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HEAT SHOCK PROTEINS IN BREAST CANCER PROGRESSION- a suitable case for treatment?

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Abstract

Heat shock proteins (HSP) and heat shock factor 1 (HSF1), key factors in the heat shock response (HSR) have been implicated in the etiology of breast cancer. At least two members of the HSP family, Hsp27 and Hsp70 undergo significant increases in cellular concentration during the transformation of mammary cells. These changes result in HSP-mediated inhibition of tumor cell inactivation through blockade of the apoptosis and replicative senescence pathways. The increases in HSP thus mediate two of the common hallmarks of cancer and favor cell birth over cell death. In addition, Hsp90 plays a role in facilitating transformation by stabilizing the mutated and overexpressed oncoproteins found in breast tumors, and permitting the activation of growth stimulatory and transforming pathways in the absence of growth factors. HSF1 appears to play a similar role as a facilitator of transformation in mammary carcinoma. Induction of some facets of the HSR in breast cancer cells therefore leads to growth stimulation and inhibits cell death. Pharmacological targeting of HSP and HSF1 is therefore indicated and in the case of Hsp90, inhibitory drugs are undergoing clinical trial for treatment of breast carcinoma and other cancers.

Keywords

heat; shock; protein; HSF1; breast; cancer

(1) INTRODUCTION

Heat shock proteins (HSP) were first discovered as mediators of resistance to hyperthermia in all cellular organisms¹. They have subsequently been characterized as members of the molecular chaperones, a group of proteins that play essential roles in the correct folding of a large proportion of cellular proteins^{2,3}. The development of thermotolerance involves this property of HSPs and is associated with the resolution of toxic protein aggregates that accumulate in hyperthermia⁴⁻⁶. Thermotolerance involves several mechanisms including: (1) the refolding of some proteins in pathway facilitated by HSPs as well as (2) degradation of proteins that are not refolded through the proteasome pathway, another HSP-mediated process⁷. In addition HSPs participate directly in cell survival during hyperthermia by inhibiting programmed cell death and cell senescence⁸⁻¹¹. The transcription of *HSP* genes is regulated by transcription factor HSF1, that senses cellular exposure to stress and turns on rapid induction of HSPs^{12,13}. These properties of the HSR appear to have been hijacked during malignant progression and aid in the development of cancer^{14,15}. HSP levels become elevated in a wide spectrum of malignant cells including mammary carcinoma cells¹⁶. Hsp27 and Hsp70 appear to foster mammary tumorigenesis by inhibiting apoptosis and senescence. Hsp90 appears to play a key role in facilitating tumor progression by

chaperoning the mutated and overexpressed oncogenes that fuel transformation and tumor progression^{17, 18}. Indeed Hsp90 may contribute to the evolution of treatment resistant cell populations by permitting the emergence of variant proteins that can overcome the stresses of cancer therapy^{17–20, 21}. It has also been shown recently that the mitochondrial Hsp90 family member TRAP-1 plays a versatile role in transformation by mediating membrane transitions within this organelle and inhibiting apoptosis at source²². Mitochondria possess a subclass of HSPs including the Hsp70 homolog mortalin and TRAP-1 as well as Hsp60, each of which appear important in progression of a wide class of cancers^{22, 23}. In addition, recent studies indicate a key role for HSF1 in breast carcinoma. HSF1 may function in breast cancer progression by inducing HSPs²¹. However, HSF1 appears to play additional roles in addition to HSP induction, including activation of metastasis by the silencing of anti-metastatic processes, activating pro-malignant signaling cascades and regulating the mitotic spindle checkpoint^{24–26}. These multiple components of the heat shock response appear to be utilized in tumorigenesis in order for cells to escape the pathways of tumor suppression, to promote evolution into advanced and treatment-resistant modes, and to facilitate metastasis. They would therefore seem suitable candidates for molecular targeting (summarized in Fig. 1).

