PROSPECTS
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Molecular Chaperones in Mammary Cancer Growth and Breast Tumor Therapy

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ABSTRACT

Heat shock protein (HSP) levels are elevated in breast cancer and are molecular targets for novel therapies. HSPs were first observed as proteins induced in massive amounts in normal cells exposed to stresses that lead to protein denaturation. Their expanded expression in mammary carcinoma appears to be largely due to the proliferation of malfolded mutant proteins and overexpressed oncoproteins that trigger transcription of *HSP* genes. HSPs play major roles in malignant transformation and progression mediated through their intrinsic molecular chaperone properties. These permit the emergence of new malignant traits through the facilitated accumulation of altered oncoproteins. The elevation of HSP concentrations in mammary carcinoma is at least partially dependent on heat shock transcription factor 1 (HSF1), a protein that responds to unfolded proteins and leads to HSP transcription. HSF1 activation has additional downstream activities, crucial for emergence of the breast cancer phenotype and these include activated cell signaling, HSP-mediated ability to evade apoptosis and senescence and an HSF1-dependent bias in transcriptional activity towards a metastatic phenotype. The HSPs are currently being targeted in breast cancer therapy and effective drugs for Hsp90 have been synthesized and evaluated in clinical trial. Mammary carcinoma cells also contain abundant quantities of HSP–tumor antigen complexes and these complexes are being used to develop effective tumor vaccine approaches that provide personalized therapy for each individual's cancer. J. Cell. Biochem. 113: 1096–1103, 2012. © 2011 Wiley Periodicals, Inc.

KEY WORDS: MOLECULAR; CHAPERONE; BREAST; CANCER; GROWTH; THERAPY

Heat shock proteins (HSPs) are expressed at high levels in mammary carcinoma, play key roles in carcinogenesis and tumor progression and are becoming important targets for therapy. The HSPs were originally detected as highly abundant cellular proteins in cells exposed to proteotoxic stresses such as heat shock and are key components of the heat shock response (HSR) that leads to profound stress resistance [Lindquist and Craig, 1988]. HSPs were subsequently ascribed a novel cellular role as molecular chaperones, proteins that fold other cellular proteins into conformations that permit their structural, enzymatic, and signaling properties [Lindquist and Craig, 1988; Ellis, 2007]. HSPs carry out the housekeeping functions of folding polypeptides after translation, but are also induced in stress crisis conditions in order to refold proteins that become denatured in the cytoplasm [Ellis, 2007]. There are at least four distinct families of molecular chaperones, each with distinct roles in client protein folding, including the *hspa*, *hspb*, *hspc*, and *hspd* families. For some HSP client proteins, particularly those with enzymatic function, folding occurs stepwise with the initial steps mediated by *hspa* family chaperones such as Hsp70 and folding to the ultimate functional state accomplished by *hspd* protein Hsp90. Cellular proteins with highly dynamic functions that require flexible conformations are often stably bound to Hsp90 [Gray et al., 2008]. *HSP* genes have in common the dependence on transcriptional induction by a powerful, sequence-specific factor—*h*eat *s*hock *f*actor 1 (HSF1) [Calderwood et al., 2010]. HSP genes all contain stress inducible, *cis*-acting elements in the 5′ promoter regions (HSE). Triggering of this response thus leads to HSF1 binding to HSE, vigorous transcription of the HSE-containing *HSP* genes, accumulation of high levels of the HSP protein products and resistance to stress [Calderwood et al., 2010]. HSF1 has recently been shown to be a key prognostic factor in breast cancer [Santagata et al., 2011].

