The Role of Heat Shock Proteins in Mammary Neoplasms: A Brief Review

Leonardo Della Salda, Mariarita Romanucci
Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, University of Teramo, Italy.
Email: ldellasalda@unite.it

Received July 13th, 2012; revised August 15th, 2012; accepted August 26th, 2012

ABSTRACT
Research into heat shock proteins (HSPs) for the clinical management of tumours has intensified as new evidence shows they can be used as biomarkers in carcinogenesis and are related to poor prognosis in some cancer types. Members of small HSP, HSP70 and HSP90 families have been studied extensively in breast cancer. This article reviews current understanding of the role of HSP and HSF-1 (Heat shock factor 1) expression in human breast cancer and looks at its potential diagnostic, prognostic and therapeutic value. The exciting progress that has been made using HSP 90 inhibitors in breast cancer treatment is examined and the results of preliminary studies on the expression of stress proteins in the animal model canine mammary tumours are also presented.

Keywords: Cancer; Stress Proteins; Stress Response; Mammary Tumour; Heat Shock Proteins

1. Introduction
Heat shock proteins (HSPs) are a highly conserved class of proteins normally expressed at low levels by all known eukaryote and prokaryote cells [1]. Their induction is mainly dependent on the activation of heat shock factor 1 (HSF-1) and its interaction with heat-shock regulatory elements (HSEs) present in the promoters of all HSP genes. HSPs are classified into several families according to their approximate molecular weight although a new nomenclature has recently been proposed [2,3]. HSPs often act in concert, in large multiprotein complexes known as molecular chaperones guiding the normal folding, intracellular disposition and proteolytic turnover of many of the key regulators of cell growth, differentiation and survival [4]. However, these functions are altered in oncogenesis allowing malignant transformation [5] with upregulation of stress-related genes and increased synthesis of intracellular and extracellular HSPs [6]. This results in HSPs being tumour-protective through mechanisms such as anti-oxidative processes, the prevention of protein denaturation, anti-apoptotic activity, and possibly the direct suppression of the immune system [7]. It has also been shown that stress proteins, including HSP70, participate in the folding of numerous protooncogene and oncogene products [8].

Small HSP, HSP70 and HSP90 families are involved in the regulation of oestrogen receptors (ER) and hence have been extensively studied in human breast cancer where they play a role in tumour cell proliferation, differentiation, invasion, metastasis, cell death and tumour immune response [9,10]. In particular, HSP27 and HSP70 have been shown to exert a pro-malignant effect in breast cancer, by blocking programmed cell death and senescence, while HSP 90 fosters the accumulation of mutated or overexpressed oncoproteins. Elevated expression of HSPs has also been observed in canine mammary tumours [11,12] and given that these tumours share many of the epidemiological, clinical-pathological and biochemical features of human breast cancer, study of the canine model may prove useful in understanding the molecular mechanisms involved in mammary carcinogenesis [13,14].

