

The role of HSPs in mammary neoplasms. A short review

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Heat shock proteins (HSPs, stress proteins) are an ancient and evolutionarily conserved class of proteins that act as molecular chaperones guiding the normal folding, intracellular disposition and proteolytic turnover of many of the key regulators of cell growth, differentiation and survival. These functions are subverted during oncogenesis to allow malignant transformation. Being regulators of oestrogen receptor (ER), members of HSP27, HSP70 and HSP90 families have been extensively studied in human breast cancer, in which they appears to be implicated in all the cancerogenetic mechanisms (tumor cell proliferation, differentiation, invasion, metastasis, death, and tumor immune response).

What is the role of stress proteins in breast carcinogenesis? The expression of HSPs in breast cancers is correlated with increased cell proliferation. On the other hand it has been shown that selective depletion of several HSP results in activation of the apoptotic event. Most HSPs such HSP70 and Hsp90 alpha isoform has demonstrated to play many important functions in the regulation of the cell cycle, by controlling the activity of several signalling proteins, specially cyclins and retinoblastoma protein (pRb), binding to these clients and regulating their stability and function. Moreover most of its known substrates are key components of the cellular apoptotic and signal transduction pathways (Wnt, ERBB e Notch), such as steroid hormone receptors, Raf-1, Wee-1 and Akt kinases, which are critically dependent on Hsps for their maturation and conformational maintenance. Many of the proteins chaperoned by Hsp90 are involved in breast cancer progression and resistance to therapy, including the estrogen receptor, receptor tyrosine kinases of the erbB family, Akt, and mutant p53. HSPs function at multiple points in the apoptotic signalling pathways, modulating both the intrinsic and extrinsic pathways. HSP 27 binds to cytochrome c, HSP 70 and HSP90 bind to Apaf1 preventing caspase 9 maturation. They can influence the events further downstream. In contrast HSP 60 and HSP 10 can directly promote the proteolytic maturation of caspase 3. HSP 27 inhibits Daxx apoptotic pathway, while HSP 70 binds to JNK1 resulting in inhibition of JNK activation. HSP 90 interacts with RIP 1 kinase and AKT resulting in both cases in promotion of NF- κ B mediated inhibition of apoptosis. Hsp70 is abundantly expressed in human tumors and tumor cell lines, and its specific depletion resulted in massive cell death of all tumorigenic cells. These results indicate that the high expression of Hsp70 is a prerequisite for the survival of human cancer cells.

HSPs expression in breast cancer are not particularly informative at the diagnostic level, although their expression has been analyzed in relation to the histopathological characteristics of the tumor tissues (e.g. tumor type, grade of differentiation, with the degree of proliferation), and with patient parameters (sex and age). In addition, Hsps can be significantly associated with other molecules. (e.g. estrogen receptors, cmyc, mutated p53). Regarding the latter, Hsps are generally coexpressed in breast cancer tissues and this is mainly due to their physiological role in the assembly and trafficking of steroid receptors. Hsp27 expression has been extensively studied in human breast cancer, and given the identity between this HSP and a cytosolic estrogen receptor-associated protein there is correlation with estrogen receptor levels, while this protein did not appear associated with PR in female cancers or with ER α in male breast carcinomas. However, other findings indicate that some but not all ER-positive breast tumours express Hsp27. There is no significant or marginal correlation between hsp27 expression and histological grade with well differentiated tumors or with the proliferation marker ki-67. The inducible form of the Hsp70 family result associated with poor differentiation and the presence of mutated p53 in breast cancers. Its nuclear staining pattern was reported to be correlated with tumor size. Moreover, a strict correlation between Hsp70 levels and elevation of estrogen receptor has been detected. HSP 90, as fundamental component of steroid receptor multimolecular complex, show positive relationship with ER and it seems to be more expressed in poorly differentiated carcinomas. This HSP seems also involved in proliferation of human breast cancer, since levels of Hsp90 α , appear positively correlated with cyclin D1 expression in this type of tumour.