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ELEVATED HSP LEVELS AND THE EVOLUTION OF MAMMARY CARCINOMA

It is evident that mammary cancer cells share similar gene expression profiles with heat shocked cells in that both express abundant levels of HSPs [Ciocca and Calderwood, 2005]. On initial reflection, one might look to the inhospitable tumor microenvironment, low in O_2 and glucose and high in CO_2 and metabolic acids as likely stressors to induce of HSPs in breast cancer. However, these deficits in the tumor milieu are more likely to activate the ER stress response, (triggered by low O_2 and high glucose) than the HSR. One may therefore have to look to more fundamental levels of oncogenesis to develop a hypothesis for HSP activation in cancer. Mammary cell transformation and tumor progression involve escaping from powerful regulatory influences that maintain the differentiated state in normal cells. Breaking these regulatory bonds requires novel genetic and epigenetic changes [Hanahan and Weinberg, 2011]. These alterations likely involve the reprogramming of gene expression in the malignant cell. Genetic alterations such as mutations and duplications are encountered in mammary carcinomata and accelerated during tumor progression; indeed cancer cells have been ascribed a "mutate phenotype" in which DNA repair mechanisms are progressively sacrificed to fuel growth, survival, and evolution [Fishel et al., 1993]. For instance the Tp53 tumor suppressor is inactivated in a wide range of cancers, a change that permits escape from programmed cell death at the cost of removing an important arm of the DNA damage response. It is thought that initial lesions in most cancers include mutations in genes whose products can activate growth (oncogenes) or inactivating mutations in tumor suppressor genes [Hanahan and Weinberg, 2011]. Most mutations are deleterious and can compromise cell viability due to expression of functionally inactive and potentially toxic proteins. However, the HSPs may be able to foster genetic variety by chaperoning these quasi-stable protein products of mutant genes. Hsp90 for instance has been ascribed a capacitator role in evolution due to its ability to stabilize the structures of mutated proteins [Queitsch et al., 2002; Whitesell and Lindquist, 2005]. Chaperones may thus permit accumulation of the available phenotypes required for cancer cell populations to escape physiological control mechanisms, become transformed and grow into tumors, escape limiting environments, and colonize new tissues and organs [Queitsch et al., 2002; Whitesell and Lindquist, 2005]. Mammary tumor cells may thus be selected for ability to express high levels of the molecular chaperones required to evolve into cancer cells. Indeed it has been observed that free Hsp90 levels seem to decrease in tumor cells presumably due to the expansion of chaperone clients [Kamal et al., 2003]. In addition the availability of resistant cohorts of cells bearing HSP-chaperoned mutant proteins may permit survival, by a minority population of treatments such as chemotherapy or radiation therapy. Such survivors can repopulate tumors and take on profound resistance to further treatments. This hypothesis provides a rationale for the elevated HSP overexpression in breast cancer as well as evolutionary processes in tumor progression [Whitesell and Lindquist, 2005] (Fig. 1).

The concept of tumor progression proposes the gradual evolution from normal cells obeying regulatory signals—to minimally transformed cells—to highly malignant cancer cells triggered by an expanding mutation repertoire and fostered my molecular chaperoning of resultant unstable variants [Vogelstein and Kinzler, 1993]. The hypothesis envisions an early transformed state in which cells begin to proliferate and form a population large enough for chance mutation to drive progression. There is considerable evidence in favor of the hypothesis including accelerated development of cancer in patients with inherited mutations in known tumor suppressor genes [Hanahan and Weinberg, 2000]. However, breast tumor progression is not necessarily a linear process and tumors may contain multiple competing subpopulations that attain the properties of independent growth, evasion of death, invasion, and metastasize in any temporal order. Subpopulations may thus compete for growth within the tumor milieu, and chaperone-mediated genetic variety may play an important part in this process (Fig. 1). Indeed there is growing evidence that initiation of mammary cancer may be a property of minority tumor cell subpopulations [Visvader, 2009]. Such tumor initiating cells (TIC) may be derived from tissue stem cells or pluripotent progenitors. Indeed in breast cancer, a population of TIC with a cell surface marker pattern found in tissue stem cells ($CD44^{high}CD24^{low}$) have been discovered. However, transformation of such mammary tissue stem cells to cancer stem cells likely requires genetic or epigenetic

