HSP90: A New Recruit in the War on Cancer

Thus, when HSP90 (along with another 50 kDa cellular protein) were described to co-immunoprecipitate with the transforming protein expressed by Rous sarcoma virus, pp60src (v-src), investigators were puzzled. How and why was a cellular protein like HSP90 being recruited by one of the first discovered oncogenic proteins?

While the relevance of this HSP90-pp60src interaction remained elusive, papers reporting HSP90 as a partner for a large cadre of other important cellular proteins began to populate the literature. The list of these so-called HSP90 "clients" is now quite large, but can be grouped into three broad classes: i) transcription factors and in particular, members of the steroid hormone receptor family; ii) Protein kinases, many of which are involved in cellular proliferation pathways; iii) other seemingly unrelated proteins (at least at the structural level) involved in viral replication and innate immunity.

Investigators probing the connection of HSP90 with steroid hormone receptors paved the way for much of our current understanding of HSP90 function. Work performed in a number of different laboratories showed that the steroid hormone receptors, during or shortly following their synthesis in the cytoplasm, rapidly associated with HSP90. Somehow HSP90 appeared to ready the receptor for its subsequent hormone-dependent activation. Now 25 years later, the details of how HSP90 and its host of co-chaperones facilitate hormone receptor maturation have emerged. The newly synthesized steroid receptor is first targeted by the HSP40/HSP70 chaperone machinery. All of the available evidence indicates that these early binding chaperones target the hormone-binding site of the receptor, thereby preventing its premature interaction with the ligand. This ternary complex is then presented to HSP90 and a co-chaperone Hop. Shortly thereafter additional HSP90 co-chaperones are also recruited to the complex (e.g., p23, a family of tetratricopeptide repeat (TPR) proteins, and likely others). This complex series of assembly events, all requiring ATP binding/hydrolysis, result in a folded receptor now competent to bind to its activating steroid hormone ligand. Binding of the hormone results in a conformational change of the receptor, release of at least some of its chaperone partners, and the ability of the now "transformed" receptor to bind to DNA. Receptor binding to its target gene(s) requires additional interactions with transcriptional co-regulator proteins and various posttranslational modifications, all mediated at least in part by various members of the chaperone family.

This model wherein HSP90, along with its cadre of co-chaperones, facilitates the synthesis and activation of steroid hormone receptors appears to apply to other HSP90 clients. In addition, other co-chaperones have been identified and may help to explain the diversity of client proteins that bind to HSP90. For example, in the case of pp60src, the normal cellular form of src (c-src) likely interacts first with the HSP40/HSP70 machinery. Cdc-37, a protein initially identified as being important in cell division, binds and presents the kinase to HSP90. Finally, the co-chaperone, p23, enters the fray. Through its influence on the rate of HSP90 ATPase activity, p23 presumably serves to stabilize the complex. Eventually src is released from the complex and is now fully active as a tyrosine-specific protein kinase. In the case of mutant and oncogenic forms of src (e.g. v-src), the HSP90/Cdc37/p23 complex containing the kinase appears longer-lived.

Although still not fully understood, the prolonged interaction of v-src with the HSP90 chaperone machinery appears to be essential for its enhanced oncogenic activity.

Protein kinases are now known to represent the single largest group of HSP90 clientele. Relevant examples include numerous receptor tyrosine kinases (e.g. EGFR, MET), non-receptor tyrosine kinases such as SRC and its relative LCK, many intracellular ser/thr kinases such as RAF, and a large number of kinases important for cell-cycle entry and progression. HSP90 also serves a role in regulating the activities of important protein kinases associated with apoptosis (e.g. AKT), tissue invasion/metastasis, and angiogenesis (e.g. MET). Although so many protein kinases have been shown to be dependent on HSP90 (and Cdc-37) for their function, the precise biochemical details by which HSP90 activates or influences the particular kinase still remains obscure. In vitro systems, like those developed to sort out HSP90's role in steroid receptor maturation, will be necessary to define how HSP90 and its horde of co-factors ready protein kinases for their activation and regulation, both under normal growth conditions as well as during the development and/or maintenance of the transformed phenotype.

