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Invited Commentary

The Hsp90 chaperone complex-A potential target for cancer therapy ?

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ORIGINAL ARTICLE

Down-regulation of Hsp90 could change cell cycle distribution and increase drug sensitivity of tumor cells.

MAJOR POINTS OF THE COMMENTED ARTICLE

Using an antisense RNA approach, Liu *et al* studied the consequence of lowering Hsp90 β expression in two human gastric (SGC7901, SGC7901/VCR), one hepatic (HCC7402) and one esophageal (Ec109) cancer cell line. For two of the investigated cell lines (SGC7901/VCR and Ec109) cell growth slowed down upon decrease of the Hsp90 β level due to an increase in G1 cell phase. The growth rate of the SGC7901 cell line was unaffected by lowering the concentration of Hsp90 β, however the duration of G1 was decreased while G2 increased. No Hsp90 β dependent change in the growth was detectable for the hepatic cancer cell line HCC7402, which expressed Hsp90 β in lower levels than the other cell lines. Upon lowering the Hsp90 β concentration, all cell lines became more sensitive to chemotherapeutic drugs. Increases in the efficacy of mitomycin C (MMC) and cyclophosphamide (CTX) were generally modest (0-5 fold), although the SGC7901 cell line exhibited a 24.8 fold increased sensitivity to MMC. With the exception of the cell line SGC7901/VCR, dramatic effects were observed on the sensitivity of the cell lines to ad riamycin (ADR) and vincristine (MMC) with a 10^4 fold and 3 $\times 10^4$ fold increase in sensitivity of SGC7901 to VCR, or Ec109 to ADR, respectively.

COMMENTARY

Molecular chaperones are one of the life-guards of a living cell. They coordinate and execute basic and essential cell functions, such as facilitation of protein folding and oligomeric assembly of proteins, as well as the regulation of ligand binding and release, subcellular localization and turnover of proteins[1,2]. They are important for cell viability, and have been proposed to act as evolutionary tools, that produce a pool of mutant proteins under stress conditions[3,4]. Not surprisingly, aberrant chaperone action has been linked to numerous diseases[5-8], and the clinical interest in chaperones as targets for drug based treatments is increasing.

Hsp90 assembles into multiprotein complexes

Hsp90, a highly conserved and ubiquitously expressed chaperone of animal and plant cells, is one of the most abundantly expressed proteins (1%-2% of the cytoso lic protein in unstressed mammalian cells)[2,9-11]. Most eukaryotic cells contain at least two Hsp90 isoforms-the heat shock induced Hsp90 β and the usually less regulated Hsp90 β[12] Another close relative, Grp94, is expressed in the endoplasmatic reticulum[11,13]. Hsp90 assembles into large multiprotein complexes, that have partially overlapping compositions and include other chaperones such as Hsp70, Hip ("Hsp70-interacting protein"), Hop ("Hsp90-Hsp70 organizing protein", also called p60, Sti1), p23, and one of three large immunophilins FKBP51, FKBP52 (Hsp56), or Cyp40, which are peptidyl prolyl isomerases[2,10].

Hsp90 folds and controls the activity of regulatory proteins involved in signaling

The predominant role of these complexes may be to facilitate the maturation, fun ctional regulation, cellular localization and stress-dependent protection and repair of proteins rather than to assist the folding of de novo synthesized proteins[14-16]. Interestingly, many of the substrates of these Hsp90 chaperone complexes are regulatory proteins, or proteins involved in structural organization such as actin and tubulin[2]. One of the best studied substrates of Hsp90 chaperone complexes are intracellular receptors, especially but not exclusively steroid receptors[10,17-22]. *In vivo* association of unliganded steroid receptors with Hsp90 chaperone complexes is required for optimal steroid binding[23-25], and may also affect receptor subcellular traffick ing[26]. Hsp90 chaperone complexes also assist in the folding, maturation, membrane localization and degradation of many

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protein kinases[2,10,20] , such as v-Src[27], Raf[28], eIF-2-α-kinase[29], casein kinase II (CK II)[30], mitogen-activated protein kinase (MEK)[31], cyclin-dependent kinase 4 (CDK4)[32,33], and the cyclin-dependent kinase regulator Weel[34]. Association of these kinases with Hsp90 chaperone complexes is mediated by p50 cdc37, an homolog of the yeast cell cycle control protein, cdc37[33, 35]. The ability to interact functionally with a wide variety of regulatory proteins suggests that Hsp90 chaperone complexes may also coordinate and establish crosstalk between different signal transduction pathways. A recent study by Le Bihan *et al*[36] gave evidence for modulation of progestin-and glucocorticosteroid receptor-mediated transcription by calcium/calmodulin kinases (CaMK types II and IV), presumably through Hsp90 chaperone complexes.

The role of Hsp90 in cell cycling and cancer

Hsp90 action has been connected to cell cycle and cell differentiation[2,37,38], most likely as a consequence of their role in folding and functional regulation of intracellular receptors, protein kinases and other potential substrates such as p53 [9,39]. Moreover, the expression pattern of Hsp90 itself can be cell cycle dependent[40]. Increased levels of Hsp90 (mostly Hsp90 α) have been found in various malignant cell lines and cancers and usually correlate with vigorous proliferation of the malignant cells[2,41-44].