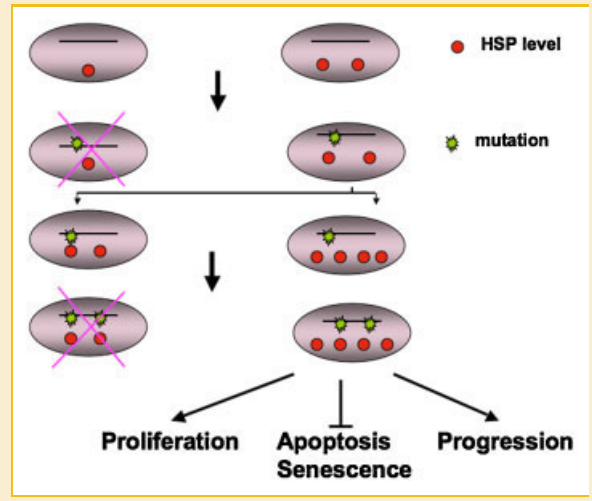

Fig. 1. Role of HSPs in the evolution of breast carcinogenesis. We show a normal population of mammary cells containing range of HSP concentrations. In a small percentage of the population a tumor initiating mutation occurs, leading to the expression of mutant oncoproteins. Higher levels of HSPs will be required to chaperone the aberrant conformation of the oncoprotein, favoring outgrowth of cells with elevated HSPs. Tumorigenesis often involves multiple mutational events and these will select for cells with progressively higher HSP levels to deal with the protein stress. Elevated levels of HSPs thus permit the build up of genetic diversity in the mammary tumor cell population, helping to drive evolution of the tumor phenotype and permit response to adverse conditions and tumor therapy. Elevated levels of HSPs protect the emerging tumor cells from apoptotic death and senescence, drive proliferation by chaperoning oncogenes. In addition, elevated levels of HSPs and HSF1 will promote tumor progression by permitting cell survival in the circulation and repressing regulatory genes that may inhibit invasion and metastasis. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

changes similar to those mooted in the tumor progression hypothesis and thus implies a role for elevated HSPs in the process. Tumor-derived TIC offer a significant challenge to tumor therapies and may evade killing due to their slow growth rates, ability to expel cytotoxic drugs due to high levels of ABC-cassette family surface transporters, activation of DNA repair pathways, and resistance to immunotherapy. We have found that in spontaneous mouse mammary carcinomas that cells with a TIC phenotype metastasize early, are profoundly radioresistant phenotype and express elevated levels of HSPs (Gong and Calderwood, in preparation). A fully inclusive hypothesis for mammary cell transformation, treatment resistance, and HSPs in mammary carcinoma at this stage is thus uncertain and a hypothesis involving breast cancer population biology and the genetic changes associated with the bulk population would be desirable.

HEAT SHOCK FACTOR 1: A KEY PROTEIN IN BREAST CANCER

As the molecule deployed at the head of the HSP induction cascade, HSF1 might be expected to play a key role in mammary oncogenesis. Indeed elevated HSF1 mRNA and protein levels have recently been shown to be associated with reduced breast cancer survival [Santagata et al., 2011]. HSF1 appears to play a key role downstream in carcinogenesis induced by a range of oncogenic conditions and hsf1−/− mice are resistant to developing multiple types of cancer [Dai et al., 2007; Min et al., 2007]. Thus we envisage a scenario in which HSF1 is activated by the proliferation of chaperone clients such as mutated, overexpressed oncoproteins within mammary cells and cooperates directly with oncogenic factors in transformation. HSF1 is repressed under physiological conditions by a complex containing Hsp90 and accessory co-chaperones, and the increases in HSP90 clients may de-repress HSF1 as in the HSR. In addition, we have shown that HSF1 can be induced by a cascade response downstream of the HER2 pathway by oncogenic cytokine heregulin [Khaleque et al., 2005]. A third mechanism that could contribute to HSP expression is loss of p53 proteins. The *HSP70* promoter is repressed by proteins such as Tp53 and loss of these proteins, a common event in cancer may contribute to chaperone increases in mammary carcinoma [Agoff et al., 1993]. These mechanisms may thus operate singly or additively in increasing molecular chaperones in breast cancer (Fig. 2).

