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Review

Heat Shock Proteins as Novel Therapeutic Targets in Cancer

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Abstract. *Heat shock proteins (HSPs) are evolutionarily conserved molecules synthesised by cells exposed to sub-lethal stresses. Acting as molecular chaperones, HSPs protect cells from environmental stress damage by assisting in proper folding and stabilisation of proteins. In addition, they help to sequester severely damaged proteins for degradation. Owing to the nature of their function, HSPs are often found to be overexpressed in a wide range of cancers. Members of the HSP family have been implicated in cancer growth as promoting tumour cell proliferation as well as inhibiting cellular death pathways. In recent years, several HSP90 client proteins have been validated as clinically important therapeutic targets for treatment of cancer, and inhibitors of HSP90 have emerged as potentially beneficial anticancer agents. This review explores the involvement of HSPs in cancer and the development of several anticancer agents with promising therapeutic applications.*

Heat shock proteins (HSPs) or stress proteins were first discovered by Ferruccio Ritossa in 1962, who observed that a transient increase in temperature induced puffing patterns in the chromosomes in salivary glands of *Drosophila melanogaster* larvae (1). This increase in temperature stimulated the expression of proteins with molecular masses of 26 and 70 kDa (2). As such, the original definition of HSPs was based on their enhanced expression in response to cellular insults, such as raised temperature, oxidative stress, chemical exposure and irradiation (3). Under normal physiological conditions, a complete set of functionally competent proteins are maintained in the cell. When exposed to cellular stressors, disturbance of the intracellular milieu induces a stress response in the cell, which inhibits the activity of many housekeeping genes whilst activating stress genes (4). This leads to increased levels of stress protein and their chaperones in the cell in a concerted effort to maintain protein homeostasis.

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HSPs as Molecular Chaperones

Most HSPs function as molecular chaperones. They constitute up to 5-10% of the total protein content in a cell under healthy growth conditions. However, when exposed to cellular insults that induce protein misfolding or aggregation, the affected proteins bind to chaperones and release heat shock factor (HSF) (5). HSF acts as a transcription factor and binds to heat shock elements within the promoter of *HSP* genes, resulting in a two- to threefold increase of cellular HSP concentration (6, 7). HSPs are known to afford protection against protein aggregation, induce solubilisation of loose protein aggregates, facilitate folding of nascent polypeptides, participate in refolding of proteins which have been damaged, and sequester damaged proteins and target them for degradation (8-10).

HSPs are generally classified based on their approximate molecular size *e.g.* HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs (sHSPs) with molecular sizes ranging from 15 to 30 kDa (11). High molecular weight HSPs are also known as adenosine triphosphate (ATP)-dependent chaperones. They assist in the folding of newly synthesised or damaged proteins in an ATP-dependent active process. In contrast, sHSPs work in an ATP-independent fashion (12). HSPs almost never act alone as they are often aided by other molecules such as other chaperones or several smaller co-chaperones (such as HSP60 with HSP10, and HSP90 with HSP70) (13).

HSPs in Cancer

HSPs have been found to be overexpressed in a wide range of human carcinomas, including both solid tumours and haematological malignancies (14-20). This may be an adaptive response by cancer cells to maintain protein

homeostasis and promote cell survival in an unfavourable environment, as well as to stimulate cell proliferation and inhibit cell death (14). Increased amounts of HSPs allow cancer cells to tolerate changes from within, such as potentially lethal mutations that have a role in oncogenesis (14, 15). Chaperones, such as HSP90, are known to be highly expressed in most tumour cells, including MCF7 breast cancer cells (Figure 1). HSP90 also acts as a biochemical buffer for genetic lesions found in cancer, allowing mutated proteins to perform their malignant functions while conferring cellular tolerance to the imbalanced signalling produced by these oncoproteins (14). Indeed, HSPs are seen to participate in the six essential alterations in cell physiology proposed by Hanahan and Weinberg to define cancerous growth (21) and described below:

Self-sufficiency in generating growth signals in cancer cells. HSP90 is needed to stabilise the fragile structures of many transcription factors and protein kinases that are involved in normal cellular growth pathways (22). This molecular chaperone is also required to maintain signalling molecules in an active conformation so as to allow rapid triggering by growth signals. In cancer, HSP90 maintains the activities of the HER2 proto-oncogene and the protein kinases Akt, c-Src and Raf-1 to promote tumour growth and survival (16, 22, 23). It also stabilises the conformations of mutant proteins such as v-Src, as well as molecules with gross structural alterations such as Bcr-Abl (formed by chromosomal translocation between chromosomes 9 and 22), thus allowing these mutated molecules to accumulate within the cancer cell (24).

Insensitivity to anti-proliferative signals. HSP70 has been shown to bind to p53 and other tumour suppressor proteins (25, 26). Mutation in the p53 protein is one of the most common events in cancer development (25, 27). However, although HPS70 has been shown to accumulate in large amounts in association with mutant p53 protein in cancer cells, there is no conclusive evidence to indicate that increased HSP levels are necessary to inactivate tumour suppressor molecules for malignant transformation to take place (25, 27).