2. The Role of HSPs in Mammary Tumour Cell Proliferation
As mentioned earlier, recent data have shown that chaperones facilitate the malignant transformation of mammary cells at a molecular level and their altered utilization during oncogenesis is critical in the development of human breast cancer [15]. The expression of HSPs in breast cancer is correlated with increased cell proliferation and it has been shown that selective depletion of several HSPs results in activation of the apoptotic event [16]. Multi-protein complexes containing isoforms of HSP70 and HSP90 and other HSPs or molecular cofactors such as CDC37, P23, CHIP, Tah1, Pih1p and im-
munophilins [17,18], have been shown to play an important role in the regulation of the cell cycle, controlling the activity of several signalling proteins [19], especially cyclins [20] and retinoblastoma protein (pRb), by binding to these clients and regulating their stability and function [21]. This interaction is transient in nature and driven by rounds of adenosine triphosphate (ATP) hydrolysis [22]. The high expression of the HSP72/73 in nucleus of canine mammary tumour cells characterised by intense proliferation activity and in mitotic cells corroborates the roles exerted by these chaperones in cell cycle control and in regulating the assembly of mitotic apparatus [12]. HSP90 client proteins include known substrates that are key components of the cellular apoptotic and signal transduction pathways involved in breast tumour (Wnt, ErbB and Notch), such as mutated p53, Bcr-Abl, HER2/Neu (ErbB2) and HIF-1α [23] steroid hormone receptors, Raf-1, Wee-1 and serine/threonine and tyrosine protein kinases (e.g. Akt kinases), which are critically dependent on HSPs for their maturation and conformational maintenance [24,25] (Figure 1(a)). HSP27 and heat shock transcription factor 1 (HSF-1) have been shown to specifically interact with betacatenin, a pivotal member of molecular pathways involved in tumour cell survival [26]. HSF-1 plays a key role in the development of tumours associated with activation of Ras or inactivation of p53 and is also critical in the proliferation of HER2-expressing breast cancer cells, probably because it maintains the levels of HSPs (HSP72 and HSP27 in particular), which in turn control regulators of senescence p21 and survivin [27]. HSPs inhibit apoptosis by functioning at multiple points in the apoptotic signalling pathways, modulating both intrinsic and extrinsic pathways [28-30]. HSP27 binds to cytochrome c [31] whilst HSP70 and HSP90 bind to Apaf-1 preventing caspase 9 maturation [32]. In contrast, HSP60 and HSP10 promote the direct proteolytic maturation of caspase 3 (prapoptotic function). HSP27 inhibits the Daxx apoptotic pathway [33], while HSP70 binds to JNK1 resulting in inhibition of JNK activation. HSP90 interacts with RIP 1 kinase and AKT [34,35] resulting, in both cases, in the promotion of NF-κB mediated inhibition of apoptosis. A similar pattern of change in HSP70, HSP90 and caspases 3 and 8 or other apoptosis-associated proteins, such as Bcl-2, Bcl-XL, Bax, in both human and canine mammary tumours has also been demonstrated [11]. A direct relation between HSPs and BRCA1 (BReast-Cancer susceptibility gene 1) was also highlighted when a DU-145 cell culture expressing exogenous wild-type BRCA1 (wtBRCA1) showed two to four-fold increased expression of the HSP27 [36]. Breast cancer metastasis suppressor 1 (BRMS1), a protein that suppresses metastasis in multiple systems without blocking tumour genesis, is stabilized by the HSP40, -70, and -90 chaperone complex [37].

3. Diagnostic and Prognostic Implications of HSP Expression in Mammary Cancer

HSP expression in breast cancer has been analyzed in relation to the histopathological characteristics of tumour tissues e.g. tumour type, grade of differentiation, degree of proliferation and patient parameters [38-43] but this has not proved to be particularly informative on a diagnostic level and cannot be relied upon for the recognition of a specific tumour histological type also in canine mammary tumors [12].

HSP27 expression has been extensively studied given its relationship with a cytosolic oestrogen receptor-associated protein, its physiological role in the assembly and trafficking of steroid receptors and correlation with oestrogen receptor levels [44,45]. However this protein has not been associated with progesteron receptor in female cancers or with ERα in male breast carcinomas [46,47]. In addition, other findings indicate that not all ER-positive breast tumours express HSP27 [44]. It has been reported by some authors that there is no significant or marginal correlation between HSP27 expression and histological grade or with the proliferation marker ki-67 [48] in well differentiated tumours, however other authors have reported that HSP27 can be directly correlated to the grade of differentiation both in human and canine breast tumour [12, 49,50]. In breast cancer the increased expression of HSP27, apart from the transcriptional activation via HSF-1, is directly and indirectly (via interaction with the oestrogen receptor) activated by Brn-3b [51], which is responsible for an increased growth rate and higher proliferative activity in mammary cancer cells [52].

Clinical-pathological studies have shown that the inducible form of the HSP70 family (HSP72) is associated with poor differentiation and the presence of mutated p53 in breast cancers [53], and its nuclear staining pattern has been reported to be correlated to tumour size [54]. A strict correlation between HSP70 levels and increased oestrogen receptors has also been detected [53]. Significant increases in HSP27 and HSP70 in its inducible form have also been observed in canine mammary tumour, particularly in the more invasive neoplastic cells, therefore these proteins (and HSP90) play a meaningful role in the multiple processes leading to malignant transformation and tumour progression in the canine mammary gland [12]. Over-expression of the glucose-regulated stress gene GRP78 has been observed in most of the more aggressive ER-tumours but not in benign human breast lesions [55].