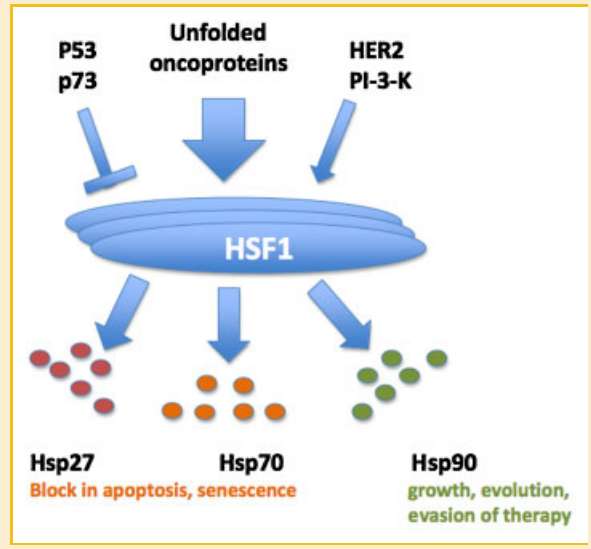

Fig. 2. Induction of HSP expression in mammary carcinoma. Elevated expression of mutated/overexpressed oncoproteins activates HSF1 and leads to elevated HSP expression in mammary carcinomata. HSP expression may also be activated through breast cancer signaling pathways altered in cancer, including the HER2/PI-3-kinase pathway. The Hsp70 gene is repressed by p53 family proteins either through HSF1 or by direct effects on the HSP70 promoter and these effects are reversed in cells in which p53 proteins are inactivated. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

MOLECULAR CHAPERONES AND THE DEFINING PROPERTIES OF MAMMARY CARCINOMA CELLS

A number of key traits permit normal cells to acquire a malignant phenotype. We will discuss the roles of HSP molecular chaperones elevated during tumorigenesis in mediating these properties.

HSPs AND UNPROMPTED MAMMARY CELL PROLIFERATION

Malignant cells can become independent of stromally derived growth factors through processes such as the direct expression/secretion of growth factors by the cancer cell or molecular changes in receptors that make them independent of ligand binding. For instance, overexpression or mutation of the cell surface protein HER2 makes breast cancer cells independent of HER2 activating cytokines such as epidermal growth factor (EGF) or heregulin. Receptor tyrosine kinases contain conserved catalytic phosphotransferase domains that can be prone to unfolding and dependent on molecular chaperones [Whitesell and Lindquist, 2005; Gray et al., 2008]. Super-expression and mutation of oncogenic proteins including protein kinases, during transformation thus leads to mammary tumor cells dependent on elevated levels of chaperones, with Hsp90 of particular note [Whitesell and Lindquist, 2005]. A fostering role for Hsp90 has been proven in principle in studies of oncogenic EGF receptor mutant proteins that become dependent on Hsp90 for expression in cancer cells [Trepel et al., 2010]. Hsp90 plays a broad role in chaperoning the structures of over 200 client proteins, many of which are key regulatory proteins in growth control and chaperone fosters tumor cell proliferation through this mechanism. Although the levels of Hsp90 are already very high in normal and malignant cells (approximately 2% of cell protein) malignant cells appear to have a critically reduced level of free Hsp90 due to the expansion of oncogenic clients [Kamal et al., 2003]. Hsp90 thus appears to act as facilitator of mammary tumor growth by chaperoning the proteins involved with stimulus-independent growth.

ELEVATED HSP LEVELS AND ESCAPE OF MAMMARY CANCER CELLS FROM APOPTOSIS

Although the HSPs participate in the HSR largely through maintaining protein quality control, they also play other protective

roles including inhibition of programmed cell death pathways such as caspase-dependent apoptosis [Garrido et al., 2006]. Most notable in this regard are the *hspb* family member Hsp27 and Hsp70 which, when expressed at high levels, as in mammary carcinoma block progression towards apoptotic death during stress [Garrido et al., 2006]. Inhibition of apoptosis is required in the HSR for cells to survive transient stress-induced apoptotic triggers while HSP-mediated refolding of the proteome and removal of such triggers occur. Diminution of apoptotic rate is a common finding in mammary carcinoma and appears to be required, among other things to counteract the effects of expressing transforming oncogenes, alterations that can trigger death in normal mammary cells [Hanahan and Weinberg, 2011]. Tumor cells invoke a range of strategies to avoid apoptosis, including reduced expression of the pro-apoptotic Tp53 pathway, increased expression of anti-apoptotic Bcl_2 family proteins and elevated HSP expression [Hanahan and Weinberg, 2011]. Thus oncogene-dependent cell death is evaded, cell birth begins to dominate and clonal expansion within tumors takes place. At high intracellular levels, Hsp70 has been shown to bind and inhibit a number of mediators of the apoptotic cascade including c-*jun* kinase, apaf-1, and caspase 8, while Hsp27 can block cytochrome *C* release [Paul et al., 2002; Garrido et al., 2006]. This phenomenon has been linked directly to HSF1 whose activity is induced by the transforming cytokine heregulin in breast carcinoma, leading to elevated Hsp60, 70, and 90 expression and escape from apoptotic cell death [Khaleque et al., 2005]. Thus we can envisage a scenario in which protein stress in the evolving mammary cancer cell triggers HSF1 activation, HSP transcription, and dampens the apoptotic cascade.

