic cycle (Hartl 1996).

Nadeau et al utilized affinity capillary electrophoresis to obtain Kd values for DSG. Kd values are 4 μM for DSG binding to Hsc70 and 5 μM for DSG binding to Hsp90. (Nadeau et al 1994) Recently, a small molecule inhibitor Pifithrin-μ (2-phenylethynesulfonamide or phenylacetylenylsulfonaminde, PES) was reported to interact with the substrate binding domain of Hsp72 and to reduce the association between Hsp72 and its cochaperones Hsp40 and Bcl_2-associated athanogene-1(BAG-1) (Leu et al 2009). Pifithrin-μ is an inhibitor of p53 binding capability and also an antiapoptotic protein. It can directly prevent p53 binding to mitochondria as well as to Bcl-xL and Bcl-2 as a temporary inhibition of p53 transcriptional activity to protect normal tissues from side effects by chemotherapy without affecting p53-mediated transactivation in cancer treatment. Moreover, *in vivo* study also indicated that administration of Pifithrin-μ inhibited spontaneous tumor development and enhanced survival in the Eμ-Myc induced-lymphomagenesis mouse model (Leu et al 2009). However, it would be interesting to investigate the effect on Hsp90 activity using Pifithrin-μ because previous study showed dual siRNA knockdown of Hsc70 and Hsp72 led to the proteasomal degradation of the Hsp90 client proteins CRAF, CDK4 and ERBB2 in human colon and ovarian cancer cells (Powers et al 2008). Regarding the multiple functions of Hsp70 protein for tumor growth, small molecule inhibitors targeting Hsp70 represents a promising approach

www.manaraa.com

to selectively target critical chaperone involvement in multiple oncogenic signaling pathways.

C. Roles of HSPs in Other Diseases

Adaptation and survival to changeable environmental states requires a cell's ability to sense misfolded proteins and to activate the corresponding protective response, signaling pathways, and functional molecular chaperones to restore intracellular homeostasis. Failure to do so results in a cell that is poorly adapted to proteotoxic stimuli, so that when dysfunctional aggregation of proteins occurs, the consequence is protein accumulation rather than degradation of protein complexes. There are many protein conformation diseases including cancer, neurodegenerative disease, aging, and chronic metabolic disease, some of which will be discussed.

C1. Aging and Senescence

Misfolding and aggregation of proteins are now considered as common molecular events in many human diseases. Conformational diseases have in common that aggregation-prone proteins cause "gain-of-function" proteotoxicity and lead to several diseases due to improper trafficking of proteins, misfolding or malfolded proteins, and inappropriate degradation of proteins. Aging and protein synthesis homeostasis are intertwined closely. The molecular interactions between the genetic pathways that regulate life span and inhibition of proteotoxicity are promoted partially by factors including heat shock factor-1 (HSF-1), molecular chaperones, and DAF-16 (a forkhead transcription factor found in *C. elegans*),

www.manaraa.com

which is closely related to mammalian transcriptional factor forkhead box O3A (FOXO3A).

HSF-1 is a transcription factor binding to Hsp40/Hsp70 and Hsp90 in a complex formation under unstressed conditions, where Hsp90 is found as a suppressor of HSF-1 activation. Upon stress, HSF-1 forms trimers and becomes transcriptionally active because Hsp90 needs to compete with misfolded proteins and dissociates from HSF-1. With age in animal models, HSF-1 level does not change but a decline in the activation and binding of DNA binding motif (Heydari *et al* 2000). Results from cultured cells suggest that the age-related reduction in Hsp70 protein expression is due to decreased binding of HSF-1 to the heat shock element (HSE) and declined Hsp70 transcription level. Overall, these reductions of Hsp proteins and increase in misfolded protein all contribute to the damage of molecular chaperone functions with age. (Horowitz *et al* 2007)

Dauer formation abnormal (DAF)-16 is a key FOXO transcription factor that regulates innate immunity in Caenorhabditis elegans (Singh *et al* 2009). The human FOXO3A has been linked to inflammation in response to infection. Recently, variants in FOXO3A have consistently been confirmed with human longevity in different ethnic populations worldwide (Flachsbart *et al* 2009). FOXO3A encodes an evolutionarily conserved key regulator of the insulin-IGF1 signaling pathway that is known to influence metabolism and lifespan in model organisms including worms, flies, mice in addition to human (Ziv et al 2011). Like FOXO3a, the activity of DAF-16 is tightly regulated by a wide variety of external stimuli such as nutrients, oxidative stress, and heat stress. Both HSF-1 and DAF-16 are essential partners and play in concert to promote longevity and to maintain protein homeostasis (Kleindorp et al 2011).

www.manaraa.com

C2. Neurodegenerative Disease

Accumulation and deposition of misfolded proteins in the brain (inside and outside neurons) and selective neuronal loss in the central nervous system (CNS) have been implicated as a common molecular mechanism of various neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and the polyglutamine (PolyQ) diseases. Hsp27 protects motor neurons from apoptosis induced by mechanical injury likely through intervention between cytochrome C release and caspase-3 activation. Hsp70 has also been reported to associate with polyglutamine proteins thereby prevention of its aggregation *in vivo* (Nagai *et al* 2010). The PolyQ diseases are a group of nine hereditary neurodegenerative diseases, including Huntington's disease (HD) and various types of spinocerebellar ataxia (SCA), which are caused by abnormal expansions of the polyQ stretch (>35-40 repeats) in unrelated disease-causative proteins. Misfolding and aggregation of the polyQ protein are the most ideal therapeutic targets because they are the most upstream events in the pathogenic progression, so that therapeutic approaches focusing on chaperones to prevent protein aggregation and assist in the refolding of misfolded proteins, are being extensively studied. Actually a variety of molecular chaperones such as Hsp70 and Hsp40 have been demonstrated to exert therapeutic effects against various experimental models of the polyQ diseases (Turturici *et al* 2011). Autosomal recessive juvenile Parkinsonism (ARJP) is caused by *parkin* gene mutations, where parkin protein cooperates with Hsp70 and the co-chaperone the E3 ubiquitin ligase C-terminus of Hsc70-interacting protein (CHIP) to suppress Parkin-associated endothelin receptor-like receptor (Pael-R) function (Takahashi *et al* 2003).

www.manaraa.com

CHIP is believed to be a central player in the cellular triage decision, as it links the molecular chaperones Hsp70/Hsc70 and Hsp90 to the ubiquitin proteasomal degradation pathway.

Loss of function of parkin, an ubiquitin ligase, is responsible for AR-JP. Pael-R was identified using a yeast two-hybrid system and identified as a putative G protein-coupled receptor protein is an authentic substrate of parkin (Takahashi *et al* 2003). It is thought to accumulate abnormally following loss of parkin activity, causing neurodegeneration of nigral dopaminergic neurons in AR-JP patients. Pael-R located in the ER membrane and promoted ER stress-induced cell death through protein degradation and the ubiquitin-proteasome pathway. When overexpressed in cells, this receptor becomes unfolded, insoluble, and finally ubiquitinated. Accumulation of the insoluble Pael-R leads to ER stress-induced cell death. Parkin specifically degrades the unfolded Pael-R, preventing cell death induced by the aggregation of unfolded Pael-R (Dusonchet *et al* 2009).

C3. Kidney Diseases

The role of HSPs in chronic kidney disease (CKD) is relatively limited, though Hsp72 inhibits the proliferation and apoptosis in tubular cells in rats, decreases the accumulation of fibroblasts and type I collagen in renal parenchyma, thereby delaying the fibrotic process. Elevated Hsp90α in children with CKD indicates activated oxidative stress and inflammation in CKD, which may ultimately trigger atherosclerosis. Clearly much more detailed molecular and cellular analysis is needed, but current data suggests that the intracellular forms of Hsp70 slowed the progression of CKD through Hsp70's anti-apoptotic and cytoprotective functions. A variety of HSPs have shown their differing roles in CKD by protecting against stress

www.manaraa.com

conditions (Hsp70 and Hsp27), serving as a predictor of the cell damage (Hsp90), or acting detrimentally on kidney function, glomerular necrosis, and anuria (Hsp60 and anti-Hsp60). Thus, there is some indication that chaperones play an important role in CKD, although the exact mechanistic functions attributed to each HSP still remain to be elucidated.

www.manaraa.com

CHAPTER THREE

THERAPEUTIC POTENTIAL OF HSP INHIBITION

A. Targeting Hsp90-mediated transformational signaling pathways in malignant cells will comprehensively affect all the classic hallmarks of cancer progression.