Key to our understanding of HSP90 structure and function, and helping in the identification of HSP90 clients, are a class of naturally occurring compounds that bind to and inhibit HSP90 function. Herbimycin and geldanamycin, benzoquinoid ansamycins, initially were observed to reverse the transformed phenotype in cells transformed by oncogenic associated tyrosine kinases (e.g. v-src) and therefore were suggested to be kinase inhibitors. Subsequent analysis however, established that these compounds instead bound to and inhibited HSP90. Interaction of these drugs with HSP90 was mapped to a novel ATP binding site near the N-terminus of HSP90. We now know that these and related compounds block the ATPase coupled chaperone cycle of HSP90 that is necessary for client maturation and function. In the absence of this chaperone cycle, the client protein is almost always destined for ubiquitination and subsequent degradation via the proteasome pathway. Thus, these drugs, along with genomic and proteomic approaches, have proven useful in helping identify the vast array of HSP90 clients in the cell. In addition, HSP90 inhibitors have proven most useful in deciphering important details regarding the structure and function of HSP90, and especially the role of ATP dependent cycling for client maturation.

The association of HSP90 with so many important proteins involved in signal transduction pathways has led to suggestions that it may be a target for cancer therapy. At first glance this suggestion seemed unwise due to concerns that the inhibition of such a ubiquitous chaperone involved in so many biological pathways likely would have an acutely toxic effect. On the other hand, the necessity of HSP90 for the activity of such a wide variety of proteins involved in oncogenesis suggested that it might represent a novel target for the treatment of many different types of cancer. Further encouraging this idea were studies showing that HSP90 inhibitors tended to accumulate at much higher levels in tumor tissues compared to normal tissues. Based on these observations, clinicians have recently begun to turn their attention to HSP90 as a potential target for the treatment of numerous types of neoplasia.

HSP90 and Co-Chaperones

Antibodies

Product	Specificity	Application	Prod. No.
Grp94, mAb (9G10)	H, M, R, B, C, CH, HA, GP, MO, P, RB, S, X	FC, IHC, WB	ADI-SPA-850
Grp94, mAb (9G10) (DyLight™ 488 conjugate)	H, M, R, B, C, CH, HA, GP, MO, P, RB, S, X	FC	ADI-SPA-850-488
Grp94, mAb (9G10) (R-PE conjugate)	H, M, R, B, C, CH, HA, GP, MO, RB, P,S, X	FC	ADI-SPA-850PE
Grp94, pAb	H, M, R, B	IP, WB	ADI-SPA-851
HSP84, pAb	H, M, R	ICC, IP, WB	ALX-210-138
HSP86, pAb	H, M, R, S	ICC, IHC, IP, WB	ALX-210-139
HSP90 co-chaperone, mAb (JJ3)	M, H	ICC, IP, WB	ALX-804-023
HSP90, mAb (16F1)	H, M, R, B, BE, C, CH, D, F, GP, HA, MO, MU, P, PL, RB, S, SC, X	WB	ADI-SPA-835
HSP90, mAb (16F1) (biotin conjugate)	H, M, R	WB	ADI-SPA-835B
HSP90, mAb (2D12)	H, M, R, BE, B, C, CH, F, GP, HA, MO, RB, S	WB	ADI-SPA-845
HSP90, mAb (3B6)	H, M, R, B	WB	ALX-804-078
HSP90, mAb (3G3)	H, M, R, CH, F	IP	ALX-804-079
HSP90, mAb (AC88)	H, M, R, BA, BE, C, CH, CE, F, GP, HA, MO, MU, P, RB, S, SC, WM	WB	ADI-SPA-830
HSP90, mAb (AC88) (DyLight™ 488 conjugate)	H, M, R, BE, C, CE, CH, F	FC	ADI-SPA-830-488
HSP90, mAb (AC88) (R-PE conjugate)	H, M, R, B, C, CH, F	FC	ADI-SPA-830PE
HSP90, pAb	H, M, R, RB	WB	ADI-SPA-836
HSP90, pAb	H, M, R, B	WB	ADI-SPA-846
HSP90α, mAb (9D2)	H, CH	FC, IHC, WB	ADI-SPA-840
HSP90α, mAb (9D2) (HRP conjugate)	H, CH	WB	ADI-SPA-840HRP
HSP90α, pAb	H, M, R, B, BE, C, F, GP, HA, MO, P, RB, S, X	WB	ADI-SPS-771
HSP90α/β, mAb (H90-10)	H, M, RB	ICC, IP, WB	ALX-804-808
HSP90β, mAb (K3701)	H, M, R, B, C, GP, HA, P, RB, S	FC, WB	ADI-SPA-843
HSP90β, mAb (K3705)	H, M, R, B, C, CH, HA, GP, P, S	IHC, WB	ADI-SPA-842
TRAP1 (human), mAb (TRAP1-6)	H	ICC, IP, WB	ALX-804-368
UNC45, mAb (AbS1)	H, M, R	WB	ADI-SRA-1800