INCREASES IN HSP70 AND HSP27 PERMIT MAMMARY CANCER CELLS TO EVADE SENESCENCE

Normal cells have a limit on the number of cell divisions permitted and on exceeding this number undergo senescence, a permanent state of dormancy [Campisi, 2005]. For tumor cells to proliferate in an unlimited manner and undergo clonal expansion, they must therefore overcome this barrier. Senescence is governed by the length of chromosomal telomeres that shorten with succeeding cell divisions until reaching a limiting length that triggers the senescence pathways. These pathways involve the participation of Tp53 and Rb-dependent inhibition of proliferation [Campisi, 2005; Hanahan and Weinberg, 2011]. Senescence can be avoided by the expression in breast cancer cells of the enzyme telomerase that can rebuild the telomere and maintain prolific levels of proliferation [Stewart et al., 2002; Jaskelioff et al., 2009]. Molecular chaperones are involved in promoting this process, as Hsp90 is an essential accessory protein of telomerase. In addition, Hsp70.2, a member of the Hsp70 family expressed normally during spermatogenesis is observed at high levels in mammary carcinoma and inhibits senescence by blocking the p53- and p21-dependent pathways of senescence [Rohde et al., 2005]. In a further series of studies Sherman et al. [2010] have shown the key importance of other Hsp70 family members in mammary tumor progression by deterring senescence. Hsp70 was shown to regulate both p53-dependent and -independent senescence downstream of the HER2 and Ras signaling pathways. In the absence of Hsp70, expression of Her2 failed to transform normal mammary epithelial cells and cells underwent senescence both in vitro and in vivo in a pathway that involved upregulation of Cdk kinase inhibitor p21 and down regulation of survivin [Sherman et al., 2010]. Hsp70 family members including Hsp70.2, Hsp72, and the mitochondrial Hsp70 chaperone mortalin appear to have a general inhibitory effect on senescence that seems to be of key importance in mammary carcinogenesis [Wadhwa et al., 2002; Rohde et al., 2005; Sherman et al., 2010]. In addition, Hsp27 plays a similar role in deterring senescence in breast cancer [O'Callaghan-Sunol et al., 2007].

INVASION, METASTASIS AND TUMOR PROGRESSION

Mammary epithelial cells are maintained in the appropriate tissue location determined by a cell surface "area code" and tightly bound by the extracellular matrix [Hanahan and Weinberg, 2011]. Thus escape from this type of regulation; invasion through the imprisoning extracellular matrix, entry into the circulation, and colonization of distant organs requires a profound alteration in cellular properties. Enhanced metastasis may involve the increased evolutionary potential offered by elevated HSP expression permitting increased proliferation, reduced cell death during transit through the microcirculation, and expression of pro-metastatic genes (Fig. 1). Our studies additionally show that HSF controls the activity of the co-repressing factor *m*etastasis *a*ssociated *p*rotein 1 (MTA1), a factor that promotes metastasis in breast cancer [Khaleque et al., 2008] (Fig. 3). HSF1 is known to possess gene repression activity in addition to activating HSP expression. MTA1 is a component of the NuRD co-repressor complex containing multiple proteins such as the DNA remodeling protein Mi2a as well as histone deacetylases 1 and 2 [Mazumdar et al., 2001]. This complex has been shown to decrease anti-metastatic gene transcription in breast cancer [Mazumdar et al., 2001]. MTA1 is coordinately regulated with HSF1 in breast cancer and the factors become co-associated on chromatin at *loci* such as *presenilin 1* and *c-Myc* which are associated with decreased metastasis [Khaleque et al., 2008]. It is likely that HSF1 becomes activated by the protein stress encountered in breast cancer cell and that the effects of HSF1/MTA1 on pro-metastatic transcription are "collateral damage" due to HSR

