

*Full Length Research Paper*

## Heat shock proteins, estrogen and progesterone receptors expression in breast carcinomas

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Human breast cancers may over express heat shock protein (HSP) members, proteins which are involved in cell proliferation and differentiation as well as with disease prognosis and drug resistance. The expression of heat shock protein 27 (HSP27) in breast cancers correlates with stage of disease, the lower the stage, the higher the expression. Here, we studied the expression of HSP27 and HSP70 in 39 breast tumors to determine any correlation between HSPs expression and both grade and estrogen (ER) and progesterone (PR) receptors. Proteins expressions were determined immunohistochemically with specific monoclonal antibodies. Results showed that no significant association between tumor grades and the HSP27 expression, a weak HSP27 cytoplasmic immunostaining was visible in 13% of the tumors, moderate immunostaining in 31%, high in 15% and 41% of the tumors did not show any immunoreaction, on the other hand very significant relationship was found between tumor grades and the HSP70 expression ( $P < 0.005$ ). Weak and moderate staining was detected in 10% each, strong nuclear and cytoplasmic staining was observed in 77%. The expression of HSP 27 was not associated with the expression of ER or PR receptors ( $p < 0.05$ ). HSP70 expression associated well with the expression of ER ( $P < 0.001$ ) but not with the expression of PR ( $p < 0.05$ ). No significant relationship was found between tumor grade and steroid receptors. Our findings contribute to the better understanding of the role of heat shock proteins in breast cancer. Also, the role of steroid receptors in the induction of heat shock proteins.

**Keywords:** Heat shock proteins, estrogen and progesterone receptors, monoclonal antibodies, immunohistochemistry, breast cancer

### INTRODUCTION

Heat shock proteins (HSPs) are powerfully induced by heat shock and other chemical and physical stresses in a

wide range of species (Georgopolis and Welch, 1993). The HSPs have been subsequently characterized as molecular chaperones, proteins, which have in common the property of modifying the structures and interactions of other proteins (Freeman and Yamamoto, 2002). HSP27 and HSP70 undergo significant increases in cellular concentration during the transformation of mam-

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many 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 favor cell birth over cell death (Calderwood, 2010). A variety of different functions has been described for HSP27, including thermotolerance, cell proliferation, drug resistance, actin polymerization and chaperone processes. HSP27 promotes estrogen receptor transport from cytoplasm to nucleus in estrogen-sensitive target organs. HSP27 overexpression in breast cancer cells has been associated with tumor growth, resistance to chemotherapeutic drugs and a shorter disease-free survival period (Coronato et al., 1999). HSPs are over expressed in a wide range of human cancers and are implicated in tumor cell proliferation, differentiation, invasion, metastasis, death, and recognition by the immune system. Several HSPs are implicated with the prognosis of specific cancers, most notably HSP27, whose expression is associated with poor prognosis in gastric, liver, and prostate carcinoma, and osteosarcomas. HSP70 is correlated with poor prognosis in breast, endometrial, uterine cervical, and bladder carcinomas (Cappello et al., 2003). Increased HSPs expression may also predict the response to some anticancer treatments. For example, HSP27 and HSP70 are implicated in resistance to chemotherapy in breast cancer; HSP27 predicts a poor response to chemotherapy in leukemia patients, whereas HSP70 expression predicts a better response to chemotherapy in osteosarcomas (Assimakopoulou et al., 1997). Cells or tissues from a wide range of tumors have been shown to express atypical levels of one or more HSPs (Fuller et al., 1994). Increased levels of HSP27, relative to its level in nontransformed cells, have also been detected in breast cancer, endometrial cancer, and leukemia (Ciocca and Calderwood, 2005). In breast tumors, elevated expression of HSP70 is associated with short-term disease-free survival, metastasis, and poor prognosis among patients treated with combined chemotherapy, radiation therapy, and hyperthermia (Liu et al., 1996). A long line of experimental evidence positions HSP70 as a cancer-relevant survival protein. Numerous experimental cancer models have demonstrated the tumorigenic potential of HSP70 in rodents (Gurbuxani et al., 2001). In support of the hypothesis that HSP70 promotes tumorigenesis via its pro-survival function, it can effectively inhibit cell death induced by a wide range of stimuli including several cancer-related stresses like hypoxia, inflammatory cytokines, monocytes, irradiation, oxidative stress, and anticancer drugs (Nylandsted et al., 2004). Other members of the HSP family, including HSP90 $\alpha$ , HSP90 $\beta$ , and HSP60 are also overexpressed in breast tumors, lung