Hanahan and Weinberg have proposed the six hallmarks of cancer a decade ago to provide a logical and solid framework for understanding the biology of cancer (Hanahan *et al* 2000). With considerable progression made in cancer research after this publication, two emerging hallmarks including reprogramming of energy metabolism and evading immune destruction have become additional highlights in the study of tumorigenesis. In addition, two enabling characteristics including tumor-promoting inflammation as well as genome instability and mutation have been proved to enhance the six core and emerging hallmark capabilities (Hanahan *et al* 2011). More importantly, in clinic pathological term "desmoplasia" initially describing the growth of fibrous or connective tissue around the tumor lesion has been proven and investigated in the mechanistic study of tumor progression, which further complets our overall consideration of cancer cells not as an "isolated island", which means they have to communicate with and depend on surrounding non-cancerous cells to facilitate tumor microenvironment. Microcommunity of cancer is not simply understood by passive composition of bystanders but a dynamic microenvironment communication of multiple cell types reciprocally interacting with each other to facilitate tumor progression, especially tumor-associated fibroblasts or cancer-associated fibroblasts (TAFs or CAFs), a

www.manaraa.com

major and critical component of tumor stroma tissue (Tlsty *et al* 2006; Beacham *et al* 2005; Kunz-Schughart *et al* 2002).

Appreciation of these critical hallmarks and characteristics for the development of cancer will definitely modulate the future direction of cancer research and promote our exploration of anti-tumor therapy with potential paradigm-shifting strategies (Hanahan *et al* 2011). As shown in Figure 1, Hsp90 plays a multifaceted part involved in the acquisition and development of the hallmarks of cancer through interacting with many client proteins responsible for essential oncogenic transformation. If these client proteins fail to bind a specific ligand or receptor to form a meta-stable chaperone-client complex, then they are subjected to ubiquitination and finally degraded by proteasome, providing the major theoretical basis of pharmaceutical inhibition of Hsp90-mediated oncogenic signaling pathways. For example, Hsp90 inhibitor was reported to inhibit angiogenic signaling pathways either through HIF-dependent or -independent manner in the tumor vascularization process.

Tumor cells activate endothelial cells through secretion of various proangiogenic growth factors including VEGF, COX-2, Ang-2, HGF, FGF as well as Tie-2, which are regulated by hypoxia through the hypoxia inducible factor (HIF) and bind to corresponding RTK on dormant endothelial cells. Once endothelial cells become activated, they migrate and proliferate to form novel branches from the preexisting blood vessels by secreting the matrix metalloproteinases to detach from the extracellular matrix and basement membrane (Harris 2002). Major anti-angiogenic effects of Hsp90 inhibitors are most likely associated with down-regulation of HIF activity, since the half life of HIF-1α is also controlled in an

www.manaraa.com

oxygen-independent way by the competitive binding of either Hsp90 to stabilize the protein, or the anchoring protein Receptor for activated C kinase 1 (RACK1) to interact with Elongin C and mediate prolyl hydroxylase domain protein 2 (PHD2) and von Hippel-Lindau protein (VHL)-independent ubiquitination and degradation of HIF-1α (Semenza 2007). IL-8/NF-κB signaling axis has been reported to upregulate VEGF expression through HIF-independent proangiogenic processes, while suppression of NF-κB signaling in animal model of ovarian cancer destroyed tumor angiogenesis with suppression of VEGF and IL-8 (Huang *et al* 2000). Furthermore, Hsp90 inhibitors-mediated suppression of NF-κB was observed in several studies. Taken together, these data suggested that Hsp90 inhibition reduced NF-κB activation in tumor angiogenesis through IL-8 mediated signaling pathway.

RTKs are transmembrane proteins that transport the extracellular signals to the intracellular context thereby regulating certain critical cellular events and tumor hallmark such as angiogenesis. Given that RTKs comprise the largest category of Hsp90 client proteins, it is promising to suppress angiogenic signalings and ameliorate therapeutic resistance using Hsp90-targeted inhibitors, since acquired chemoresistance is a common challenge for application of tyrosine kinase inhibitors (TKIs) as anti-angiogenic approach because of activation of compensatory and redundant signaling in several cancer types (Bohonowych *et al* 2010). For example, the family of platelet-derived growth factor (PDGF)/fibroblast growth factor (FGF)/VEGF signaling axis has been reported to play a critical role for angiogenesis in several transformed cells of malignancy. Mutations and upregulation of PDGF-receptor α and β have been observed in human cancers to regulate vascular permeability and VEGF expression. Crawford et al reported PDGF-C as a key mediator of tumor-associated

www.manaraa.com

fibroblasts (TAFs)-induced angiogenesis from resistant EL4 tumors, which are refractory to anti-VEGF treatment. In the same study, neutralizing antibody against PDGF-C blocked angiogenesis in such TAFs and slowed down the tumor growth with anti-VEGF resistance (Crawford *et al* 2009). These findings suggested that a combination therapy utilizing anti-PDGF-C and anti-VEGF antibodies may be more effective and synergistic than anti-VEGF treatment alone. Moreover, this study also indicated that exposure of tumor cells to anti-VEGF therapy may stimulate them switch to another survival signaling, in this case, upregulation of PDGF-C inducing angiogenesis *in vivo* produced by those TAFs from anti-VEGF-refractory tumors.

Several chemo-resistance molecular mechanisms have been uncovered in both preclinical and clinical studies that activate partially redundant signaling pathways regulating the hallmarks of cancer, including EGF/IGF/HGF/VEGF proangiogenic signaling pathways, and PDGF/FGF/VEGF signaling module (Bohonowych *et al* 2010). In response to therapeutic killing, cancer cells develop a large scope of compensatory and interconnected mechanisms to cope with a variety of individually-targeted treatment. These evidences strongly support the use of combinational therapy as a complementary means to combat chemo-resistance induced by redundancy of oncogenic signalings in human cancers. Given that the majority of these oncogenic proteins identified as Hsp90 client proteins, Hsp90-directed pharmacological intervention becomes promising for broad suppression of these signaling interactions within the tumor progression.

TAFs/CAFs is a pivotal component in the TAFs/CAFs-rich stroma contributing to tumorigenesis. Accumulating evidences indicate targeting those fibroblasts may facilitate

www.manaraa.com

anti-cancer therapy by affecting oncogenic interactions between cancer cells and the assorted cell types constituting the tumor microenvironment. Intriguingly, Hsp90 inhibitors suppressed the reactive stroma phenotype in hepatic stellate cells and induced caspase-8-mediated apoptosis via sphingomyelinase- and NF-κB-dependent pathways (Myung *et al* 2009). In addition, Akt/PI3K and MAPK signaling pathways were activated in pancreatic stellate cells and colon cancer through upregulation of periostin, a component of extracellular matrix to promote tumor metastasis and invasion (Erkan *et al* 2007;Bao *et al* 2004), suggesting targeting Hsp90-client oncogenic signalings may represent a novel approach for anti-stroma regimen in the future even though these area are still at rudimentary stage and await to be investigated further.

B. Exploration of small molecule inhibitors of Hsp90 activity: Small molecule inhibitors targeting Hsp90 with minimum off-target effects should be considered for cancer treatment.

