

Heat Shock proteins in Cancer

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ABSTRACT: Heat shock proteins (HSPs) are highly conserved and inhabit nearly all subcellular locations where they perform a variety of chaperoning functions including folding and unfolding of nascent polypeptides, proteins, transport of proteins and support of antigen presentation processes. Apart from their intracellular location HSPs with a molecular weight of 70 kDa (HSP70) also have been found on the plasma membrane of malignantly transformed cells, on virally/bacterial infected cells and in the extracellular space. Depending on their intra- and extracellular location HSPs exert either protection against environmental stress or act as potent stimulators of the immune response. In this review we address the dual function of intracellular and extracellular located small HSPs and members of the HSP70 family and its immunological consequences for cancer immunity.

KEYWORDS: Heat shock proteins; adaptive/innate immune response; cancer

INTRODUCTION

The major groups of molecular chaperones have been implicated in cancer development. Here we will focus on the role of HSP70 and small heat shock proteins family members in cancer, because Hsp90 and ER chaperone Grp78, Grp74 (gp96) are subjects of different symposia.

There are many reports indicating that the major inducible chaperones Hsp70 (member of the HSP70 family) and Hsp27 (member of the small heat shock proteins family) are present at elevated levels in various human tumors, especially of epithelial origin or gliomas. For example, these heat shock proteins are expressed at high levels in a large fraction (up to 50%) of breast, endometrial, lung, prostate and other types of tumor biopsies¹⁻⁵, and their expression often correlates with increased cell proliferation, lymph node metastases, poor response to chemotherapy, and poor survival (^{4,6,7} for review). In addition to their intracellular location HSPs are also found on the plasma membrane and in the extracellular space where they can activate the immune system.

Beside correlation studies with human tumor biopsies, there have been many works that attempt to address the role of these HSPs in cancer using cell culture and animal experiments. Overall these studies indicate that HSP70 and Hsp27 have dual effects on cancer – (1) they promote cancer development by suppression various anti-cancer mechanisms, like apoptosis and senescence, as well as by facilitating expression of metastatic genes; and (2) they facilitate tumor rejection by immune system. Accordingly, there have been attempts to inhibit or down-regulate intracellular Hsps to facilitate apoptosis or senescence of cancer cells. On the other hand, extracellular and membrane-associated HSPs have been utilized for cancer immunotherapy. Here, we will address both opposing activities of HSP70 and small heat shock protein family members in cancer.

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Intracellular HSP70 family members in cancer

Earlier experiments with permanent overproduction of Hsp70 in several cell types supported the idea that Hsp70 facilitates cell's tumorigenicity, including the ability to form tumors in nude mice⁸, formation of foci, anchorage-independent growth and formation of tumors in mice xenografts⁹, as well as development of multiple lymphomas¹⁰. Furthermore, in human breast cancer MCF-7 cells overproduction of recombinant Hsp70 led to a strong acceleration of cell growth by shortening of G0/G1 phases¹¹. This effect could be related to stabilization of the cyclin D1 upon overproduction of Hsp70¹².

On the other hand, experiments with a specific depletion of Hsp70 in cancer cells indicated that Hsp70 is important for the tumor cell survival. For example, depletion of Hsp70 led to an apoptosis-like death of a variety of tumor cell types, including human oral carcinoma cells isolated from primary tumors, HSC-2, MCF-7, Molt-4, PC-3 and others^{13,14,15}. On the other hand, untransformed cells (e.g. primary fibroblasts, Rat1, CM, MIN-6) did not lose their viability upon Hsp70 depletion^{15,16} suggesting that, in contrast to untransformed cell types, many tumor cell lines cannot survive endogenously activated death if they lack Hsp70.

An interesting extension of these studies was recent finding reported in this symposium that depletion of Hsp70 leads to rapid premature senescence in several cancer cell lines¹⁷. Originally cellular senescence was described as a limit to a number of divisions that a normal cell can undergo. For example, normal fibroblasts can undergo about 60 divisions in culture before acquiring a specific "flat" morphology and becoming permanently growth arrested¹⁸. While originally it was thought that the replicative senescence is an ultimate result of the telomeres shortening, at present it is commonly accepted that senescence could be triggered by the cell cycle inhibitors p16 and p21 (see ref¹⁹) for review). Senescence is a very complex program with multiple end points that include not only growth arrest, but also enlargement of cells, extensive vacuolization, repression and de-repression of certain sets of genes, secretion of various signaling molecules, inhibition of the heat shock response, and other manifestations.