cancer, leukemias, and Hodgkin's disease (Jamee et al., 1992; Yufu et al., 1992;). 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 (Beliakoff et al., 2004). The molecular basis for overexpression of HSPs in tumor cells has not been well studied and may have multiple molecular etiologies, for example, associated with the complexity of the promoter region of the human HSP70 gene (Morimoto, 1991). The suggestion that heat shock proteins interfere with apoptotic signaling is consistent with observations that high levels of HSPs are often detected in tumors (Jaattela, 1999). Apoptosis is the negative counterpart of proliferation; therefore, defects in apoptosis are associated with maintenance of the transformed state and cancer (Evan and Littlewood, 1998). In tumor cells, the intricate balance between proliferation and cell death shifts toward continued cell growth as a result of the expression of antiapoptotic proteins. Such proteins include members of the Bcl-2 family, members of the inhibitory of apoptosis protein family, and members of the HSP family in particular, HSP70 and HSP27 that render tumor cells resistant to apoptosis (LaCasse et al., 1998). How does HSPs protect cells against apoptosis? A possible mechanism of action could be through binding of HSP70 to proapoptotic proteins, such as p53 and c-myc (Pinhasi-Kimhi et al., 1986). In this study we assessed the expression of HSP27 and HSP70 in a series of invasive breast cancers in an attempt to clarify their potential clinical importance. Their expression was correlated with the expression of steroid receptors (ER and PR) and tumor grade. We also investigated the interrelationship of the expression of these proteins, as they have some common functions, with the aim of elucidating the role of these molecules in tumor development.

## MATERIALS AND METHODS

### Patient and tumor characteristics

The mean age of patients was 47 years old. Paraffin sections of 39 invasive breast tumors were collected from the pathological archives of King Abdulaziz Hospital and Tumor Center in Jeddah, Saudi Arabia. Three of the tumors were grade I, 14 tumors were grade II, 16 tumors were grade III and 6 tumors had no data. Grading was done according to the Modified Bloom–Richardson grading system (Elston, 1987) (Table 1).

**Table 1:** Patients characteristics and immunohistostaining results

Case No.	Grade	Estrogen receptor (ER)	Progesterone receptor (PR)	HSP27	HSP70
1	II	—	+	—	+++
2	I	+	+	+	+++
3	II	+	++	++	+++
4	II	+	+	++	+++
5	II	ND	ND	—	+
6	III A	ND	ND	++	++
7	II	++	++	++	+++
8	III B	ND	ND	—	+
9	III A	ND	ND	—	-ve
10	II	ND	ND	+	+++
11	II	ND	ND	+++	+++
12	II	+	+	++	+++
13	II	—	+	—	+++
14	III	ND	ND	+++	+++
15	II	+	+	+++	+++
16	III	—	-	—	+++
17	-	+++	++	+++	+++
18	III	—	-	—	+++
19	-	+	+++	—	+++
20	III	ND	ND	++	+++
21	II	+	+	++	++
22	III	+	+	++	+++
23	III	+	++	+	+++
24	III	+	+	+++	+++
25	I	—	++	—	++
26	III	ND	ND	++	+++
27	II	—	+	—	++
28	III	ND	ND	—	+++
29	III	ND	ND	+	+++
30	-	+	+++	—	+
31	II	ND	ND	--	+++
32	III	++	++	—	+++
33	III	—	-	—	+++
34	III	ND	ND	++	+++
35	I	—	+	—	+
36	II	+	+	++	+++
37	ND	ND	ND	+	+++
38	ND	ND	ND	+++	+++
39	ND	ND	ND	++	+++

ER=estrogen receptor; PR = progesterone receptor; + = weakly expressed; ++ = moderately expressed; +++ = strongly expressed and ND = no data.