Almost two decades ago, Whitesell et al firstly demonstrated Hsp90-involved heteroprotein complex formation was required for v-src-mediated morphologic transformation and benzoquinone ansamycins such as Geldanamycin (GA), can inhibit Hsp90-src through competitive binding to Hsp90 (Whitesell *et al* 1994). Subsequently studies of HSPs-targeted anti-tumor therapy proliferated in both preclinical and clinical stages (Table I, II and III). Recently, Roué et al reported the Hsp90 inhibitor IPI-504 (retaspimycin hydrochloride) restored drug sensitivity in proteasome inhibitor bortezomib-resistant aggressive mantle cell lymphoma (MCL) (Roué *et al* 2011). In their study, one of Hsp70 family members,

www.manaraa.com

BiP/GRP78 was up-regulated in aggressive B-cell malignancies including MCL and was responsible for constitutive or induced-bortezomib resistance. IPI-504 in combination with bortezomib dissociated Hsp90-BiP/GRP78 complex, causing the latter to be depleted thus affecting the UPR and restoring apoptosis (Roué *et al* 2011). These findings added a novel function of Hsp90 in cancer treatment by inhibition of UPR-related oncogenic phenotypes including drug resistance and evasion of apoptosis. With the help of X-ray crystallography and structure-based drug design to improve potency, a second class of synthetic Hsp90 inhibitors have been reported, NVP-AUY922 (Novartis) is a novel Hsp90 inhibitor and has the highest affinity for the NH_2-terminal ATP-binding site among synthetic small molecule inhibitors. Effects of NVP-AUY922 include inhibition and/or repression of tumor growth in tumor xenografts with a variety of types of human cancers, blockage of tumor cell invasion and metastasis both *in vitro* and *in vivo*, and depletion of client proteins including BCR-ABL, ERBB2, CRAF, CDK-4, AKT and HIF-α (Eccles *et al* 2008).

Recent studies show impressive synergistic action of NVP-AUY922 with melphalan, doxorubicin, NVP-LBH589, and suberoylanilide hydroxamic acid (SAHA) in multiple myeloma and build the experimental foundation for clinical trials (Kaiser *et al* 2010). Of notice, cell surface Hsp90 was found on melanoma cells, fibrosarcoma cells, bladder cancer cells, prostate cancer cells as well as neuronal cells and play an important role to control cancer cell migration independent of intracellular Hsp90 pool function (Tsutsumi *et al* 2008). Intriguingly, extracellular Hsp90α once hyperacetylated by HDAC inhibitor acted as a chaperone for MMP-2 to promote tumor cell invasion, suggesting inhibition of extracellular hyperacetylated Hsp90α may affect tumor invasion and metastasis (Yang *et al* 2008).

www.manaraa.com

Table 2. Hsp90 Inhibitors in Clinical Trials

Hsp90 Inhibitor	Clinical Trial Phase	Cancer	References
Geldanamycin	I*	thyroid (hepatotoxicity *in vivo*)	Supko 1995; Park 2003;Kim 2009; Samuni 2010
17-AAG (Tanespimycin)	II/III	breast, leukemia, prostate	Janin 2005; Grem 2007; Erlichman 2009
17-DMAG	I	breast, leukemia	Palacios 2010
Retaspimycin hydrochloride	I/II/III	NSLC, GIST, pancreatic, MCL	Hanson 2009; Dewaele 2008
IPI-493	I	advanced solid tumors	Porter 2009; Croasdell 2010
Radicicol	I	*in vitro* (no + *in vivo* results)	Delmotte 1953; Soga 2003
Purine-scaffold inhibitors	I	Hodgkin's lymphoma, CLL	Zhang 2010
Shepherin	-	leukemia (animal model)	Plescia 2005
Pyrazoles (CCT-018159)	-	prostate cancer (*in vitro*)	Rowlands 2004; Cheung 2005; Barril 2006; Sharp 2007
PU24FCI	II	breast, CLL, SCLC	Vilenchik 2004
Mesylate (SNX-5422)	I	HT-29 model	Huang 2009; Fadden 2010
STA-9090	I	mast cell tumors	Lin 2008;Wang 2010
NVP-AUY922	-	breast, prostate, GBM, MM	Eccles 2008;Tauchi 2011;Gaspar 2010
Oxime derivative KF58333	-	breast (*in vitro*)	Soga 2001; Kurebayashi 2001
Novobiocin	II	breast and melanoma (*in vitro*)	Donnelly 2008; Yang 2003; Nordenberg et al 1992
Coumermycin A1	-	breast (*in vitro*)	Marcu 2000
Cisplatin	I/II/III/IV	head and neck	Donnelly 2008; Ashan 2010
AR-42 (HDAC6)	I	mast cell tumors, MM, B-cell	Lin 2010; Zhang 2010; Lucas 2010
Vorinostat/SAHA (HDAC6)	I/II	Leukemia, head and neck	Kovacs 2005; Yang 2008; Blumenschein 2004
Romidepsin/FK228 (HDAC6)	II	T-cell lymphoma	Chen 2005; Piekarz 2011

*Clinical Trial suspended

www.manaraa.com

Table 3. Chemotherapy Drugs that Synergize with Hsp90 Inhibitors

Drug Combinations	Clinical Trial Phase	Disease	References
17-DMAG + arsenic oxide	-	leukemia	Wu et al 2009
17-AAG + Gleevec/imatinib	-	leukemia, breast cancer	Radujkovic et al 2005
17-AAG + bortezomib	II	multiple myeloma	Richardson et al 2010
17-AAG + HDAC-6 siRNA	-	leukemia	Rao et al 2008
IPI-504 + Gleevec/imatinib	-	leukemia (mouse model)	Peng et al 2007

www.manaraa.com

Definition of four phases clinical trial from NIH:

Phase I clinical trials test a new biomedical intervention in a small group of people (e.g., 20-80) for the first time to evaluate safety (e.g., to determine a safe dosage range and to identify side effects).

Phase II clinical trials study the biomedical or behavioral intervention in a larger group of people (several hundred) to determine efficacy and to further evaluate its safety.

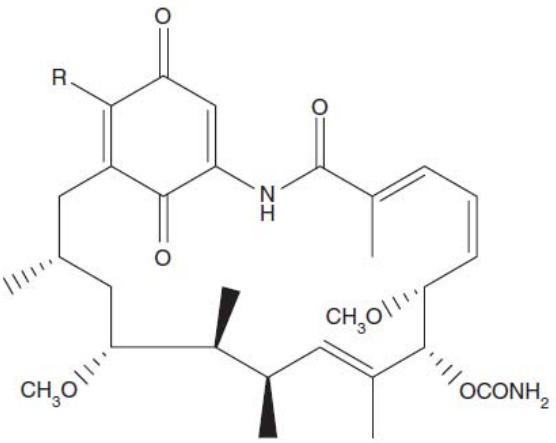
Phase III studies investigate the efficacy of the biomedical or behavioral intervention in large groups of human subjects (from several hundred to several thousand) by comparing the intervention to other standard or experimental interventions as well as to monitor adverse effects, and to collect information that will allow the intervention to be used safely.

Phase IV studies are conducted after the intervention has been marketed. These studies are designed to monitor effectiveness of the approved intervention in the general population and to collect information about any adverse effects associated with widespread use.