The senescence program seems to represent one of the major breaks on cancer emergence at the cellular level in addition to activation of apoptosis. Indeed, limiting cell divisions seems to be a perfect way of preventing tumor growth, and mammalian cells appear to utilize both apoptotic and senescence programs to counteract tumor-inducing action of the major oncogenes. In fact, surprisingly, overexpression of major oncogenes could either activate apoptosis, as seen with *myc* or *E1A*²⁰⁻²⁴, or trigger senescence as seen with *Ras*, *Her-2*, *PTEN*, *Raf*, and others oncogenes of the *Ras* pathway²⁵⁻²⁷. Under these conditions, both apoptosis and senescence are associated with activation of the p53 pathway²⁷⁻²⁹. If a p53 target gene p21 is induced, senescence is initiated, however if p21 induction is abrogated, e.g. by *myc*, apoptosis is activated^{28,27,29}. Importantly, in recent experiments where senescence of cancer cells was seen upon depletion of Hsp70, a strong activation of p53 and p21 was observed under these conditions. Furthermore, induction of p21 was dependent on p53. These data indicate that endogenous high levels of Hsp70 in these cancer lines are critical for control of the p53 pathway, and thus for cell proliferation.

Irina Guzhova and her colleagues (Institute of Cytology RAS, St Petersburg, Russia) report in this symposium that Hsp70 can also suppress apoptosis stimulated by *myc* oncogene. In fact, they observed that introduction of *v-myc* into U-937 cells or activation of *myc* in Rat1 cells sensitizes them to camptothecin and etoposide by enhancing apoptosis. Overexpression of Hsp70 suppressed the *myc*-induced sensitization, which represents the first example when Hsp70 interferes with *myc*-facilitated apoptosis. Interestingly, *myc* can promote apoptosis via stimulation of p53³⁰, and furthermore, both drugs used in this study also activate p53. Therefore, it is likely that there is a unifying mechanism of suppression of both apoptosis and senescence in cancer cells by Hsp70, which involves down-regulation of the p53 pathway, and the choice of a specific pathway of cell demise appears to be dependent on the ability of p53 to induce p21.

These data suggest a simple model that explains overexpression of Hsp70 (as well as Hsp27, see below) in various cancers. In fact, since oncogenes initiate apoptosis or senescence, emergence of cancer clones indicate that they somehow by-pass these breaks on cancer development. One of the mechanisms is accumulation of mutations in the p53 pathway, which was reported for many cancers. On the other hand, there must be alternative mechanisms of suppression of the p53-dependent cell demise, which take over in

cancers with normal p53 pathway. Hsps appear to be ideal candidate factors that can provide such a suppression, which can explain their accumulation in cancers.

Interestingly, as reported in the symposium by Marja Jäättelä (Institute of Cancer Research, Copenhagen, Denmark), another member of the Hsp70 family, a less abundant but ubiquitously expressed protein Hsp70-2, which is essential for testis development, also protects from senescence as seen with various cancer cell lines.³¹ Depletion of this protein using siRNA approach caused up-regulation of p53 followed by permanent G1 arrest, cell enlargement and flattening typical for senescent cells.³¹ Therefore, as with Hsp70, the primary effect of Hsp70-2 depletion seems to involve p53 activation, which in turn triggers the senescence program. Accordingly, Hsp70-2 seems to play a role in keeping p53 pathway suppressed, and Hsp70-2 depletion leads to the abrogation of this control and reactivation of the default senescence program.

In addition to controlling the p53 system Hsp70-2 controls lysosome stability and a lysosome-dependent apoptosis-like process. This control appears to involve expression of a number of genes, including a lens epithelium-derived growth factor (LEDGF)³². Accordingly, down-regulation of either Hsp70-2 or LEDGF led to lysosomal destabilization and a cathepsin-dependent cell death. Interestingly, overexpression of LEDGF increased the tumorigenic potential of human cancer cells, and both LEDGF and Hsp70-2 are found at high levels in breast and bladder carcinomas.³² Therefore, it appears that Hsp70-2 has multiple tumor-promoting activities, including suppression of p53, induction of LEDGF, and possibly others.