Inhibitors

Product	Description	Prod. No.
17-AAG	Less toxic, more potent synthetic derivative of geldanamycin	BML-EI308
17-DMAG	A water soluble, less metabolized analog of 17-AAG	BML-EI337
17-GMB-APA-GA	A maleimido-containing geldanamycin analog suitable for conjugation	BML-EI338
Geldanamycin	A benzoquinoid ansamycin inhibitor of HSP90, which inhibits ATP binding	BML-EI280
Geldanamycin, (biotin conjugate)	Useful for affinity purification of HSP90 client proteins	BML-EI341
Geldanamycin, (FITC conjugate)	Fluorescent HSP90 probe suitable for fluorescent polarization assays	BML-EI361
Herbimycin A	A benzoquinoid ansamycin inhibitor of HSP90, which inhibits ATP binding	BML-EI227
Novobiocin	Binds HSP90 C-terminus rather than the ATP-binding site	BML-A256
Radicicol	Macrocyclic lactone inhibitor of HSP90 with nM affinity	BML-EI285

Kits, ImmunoSets and Sample Packs

Product	Size	Sample Type	Specificity	Prod. No.
ImmunoSet™ Grp94 ELISA development set	5 x 96 wells	CL, T	H, M, R, C	ADI-960-077
HSP90α (human), EIA kit	1 x 96 wells	CL, S, T	H	ADI-EKS-895
HSP90, Ab sample pack	8 x 25 µg	Not applicable	Multiple species	ADI-PAK-010
HSP90, Ab sample pack with protein standards	10 x 25 µg	Not applicable	Multiple species	ADI-PAK-011

Proteins

Product	Application	Prod. No.
Activator of HSP90 ATPase 1 (human), (rec.)	Not available	ALX-201-275
Grp94 (canine), (rec.)	WB control	ADI-SPP-766
HOP (human), (rec.)	WB	ALX-201-218
HOP (human), (rec.)	WB control	ADI-SRP-1510
HSP90 (human), (native)	WB	ADI-SPP-770
HSP90 (yeast), (rec.) (His-tag)	WB	ALX-201-138
HSP90α (human), (rec.)	WB control	ADI-SPP-776
HSP90β (human), (rec.)	WB control	ADI-SPP-777
HSP90β (human), (rec.)	WB	ALX-201-147