(2) Targeting Hsp90

Hsp90 has emerged as important agent in cancer therapy and a number of drug classes (including the ansamycins and novobiocin homologs) appear able to target its ATP binding domain and inhibit activity^{27, 28}. Binding and hydrolysis of ATP is essential for its molecular chaperone function²⁹. Hsp90 is not generally expressed at elevated levels in cancer although its basal levels are already high in the majority of cells. However Hsp90 activity appears essential for growth of breast and other cancers due to properties that include its ability to chaperone a wide spectrum of oncogenic proteins. These include proteins important in breast cancer progression such as Her2 / *neu* and c-src^{17, 18}. Many such proteins become overexpressed or mutated in mammary cancer progression and such tumors appear to be dependent on, or “addicted to”, Hsp90 to maintain the levels of these proteins and drugs that inactivate the chaperone target this dependency^{17, 18}. Hsp90 drugs have the advantages of multi-targeting, in that many of the oncogenic proteins depend on Hsp90 activity and a dynamic mechanism of inhibiting the evolution of treatment resistant cells^{17, 18, 30}. Hsp90 is known to maintain pools of mutant / polymorphic molecules in the cell that could potentially be recruited for the evolution of new traits¹⁹. Inhibition of the protein is predicted to cause loss of the mutant pool and inability to respond to changes in microenvironment and to anticancer treatments. Clinical trials are beginning on a number of the ansamycin-derived Hsp90 drugs including 17-AAG and others³¹.

Hsp90 carries out its molecular chaperone role in cells in association with a cohort of accessory proteins called co-chaperones. One of these proteins in particular, p50 / Cdc37 is expressed to high level in multiple cancers and is required in the maturation of a wide spectrum of oncogenic protein kinases. Targeting Cdc37 in cancer has been attempted and appears highly effective at least in prostate cancer³². Cdc37 offers the potential of a therapeutic gain due to its elevated expression in cancer and experiments are underway to target the protein in breast cancer³³.

(3) A role for Hsp70

Hsp70, like Hsp90 mediates the chaperoning of a wide spectrum of cellular proteins in an ATP-dependent manner³. Hsp70 is expressed at high levels in a wide spectrum of cancer cells and is induced through activation of the Her2 / *neu* pathway that is important in advanced breast cancer²¹. Hsp70 is involved in the early stages of protein folding,

chaperoning a proportion of polypeptides undergoing synthesis on ribosomes before passing on some clients to Hsp90. Hsp70 does not, however seem required for the long-term stabilization of a wide spectrum of clients as observed with Hsp90. Thus Hsp70 addition in cancer cells is not as commonly observed as with the more ubiquitous Hsp90. Hsp70 appear to function in breast cancer due to its ability to inhibit programmed cell death and senescence when expressed at high levels, important features of malignant transformation^{10, 11}. Thus targeting Hsp70 can lead to cell inactivation by permitting programmed cell death. However, unlike Hsp90, really effective drugs are not currently available for targeting the ATP binding domain of Hsp70. However, since Hsp70 blocks apoptosis at the post-mitochondrial level by inactivating the apoptosome as well as AIF, strategies targeting Hsp70 appear to be effective in overcoming tumor cell resistance. It has recently been shown that decoy targets of Hsp70 derived from AIF can sensitize cancer cells to apoptosis induction by neutralizing Hsp70 functions. AIF-derived peptides mimic a domain of the AIF protein (amino acids 150 to 228) required for Hsp70 binding³⁴. Hence, they bind to Hsp70 but lack an autonomous pro-apoptotic function. Experiments using different cancer cell lines (leukemia, colon cancer, breast cancer and cervix cancer) demonstrate that several among these peptides strongly increase the sensitivity to chemotherapy *in vitro*. This effect was related to their ability to neutralize endogenous Hsp70, because this pro-apoptotic activity was lost in Hsp70-negative cells³⁵.