In promoting cancer development, Hsp70 family members may cooperate with cofactors. For example, Vince Guerriero and his colleagues (University of Arizona, Tucson) report in the symposium that HspBP1, a co-chaperone with nucleotide exchange activity that binds to and regulates Hsp70, is highly overexpressed in a variety of tumors. Although this group does not identify a role of HspBP1 in cancer, they found an interesting correlation between expressions of HspBP1 and Hsp70, where the ratio between these proteins is constant in various biopsies. These data suggest that HspBP1 cooperates with Hsp70 in promoting tumors.

Intracellular small heat shock proteins in cancer

Hsp27, the major member of the small heat shock proteins family, is overexpressed in a variety of cancers, and was suggested to play a major role in tumor development. These findings are discussed in details in recent reviews by Calderwood & Ciocca, and Arrigo. In part implication of small Hsps in cancer is based on the well-described anti-apoptotic activity of Hsp27, which upon over-expression blocks apoptosis caused by a variety of stimuli (see ref^{4,7,33} for review). There are multiple steps in the apoptotic process that can be controlled by Hsp27, but this discussion is beyond the scope of this assay.

In addition to suppression of apoptosis, Hsp27 can suppress the senescence program, as reported by Michael Sherman and his colleagues (Boston University, USA) at this symposium. In fact, depletion of Hsp27 in highly transformed cells caused activation of the p53 pathway, induction of p21 and expression of typical signs of cell senescence. On the other hand, overexpression of Hsp27 in immortalized mammary epithelium cells caused suppression of the senescence program activated by genotoxic drugs and oxidants. Therefore, the function of Hsp27 in cancer appears to be suppression of the default senescence program that involves p53. In that sense the role of Hsp27 is similar to that of Hsp70. These two proteins may serve as two alternative factors responsible for suppression of the oncogene-induced senescence in cells with normal p53 pathway. Furthermore, these data suggest that suppression of p53 may be a novel factor in the anti-apoptotic activity of Hsp27.

In addition to anti-apoptotic and anti-senescence activity of Hsp27, this chaperone appears to play a major role in cell migration and metastases. These functions appear to be unrelated to p53, since they were detected in cell lines with defective p53. In part, the role of Hsp27 in cell migration can be explained by its interaction with the actin cytoskeleton.^{34,35} In line with these findings, recently it was reported that a novel inhibitor of phosphorylation of Hsp27 suppresses tumor cell migration and invasion.³⁶ On the other hand Hsp27 appears to be critical for expression of metalloproteases MMP2 and MMP9 that are essential for metastases.³⁷ In fact, it was found that in PC-3 cells activation of metastases by TGF-alpha required

Hsp27, where this Hsp serves as a regulator of transcription of the metalloproteases³⁷. In this pathway, TGF- α activates p38 kinase, which in turn activates the MAPKAP-2 kinase, which phosphorylates and activates Hsp27.

Surprisingly, a close homolog of Hsp27 α B-crystallin appears to be sufficient for cancer transformation³⁸. Vince Cryns and his colleagues (Northwestern University, Chicago, USA) report at this symposium that overexpression of α B-crystallin transforms immortalized human mammary epithelial cells. It induces EGF- and anchorage-independent growth, increases cell migration and invasion, and constitutively activates the MAPK kinase/ERK (MEK/ERK) pathway. The transformed phenotype required activity of this signaling pathway. In addition, cells overexpressing α B-crystallin formed invasive mammary carcinomas in nude mice that recapitulated aspects of human basal-like breast tumors. These results indicate that α B-crystallin is a novel oncoprotein.