HSP90 is a fundamental component of the steroid re-
ceptron complex and is positively related to ER and c-erbB-2 and appears to be expressed more in poorly differentiated carcinomas [56], while a significantly decreased HSP90 expression has been observed in triple-negative tumours and seems not to be triggered in precursor and pre-invasive lesions [57].

This HSP also seems to be involved in the proliferation of human breast cancer as levels of HSP90α, appear to be positively correlated to cyclin D1 expression in this type of tumour [20].

HSP27 cannot be considered a useful prognostic factor in breast cancer [58] as numerous studies have produced conflicting results. In fact, even though the positive link with ER suggests a correlation between high levels of HSP27 and a better prognosis, an association between HSP27 over-expression and more aggressive tumours has also been detected, particularly in the early stages of breast cancer [41].

HSP27 levels have been correlated to different biological features in early and advanced breast cancer, being linked with short disease-free survival (DFS) in node-negative patients but with prolonged survival from first recurrence [38,45,48]. In fact high expression of HSP27 gene has been found to be associated with increased anchorage-independent growth, invasion, metastasis and resistance to chemotherapeutic drugs [51,59]. A similar correlation between HSP27 expression and tumour invasiveness, in association with reduced overall survival (OS) has also been observed in malignant canine mammary neoplasms [12], supporting the theory that HSP27 overexpression may influence the invasive and metastatic potential of both canine and human breast cancer cells [14]. It was thought that high levels of HSP27 in advanced cancer were indicative of long survival because of the link with hormone response; however, the biological explanation for the switch from HSP27 being a bad to good prognostic factor in early and advanced breast cancer remains to be defined. Moreover, HSP27 seems to sort out cases with a better prognosis from the ER negative group of patients, with a poor prognosis [60]. Nevertheless, subsequent other studies have failed to detect a correlation between HSP27 expression and response to hormone therapy or with DFS or OS [61]. High expression of Hsp 27 and HSP70 in breast cancer correlates with lymph node involvement [48,62-65]. The surface expression of HSPs differentially regulates metastasis; murine breast carcinoma cells sorted for high HSP25 (the murine homologue of human HSP27) surface expression metastasized to the lungs more aggressively than wild-type HSP25 cells and HSP72 positive cells [6]. αβ-crystallin (small HSP family) expression is also closely tied to lymph node involvement, and increased intensity has been correlated to shorter survival [66]. High stress-inducible HSP70 (HSP72) expression is correlated to poor prognosis in breast cancer [54,62,67], in particular with nuclear cytoplasmic localization [68]. This is consistent with the association of HSP70 with some of the diagnostic parameters of malignancy (poor differentiation, lymph node metastasis, increased cell proliferation, block of apoptosis, and higher clinical stage) [64]. Investigations into the genetic polymorphism of HSP genes indicate that homozygosity for HSP70-2 genes is significantly associated with increased OS but not with DFS in breast carcinoma [67,69]. HSP90 expression in breast cancer tissues [20] and the presence of auto-antibodies to HSP90 have been correlated with poor prognosis in breast cancer [70,71]. In canine malignant mammary tumours, HSP70 and HSP90 do not appear to be of significant prognostic value but the high levels of HSP90 expression detected in neoplastic tissues, independently of tumour histological type or aggressiveness, suggest that this protein plays a fundamental role in malignant transformation and tumour progression in the canine mammary gland [12].