### Monoclonal antibodies

Human HSP-27, HSP-70, ER, and PR were identified using a specific anti-HSP-27 mouse monoclonal antibody clone G3.1, the anti-HSP-70 mouse monoclonal antibody clone BRM-22, the anti-ER mouse monoclonal antibody clone 1D5 and the anti-PR mouse monoclonal antibody clone 1A6 respectively. All were obtained from Biogenex, 4600 Norris Canyon Road, San Ramon, CA 94583, USA.

### Immunohistostaining

Immunohistochemical staining was performed on 3  $\mu$ m thickness paraffin sections. Slides were dewaxed and antigen retrieval was performed by 2 min. cycles in 0.01 M citrate buffer using a Retriever Autoclave. The demonstration of antigens by immunohistochemistry is a two-step process involving first, the binding of a primary antibody to the antigen of interest, and second, the detection of bound antibody by a chromogen. The positive reaction was evaluated considering the location of the specific immunostaining on the tumor cells (nuclear or cytoplasmic), using a semiquantitative grade according to the intensity of immunostaining and the proportion of stained cells (Vargas-Roig et al., 1998). At least 5 fields per case were analyzed by light microscope and expressed as a percentage of the total epithelium present. The mean percentage was calculated for every case. HSP-27, HSP-70 ER, and PR were scored as positive if >10% staining was found. Immunohistochemical scoring of signaling molecules was based on a semiquantitative method according to the percentage of positive cells and the intensity of staining. Staining in either the cytoplasmic or the nuclear compartment was all considered positive. The percentage of positive cells was scored as follows: -ve, no staining or staining in <1% of the tumor cells; +, staining in 5% to 30% of the cells; ++ staining in 31% to 60% of the cells; +++, staining in > 61% of the cells. Immunohistochemical staining of negative and positive control slides for HSP27 and HSP70 were carried out along with the patient's slides to evaluate the assay (Figure 1).

### RESULTS

Results of immunohistostaining and patients characteristics are shown in table 1. Immunohistochemical staining analysis of patient's slides showed that HSP27

was expressed in 59% and HSP70 in 97.4% of the 39 samples of the invasive breast cancers examined. ER was expressed in 65% (n=23) and PR in 86.4% (n=22) of the samples.

### HSP27 and HSP70 expression

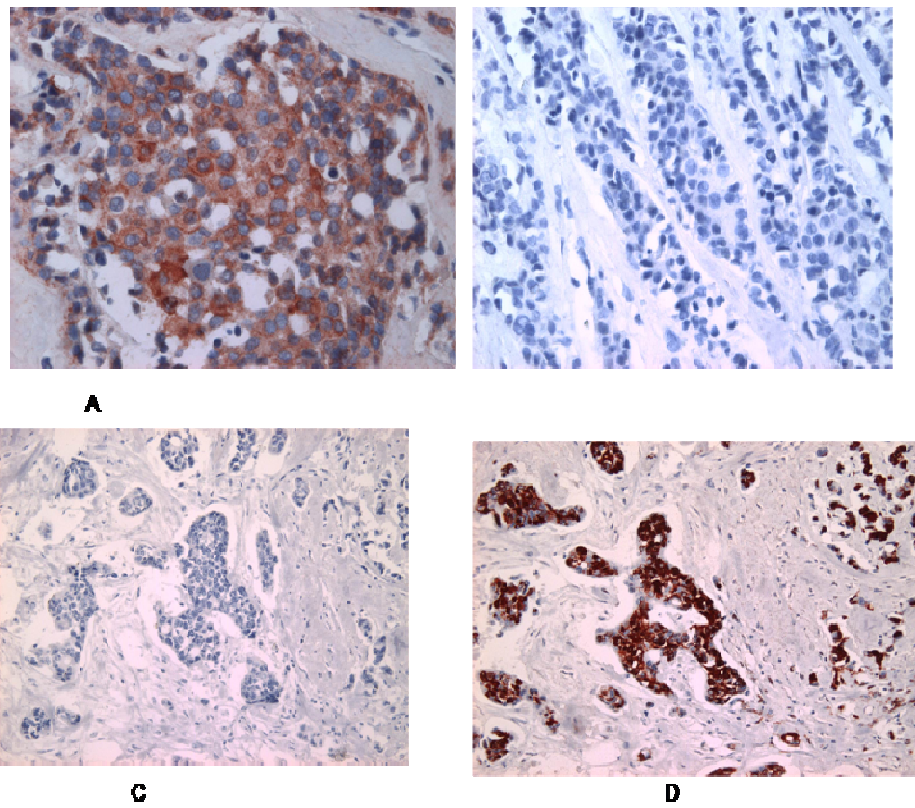
HSP27 was Highly expressed in 6/39 of the tumors, moderately expressed in 12/39, weakly expressed in 5/39, and not expressed in 16/39 (Figure 2). Whereas HSP70 was highly expressed in 30/39 tumors, moderately in 4/39 and weakly in 4/39 (Figure 3). The immunoreactions of HSP27 were mainly cytoplasmic meanwhile positive HSP70 immunoreaction was visible in cytoplasm and nuclei of the majority of cells (Figure 4). This is in agreement with other people findings (Caroline and Morimoto, 2000). There was a significant positive correlation between HSP-70 expression and high histologic grade ( $P < 0.005$ ) unlike HSP27.