In a complementary study Liu *et al*[45] (this issue) investigate the consequence of lowering Hsp90 β expression in several human cancer cell lines using an antisense RNA approach. In agreement with the trend seen in other studies[41-44], they find that in some but not all cancer cell lines growth slows upon decrease of the Hsp90 β level, with various changes in cell cycle phasing.For one cell line, the hepatic cancer cell line HCC7402, growth and cell cycle phasing is not affected by reduced expression of Hsp90 β. Thus, overexpression of Hsp90 is not essential for cancerous growth. In fact, in an invasive and tumorigenic subline of 8701-BC breast cells down-regulation of Hsp90 β has been observed[46]. In view of the differences in the regulation of Hsp90 α and Hsp90 β, it would be interesting to extend the studies of Liu *et al* to Hsp90 α, whose expression is usually more directly linked to the cell cycle than that of Hsp90 β.

Hsp90 as target for anti-tumor drugs

Pharmacologically, the influence of Hsp90 activity on tumor growth is well established. Hsp90 chaperone complexes are targets for several pharmacological drugs[2,9,47]. The antibiotic geldanamycin is an anti-tumor drug that binds to the ATP/ADP binding site in the N-terminal domain of Hsp90[48]. Geldanamycin interferes with the folding, maturation, cellular localization and degradation of various intracellular receptors and kinases[2,9,47], and initially was described as an inhibitor for cell cycle kinases[49]. Another, unrelated antibiotic, Radicicol, also binds to the ATP-ADP-binding site of Hsp90 and suppresses transformation by diverse oncogenes such as Src, Ras and Mos[50,51]. Geldanamycin prevents binding of p23 to Hsp90[19], however whether its anti-tumor activity is due directly to this interference remains to be investigated. Although the role of ATP/ADP in the function of Hsp90 is not fully understood, the functional consequence of the binding of these structurally unrelated antibiotics to the Hsp90 ATP/ADP-binding site marks this site as an interesting target for drug design. Other potential and probably more selective drug targets in the Hsp90 chaperone complex are the immunophilins FKBP51, FKBP52 or Cyp40 that bind the immunosuppressants FK506, rapamycin or cyclosporin A[10,19].

Hsp90 mediated multidrug resistance

In addition of being target for several pharmacological drugs, Hsp90 chaperone complexes influence the sensitivity of cells to many drugs, and high Hsp90 expression is often associated with multidrug resistance[52], a major impediment of successful cancer chemotherapy. In their present study, Liu *et al*[45] demonstrate that upon lowering the Hsp90 β concentration the sensitivity of cancer cell lines to chemotherapeutic drugs increases, however the extent of these changes was strongly dependent on the drug. With exception of some cancer cell lines, their Hsp90 β dependent increases in the efficacy are generally modest (0-5-fold) for the drugs mitomycin C and cyclophosphamide, and more dramatic (up to 3×10^4-fold) for the drugs adriamycin and vincristine.

The mechanisms underlying multidrug resistance appear to be complex and are not well understood. In many tumor cells multidrug resistance is associated with overexpression of either the 170 kDa P-glycoprotein (Pgp) or members of the ATP-binding cassette transporter superfamily, such as the multidrug resistance protein (MRP) or the breast cancer resistance protein (BCRP), that act as drug export pumps[53-55]. A third form of multidrug resistance (atypical MDR) correlates with quantitative or qualitative alterations in topoisomerase ii α, that actively participates in the lethal action of cytotoxic drugs[56,57]. The mechanism of Hsp90 mediated multidrug resistance remains largely to be characterized. The contribution of Hsp90 might be a general strengthening of the stress response and the cellular

resistance to cytotoxic drugs. However, in some drug resistant cell lines Hsp90 β was found to stabilize and enhance the function of Pgp[52] suggesting a more direct role of Hsp90 in regulating multidrug resistance.

CONCLUSIONS AND PERSPECTIVES

Hsp90 chaperone complexes are vital and versatile coordinators and regulators of multiple signal transduction pathways. In higher organisms their action goes beyond that of a single cell and also affects complex regulatory systems such as the immune response. Hsp90, Grp94 and Hsp70 bind peptides and deliver them to MHC class I molecules, which increases the efficiency of the immune response[58] and often enhance tumor immunogenicity [59]. These pleiotropic functions make Hsp90 chaperone complexes ideal targets for the treatment of cancers.

Antisense Hsp90 mRNA expression, as used by Liu *et al*[45] is a powerful tool for regulating Hsp90 expression and reducing proliferation in some cancer cell lines. However, the inherent difficulty of selectively targeting antisense constructs to tumor cells impedes the usage of this strategy for clinical therapy. In contrast, the role of Hsp90, Grp94 and Hsp70 in tumor immunogenicity may offer new strategies for anti-tumor vaccination. Presently, the most promising strategy for an Hsp90 targeted therapy is the functional regulation of Hsp90 by drugs such as geldanamycin or radicicol. The solution of the molecular structures of Hsp90 : ATP/ADP and Hsp90 : geldanamycin complexes[48,60] allows the identification of structural features required for Hsp90 binding, and to develop new drugs with different pharmacological properties by structure based design. Other attractive targets for drug based regulation of Hsp90 chaperone complexes are the sites for the interaction with the kinase specific $p50^{cdc37}$ or immunophilins that appear to mediate specificity by directing Hsp90 chaperone complexes to particular substrates. Future elucidation of the composition, structure and function of those complexes will certainly open new possibilities for the treatment of cancer.

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