4. Predictive and Therapeutic Implications of HSP Expression in Mammary Cancer

A growing body of evidence suggests that high intracellular HSP27 and HSP70-family expression may render mammary tumours resistant to a number of chemotherapeutic agents [72,73] which is of relevance in treatment management. However it should not be forgotten that chemotherapeutic drugs can also induce their expression as part of the cellular stress response, thus potentially increasing cancer cell resistance by up-regulating anti-apoptotic factors [74]. Although the expression of HSP27 has been correlated to ERα in breast cancer, its detection does not predict response to Tamoxifen. Overexpression of HSP27 has been correlated to shorter DFS in advanced breast cancer patients who received neoadjuvant chemotherapy [68]. In contrast, HSP70 is emerging as a predictor of resistance to chemotherapy in breast cancer [62], but, like HSP27, it has not shown predictive value for Tamoxifen administration [61]. Moreover, high HSP70 levels have been correlated to lower response of breast cancers to radiation and hyperthermia [75]. The use of HSPs in the treatment of breast cancer represents a new and very promising approach. Treatment of tumour cells with a synthetic inhibitor of HSP27 phosphorylation [30,76], as well as knocking down using transfection with short interference RNA [76,77], has been found to block tumour cell migration. Many studies have tried to establish whether HSP90-binding drugs can effectively destabilize and reduce oestrogen receptor levels, which are a prominent target for the treatment of
hormone-dependent cancer which has become refractory to classical hormonal therapy with anti-oestrogen agents [21,78]. Agents such as geldanamycin (GA) or the GA analogous 17-allylamino, 17-demethoxygeldanamycin (17-AAG) have been shown to inhibit HSP90. 17-AAG is an aminoquinone macrocyclic compound; it shares the same ability of geldanamycin to bind to HSP90 and GRP94. These drugs target the nucleotide-binding site in the N-terminal domain of HSP90, disrupt p23 containing HSP90 complexes preventing it from binding to client proteins [34], like the inhibitor of apoptosis protein (IAP) Survivin [79]. Other products such as herbimycin A, purine-scaffold derivatives, the peptidomimetic shepherdin (specifically designed to block the interaction between HSP 90 and Survivin) and the natural macrolide radicicol inhibit HSP90 function by binding to the same pocket [80,81]. Novobiocin, a cumarin-type antibiotic acts in vivo and in vitro in a similar but unique manner: it binds the C-terminal domain of HSP90 [82] and disrupts both HSP90-HSP70-p60<sup>pop</sup> and HSP90-p50-p23 complexes [83]. When GA binds to HSP90 it locks the chaperone in an alternative conformation that prevents normal cycling and the formation of mature chaperone complexes. The HSP90 client 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 (Figure 1(b)). 17-AAG has a

---

**Figure 1.** (a) Even though it is still not completely understood how HSP90 chaperone complexes recognize their substrates or affect their conformation, a widely accepted model for a steroid hormone receptors is shown (in this case oestrogen receptor). The current understanding of this complex indicate that generally the HSP90 complex holds the receptor (CBP: client bound protein) in an intermediate state until the cognate hormone (L) enters the cell, but oestrogen receptor does not require continuous interaction with the Hsp90-based chaperone machinery to maintain a high affinity hormone binding conformation. The client protein initially interacts with the “open” state of the HSP90 dimer and other co-chaperones, then, ATP binding leads to conformational changes of HSP90, which includes the transient dimerisation of the N-terminal domains and the replacement of HOP by p23 and immunophillins, converting the chaperone complex into a mature state. Upon hormone binding and ATP hydrolysis, the hormone receptor is released from the HSP90 complex and the hormone is translocated into the nucleus, where it binds specific DNA elements and activates transcription. CyP-40: cyclophilin of 40kDa; HIP: HSP70-binding co-chaperone; Hop: HSP-organizing protein; (b) Inhibition of ATP binding to HSP90 prevents the formation of the mature state and results in the proteasome-dependent degradation of associated client protein. This can occur by the recruitment of E3-Ubiquitin ligase, CHIP (carboxy-terminus of HSP70-interacting protein), which is a protein able to interact with both HSP70 and HSP90. Geldanamycin and its derivatives exert their anti-tumour effect by binding to the N-terminal ATPase domain of HSP90 to inhibit its chaperone function; other molecules interact with the N or C-terminal domain of HSP90 with similar effects.
100-times higher affinity towards the tumour-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 [84]. However preferential GA binding has been questioned by a more recent study that also indicated time-dependency for GA binding [85].