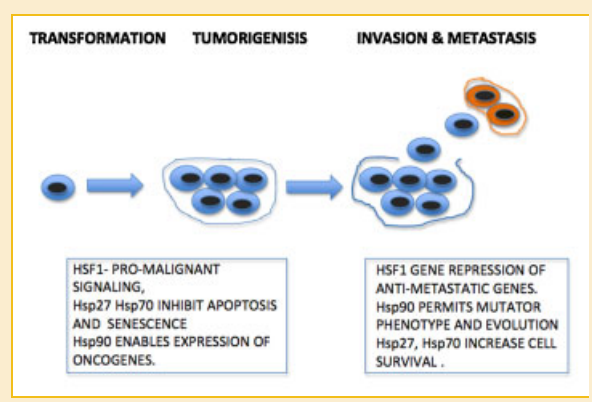

Fig. 3. Roles for HSF1, Hsp27, and Hsp90 in mammary cell carcinogenesis and breast cancer progression. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

activation (Figs. 1 and 2) with the happy consequence for the cancer cell of enhanced metastatic potential. In addition, our studies also show that HSF1 and MTA1 can be activated by heregulin-triggered signaling pathways including the HER2/*neu* and PI-3 kinase/Akt cascades [Khaleque et al., 2005, 2008]. Cells exposed to heregulin appeared to undergo changes resembling the epithelial–mesenchymal transition (EMT) in an HSF1-dependent manner [Khaleque et al., 2008]. MTA1 and the NuRD complex also play roles, along with Polycomb factors in maintaining pluripotency in normal and malignant cancer stem cells through repression of genes required for differentiated function [Liang et al., 2008]. It will be interesting to discover, in future studies whether HSF1/MTA1 complexes participate in stem cell maintenance.

EXTRACELLULAR HSPs AND ESCAPE FROM IMMUNOSURVEILLANCE

Mammary tumor cells express tumor-associated antigens which may be mutant proteins as well as re-expressed embryonoic antigens such as MUC1 and can be recognized by activated CD8+ T cells [Pardoll, 2003]. However it is apparent that such tumors escape immunosurveillance, grow and can progress to kill their hosts. Mechanisms employed by cancer cells to escape immune killing include ''loss of self'' in which surface MHC class I molecules fail to become expressed on the surface of tumor cells, secretion of immunosuppressive interleukin 10, and transforming growth factor-beta [Chouaib et al., 2002]. Recent studies have shown that extracellular HSPs have profound immunomodulatory functions and that Hsp70 can be secreted by tumor cells [Mambula and Calderwood, 2006]. Extracellular HSPs can either activate or inhibit immunity depending on the context in which immune cells encounter the proteins. The effects of constitutively secreted extracellular Hsp70 are likely to aid in the escape from immunosurveillance and include induction and release of immunosuppressive cytokine IL-10 and activation of immunoregulatory T regulatory Foxp3^{+}CD25^{+} cells that suppress cytotoxic lymphocytes. It has also been shown that tumors can secrete Hsp70 embedded in exosomes and that these structures can interact with dendritic cells (DC) to down-modulate immune responses [Chalmin et al., 2011].