(4) The small HSP family, especially Hsp27

Hsp27 is expressed to high levels in breast carcinoma and many other types of cancer, compared to normal cells in which expression is moderate in the absence of stress³⁶. Hsp27 is a member of the small HSP (sHSP) family and is a potent mediator of protein folding¹¹. The sHSP are highly pleiotropic molecules both in terms of molecular function and cell biology properties. In breast cancer, Hsp27 exerts pro-malignant effects largely due to its properties of inhibiting programmed cell death and senescence^{37, 38}. Hsp27 is activated in stress both by transcriptional activation and posttranslational modification (phosphorylation) downstream of the p38 MAPK stress kinase pathway, although it is not clear which of these processes is involved in Hsp27 up-regulation in cancer³⁹. However, HSF1 becomes activated in breast cancer and Hsp27 levels may increase secondarily to this change. Hsp27 mediates its molecular activities after activation through phosphorylation –dependent changes in oligomerization that are involved in its protein folding and cell regulatory functions³⁹. Unlike the other HSPs, the sHSP do not bind ATP, a property that may make this molecule problematic for targeting with small compounds. Many effective anti-cancer agents are aimed at the ATP binding domains of protein kinases or other molecular chaperones^{17, 40}.

(5) HSF1

As the central regulator of HSP expression, HSF1 would seem a focal point in designing anti-breast cancer approaches. HSF1 inactivation has been shown to inhibit the progression of a wide spectrum of cancers^{26, 41}. In addition, activation of the HER2 / neu pathway has been shown to induce HSF1 and HSP expression and render cells resistant to spontaneous and drug-induced programmed cell death²¹. HSF1 may also mediate tumorigenic effects through an alternative pathway involving the recruitment of the pro-metastatic gene co-repressor MTA1 which inhibits the expression of (estrogen-induced) anti-metastatic genes²⁴. MTA1 thus appears to stand at the crossroads between estrogen and Her2 / neu regulated signaling, encouraging metastasis and HSF1 appears complicit in these effects. HSF1 also possesses additional properties in cancer including the enhancement of pro-malignant signaling through the ERK, PKA and TOR pathways²⁶. Chemical inhibitors of HSF1 activation have been described, including genistein, a compound which has the interesting

property of synergizing with hyperthermia in cancer regression in vivo^{42, 43}. However, genistein is a highly pleiotropic molecule with many potential targets in cancer cells and it is not clear what proportion of its effects on tumor growth involve HSF1 inhibition. Additional HSF1 inhibitors include KNK437 and triptolide and information on their specificity, effectiveness in treatment of breast cancer is awaited^{44, 45}. In addition an inhibitor of the heat shock response termed emunin has been described⁴⁶. Emunin inhibits HSP expression in cancer cells by unknown mechanisms and potentiates mammary tumor cell killing by agents such as Hsp90 inhibitors and proteasome inhibitors⁴⁶. It is thus apparent that chemical targeting of HSF1 is at an early stage in development and advances may be expected.

(6) DISCUSSION

The heat shock response thus appears to have become “hijacked” in the processes of mammary cell transformation and progression. Transformation is accompanied by HSF1 activation and involves multiple downstream effects of this molecule (Fig. 1). These effects include the induction of Hsp27 and Hsp70, powerful antagonists of programmed cell death. However, in addition to HSR activation in cancer, tumor cells also appear to become “addicted” to basal expression of Hsp90 and its co-chaperones. Hsp90 appears to be required in order to permit accumulation of overexpressed and mutated oncogenes. However, other mechanisms may also be involved. At least in the case of Hsp90, effective classes of inhibitory drugs are available and are undergoing clinical trial in breast cancer treatment. For other arms of the HSR, agents are currently being developed although it is too early to assess their worth in treatment of breast cancer.

Cancer research is currently undergoing a major re-evaluation as its cell biological parameters come under increased scrutiny. Understanding the relative roles of cancer stem cells, progenitor cells and differentiated cancer cells in treatment response may alter our assessments of the molecular biology studies carried out on bulk populations of cells in recent years^{47, 48}. The role of HSF1 and HSPs in breast cancer stem cell growth, survival and response to treatment is currently unknown but may be highly significant in future approaches to breast cancer treatment.

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Figure 1. Role of the HSR in breast cancer

Activated nuclear HSF1 is depicted as orchestrating the expression of Hsp27 and Hsp70. These chaperones block the programmed cell death that often accompanies malignant transformation. In addition, Hsp90 carries out other functions in tumor progression including fostering the accumulation of oncoproteins. Oncoproteins may be mutated (oncogene) or overexpressed [oncogene, oncogene, oncogene]. Apoptosis can be inhibited by Hsp27 and Hsp70 in the cytosol or by TRAP-1 and / or mortalin in the mitochondria.