Summary of Hsp90 inhibitors in the current clinical trial

Hsp 90 inhibitors are composed of two major classes, natural products and synthetic products. Natural products include Geldanamycin (GA) and radicicol (RD) as well as their derivatives. They modulate Hsp90 molecular chaperone function in a similar manner and have comparable biological activity. Synthetic inhibitors include purine and pyrazoles as well as their derivatives, which have been discovered by structure-based design, fragment-based design, high throughput screening as well as virtual screening.

www.manaraa.com



Compound	R
GA	CH_3O
17-AAG	CH_2=CH-CH_2-NH
17-AG	NH_2
DMAG	NH-$(CH_2)_2$-N$(CH_3)_2$

Figure 3. Chemical structure of Geldanamycin (GA)-related compounds. The 'R' group in GA can be replaced with the indicated side group to make the following compounds: Tanespimycin (17-AAG), 17-amino-17-demethoxygeldanamycin (17-AG), 17-dimethylaminothylamino-17demethoxy-geldanamycin (DMAG) (Refer to Erlichman 2009).

www.manaraa.com

GA is a potent cytotoxic drug but has been terminated in the clinical trial due to several reasons including severe hepatotoxicity, unstable metabolism and chemical structure as well as poor solubility. A substantial effort has been devoted to modify its structure to overcome aforementioned side effects. 17-AAG (Tanespimycin) is a result of the relative ease with which the C-17 methoxy group substituted by amino and small unhindered alkylamino groups. Kosan Pharmaceuticals developed a DMSO-free formulation of 17-AAG in the combination with other drugs such as trastuzumab or bortezomib, which resulted in encouraging clinical results reported in trastuzumab-resistant HER2-positive breast cancer and in multiple myeloma patients even if refractory to bortezomib. 17-DMAG was designed by the NCI and Kosan as a more soluble analogue of 17-AAG. 17-DMAG entered clinical trials in 2005 to treat patients with acute myeloid leukemia (AML) and was discontinued at the beginning of 2008.

Retaspimycin hydrochloride (IPI-504) and Alvespimycin (IPI-493/KOS-1022) are the second generation Hsp90 inhibitors developed by Infinity to treat different types of cancer. These drugs show higher solubility exemplified by IPI-504 being 400-fold more soluble than 17-AAG. As a highly soluble hydroquinone hydrochloride derivative of 17-AAG, IPI-504 has entered the clinical trials in phase III for gastrointestinal stromal tumor (GIST), phase II for non-small-cell lung cancer (NSCLC) and breast cancer, phase I for sarcoma and multiple myeloma. IPI-493 is a water-soluble, stable GA derivative 17-DMAG with limited metabolism and higher oral bioavailability, demonstrating more potent *in vitro* and *in vivo* anti-tumor effect than 17-AAG. It has entered in phase I clinical trial for advanced solid tumors (Porter *et al* 2009; Croasdell *et al* 2010).

www.manaraa.com

Radicicol (RD) is a macrocyclic lactone antibiotic first purified from the fungus *Monosporium bonorden*. RD has no *in vivo* activity due to instability in serum. In order to enhance its *in vivo* activity, oxime derivatives have been synthesized to reduce the electrophilicity of the Michael acceptor. KF58333 demonstrated potent anti-proliferative activity against all breast cancer cell lines tested *in vitro* and depleted hsp90 client proteins such as erbB2, raf-1 and Akt in the tumor tissues from nude mice (Soga *et al* 2001).

The basis of the designing the first synthetic Hsp90 inhibitors was using ATP mimics composed of a purine structure tethered by a linker to adjacent aryl moiety to form the C-shaped conformation. This unique shape can compete with ATP for binding to the N-terminal nucleotide pocket of Hsp90. As a purine-scaffold modified drug, PU24FCI results in multiple anti-tumor activities through degradation of Hsp90-client proteins including HER-2, Akt and Raf-1 in tumors. In addition it demonstrates 10- to 50- fold higher binding affinity to Hsp90 from transformed cells and has entered phase II clinical trial for breast cancer, small-cell lung cancer and chronic lymphocytic leukemia (CLL).

Pyrazoles and isoxazoles represent another novel scaffold to be characterized and developed as Hsp90 inhibitors. CCT-018159 was discovered by a high throughput screening against a 50,000 compounds library and had good activity in a proliferation assay using HCT116 colon cancer cells. isoxazoles are chemically related to pyrazoles as a novel type of Hsp90 inhibitors by binding to the N-terminal ATP pocket. These derivatives demonstrated promising effect in the cell growth inhibition assay exemplified by NVP-AUY922, which has entered phase I clinical trial for advanced cancer.

Serenex developed an oral Hsp90 inhibitor, Mesylate (SNX-5422), which has entered

www.manaraa.com

phase I clinical trial for evaluation of some types of cancers, although the structures are not reported. Synta developed an intravenous Hsp90 inhibitror, STA-9090, which as entered phase I/II clinical trial for solid tumors and hematologic Malignancies. There are no clinical data available using aforementioned synthetic inhibitors, however, it is worthy to expect intriguing results form these studies.

Cisplatin is a FDA approved drug to be used alone or with other drugs to treat several types of cancer (http://www.cancer.gov/cancertopics/druginfo/cisplatin). It is also being investigated in the other types of cancer. Cisplatin is an inorganic platinum agent (cis-diamminedichloroplatinum) with anti-tumor activity. It can form highly reactive, charged, platinum complexes to bind to GC-rich sequences in DNA, inducing intra-strand and inter-strand DNA crosslinks, as well as DNA-protein crosslinks, finally leading to apoptosis and cell growth inhibition. It has been reported that Cisplatin prevents Hsp90 from interacting with hormone receptors, such as AR and GR. *In vitro* study in pediatric cancers also showed combinational therapy using Cisplatin and GA suppressed the ability of GA to induce a cytoprotective heat shock response and resulted in synergistic anticancer acitivity (Bagatell *et al* 2005).

HDAC inhibitors including suberoylanilide hydroxamic acid (SAHA), FK228 and AR-42 have been reported in clinical trials for evaluation of the treatment of several types of cancer. SAHA or Vorinostat is a member of a larger class of compounds that inhibit histone deacetylases (HDAC), which remove the acetyl groups on the DNA backbone, increase the positive charge of histone tails on the amine groups and high-affinity binding between the histones and DNA backbone and finally prevents transcription. The effect of Hsp90

www.manaraa.com

acetylation on chaperone function exerted by HDAC inhibitors has been extensively studied in several types of cancer models (Rao *et al* 2008; Yang *et al* 2008; Wanczyk *et al* 2011).

Novobiocin is an aminocoumarin antibiotic produced by the actinomycete Streptomyces niveus, which was also subjectively called S. spheroides. Other aminocoumarin antibiotics include clorobiocin and coumermycin A1. These compounds can bind type II topoisomerases, including DNA gyrase, thereby inhibiting the enzyme-catalyzed hydrolysis of ATP as good candidates for research of the treatment of bacterial infection.

The C-terminal binding domain of Hsp90 was reported to bind not only ATP, but also cisplatin, novobiocin, epilgallocatechin-3-gallate (EGCG) and taxol. Novobiocin binds weakly to the Hsp90 C-terminal ATP binding site but structural modification can improve this compound binding affinity with1,000 higher in anti-proliferation assay. Novobiocin entered in one phase II clinical trial when in combination with high-dose chemotherapy for the treatment of advanced breast cancer, Clinical data did not translate into a substantial increase progression-free survival (PFS) and overall survival (OS),compared with controls treated with high-dose alkylator therapy alone (Hahm et al 2000).

www.manaraa.com

CHAPTER FOUR

CONCLUSIONS & FUTURE DIRECTIONS

It was originally held that Hsp90 function was solely associated with the stabilization the nascent proteins and protein complexes in the cytoplasm, while subsequent work showed a multifaceted role for HSPs in intracellular transport, maintenance and degradation of client proteins, and participation in many biochemical signaling pathways to preserve intracellular homeostasis. An immense amount of work has been done, and continues to be done, to progress the field over the last two decades, focusing substantially on Hsp90-asscociated client proteins, which play a complex role in the maintenance of each of the core intracellular signaling networks relevant to the Hallmarks of Cancer. Many Hsp90 inhibitors capable of binding within either the N- and C-terminal domains of Hsp90 have been found, synthesized, and characterized, and many have proven useful for treating a variety of human diseases including cancer (Tables I, II).