MALIGNANT PROPERTIES OF MOLECULAR CHAPERONES AND CANCER TREATMENT

TARGETING MOLECULAR CHAPERONES WITH INHIBITORY DRUGS

HSPs appear to be induced in breast cancer and other malignancies by both HSF1-dependent and -independent pathways involving p53-family proteins and may respond both to the accumulation of incompletely folded proteins as well as being hijacked by malignant cell signaling [Agoff et al., 1993; Kamal et al., 2003; Khaleque et al., 2005]. HSP accumulation in cancer and during the HSR has a key difference: in the HSR, HSP induction is transient and decays when denatured proteins are unfolded. In breast cancer, most of the lesions involve covalent changes and such proteins cannot be refolded and require permanent chaperoning. Inhibition of chaperone function will lead to the degradation of oncoproteins requiring chaperone activity and loss of growth promoting signals. HSPs are therefore excellent candidates for chemical targeting in mammary cancer. The initial studies have involved inhibiting Hsp90 activity in cancer using drugs based largely on the natural product drug geldanomycin that inhibits the ATPase activity of Hsp90 [Neckers, 2002; Workman, 2004]. Indeed at least 13 Hsp90 inhibitors are undergoing clinical trial in a range of malignancies [Trepel et al., 2010]. Hsp90 inhibitor 17-AAG has been used to treat HER2 positive breast cancer following Trastuzumab treatment in a phase II clinical trial with positive results. In addition, Hsp90 inhibitors may find use in treating triple negative breast cancer [Trepel et al., 2010]. These drugs have the potential to cause cytostasis and cell death by down-regulating levels of multiple growth promoting proteins and to block evolutionary processes leading to the development of treatment resistance in tumors by preventing accumulation of variant proteins. The multi-targeting activity of Hsp90 drugs may thus deter the development of resistance observed in agents that target a single activity. As mentioned above, Hsp70 family proteins can inhibit programmed cell death and senescence in breast cancer and are thus strong potential candidates for pharmacological targeting [Powers et al., 2011]. In addition, as with Hsp90, Hsp70 may be involved in chaperoning mutant oncoproteins arising during transformation and may participate in mammary tumor cell evolution [Powers et al., 2011]. A number of compounds have been shown to inhibit Hsp70 proteins and this area has considerable promise, since Hsp70, being overexpressed in mammary carcinoma will render tumor cells selectively vulnerable [Powers et al., 2011]. Most cellular organisms express a range of hsp70 proteins encoded by different genes. As we mentioned earlier, Hsp72, Hsp70.2, and mortalin are elevated in breast cancer and can reduce apoptosis and replicative senescence [Wadhwa et al., 2002; Rohde et al., 2005]. In addition, Powers et al. [2008] showed recently that silencing Hsp72 and the constitutive Hsp70 protein Hsc70 selectively induce apoptosis in malignant cells as well as causing proteasomal degradation of Hsp90 clients CDK4 and Her2. Hsp70 and Hsp90 cooperate in the folding of their clients and targeting one chaperone is likely to interfere with the function of the other. In addition, in order to complete their folding cycles, Hsp70 and Hsp90 utilize accessory proteins known as co-chaperones that facilitate interaction of primary chaperones with client polypeptides and adenosine nucleotides and promote interactions between chaperones [Gray et al., 2008]. Without co-chaperones such as DNA-J family proteins, nucleotide exchange factors such as the BAG protein family and scaffold proteins such as Hop, chaperone function is reduced to a fraction of normal activity [Gray et al., 2008]. Several of these factors have been investigated as drug targets in cancer and seem promising candidates for future drug discovery [Gray et al., 2008]. Cdc37 is elevated in a number of tumor types and in fact functions as an oncogene, offering therapeutic advantage to as a tumor target [Gray et al., 2008]. Hsp27 is the most abundantly expressed chaperone in mammary carcinoma although effective drugs are not currently available [Ciocca and Calderwood, 2005]. However, studies using Hsp27 silencing suggest the potential of this approach [Kaur et al., 2011].

As the protein at the head of the HSP cascade, HSF1 could be a major target in cancer therapy and lead to loss of the whole HSR. Indeed silencing HSF1 or HSF1 inactivation prevents tumor development in breast cancer and other cancers [Dai et al., 2007; Min et al., 2007]. A number of drugs have been described as selective

for HSF1 specific and studies are underway to determine their potential effectiveness [Zaarur et al., 2006].

Targeting HSF1 and HSP products with drugs is thus an attractive proposition and offers the potential of inactivating many of the unique characteristics of tumor cells. At first evaluation, the approach would seem somewhat non-specific due to the housekeeping functions of HSPs in protein folding. However, specificity is provided by the findings that cancers often have markedly elevated levels of chaperone clients making malignant cells more dependent on HSPs and HSF1. In addition, many of the HSPs are expressed to high level in cancer, again making mammary cancer cells more susceptible to inactivation.