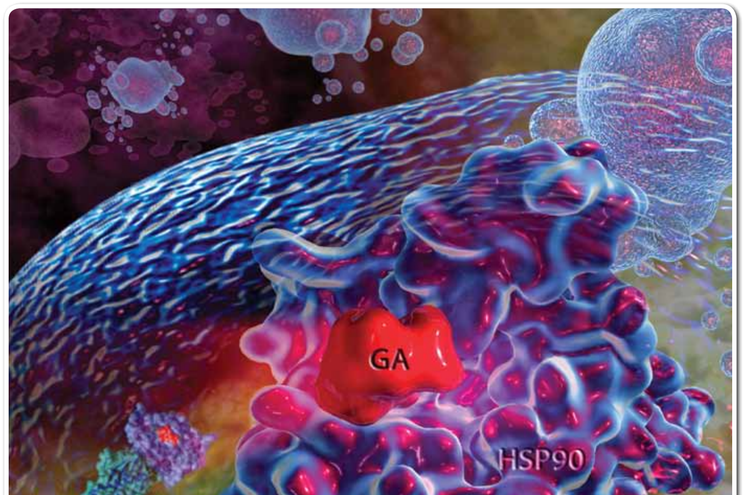

FIGURE: HSP90, a regulator of cell survival. Inhibition of HSP90 activity by drugs like geldanamycin (GA) destabilizes client proteins which ultimately lead to apoptosis.

See back for list of abbreviations

HSP90: A New Recruit in the War on Cancer

While geldanamycin and a few of the other early identified HSP90 inhibitors were found to be too toxic for clinical development, a semi-synthetic analog of geldanamycin, 17-AAG (tanespimycin), was produced and characterized. A number of phase I clinical trials have provided evidence that the compound can indeed inhibit HSP90, as evidenced by the depletion of numerous HSP90 client proteins. These early clinical results show that blocking HSP90 function in vivo results in the inactivation and/or depletion of oncogenic proteins needed to drive tumor growth. Tanespimycin is currently being tested in phase III clinical trials for the treatment of multiple myeloma and other cancers.

Recent studies describing the development and utilization of another HSP90 antagonist fuel further enthusiasm for HSP90 as a cancer target. Shepherdin is a peptidomimetic that was developed to block the interaction of HSP90 with yet another of its client proteins, survivin, an apoptotic inhibitor important in cell growth and survival. A cell permeable derivative of Shepherdin was found to selectively kill tumor-derived cells and diminish the relative amount of numerous HSP90 clients. A subsequent study revealed that Shepherdin not only interacted with HSP90, but also with an HSP90 relative, TRAP-1, located in mitochondria. Here TRAP-1 (and possibly HSP90 as well) normally provides protection against various types of stress-induced apoptosis. Disabling this protective pathway in cancer cells may be one key to the selective anti-tumor effects observed for different HSP90 inhibitors.

All of these studies, both in cell models and animal tumor models, continue to support the idea that inhibition of HSP90 (or its related family members) may prove to be an effective and novel target for cancer therapeutics. As of the early part of 2009, there were at least eight HSP90 inhibitors in clinical development to treat cancer. Some of the compounds being tested are derivatives of the previously mentioned ansamycins, while others are proprietary. Following up on the success of shepherdin, investigators have synthesized ansamycin derivatives designed to accumulate within the mitochondria (called gamitrinibs). Certain trials will test the compound alone, while others will likely test the potential efficacy of the particular HSP90 inhibitor with another more conventional anticancer modality. Already in some instances synergistic effects between HSP90 inhibitors and conventional agents like cisplatin, proteasome inhibitors, and histone deacetylase inhibitors have been observed. This latter result is particularly intriguing considering the fact that HSP90 itself is heavily acetylated and that HSP90 hyperacetylation has been reported to inhibit its chaperone functions. Additionally, HSP90 is heavily phosphorylated and it will therefore be of interest to understand how the multitude of HSP90 post-translational modifications serve to regulate its many chaperone activities and/or client selection.