However, information from animal studies and clinical trials has not shown substantial progress in the use of Hsp90 inhibition as a first line of defense, suggesting that Hsp90 inhibition would be infinitely more affective in combination with other therapies. Here, we summarize many of the current combinational therapies or treatments using Hsp90 inhibitors from both clinical trials and laboratory studies (Table III). Many combinations show a synergistic effect when compared to individual, independent drug treatment in several types of cancers. Of note, however, a synergistic effect was not observed in several murine solid

www.manaraa.com

tumor models compared to blood-related cancers, likely due to the inherent differences between these types of cancers, their tumor microenvironment, the effective delivery of the compounds, and other as yet unknown factors. Quite possibly, there exists redundant signaling pathways that may be activated in solid tumors, promoting increased survival as a consequence. For example, initial clinical studies showed that targeting angiogenic signaling in human glioblastomas allowed tumor cells to shift toward invasiveness and metastasis, resulting in the tumor cells having access to pre-existent blood vessels within the surrounding normal tissue vasculature (Ellis *et al* 2009; Verhoeff *et al* 2009; Norden *et al* 2009). Moreover, the molecular heterogeneity of cancers exemplified by the elaborate integrated signaling circuitry clearly influences the therapeutic killing effect. To complicate matters, differential client protein affinity with Hsp90 in a variety of cancer types, as well as within desmoplastic tumor microenvironment (like CAFs), can be supportive factors for inducing resistance to therapeutic treatment.

In order to consider a regimen targeting Hsp90 in a broad sense, the following suggestions should be considered for both future basic science and clinical studies, as well as when assessing the utility and effectiveness of Hsp90-targeted approaches:

1. Inhibiting Hsp90 in combination with other heat shock proteins, such as Hsp70 and Hsp27, which may be an alternative strategy to enhance synergistic cancer therapy with minimum off-target side effect. As mentioned earlier, Hsp90 inhibition was reported to upregulate Hsp70 and Hsp27 expression in tumor cells in order to prevent apoptosis.
2. Additional studies should be accomplished to define the precise molecular

www.manaraa.com

mechanism(s) of Hsp90 client proteins responsible for the unique phenotypes in different types of cancer in order to understand the exact Hsp90 biochemical functions implicated in multiple oncogenic signaling networks. This will ultimately allow for the identification of reliable biomarkers for monitoring the effects of Hsp90 suppression *in vivo*, as well as for optimizing drug transmission and treatment efficacy through various combinational therapies targeting major oncogenic signaling pathways.

3. The use of specific cellular or subcellular targeting for chaperones needs to be considered and developed. Accumulating evidence suggests alternative locations of HSPs, especially Hsp90 inside or outside of the cells, in the cytoplasm, in the nucleus, *etc*., which indicates it plays a unique role as part of a tumor-specific phenotype. Therefore, development and validation of cell-location-specific inhibitors will benefit Hsp90 targeted chemotherapy in human cancer, especially when used in combination with efficient delivery approaches.
4. Screening of novel chemical structures with increased solubility and stability *in vivo* will provide the potential for oral administration in the clinic in order to eliminate the current issues of hepatoxicity and limited solubility related to some Hsp90 inhibitors.

www.manaraa.com

REFERENCES CITED

Akalin A, Elmore LW, Forsythe HL, Amaker BA, McCollum ED, Nelson PS, Ware JL, Holt SE. A novel mechanism for chaperone-mediated telomerase regulation during prostate cancer progression. 2001Cancer Res 61;4791-4796.

Andrieu C, Taieb D, Baylot V, Ettinger S, Soubeyran P, De-Thonel A, Nelson C, Garrido C, So A, Fazli L, Bladou F, Gleave M, Iovanna JL, Rocchi P. Heat shock protein 27 confers resistance to androgen ablation and chemotherapy in prostate cancer cells through eIF4E. 2010 Oncogene 29;1883-1896.

Bagatell R, Beliakoff J, David CL, Marron MT, Whitesell L. Hsp90 inhibitors deplete key anti-apoptotic proteins in pediatric solid tumor cells and demonstrate synergistic anticancer activity with cisplatin. 2005 Int J Cancer. 113;179-188.

Bao S, Ouyang G, Bai X, Huang Z, Ma C, Liu M, Shao R, Anderson RM, Rich JN, Wang XF. Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. 2004 Cancer Cell 4;329-339.

Barginear MF, Van Poznak C, Rosen N, Modi S, Hudis CA, Budman DR. The heat shock protein 90 chaperone complex: an evolving therapeutic target. 2008 Curr Cancer Drug Targets 8;522-532.

Barril X, Beswick MC, Collier A, Drysdale MJ, Dymock BW, Fink A, Grant K, Howes R, Jordan AM, Massey A, Surgenor A, Wayne J, Workman P, Wright L. 4-Amino derivatives of the Hsp90 inhibitor CCT018159. 2006 Bioorg Med Chem Lett 9;2543-2548.

Basso, A. D. Basso AD, Solit DB, Chiosis G, Giri B, Tsichlis P, Rosen N. Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. 2002 J. Biol. Chem 277;39858-39866.

Beacham DA, Cukierman E. Stromagenesis: the changing face of fibroblastic microenvironments during tumor progression. 2005 Seminars in Cancer Biology 15;329-341.

Bohonowych JE, Gopal U, Isaacs JS. Hsp90 as a gatekeeper of tumor angiogenesis: clinical promise and potential pitfalls. 2010 J Oncol. 2010:412985

Brodsky JL. Selectivity of the molecular chaperonespecific immunosuppressive agent 15-deoxyspergualin: modulation of Hsc70 ATPase activity without compromising DnaJ chaperone interactions. 1999 Biochem Pharmacol 57;877-880.

Brugge, J., Yonemoto, W. & Darrow, D. Interaction between the Rous sarcoma virus transforming protein and two cellular phosphoproteins: analysis of the turnover and distribution of this complex. 1983 Mol. Cell. Biol 3;9–19.

www.manaraa.com

Calderwood SK, Khaleque MA, Sawyer DB, Ciocca DR. 2006. Heat shock proteins in cancer: chaperones of tumorigenesis. Trends Biochem Sci 31;164-172.

Cappello F, David S, Peri G, Farina F, Conway de Macario E, Macario AJ, Zummo G. Hsp60: molecular anatomy and role in colorectal cancer diagnosis and treatment. 2011 Front Biosci 3;341-351.

Chen G, Cao P, Goeddel DV. TNF-induced recruitment and activation of the IκK complex require Cdc37 and Hsp90. 2002 Mol. Cell 9;401–410.

Chen L, Meng S, Wang H, Bali P, Bai W, Li B, Atadja P, Bhalla KN, Wu J. 2005 Mol Cancer Ther 4;1311–1319.

Cheung KM, Matthews TP, James K, Rowlands MG, Boxall KJ, Sharp SY, Maloney A, Roe SM, Prodromou C, Pearl LH, Aherne GW, McDonald E, Workman P. The identification, synthesis, protein crystal structure and *in vitro* biochemical evaluation of a new 3,4-diarylpyrazole class of Hsp90 inhibitors, Bioorg. 2005 Med.Chem. Lett 15;3338–3343.

Chun JN, Choi B, Lee KW, Lee DJ, Kang DH, Lee JY, Song IS, Kim HI, Lee SH, Kim HS, Lee NK, Lee SY, Lee KJ, Kim J, Kang SW. Cytosolic Hsp60 is involved in the NF-kappaB-dependent survival of cancer cells via IKK regulation. 2010 PLoS One 5;e9422.

Crawford Y, Kasman I, Yu L, Zhong C, Wu X, Modrusan Z, Kaminker J, Ferrara N. PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. 2009 Cancer Cell. 15;21-34.

Croasdell G, Mason VL. 28th Annual JPMorgan Healthcare Conference--Affymax and Infinity Pharmaceuticals. 2010 IDrugs 13;142-144.

de Billy E, Powers MV, Smith JR, Workman P. Drugging the heat shock factor 1 pathway: exploitation of the critical cancer cell dependence on the guardian of the proteome. 2009 Cell Cycle 23;3806-3808.

Delmotte P, Delmotte-Plaque J. A new antifungal substance of fungal origin. 1953 Nature 171;344.

Donnelly A, Blagg BS. Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide-binding pocket. 2008 Curr Med Chem 26;2702-2717.