Avoidance of apoptosis. Cancer cell population size is determined by a balance between tumour cell proliferation and attrition. The latter is due, in large part, to cellular apoptosis (21). Due to their cytoprotective role, HSPs have been found to play extremely complex roles in the regulation of apoptosis. They are implicated in both caspase-dependent and independent apoptotic pathways, as well as in the maintenance and activation of anti-apoptotic mediators (28). For example, inactivation or knock-down of HSP70 or HSP27 has been shown to lead to caspase-dependent apoptosis (29). In contrast, up-regulated

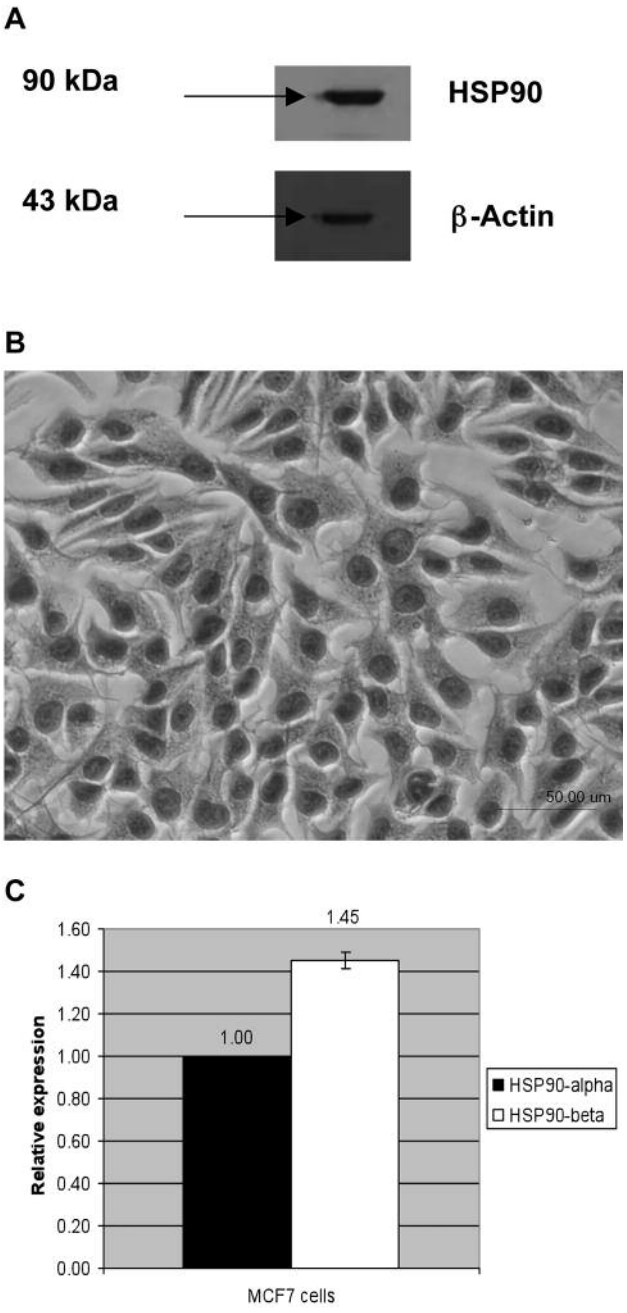

Figure 1. *Expression of HSP90 in MCF 7 breast cancer cells. A) HSP90 protein was detected in MCF7 cells by Western blotting. The separated proteins were transferred onto a nitrocellulose membrane, probed with primary rat monoclonal HSP90 antibody and exposed to anti-rat horseradish peroxidase (HRP)-conjugated secondary antibodies before visualization by chemiluminescence. Pre-stained protein standards were used to calculate the apparent molecular weight of the protein bands. β-Actin was used as an endogenous control. B) Immunostaining of MCF-7 breast cancer cells using primary rat monoclonal HSP90 antibody. HSP90 was localized in both the nucleus and cytoplasm of the breast cancer cells. Bar = 50 μm. C) HSP90-α and HSP90-β isoforms were found to be expressed in MCF7 cells by real-time RT-PCR. Samples were performed in duplicates.*

expression of HSP70 or HSP27 results in inhibition of caspase-dependent apoptosis. Some of the targets of HSP70 and HSP27 include c-Jun kinase, Apaf-1 and caspase 8. HSP70 is also involved in caspase-independent apoptotic pathways, and inhibits a death pathway involving cathepsins (23, 28).

Unlimited replicative capacity. For cancer cells to escape senescence and have unlimited replicative potential, they must bypass the crisis state in which massive cell death occurs together with significant shortening of telomeres in chromosomes, thus preventing cell divisions from taking place (21, 23). HSP90 is essential for telomerase stability, indicating its importance in the transformation of malignant tumour cells (30). HSP75, a member of the HSP70 family, also plays a role in increasing the number of cell divisions in cancer cells and countering replicative senescence by inhibiting the activity of p53 (31).

Angiogenesis. HSP90 and HSP70 are needed to stabilise the transcription factor H1F1α, the primary sensor of cancer cell hypoxia (32). In addition, HSP90 regulates vascular cell proliferation and motility by inducing and stabilising vascular endothelial growth factor (VEGF) and nitric oxide synthase expression in endothelial cells (22, 33). Indeed, overexpression of HSP90 has been shown to enhance tumour angiogenesis (33).