Progress in our understanding of the stress response and the function of the individual stress proteins has been a remarkable journey. Rather than simply being important players during stress, HSPs serve prominent roles in some of the cell's most basic processes. Their general role as molecular chaperones, protectors of the proteome, is now featured in many areas of active research including aging, neurodegeneration, signaling, immunology and as discussed here cancer. The fascinating role of HSP90 as a key regulator and integrator of signal transduction events, from growth and differentiation to stress and apoptosis, continues to reinforce the idea that this very abundant, heavily modified protein is in fact a type of "biological buffer." Consequently, the idea that its buffering capacity may be simply overwhelmed in diseases like cancer makes HSP90 an ideal target for further drug discovery efforts aimed at controlling malignant transformation. As basic research continues to sort out its many client proteins and how they are regulated, and as clinicians continue to design compounds to inhibit its central role in signal transduction events, HSP90 likely will remain a top recruit in the development of new approaches for cancer therapeutics.

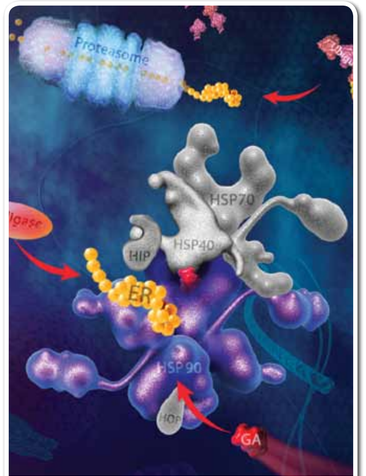

FIGURE: HSP90 a 'drugable' target. Inhibition of HSP90 by geldanamycin (GA) favors the ubiquintination and degradation of client proteins, such as the estrogen receptor (ER).

Suggested Reading:

Whitesell L and Lindquist S. **(2005).** HSP90 and the chaperoning of cancer. Nature Reviews Cancer 5, 761-772.

Pearl L. H., Prodromou C., and Workman P. **(2008).** The HSP90 molecular chaperone: An open and shut case for treatment. Biochem J. 410, 439-453.

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See back for list of abbreviations

HSP90 Reagents

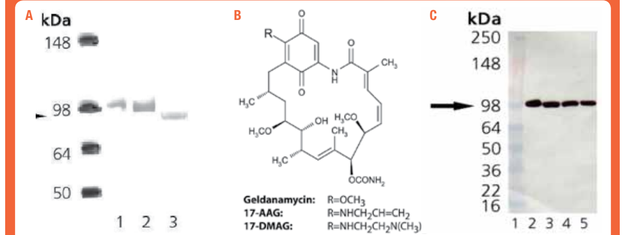

FIGURE A: Western blot analysis of HSP90 (human), (native) (ADI-SPP-770) in lane 1; HSP90α (human), (rec.) (ADI-SPP-776) in lane 2; and HeLa (heat shocked), (cell lysate) (ADI-LYC-HL101) in lane 3 probed with HSP90α, mAb (9D2) (ADI-SPA-840).

FIGURE B: Structure of Geldanamycin and derivatives.

FIGURE C: Western blot analysis of Grp94 (canine), (rec.) (ADI-SPP-766) in lane 2; HeLa, (cell lysate) (ADI-LYC-HL100) in lane 3; liver (mouse), (mircosome extract) (ADI-LYT-MM100) in lane 4; and vero, (cell lysate) in lane 5 probed with Grp94, mAb (9G10) (ADI-SPA-850). (Lane 1 = molecular weight marker).

Table Abbreviations

Application:
AA: ATPase Assay
EIA: Enzyme Immunoassay
EM: Electron Microscopy
FC: Flow Cytometry
IA: Inhibition Assay
ICC: Immunocytochemistry
IF: Immunofluorescence
IHC: Immunohistochemistry
IP: Immunoprecipitation
KA: Kinase Assay
WB: Western Blot

Conjugate:
AP: Alkaline phosphatase
FITC: Fluorescein isothiocyanate
HRP: Horseradish peroxidase
R-PE: R-Phycoerythrin

DyLight 488: DyLight™ 488 is a trademark of Thermo Fisher Scientific, Inc. and its subsidiaries.