Dong D, Ni M, Li J, Xiong S, Ye W, Virrey JJ et al. Critical Role of the Stress Chaperone GRP78/BiP in Tumor Proliferation, Survival, and Tumor Angiogenesis in Transgene-Induced Mammary Tumor Development. 2008 Cancer Res 68;498–505.

www.manaraa.com

Dudek J, Benedix J, Cappel S, Greiner M, Jalal C, Müller L, Zimmermann R. Functions and pathologies of BiP and its interaction partners. 2009 Cell Mol Life Sci 66;1556-1569.

Dusonchet J, Bensadoun JC, Schneider BL, Aebischer P. Targeted overexpression of the parkin substrate Pael-R in the nigrostriatal system of adult rats to model Parkinson's disease. 2009 Neurobiol Dis 1;32-41.

Easton DP, Kaneko Y, Subjeck JR. The Hsp110 and Grp170 stress proteins: newly recognized relatives of the Hsp70s. 2000 Cell Stress & Chaperones 5;276-290.

Eccles SA, Massey A, Raynaud FI, Sharp SY, Box G, Valenti M, Patterson L, de Haven Brandon A, Gowan S, Boxall F, Aherne W, Rowlands M, Hayes A, Martins V, Urban F, Boxall K, Prodromou C, Pearl L, James K, Matthews TP, Cheung KM, Kalusa A, Jones K, McDonald E, Barril X, Brough PA, Cansfield JE, Dymock B, Drysdale MJ, Finch H, Howes R, Hubbard RE, Surgenor A, Webb P, Wood M, Wright L, Workman P. NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. 2008 Cancer Res 68;2850-2860.

Ellis, L.M., and Reardon, D.A. Cancer: The nuances of therapy. 2009 Nature 458;290–292.

Erkan M, Kleeff J, Gorbachevski A, Reiser C, Mitkus T, Esposito I, Giese T, Büchler MW, Giese NA, Friess H. Periostin creates a tumor-supportive microenvironment in the pancreas by sustaining fibrogenic stellate cell activity. 2007 Gastroenterology. 132;1447-1464.

Fadden P, Huang KH, Veal JM, Steed PM, Barabasz AF, Foley B, et al. Application of chemoproteomics to drug discovery: identification of a clinical candidate targeting Hsp90. 2010 Chem Biol 7;686-694.

Flachsbart F, Caliebe A, Kleindorp R, Blanché H, von Eller-Eberstein H, Nikolaus S, Schreiber S, Nebel A. Association of FOXO3A variation with human longevity confirmed in German centenarians. 2009 Proc Natl Acad Sci U S A 8;2700-2705.

Forsythe HL, Jarvis JL, Turner JW, Elmore LW, Holt SE. Stable association of Hsp90 and p23, but Not hsp70, with active human telomerase. 2001 J Biol Chem 276;15571-15574.

Fu Y, Li J, Lee AS. GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen-starvation induced apoptosis. 2007 Cancer Res 67;3734–3740.

Gao T, Newton AC. The turn motif is a phosphorylation switch that regulates the binding of Hsp70 to protein kinase C. 2002 J Biol Chem 277;31585-31592.

Ghosh JC, Siegelin MD, Dohi T, Altieri DC. Heat shock protein 60 regulation of the mitochondrial permeability transition pore in tumor cells. 2010 Cancer Res 15;8988-8993.

www.manaraa.com

Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV. GRP78: a multifunctional receptor on the cell surface. 2009 Antioxid Redox Signal 11;2299-2306.

Hahm HA, Armstrong DK, Chen TL, Grochow L, Passos-Coelho J, Goodman SN, Davidson NE, Kennedy MJ. Novobiocin in combination with high-dose chemotherapy for the treatment of advanced breast cancer: a phase 2 study. 2000 Biol Blood Marrow Transplant 6;335-343.

Hartl F.U. Molecular chaperones in cellular protein folding. 1996 Nature 381;571–579.

Hendrick JP, Hartl FU. Molecular chaperone functions of heat-shock proteins. 1993 Ann Rev Biochem 62;349-384.

Heydari, A.R., You, S., Takahashi, R., Gutsmann-Conrad, A., Sarge, K.D., Richardson, A. Age-related alterations in the activation of heat shock transcription factor 1 in rat hepatocytes. 2000 Exp. Cell Res 256;83–93.

Horowitz M, Robinson SD. Heat shock proteins and the heat shock response during hyperthermia and its modulation by altered physiological conditions. 2007 Prog Brain Res 162;433-446.

Jego G, Hazoumé A, Seigneuric R, Garrido C. Targeting heat shock proteins in cancer. 2010 Cancer Lett doi:10.1016/j.canlet.2010.10.014.

Kahali S, Sarcar B, Fang B, Williams ES, Koomen JM, Tofilon PJ, Chinnaiyan P. Activation of the unfolded protein response contributes toward the antitumor activity of vorinostat. 2010 Neoplasia 12;80-86.

Jiang Q, Wang Y, Li T, Shi K, Li Z, Ma Y, Li F, Luo H, Yang Y, Xu C. Hsp90-mediated inactivation of NF{kappa}B switches autophagy to apoptosis through becn1 transcriptional inhibition in selenite-induced NB4 cells. 2011 Mol Biol Cell PMID: 21346199

Kaiser M, Lamottke B, Mieth M, Jensen MR, Quadt C, Garcia-Echeverria C, Atadja P, Heider U, von Metzler I, Türkmen S, Sezer O. Synergistic action of the novel HSP90 inhibitor NVP-AUY922 with histone deacetylase inhibitors, melphalan, or doxorubicin in multiple myeloma. 2010 Eur J Haematol 4;337-344.

Kim W, Lee D, Hong SS, Na Z, Shin JC, Roh SH, Wu CZ, Choi O, Lee K, Shen YM, Paik SG, Lee JJ, Hong YS. Rational biosynthetic engineering for optimization of geldanamycin analogues. 2009 Chembiochem 10;1243-1251.

Kleindorp R, Flachsbart F, Puca AA, Malovini A, Schreiber S, Nebel A. Candidate gene study of FOXO1, FOXO4 and FOXO6 reveals no association with human longevity in Germans. 2011 Aging Cell doi: 10.1111/j.1474-9726.2011.00698.x.

www.manaraa.com

Kovacs, J. J. et al. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. 2005 Mol Cell 18;601–607.

Kunz-Schughart LA, Knuechel R. Tumor-associated fibroblasts (part I): Active stromal participants in tumor development and progression? 2002 Histol Histopathol 17;599-621.

Kunz-Schughart LA, Knuechel R. Tumor-associated fibroblasts (part II): Active stromal participants in tumor development and progression? 2002 Histol Histopathol 17;623-637.

Kurebayashi J, Otsuki T, Kurosumi M, Soga S, Akinaga S, Sonoo H. A radicicol derivative, KF58333, inhibits expression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor, angiogenesis and growth of human breast cancer xenografts. 2001 Japan J Cancer Res. 12;1342-1351.

Lee AS. GRP78 induction in cancer: Therapeutic and prognostic implications. 2007 Cancer Res 67;3496–3499.

Lee AS. The glucose-regulated proteins: Stress induction and clinical applications. 2001 Trends Biochem Sci 26;504–510.

Leu JI, Pimkina J, Frank A, Murphy ME, George DL. A small molecule inhibitor of inducible heat shock protein 70. 2009 Mol Cell; 36:15-27.

Li CY, Lee JS, Ko YG, Kim JI, Seo JS. Heat shock protein 70 inhibits apoptosis downstream of cytochrome c release and upstream of caspase-3 activation. 2000 J Biol Chem 275;25665-25671.

Li J, Lee A.S. Stress Induction of GRP78/BiP and Its Role in Cancer. 2006 Curr Mol Med 6;45-54.

Lin TY, Fenger J, Murahari S, Bear MD, Kulp SK, Wang D, Chen CS, Kisseberth WC, London CA. AR-42, a novel HDAC inhibitor, exhibits biologic activity against malignant mast cell lines via downregulation of constitutively activated Kit. 2010 Blood 115;4217–4225.