High expression of heat shock protein 27 and HSP 70 in breast cancers correlates with lymph node involvement. The surface expression of HSPs differentially regulates metastasis having cells with high Hsp25 surface expression metastasized to the lungs more aggressively than either wild-type Hsp25 cells or Hsp72 (+) cells. Also $\alpha\beta$ crystallin expression is strongly associated with lymph node

involvement, and increased intensity correlate with shorter survival, but it is not an independent of node status as predictor of survival

Regarding the prognostic implications of Hsps in breast cancer, Hsp27 is not among the list of useful prognostic factors in breast cancer since the many studies have produced conflicting results. In fact, both the association between Hsp27 overexpression and more aggressive tumour or better prognosis has been detected, particularly in the early stage of breast cancer. Hsp27 levels appear to correlate with different biological features in early and advanced breast cancer, being linked with short disease-free survival in node-negative patients but with prolonged survival from first recurrence. Nevertheless, other studies have failed to detect a correlation between Hsp27 expression and response to hormone therapy or with DFS or OS. At the contrary, High Hsp70 expression is correlated with poor prognosis in breast cancer. This is consistent with the Hsp70 associations with some diagnostic parameters of malignancy. Hsp90 expression in breast cancer tissues and presence of autoantibodies to Hsp90 have been correlated with poor prognosis in breast cancer. It is evident that we need more studies on Hsps to confirm whether they have prognostic value in breast cancers.

A growing body of evidences point out that high HSP27 and HSP 70 expression may render tumours strongly resistant to a number of chemotherapeutic agents (for example, doxorubicin), so they can be useful predictive factors. In this respect, it has to be remarked that many chemotherapeutic drugs can also induce their expression, thus potentially increasing cancer cells resistance by up-regulating anti-apoptotic factors. Although the expression of Hsp27 correlated with that of ER α in breast cancer, its detection does not predict the response to tamoxifen. Moreover, high Hsp70 levels predicted lower response of breast cancers to radiation and hyperthermia.

Treatment implication of Hsps represents a new and very promising approach in the treatment of breast cancer. The greatest number of study has been directed to investigate if Hsp90-binding drugs could be effective in destabilization and reduction of estrogen receptor levels, which are a prominent target for the treatment of hormone-dependent cancer become refractory to classical hormonal therapy with anti-estrogens agents. Regarding agents that modify the molecular levels or molecular capabilities of the Hsps this is achieved by the inhibition of Hsp90 by the natural product such as geldanamycin or the geldanamycin analogous 17AAG. In the phase I clinical trials on cancer patients, 17AAG produced in some patients less proliferation of the tumors but with lower potency than radiotherapy or chemotherapy with which can be used in combination. These drugs target the nucleotide-binding site in the N-terminal domain of Hsp90 that is required for his function, causing inhibition of the binding of Hsp90 to the client proteins. Several other natural products, herbimycin A and radicicol bind to this pocket and inhibit HSP90 function. 17AAG has a 100-times higher affinity towards the tumor-specific Hsp90 complexed by a large number of co chaperones than to the Hsp90 dimer, which is the predominant form of this chaperone in normal cells. Geldanamycin binding to HSP90 locks the chaperone in an alternative conformation that prevents normal cycling and the formation of mature chaperone complexes. The ER accumulates in an intermediate complex that recruits E3 ubiquitin ligase and drives proteasome-mediated degradation of the protein, thereby dramatically lowering cellular levels of the receptor and disrupting its function. Hsp90 inhibitors completed five Phase I clinical trials and are entering to phase II trials. Nevertheless, it seem clear that combination therapies, applying low doses of Hsp90 inhibitors together with conventional chemotherapeutic agents (such as Taxol), seem to be an effective way to target various cancers. Besides, the availability of an in vivo model for further testing Hsp90-targeted cancer therapy appears to be essential, because of recent evidence of potential contraindication to this therapy, since the 17-AAG appeared to enhance bone metastasis of a human breast cancer cell line following intracardiac inoculation in the nude mouse.

HSF-1, Hsp27, Hsp70, and grp78 are also targets of antisense oligonucleotide therapies or other manipulations with possibilities for anticancer therapies. All elements of the HSF-1 activation and down regulation cascade, or the additional proteins Ralbinding protein 1, tubulin and p23 are of great interest as potential drug targets. These approaches are still at the preclinical level.