Product:
pAb: Polyclonal Antibody
mAb: Monoclonal Antibody

Sample Type:
CL: Cell Lysate
CS: Cell Culture Supernatant
P: Plasma
S: Serum
T: Tissue

Specificity:
A: Avian
B: Bovine
BA: Bacteria
BE: Beluga
C: Canine
CE: *C. elegans*
CH: Chicken
D: *Drosophila*
F: Fish
FE: Feline
FN: Fungus
G: Goat
GP: Guinea Pig
H: Human
HA: Hamster
HO: Horse
I: Insect
M: Mouse
MO: Monkey
MU: Mussel
P: Pig
PL: Plant
R: Rat
RB: Rabbit
S: Sheep
SC: Scallop
WM: Water Mold
X: *Xenopus*
Y: Yeast

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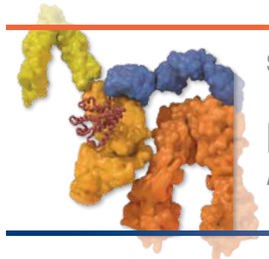

Scientific Review

HSP90
A New Recruit in the War on Cancer

HSP90: A New Recruit in the War on Cancer

By William J. Welch, Ph.D.
Professor Emeritus, University of California at San Francisco

Heat shock proteins (HSPs) feature in a multitude of important biological pathways and have come to the forefront of the research field today. Initially described as a curious group of proteins whose levels increased in fruit flies subjected to elevated growth temperatures, HSPs are in fact expressed under most conditions and are essential for growth and survival. Their most important role is to serve as guardians of the cellular proteome. Acting as "molecular chaperones" the various HSPs identify and bind to other proteins that have not yet reached their native folded state. The transient interaction of the chaperone with its target helps to insure against premature folding and/or aggregation of the polypeptide with other macromolecules. Acting together, molecular chaperones increase the fidelity of protein folding and thereby serve to maintain the integrity of the proteome.

Whenever conditions for protein folding/assembly or stability appear unfavorable (e.g. heat shock), the cell down-regulates overall protein synthesis and selectively upregulates chaperone expression to help deal with the problem at hand. Proteins that begin to unfold or aggregate are stabilized and in some cases refolded by the action of the chaperones. In other cases, proteins irreparably damaged are targeted by the chaperones to the ubiquitin-proteasome pathway for degradation. Over the past few years however, investigators have begun to realize there is much more to the story. Specifically, in their role as molecular chaperones, many of the HSPs appear to influence critical events controlling growth, survival and death. In what follows, the biology of one particular stress protein, HSP90, and its role in the development of cancer is presented. Indeed, its participation in so many pathways necessary for tumor formation has made HSP90 (and related family members) a new and exciting target for cancer therapeutics.

As far back as the 1970s, investigators began to realize that despite their designation, most of the HSPs were expressed at high levels in cells maintained under normal growth conditions or from healthy animal tissue. Both genetic and biochemical approaches revealed that deletion of certain HSPs compromised cell growth. Moreover, the expression of some of the HSPs was found to be critical for cell survival under most conditions. One particular stress protein, HSP90, constituted as much as 1-2% of the total cellular protein. Many suspected that such an abundant protein likely served some kind of structural role, perhaps analogous to cytoskeletal components like actin and tubulin.

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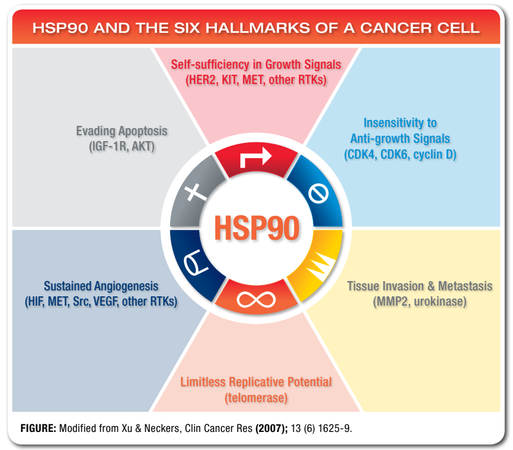

FIGURE: Modified from Xu & Neckers, Clin Cancer Res **(2007);** 13 (6) 1625-9.