### Relation of HSPs to steroid receptors expression

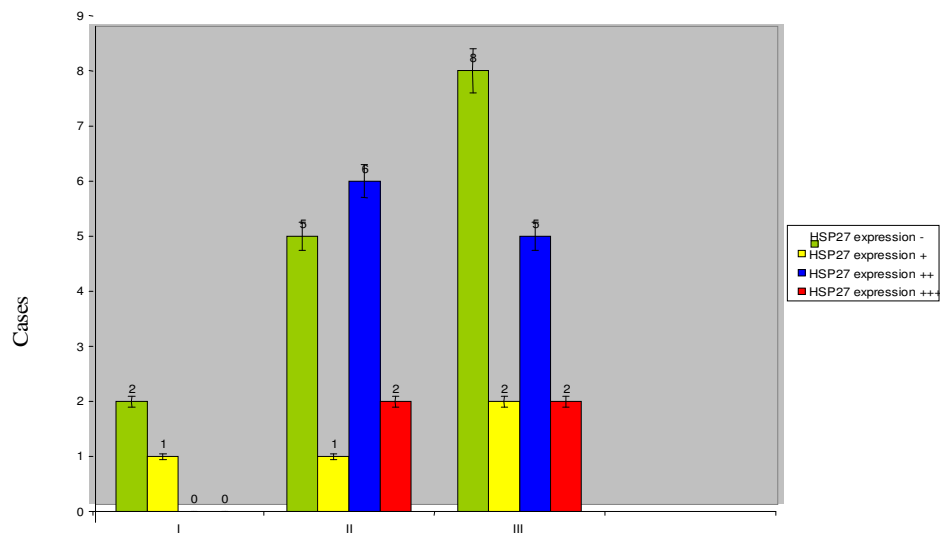
The expression of HSP 27 was not associated with any of estrogen or progesterone receptors expression ( $p < 0.05$ ). HSP70 expression associated well with the expression of estrogen receptor ( $P < 0.001$ ) but not with the expression of progesterone receptor ( $p < 0.05$ ). Although there was no significant association between estrogen receptors expression and HSP27, eight of the tumors investigated negative for estrogen receptor were also negative for HSP27 confirming the possible relation of estrogen receptor and HSP27 induction. However, the relationship between estrogen regulation of HSP27 and its expression in hormone response tumors, is not a consistent predictor of response to hormonal treatment, since only a subset of estrogen-positive breast tumors expresses high levels of HSP27. Although the number of tumor investigated is very small, studies with larger number of tumors could help determine if the expression of HSP27 and HSP70 can be used to predict clinical outcome and to better understand the relationships between HSPs and other clinicopathological features. Expression of Progesterone and estrogen receptors did not correlate with histologic grade.