Chaperone-based inhibitors offer the advantage of diminishing the level of many protein targets in parallel. 17-AAG, by simultaneously and durably inhibiting multiple signalling activators including ErbB and Src kinases, does not permit the re-activation of signalling pathways by one or more redundant upstream activators (“oncogene switching”) and results in a more prolonged and robust inhibition of downstream signalling pathways in breast cancer cells than do individual tyrosine kinase inhibitors, such as gefitinib, which appear to lose the ability to modulate ErbB-driven signalling pathways over time [86]. It has also proved of use in the treatment of trastuzumab-resistant ErbB2-overexpressing tumours [87]. The anti-proliferative effect of 17-AAG positively correlated with phosphorylation and downregulation of ErbB2 with a marked increase in apoptosis, although, necrosis was also present especially at higher doses [88]. A second more soluble and less hepatotoxic generation analogue of GA is 17-(dimethylaminoethy-lamo)-17-demethoxygeldanamycin (17-DMAG) [89]. In vivo and in vitro, 17-DMAG exerts anti-angiogenic activity interfering with HSP90 chaperone performance on VEGF induced expression in endothelial cells, and regulating HIF-1α activation [90]. Clinical development of the geldanamycin derivatives 17-AAG and 17-DMAG was discontinued some time ago [15,91-97]. In phase I trials, 17-DMAG [98,99] exhibited an unfavourable toxicity profile. Clinical trials have been done with an optimized 17-AAG formulation KOS-953 (Tanespimycin) being used alone and in combination with other chemotherapeutic agents [100]. The wide array of HSP90 inhibitors and their clinical applications have been reviewed extensively elsewhere [97,101-106], and a summary of trials currently in progress provided by the US National Cancer Institute (http://clinicaltrials.gov/ct2/results?term=hsp90+inhibitor &pg=1).

A different approach to inhibiting HSP90 function is disrupting its interaction with co-chaperones, such as HIP (HSP organizing protein) [120] or AHA1 (activator of HSP90 ATPase) [121].

Different synthetic HSP90 inhibitors based on the 4,5-diarylisoxazole scaffold (NVP-AUY922) inhibits HSP90 in vitro and exhibits potent anti-tumour activity at tolerated doses in an ER- and ErbB2-positive human breast cancer model [119]. A summary of the HSP90 inhibitors clinical trials currently in progress is provided by the US National Cancer Institute (http://clinicaltrials.gov/ct2/results?term=hsp90+inhibitor &pg=1).

A different approach to inhibiting HSP90 function is disrupting its interaction with co-chaperones, such as HIP (HSP organizing protein) [120] or AHA1 (activator of HSP90 ATPase) [121].

Treatment of human breast cancer cell lines with these compounds results in a drop in—the levels of the HSP90-dependent client protein HER2, or in an increase in sensitivity to 17-AAG with consequent cell death. Treatment with hydroxamic acid analogue pan-HDAC inhibitors (HA-HDI) induces HSP90 hyperacetylation, this inhibits its chaperone function decreasing its binding to ERalpha, and sensitizes ERalpha-positive breast cancer cells to Tamoxifen [122]. Inhibition of cell-surface HSP90 with antibodies or cell-impermeable HSP90 inhibitors blocks cell motility and invasion in vitro and cancer metastasis in vivo [123].

HSP-1, HSP27, HSP70, and GRP78 are also the targets of antisense oligonucleotide therapies [10]. All elements of the HSF-1 activation and down regulation cascade and Ralbinding protein 1, tubulin and p23 are of great interest as potential drug targets [35]. Chemical inhibitors of HSF-1 activation (genistein, KNK437 and Triptolide) are in a very early stage of development but their effective- ness in breast cancer treatment has yet to be proven [106,124].