Hsps may also provide a tempting target for immunotherapy protocols because they are able to chaperone tumor antigens and act as biological adjuvants to break immune tolerance to tumor antigens and cause tumor regression. The purpose is to elicit in a cancer patient a specific immune response against its own tumor using tumor-derived Hps (gp96, Hsp70, HSP90 and calreticulin) covalent binding with the specific tumor peptides as natural adjuvants that present to the immune system the molecules that have shielded the potential epitopes from immune recognition. When injected the Hsps interact with receptors on the antigen presenting cells (dendritic cells, macrophages) as CD91, which act as a global receptor for HSPs. These cells processed the antigen(s) inducing a specific cytotoxic T lymphocyte response and the production of proinflammatory cytokines.

In veterinary medicine as concerns HSPs, besides the researches carried out on laboratory animals, only two studies directed to investigate HSPs expression in neoplastic canine mammary tissues have been reported. In the first Hsp60 and Hsp70 were undetectable while a recent Western Blotting study has demonstrated a similar pattern of changes in Hsp70 and Hsp90 and apoptosis-associated proteins, in both human and canine mammary tumours. In our recent unpublished studies we evaluate the immunohistochemical expression of different HSPs in canine malignant mammary tumours at different histological stages, trying also to correlate this expression with both the histological stage of the neoplasms and the overall survival of the animals. These studies demonstrated a significant increase of HSP27, HSP72 and HSP90 expression, as well as a significant reduction of HSP73 in comparison to normal mammary gland. In particular, HSP27 and HSP72 appeared to be strongly positive in infiltrating tumour cells of invasive stages, where HSP27 appeared to be significantly correlated with a shorter OS. HSP90 expression was high in all stages and, like HSP73, it showed an intense positivity in lymphatic emboli.

These results suggest that HSP27, HSP72 and HSP90 could be involved in carcinogenesis of canine mammary gland, and the similar pattern of changes in Hsps in human and canine mammary tumors validate use of the canine model of study for human breast cancer.

Selected references. (A more detailed list of references is available from the author)

1. Beere HM: Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *J Clin Invest* 2005, 115:2633-2639.
2. Beliakoff J, Bagatell R, Paine-Murrieta G, Taylor CW, Lykkesfeldt AE, Whitesell L: Hormone-refractory breast cancer remains sensitive to the antitumor activity of heat shock protein 90 inhibitors. *Clin Cancer Res* 2003, 9:4961-4971.
3. Chu RM, Sun TJ, Yang HY, Wang DG, Liao KW, Chuang TF, Lin CH, Lee WC: Heat shock proteins in canine transmissible venereal tumor. *Immunol Immunopathol.* 2001, 82:9-21.
4. Ciocca DR, Calderwood SK: Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress Chaperones* 2005, 10:86-103.
5. Ciocca DR, Clark GM, Tandon AK, Fuqua SAW, Welch WJ, McGuire WL: Heat shock protein Hsp70 in patients with axillary lymph node-negative breast cancer: prognostic implications. *J Natl Cancer Inst* 1993, 85:570-574.
6. Ciocca DR, Green S, Elledge RM, Clark GM, Pugh R, Ravdin P, Lew D, Martino S, Osborne CK: Heat shock proteins hsp27 and hsp70: lack of correlation with response to tamoxifen and clinical course of disease in estrogen receptor-positive metastatic breast cancer (A Southwest Oncology Group Study). *Clin Cancer Res* 1998, 5:1263-1266.
7. Conroy SE, Latchman DS: Do heat shock proteins have a role in breast cancer? *Br J Cancer* 1996, 74:717-721.
8. Fuqua SAW, Oesterreich S, Hilsenbeck SG, Von Hof DD, Eckardt J, Osborne CK: Heat shock proteins and drug resistance. *Breast Cancer Res Treat* 1994, 32:67-71.
9. Goetz MP, Toft D, Reid J, Ames M, Stensgard B, Safgren S, Adjei AA, Sloan J, Atherton P, Vasile V, et al. Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. *J Clin Oncol* 2005, 23:1078-1087.
10. Helmbrecht K, Zeise E, Reinsing L: Chaperones in cell cycle regulation and mitogenic signal transduction: a review. *Cell Prolif* 2000, 33:341-365.
11. Kumaraguruparan R, Karunagaran D, Balachandran C, Manohar BM, Nagini S: Of humans and canines: A comparative evaluation of heat shock and apoptosis-associated proteins in mammary tumors. *Clin Chim Acta* 2005, 365:168-176.
12. Miyata Y: Hsp90 inhibitor geldanamycin and its derivatives as novel cancer chemotherapeutic agents. *Curr Pharm Des* 2005, 11:1131-1138.
13. Nylandsted J, Brand K, Jaattela M: Heat shock protein 70 is required for the survival of cancer cells. *Annals of NY Accademy of Sciences* 2000, 926:122-125
14. O'Neill PA, Shaaban AM, West CR, Dodson A, Jarvis C, Moore P, Davies MPA, Sibson DR, Foster CS: Increased risk of malignant progression in benign proliferating breast lesions defined by expression of heat shock protein 27. *Br J Cancer* 2004, 90:182-188.
15. Oesterreich S, Hilsenbeck SG, Ciocca DR, Allred DC, Clark GM, Chamness GC, Osborne CK, Fuqua SAW: The small heat shock protein Hsp27 is not an independent prognostic marker in axillary lymph node-negative breast cancer patients. *Clin Cancer Res* 1996, 2:1199-1206.