Lindquist S. The heat-shock proteins. 1988 Annu Rev Genet 22;631-677.

Lucas DM, Alinari L, West DA, Davis ME, Edwards RB, Johnson AJ, Blum KA, Hofmeister CC, Freitas MA, Parthun MR, Wang D, Lehman A, Zhang X, Jarjoura D, Kulp SK, Croce CM, Grever MR, Chen CS, Baiocchi RA, Byrd JC. The novel deacetylase inhibitor AR-42 demonstrates pre-clinical activity in B-cell malignancies *in vitro* and *in vivo*. 2010 PLoS One 5(6):e10941.

www.manaraa.com

Maria Vilenchik, David Solit, Andrea Basso, Henri Huezo1, Brian Lucas, Huazhong He, Neal Rosen, Claudia Spampinato, Paul Modrich, and Gabriela Chiosis. Targeting Wide-Range Oncogenic Transformation via PU24FCl, a Specific Inhibitor of Tumor Hsp90. 2004 Chem & Bio 11;787-797.

Marozkina NV, Yemen S, Borowitz M, Liu L, Plapp M, Sun F, Islam R, Erdmann- Gilmore P, Townsend RR, Lichti CF, Mantri S, Clapp PW, Randell SH, Gaston B, Zaman K. Hsp 70/Hsp 90 organizing protein as a nitrosylation target in cystic fibrosis therapy. 2010 Proc Natl Acad Sci U S A 25;11393-11398.

Martins VR, Graner E, Garcia-Abreu J, de Souza SJ, Mercadante AF, Veiga SS, Zanata SM, Neto VM, Brentani RR. Complementary hydropathy identifies a cellular prion protein receptor. 1997 Nat Med 12;1376-1382.

Misra, U K, Deedwania R, Pizzo SV. Activation and cross-talk between Akt, NF-κB, and unfolded protein response signaling in 1-LN prostate cancer cells consequent to ligation of cell surface-associated GRP78. 2006 J Biol Chem 281;13694-13707.

Misra U K, Pizzo SV. Ligation of cell surface GRP78 with antibody directed against COOH-terminal domain of GRP78 suppresses Ras/MAPK and PI 3-kinase/AKT signaling while promoting caspase activation in human prostate cancer cells. 2010 Cancer Biology & Therapy 9;142-152.

Martins VR, Graner E, Garcia-Abreu J, de Souza SJ, Mercadante AF, Veiga SS, Zanata SM, Neto VM, Brentani RR. Complementary hydropathy identifies a cellular prion protein receptor. 1997 Nat Med. 12;1376-1382.

Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, Massie B. The chaperone function of hsp70 is required for protection against stress-induced apoptosis. 2000 Mol Cell Biol 20;7146-7159.

Myung SJ, Yoon JH, Kim BH, Lee JH, Jung EU, Lee HS. Heat shock protein 90 inhibitor induces apoptosis and attenuates activation of hepatic stellate cells. 2009 J Pharmacol Exp Ther. 330;276-282.

Nadeau K, Nadler SG, Saulnier M, Tepper MA, Walsh CT. Quantitation of the interaction of the immunosuppressant deoxyspergualin and analogs with Hsc70 and Hsp90. 1994 Biochemistry 33;2561-2567.

Nadler SG, Cleaveland J, Tepper MA, Walsh C, Nadeau K. Studies on the interaction of the immunosuppressant 15-deoxyspergualin with heat shock proteins. 1993 Ann NY Acad Sci 696;412-414.

Nagai Y, Fujikake N, Popiel HA, Wada K. Induction of molecular chaperones as a therapeutic

www.manaraa.com

strategy for the polyglutamine diseases. 2010 Curr Pharm Biotechnol 2;188-197.

Norden, A.D., Drappatz, J., and Wen, P.Y. Antiangiogenic therapies for high-grade glioma. 2009 Nat. Rev. Neurol. 5;610–620.

Nordenberg J, Albukrek D, Hadar T, Fux A, Wasserman L, Novogrodsky A, Sidi Y. Novobiocin-induced anti-proliferative and differentiating effects in melanoma B16. 1992 Br J Cancer 65;183-188.

Odunuga OO, Longshaw VM, Blatch GL Hop: more than an Hsp70/Hsp90 adaptor protein. 2004 Bioessays 10;1058-1068.

Oppermann, H., Levinson, W. & Bishop, J. M. A cellular protein that associates with the transforming protein of Rous sarcoma virus is also a heat-shock protein. 1981 Proc Natl Acad Sci USA 78;1067–1071

Pacey S, Banerji U, Judson I, Workman P. Hsp90 inhibitors in the clinic. 2006 Handbook Exp Pharmacol 331–358.

Park HS, Lee JS, Huh SH, Seo JS, Choi EJ. Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. 2001 Embo J 20;446-456.

Park JW, Yeh MW, Wong MG, Lobo M, Hyun WC, Duh QY, Clark OH. The heat shock protein 90-binding geldanamycin inhibits cancer cell proliferation, down-regulates oncoproteins, and inhibits epidermal growth factor-induced invasion in thyroid cancer cell lines. 2003 J Clin Endocrinol Metab 88;3346-3353.

Peng C, Brain J, Hu Y, Goodrich A, Kong L, Grayzel D, Pak R, Read M, Li S. Inhibition of heat shock protein 90 prolongs survival of mice with BCR-ABL-T315I-induced leukemia and suppresses leukemic stem cells. 2007 Blood 110;678-685.

Piekarz RL, Frye R, Prince HM, Kirschbaum MH, Zain J, Allen SL, Jaffe ES, Ling A, Turner M, Peer CJ, Figg WD, Steinberg SM, Smith S, Joske D, Lewis I, Hutchins L, Craig M, Fojo AT, Wright JJ, Bates SE. Phase II trial of romidepsin in patients with peripheral T-cell lymphoma. 2011 Blood PMID: 21355097

Pirkkala L, Nykänen P, Sistonen L. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. 2001 FASEB J 7;1118-1131.

Porter JR, Ge J, Lee J, Normant E, West K. Ansamycin inhibitors of Hsp90: nature's prototype for anti-chaperone therapy. 2009 Curr Top Med Chem 9;1386-1418.

Powers MV, Clarke PA, Workman P. Dual targeting of HSC70 and HSP72 inhibits HSP90 function and induces tumor-specific apoptosis. 2008 Cancer Cell 14;250-262.

www.manaraa.com

Rao R, Fiskus W, Yang Y, Lee P, Joshi R, Fernandez P, Mandawat A, Atadja P, Bradner JE, Bhalla K. HDAC6 inhibition enhances 17-AAG-mediated abrogation of Hsp90 chaperone function in human leukemia cells. 2008 Blood 112;1886–1893.

Rauschert N, Brandlein S, Holzinger E, Hensel F, Muller-Hermelink HK, Vollmers HP. A new tumor-specific variant of GRP78 as target for antibody-based therapy. 2008 Lab Invest 88;375-386.

Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. 2003 J Biol Chem 278;20915-20924.

Romani AA, Crafa P, Desenzani S, Graiani G, Lagrasta C, Sianesi M, Soliani P, Borghetti AF. The expression of HSP27 is associated with poor clinical outcome in intrahepatic cholangiocarcinoma. 2000 BMC Cancer 7;232.

Romano SA, Cordeiro Y, Lima LM, Lopes MH, Silva JL, Foguel D, Linden R. Reciprocal remodeling upon binding of the prion protein to its signaling partner hop/STI1. 2009 FASEB J. 23;4308-4316.

Roué G, Pérez-Galán P, Mozos A, López-Guerra M, Xargay-Torrent S, Rosich L, Saborit-Villarroya I, Normant E, Campo E, Colomer D. The Hsp90 inhibitor IPI-504 overcomes bortezomib resistance in mantle cell lymphoma *in vitro* and *in vivo* by down-regulation of the prosurvival ER chaperone BiP/Grp78. 2011 Blood. 117;1270-1279.