When located in the extracellular space or on the plasma membrane, HSPs may provide a target for immunotherapy protocols because they are able to chaperone tumour antigens and act as biological adjuvants to break immune tolerance to tumour antigens causing tumour regression [125,126]. The immunoregulatory functions of HSPs are represented by the ability of such chaperones to bind to several tumour-associated peptides/proteins. These complexes can be recognized by
specific receptors on the surfaces of antigen presenting cells confirmed to be CD40 or CD91 (also known as a2-macroglobulin receptors which act as global receptors for HSPs) and cell processed. The purpose is to elicit a specific immune response against its own tumour using tumour-derived HSPs (mainly gp96, HSP70, HSP90 and calreticulin) covalently binding to specific tumour peptides—i.e. tumour antigen mucin (MUC1) derived peptides [127]—as natural adjuvants that present to the immune system the molecules that have shielded the potential epitopes from immune recognition [30,128]. Preclinical studies have been conducted to design a variety of novel HSP-based tumour vaccines with improved therapeutic potential: a reproducible anti-tumour response has been reported in approximately 50% of subjects studied [129,130]. These approaches include development of HSP fusion proteins and genetic vaccines using plasmid DNA and adenoviruses [126]. This topic has been reviewed in detail [126,131,132]. Studies have demonstrated that such active-specific immunotherapy has potential for controlling mammary tumour progression. BALB/c female mice vaccinated with a repeat beta-hCG C-terminal peptide carried by mycobacterial HSP65 induced high avidity antibodies and effectively inhibited the growth of EMT6 mammary tumour cells injected subcutaneously [125]. Smith et al. (2008) [130] have recently demonstrated that perioperative vaccination with an ex vivo HSP-loaded dendritic cell vaccine abrogated recurrent tumour growth in an in vivo model of breast-conserving surgery for breast cancer (BALB/c mice).

Finally, the differing expression of HSPs in mammary carcinomas and conflicting results may be the result of the complex array of genetic (derepression/repression of specific genes) and epigenetic (methylation of genes) alterations that characterizes the process of carcinogenesis and its intrinsic genetic instability. Recent studies have implicated HSP90 in transcriptional regulation demonstrating an important role for it in buffering genetic and epigenetic variation leading to altered phenol-types. It cooperates with Trithorax (a TrxG chromatin protein) maintaining the active expression state of targets like the Hox genes [133]. Aberrant expression of Hox genes is related to the development of breast cancer and the malignant behaviour of cancer cells, and the expression of HoxC5 is lower in cancerous tissues with mutated-type p53 than in normal and cancerous tissues with wild-type p53 [134,135]. Pharmacological inhibition of HSP90 results in degradation of Trx and a concomitant downregulation of homeotic gene expression [133].

5. Conclusions

Studies on this subject clearly indicate that intracellular and extracellular HSPs play a significant role in the biological and clinical aspects of mammary cancer. Although of no particular significance on a diagnostic level, HSP27 and HSP70 are, however, useful biomarkers for carcinogenesis and can predict response to some anti-cancer therapies. HSP90, on the other hand, represents a promising target for the treatment of breast cancer. Major advances have been made in recent years in understanding the complex structural and functional relationship between HSPs and their co-chaperones, and identifying client proteins in breast cancer.

Currently the potential of HSP90 inhibitors, that have proven to be effective in killing cancer cells, lies in prolonged disease stabilization. Further study will help identify novel HSP90 N-terminal-ATPase inhibitors or more sophisticated drugs capable of taking advantage of the immunogenic properties of extracellular HSPs.

6. Acknowledgements

We would like to thank Tania Bastow for the linguistic review of the manuscript.


Copyright © 2012 SciRes.
The Role of Heat Shock Proteins in Mammary Neoplasms: A Brief Review


The Role of Heat Shock Proteins in Mammary Neoplasms: A Brief Review


[70] S. E. Conroy, P. D. Sasiemi, I. Fentiman and D. S. Latchman, "Autoantibodies to the 90kDa Heat Shock Protein and Poor Survival in Breast Cancer Patients," European


765

The Role of Heat Shock Proteins in Mammary Neoplasms: A Brief Review


The Role of Heat Shock Proteins in Mammary Neoplasms: A Brief Review


Copyright © 2012 SciRes.
The Role of Heat Shock Proteins in Mammary Neoplasms: A Brief Review


do:10.1073/pnas.0809669106


do:10.1051/medsci/200925177


do:10.1051/medsci/200925177