16. Price JT, Quinn JM, Sims NA, Vieusseux J, Waldeck K, Docherty SE, Myers D, Nakamura A, Waltham MC, Gillespie MT, *et al.* The heat shock protein 90 inhibitor, 17-allylamino-17-demethoxygeldanamycin, enhances osteoclast formation and potentiates bone metastasis of a human breast cancer cell line. *Cancer Res* 2005, 65:4929-4934.
17. Ramanathan RK, Trump DL, Eiseman JL, Belani CP, Agarwala SS, Zuhowski EG, Lan J, Potter DM, Ivy SP, Ramalingam S, *et al.* Phase I pharmacokinetic-pharmacodynamic study of 17-(allylamino)-17-demethoxygeldanamycin (17AAG, NSC 330507), a novel inhibitor of heat shock protein 90, in patients with refractory advanced cancers. *Clin Cancer Res* 2005, 11:3385-3391.
18. Solit DB, Basso AD, Olshen AB, Scher HI, Rosen N: Inhibition of heat shock protein 90 function down-regulates akt kinase and sensitizes tumors to taxol. *Cancer Res* 2003, 63:2139-2144.
19. Soti C, Nagy E, Giricz Z, Vigh L, Csermely P, Ferdinandy P: Heat shock proteins as emerging therapeutic targets. *Br J Pharmacol* 2005, 146:769-780.
20. Thanner F, Sütterlin MW, Kapp M, Rieger L, Kristen P, Dietl J, Gassel AM, Müller T: Heat-shock protein 70 as a prognostic marker in node-negative breast cancer. *Anticancer Res* 2003, 23:1057-1062.
21. Thanner F, Sutterlin MW, Kapp M, Rieger L, Morr AK, Kristen P, Dietl J, Gassel AM, Muller T: Heat shock protein 27 is associated with decreased survival in node-negative breast cancer patients. *Anticancer Res* 2005, 25:1649-1653.
22. Thor A, Benz C, Moore D, Goldman E, Edgerton S, Landry J, Schwartz L, Mayall B, Hickey E, Weber LA: Stress response protein (srp-27) determination in primary human breast carcinomas: clinical, histologic, and prognostic correlations. *J Natl Cancer Inst* 1991, 83:170-178.
23. Vargas-Roig LM, Gago FE, Tello O, Aznar JC, Ciocca DR: Heat shock protein expression and drug resistance in breast cancer patients treated with induction chemotherapy. *Int J Cancer* 1998, 79:468-475.
24. Welch WJ: Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiol Rev* 1992, 72:1063-1081.
25. Whitesell L, Lindquist SL: Hsp90 and the chaperoning of cancer. *Nat Rev Cancer* 2005, 5:761-772.
26. Workman P: Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone. *Cancer Lett* 2004, 206:149-157.
27. Yano M, Naito Z, Yokoyama M, Shiraki Y, Ishiwata T, Inokuchi M, Asano G: Expression of Hsp90 and Cyclin D1 in human breast cancer. *Cancer Lett* 1999, 137:45-51.