Invasive and metastatic capability. Tissue invasion and metastasis are hallmarks of advanced stages of cancer development. Clinical studies have demonstrated positive correlations between increased amounts of HSP27 and HSP70 with the invasive and metastatic capacities of malignant tumours (34). HSP90 has also been found to act as a molecular chaperone that assists in matrix metalloproteinase-2 activation, leading to increased cancerous invasion by cleaving constituents of the extracellular matrix (35).

HSPs as Anticancer Targets

Given the many roles that HSPs play in tumourigenesis and cancer progression, these molecules are potentially ideal therapeutic targets for cancer treatment. For example HSPs contribute to major apoptotic signalling pathways and act as chaperones for other essential molecules in apoptosis. Thus, inhibitors of HSPs could simultaneously block multiple signalling pathways, leading to both caspase-dependent and independent apoptosis of cancer cells (36, 37). Inhibitors have been developed against several HSPs (38-40). However, thus far, only inhibitors of HSP90 have shown promising results in clinical trials. One possible reason for this is that many of the HSP90 client proteins, such as epidermal growth factor receptor, Bcr-Abl fusion proteins, mutant p53, hypoxia-inducible factor 1α and matrix metalloproteinase 2 are involved in various cancer signalling pathways (41). Among the HSP90 inhibitors, the most promising compounds currently undergoing Phase I and II trials are the ansamycins: geldanamycin (GA), 17-allyl-17-dimethoxygeldanamycin (17-AAG) and 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17-DMAG). The latter is more soluble, has a higher oral bioavailability and is easier to formulate compared with 17-AAG.

GA, a benzoquinoid ansamycin antibiotic, was originally developed as a tyrosine kinase inhibitor. It induces the degradation of both tyrosine and serine/threonine kinases by the proteosome (42). However, it was shown later to bind specifically to the N-terminal ATP-binding domain of HSP90 homologues (42, 43). This discovery led to much of the understanding of the biological functions of this chaperone and stimulated interest in its use as an anticancer target. GA is unsuitable for clinical use due to its poor solubility and significant hepatotoxicity in mammals (44). However, GA analogues containing a substitution on the 17-alkylamino group have similar inhibitory effects on HSP90 with reduced hepatotoxicity. The two most prominent analogues that have been studied are 17-AAG and 17-DMAG.

Compared with GA, 17-AAG has a slightly lower binding affinity for HSP90 but possesses similar anticancer activities and displays much less toxic effects on the liver (45, 46). It was reported that cancer cell-derived HSP90 has a 100-fold higher binding affinity for 17-AAG than that derived from normal cells (47). This is most probably due to tumour-derived HSP90 being found mostly in multi-protein complexes with high ATPase activity, especially with an increased load of mutant client proteins (47). In contrast, HSP90 from normal cells is usually uncomplexed, with low ATPase activity (47). Clinical trial data for 17-AAG have demonstrated cancer stabilisation, with high apoptosis and reduced tumour proliferation at drug concentrations below the maximum tolerated dose (41).

The HSP90 clients most sensitive to ansamycin-induced degradation in preclinical models are HER2 and Met receptor tyrosine kinases, Raf-1 kinase, and oestrogen and androgen receptors (48, 49). HER2 has been found to be the HSP90 client protein that is most sensitive to 17-AAG-induced degradation. In breast cancer with amplification of the *HER2* gene, overexpression of HER2 protein often results in formation of HER2/HER3 heterodimers which activate the PI3Kinase/Akt signalling pathway and result in deregulated tumour growth and inhibition of apoptosis (50). Treatment of cancer with 17-AAG induces the degradation of HER2 and loss of its expression on the cell membrane and leads to rapid inhibition of Akt activity (51).

There is increasing interest in combining the use of 17-AAG or other HSP inhibitors with other chemotherapeutic drugs, as this may increase *in vivo* efficacy of the latter due

to the chemoprotective activity of some HSP90 client proteins (52, 53). Indeed, combination therapies appear to be effective in treating cancer. For example, a low dose of GA is sufficient to sensitize Bcr-Abl-expressing leukaemia cells to apoptosis despite using ineffective concentrations of the chemotherapeutic drug doxorubicin (54). Combination of 17-AAG with angiogenesis inhibitors has also proven highly successful in treating breast cancer (49).

Conclusion

There is currently much effort being made to develop more effective HSP inhibitors for use in cancer treatment (50). Besides ansamycins, other HSP90 inhibitors include macrolides, purine-scaffold derivatives, pyrazoles, shepherdin, cisplatin, novobiocin and other post-translational modification inhibitors, many of which are still under preclinical development. These inhibitors target different HSP90 domains specifically, such as the N-terminal domain or the C-terminal domain (41). Given the abundance of HSPs in normal cells and their fundamental physiological functions, the successful development of HSP inhibitors into clinically useful therapeutic agents will be highly dependent upon the discovery of efficacious anticancer compounds while minimising cytotoxic and other unwanted side-effects to normal cells in the body.

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