USING THE IMMUNE PROPERTIES OF MOLECULAR CHAPERONES

When used as vaccines, HSPs possess profound immunostimulatory properties based on their status as endogenous danger signals and ability to chaperone antigenic peptides in the intracellular milieu [Murshid et al., 2011]. These properties have led to their use in various approaches to immunotherapy. In one such approach HSPs such as Grp96 or Hsp70 are extracted from tumors by affinity chromatography in association with a repertoire of tumor antigens and used as autologous vaccines. This approach has the advantage of a personalized, autologous vaccine carrying a range of antigenic epitopes, apparently with some adjuvant activity based on innate immune stimulation by the HSP itself. Mammary tumors contain abundant levels of HSPs chaperoning the expanding levels of oncoproteins as in Figure 1, an ideal source for harvesting HSP-antigen complexes. Clinical trials have been carried out on HSP-based vaccines suggesting that the approach is safe and potentially powerful although no trials have currently addressed breast cancer (Fig. 4). We have used a variation on this theme, using breast cancer cells fused to DC as the source for Hsp70-peptide complexes (known as Hsp70.PC-F or *Hsp70.peptide complexes from fusion cells*) [Gong et al., 2010]. The resultant vaccines were potent growth inhibitors in murine tumors expressing the epitope MUC1 in vivo and against human breast carcinoma cell lines in vitro. This preparation has potential as a common vaccine targeting breast cancer using pooled DC and breast cancer cell lines to prepare tumor DC fusions [Gong et al., 2010]. Another variation on this theme utilizes the larger heat shock proteins Hsp110 and Grp170 that have expanded polypeptide-binding domains and can chaperone whole tumor antigens at high affinity when loaded in vitro. Hsp110 has been used to chaperone the Her2 tumor antigen and provide potent tumor immunity [Manjili et al., 2003]. This approach has the advantage of using a high concentration of tumor antigens within the peptide binding domains of Hsp110 or Grp170 in the context of innate immune activity of the HSPs [Manjili et al., 2003]. The method possesses the disadvantage of requiring pre-knowledge of tumor antigen expression in the target tumor. HSP-based vaccines are thus highly promising. Future studies will likely see the HSP vaccines deployed with adjuvant molecules such as double stranded RNA, Cpg DNA, and anti-CTLA-4 antibodies that stimulate innate immunity and activate T cell programming [Murshid et al., 2011]. In addition, we are aiming to combine Hsp70.PC-F vaccines with ionizing radiation for use in mammary carcinoma. One interesting possibility for using molecular chaperone vaccines may be the ability to target specific tumor cell populations such as CSC. We have shown that tumor–DC fusion can preferentially target CSC after selecting cancer cells with CSC surface markers for use in vaccines [Weng et al., 2010]. As previous studies in melanoma indicate that CSC are resistant to vaccines targeting tumor-associated antigens, HSP vaccines prepared from CSC may thus attack a specific antigenic vulnerability in these cells [Murshid et al., 2011]. We are currently attempting to target CSC in mammary carcinoma by the Hsp70.PC-F vaccine approach.

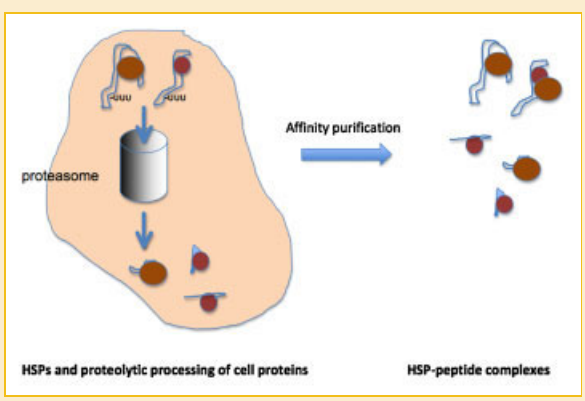

Fig. 4. Molecular chaperone vaccines. Hsp70 and hsp90 bind to intracellular proteins in mammary carcinoma cells and transport them to the proteasome, where they are hydrolyzed to smaller peptides. Intracellular Hsp70 and Hsp90 can then bind and chaperone the processed peptides. The chaperones complexed with processed and unprocessed polypeptide antigens can then be harvested from cells after lysis and used to prepare molecular chaperone anti-breast cancer vaccines. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

CONCLUSIONS AND PROSPECTS

It is increasingly evident that activation of the HSF1-mediated transcriptome plays a critical role in breast cancer initiation and progression. The next few years should give us a fuller understanding of how HSF1 activity is triggered in cancer and the role of its transcriptional products, the HSPs in the individual stages in tumorigenesis. Laboratory studies and clinical trials with Hsp90 inhibitors have established the principle of targeting members of the HSF1 transcriptome with chemical inhibitors. We may soon see the development of effective inhibitors for HSF1, Hsp27, and Hsp70, proteins regularly overexpressed in mammary carcinoma. Inhibiting HSPs effectively, targets multiple oncogenic pathways in the cell and promotes tumor apoptosis and senescence. An alternative approach to chemical inhibitors is to utilize the abundant HSP–tumor antigen complexes in immunotherapy with chaperone-mediated vaccines. Recent clinical trials have proven the vaccines safe and effective. Future treatment of breast cancer will likely involve HSP vaccines combined with conventional therapies such as ionizing radiation in order to treat both primary and disseminated disease.

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