Rowlands MG, Newbatt YM, Prodromou C, Pearl LH, Workman P, Aherne W. High-throughput screening assay for inhibitors of heat-shock protein 90 ATPase activity, 2004 Anal.Biochem 327;176–183.

Samuni Y, Ishii H, Hyodo F, Samuni U, Krishna MC, Goldstein S, Mitchell JB. Reactive oxygen species mediate hepatotoxicity induced by the Hsp90 inhibitor geldanamycin and its analogs. 2010 Free Radic Biol Med 48;1559-1563.

Serda RE, Godin B, Blanco E, Chiappini C, Ferrari M. Multi-stage delivery nano-particle systems for therapeutic applications. 2011 Biochim Biophys Acta 1810;317-329.

Sharp SY, Boxall K, Rowlands M, Prodromou C, Roe SM, Maloney A, Powers M, Clarke PA, Box G, Sanderson S, Patterson L, Matthews TP, Cheung KM, Ball K, Hayes A, Raynaud F, Marais R, Pearl L, Eccles S, Aherne W, McDonald E, Workman P. *In vitro* biological characterization of a novel, synthetic diaryl pyrazole resorcinol class of heat shock protein 90 inhibitors. 2007 Cancer Res 67;2206-2216.

www.manaraa.com

Singh V, Aballay A. Regulation of DAF-16-mediated Innate Immunity in Caenorhabditis elegans. 2009 J Biol Chem 51;35580-35587.

Soga S, Shiotsu Y, Akinaga S, Sharma SV. Development of radicicol analogues 2003 Curr Cancer Drug Targets 3;359–369.

Soga S, Sharma SV, Shiotsu Y, Shimizu M, Tahara H, Yamaguchi K, Ikuina Y, Murakata C, Tamaoki T, Kurebayashi J, Schulte TW, Neckers LM, Akinaga S. Stereospecific antitumor activity of radicicol oxime derivatives. 2001 Cancer Chemother Pharmacol 6;435-445.

Song X, Wang X, Zhuo W, Shi H, Feng D, Sun Y et al. The regulatory mechanism of extracellular Hsp90{alpha} on matrix metalloproteinase-2 processing and tumor angiogenesis. 2010 J Biol Chem 285;40039-40049.

Stankiewicz AR, Lachapelle G, Foo CP, Radicioni SM, Mosser DD. Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation. 2005 J Biol Chem 280;38729-38739.

Steele AJ, Prentice AG, Hoffbrand AV, Yogashangary BC, Hart SM, Lowdell MW, et al. 2-Phenylacetylenesulfonamide (PAS) induces p53-independent apoptotic killing of B-chronic lymphocytic leukemia (CLL) cells. 2009 Blood 114;1217-1225

Supko JG, Hickman RL, Grever MR, Malspeis L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. 1995 Cancer Chemother Pharmacol. 36;305-315.

Tapia H, Morano KA. Hsp90 nuclear accumulation in quiescence is linked to chaperone function and spore development in yeast. 2010 Mol Biol Cell 21;63-72.

Takahashi R, Imai Y. Pael receptor, endoplasmic reticulum stress, and Parkinson's disease. 2003 J Neurol. Suppl 3;III25-9.

Takeuchi S. Analytical assays of human HSP27 and thermal-stress survival of Escherichia coli cells that overexpress it. 2006 Biochem Biophys Res Commun 4;1252-1256.

Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. 2010 Nat Rev Cancer 8;537-549.

Tsai J, Douglas MG. A conserved HPD sequence of the J-domain is necessary for YDJ1 stimulation of Hsp70 ATPase activity at a site distinct from substrate binding. 1996 J Biol Chem. 16;9347-9354.

Tlsty TD., Coussens LM. Tumor stroma and regulation of cancer development. 2006 Ann Rev Pathol Mech 1;119-150.

www.manaraa.com

Turturici G, Sconzo G, Geraci F. Hsp70 and its molecular role in nervous system diseases. 2011 Biochem Res Int 2011:618127. PMID: 21403864

Ushioda R, Hoseki J, Araki K, Jansen G, Thomas DY, Nagata K. ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER. 2008 Science 5888;569-572.

Ushioda R, Nagata K. The endoplasmic reticulum-associated degradation and disulfide reductase ERdj5. 2011 Methods Enzymol 490;235-258.

Vanden Berghe, T., Kalai, M., van Loo, G., Declercq, W. & Vandenabeele, P. Disruption of HSP90 function reverts tumor necrosis factor-induced necrosis to apoptosis. 2003 J Biol Chem 278;5622–5629.

Verhoeff, J.J., van Tellingen, O., Claes, A., Stalpers, L.J., van Linde, M.E., Richel, D.J., Leenders, W.P., and van Furth, W.R. Concerns about anti-angiogenic treatment in patients with glioblastoma multiforme. 2009 BMC Cancer 9;444.

Voellmy R. On mechanisms that control heat shock transcription factor activity in metazoan cells. 2004 Cell Stress Chaperones 2:122-133.

Wang X, Song X, Zhuo W, et al. 2009. The regulatory mechanism of Hsp90alpha secretion and its function in tumor malignancy. Proc Natl Acad Sci USA 106; 21288-21293.

Whitesell, L., Mimnaugh, E. G., De Costa, B., Myers, C. E. & Neckers, L. M. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. 1994 Proc. Natl. Acad.Sci USA 91;8324–8328.

Whitesell L, Lindquist SL. HSP90 and the chaperoning of cancer. 2005 Nat Rev Cancer 10;761-772.

Williams DR, Ko SK, Park S, Lee MR, Shin I. An apoptosis-inducing small molecule that binds to heat shock protein 70. 2008 Angew Chem Int Ed Engl 47;7466-7469.

Workman P, Burrows F, Neckers L, Rosen N. Drugging the cancer chaperone HSP90: combinatorial therapeutic exploitation of oncogene addiction and tumor stress. 2007. Ann N Y Acad Sci 1113:202-216.

Wu YC, Yen WY, Lee TC, Yih LH. Heat shock protein inhibitors, 17-DMAG and KNK437, enhance arsenic trioxide-induced mitotic apoptosis, 2009 Toxicol Appl Pharmacol 236;231–238.

Xu Y, Singer MA, Lindquist S. Maturation of the tyrosine kinase c-src as a kinase and as a

www.manaraa.com

substrate depends on the molecular chaperone Hsp90. 1999 Proc. Natl Acad. Sci. USA 96;109–114.

Xu Y and Lindquist S. Heat-shock protein Hsp90 governs the activity of pp60vsrc kinase. 1993 Proc. Natl Acad. Sci. USA 90;7074–7078.

Yang CH, Chen YC, Kuo ML. Novobiocin sensitizes BCRP/MXR/ABCP overexpressing topotecan-resistant human breast carcinoma cells to topotecan and mitoxantrone. 2003 Anticancer Res 23;2519-2523.

Yang, Y. et al. Role of acetylation and extracellular location of heat shock protein 90α in tumor cell invasion. 2008 Cancer Res 68;4833–4842.

Zhang H, Neely L, Lundgren K, Yang YC, Lough R, Timple N, Burrows F. BIIB021, a synthetic Hsp90 inhibitor, has broad application against tumors with acquired multidrug resistance. 2010 Int J Cancer 126 ;1226–1234.

Ziv E, Hu D. Genetic variation in insulin/IGF-1 signaling pathways and longevity. 2011 Ageing Res Rev 2;201-204.

www.manaraa.com

Vita

Chao Li was born on April 13th, 1978 in a small town in Hu Bei province, China. He finished his M.D. in 2001 and a M.S. of Hepatology in 2006. He came to Richmond, Virginia in 2007 to attend graduate school. He married Jie Qian and has a son, David Li. His future goal is to find a postdoctoral training position in the USA in order to continue doing research in cancer-related areas.

www.manaraa.com