Extracellular and membrane-bound HSPs in cancer

Apart from their intracellular location HSPs with molecular weights of 60, 70 (Hsp70, Hsc70) and 90 (gp96) kDa have been found on the plasma membrane of malignantly transformed cells^{39, 40} and in the extracellular milieu⁴¹ (Triantafilou, University of Sussex, UK). Pioneering work of the group of Srivastava⁴² demonstrated that HSP90 as well as HSP70 peptide complexes are potent stimulators of the adaptive immune system. HSP-chaperoned peptides are taken up by professional and non-professional antigen presenting cells including monocytes, macrophages, dendritic cells (DC) and B cells via receptor mediated endocytosis and thus become cross-presented as classical antigens for CD8-positive cytotoxic T cells on MHC class I molecules. Presently, the α 2 macroglobulin receptor CD91, Toll-like receptors 2 and 4, the LPS receptor CD14^{43,44}, scavenger receptors (SR-A-H)⁴⁵ including lectin-like oxidized LDL receptor LOX-1, SR expressed by endothelial cells (SREC-1), SR-H member FEEL-1 (identical to CLEVER-1/stabilin-1), B cell receptor CD40, CD36, chemokine receptor CCR5, as well as C-type-lectin receptors NKG2A, NKG2C in association with CD94, and NKG2D^{46,47} are discussed as potential mediators of binding and uptake of members of the HSP70 and HSP90 families into antigen presenting cells.

Our group (Multhoff, Technische Universität München, Germany) demonstrated a tumor-specific cell surface location of Hsp70, the major stress-inducible member of the HSP70 family by selective cell surface iodination and by flow cytometry using the mAb cmHsp70.1 (multimmune GmbH, Munich), detecting membrane-bound Hsp70 in the plasma membrane of viable tumor cells.^{48,49} Even in the absence of chaperone-bound peptides, plasma membrane-bound Hsp70 has been determined as a tumor-specific recognition structure for pre-activated NK cells. Following incubation with the Hsp70-peptide TKDNNLLGRFELSG plus pro-inflammatory cytokines NK cells acquire migratory capacity and efficiently kill Hsp70 membrane-positive tumor cells. TKD peptide represents a 14-mer amino acid sequence of the C-terminal domain which is exposed to the extracellular milieu of tumor cells and contains the recognition epitope of the Hsp70 mAb cmHsp70.1.⁵⁰

In addition to Hsp70, extracellular located HSP60, HSP90, HSP110 and the ER chaperone glucose related protein 170 (Grp170) have been found to exhibit pro-inflammatory and pro-immune activities. However, the species from which the HSPs or Grps are derived from and the concentration can affect immune function. In contrast to stress-inducible Hsp70, BiP an ER-residing HSP70 member as well as Grp78 and Grp74 (gp96), and members of the small HSP families, including HSP27 and cpn10, have been found to exhibit anti-inflammatory (Pockley, University of Sheffield, UK) immunoregulatory activities towards the adaptive immune system^{51,52}. These data indicate that despite their homologies, members of different HSP families can mediate divergent functions on the innate and adaptive immune system.

Although the immunological consequences of membrane-bound and extracellular located HSPs are obvious the mechanism of anchorage, export and uptake of HSPs remains to be elucidated. Evidence is accumulating that similar to IL-1- β HSPs lacking a membrane translocation and anchorage sequence are released in an ER-golgi independent manner via a non-classical lysosomal pathway.⁵³ Anchorage of Hsp70 in the plasma membrane has been found to be associated with a lipid-protein rather than a receptor-protein interaction since high salt and pH changes did not affect the Hsp70 membrane expression (unpublished). In contrast, methyl- β -cyclodextrin, a cholesterol depleting agent, is highly efficient in diminishing the

amount of membrane-bound Hsp70.⁴⁵ These data might provide a first hint that Hsp70 is associated with lipid rafts in the plasma membrane of tumor cells. Another question which is presently a matter of debate is related to the physiological function of membrane-bound Hsp70 in lipid rafts and in the extracellular space. Are membrane-bound and extracellular HSPs still capable to act as chaperones and if so which co-chaperones are associated with HSPs. Last but not least it is still not clear as to whether extracellular HSPs can gain access to the plasma membrane from outside and thus might be re-integrated into the plasma membrane as already shown much earlier by Tytell and Hightower^{54,55} for neurological cells.

Although many questions still remain open, it is obvious that HSPs possess dual roles depending on their intra- and extracellular location and depending on the species from which they are derived of. As potent stimulators of the adoptive and innate immune response presently HSPs with molecular weight of 70 and 90 kDa strongly increase the interest in using them as immune-modulators in cancer therapy, as mediators of tolerance small HSPs and BiP might be useful for the cure of autoimmune diseases and as intracellular molecular chaperones they might support survival of normal cells from apoptotic cell death following environmental stress.

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