### DISCUSSION

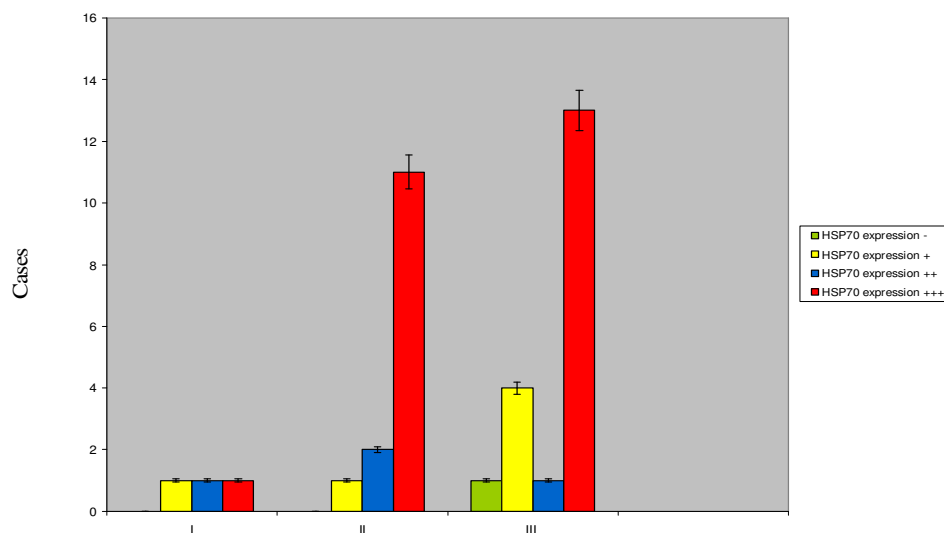
Several studies indicate that the stress inducible HSPs



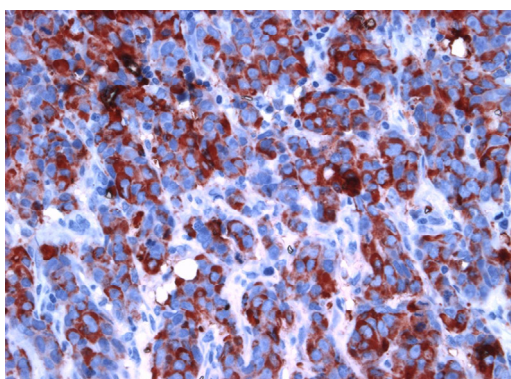
**Figure 1:** Breast carcinoma stained with anti-HSP-27 (positive control) (A); Negative control for the same tissue(B); breast carcinoma tissue, stained with anti-HSP70 (positive control) (C); Negative control for the same tissue (D). A & B observed at 40x; C & D observed at 20x.



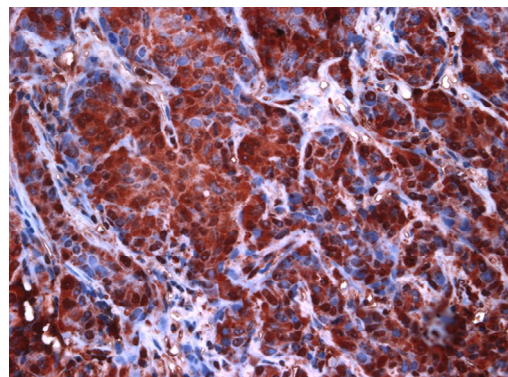
**Figure 2:** Immunostaining intensity of HSP27



**Figure 3:** Immunostaining intensity of HSP70



**A**



**B**

**Figure 4:** Breast invasive ductal carcinoma grade III stained with anti-HSP27 (A), and with anti-HSP70 (B), both expressed strongly the antigen. Observed at 40x

can influence the phenotype of cancer cells (Ciocca and Calderwood, 2005). Cancer cells that overexpress this protein are more resistant to apoptosis and tolerate higher doses of anticancer drugs. It has been reported that tumor cells exposed to sublethal heat shock before chemotherapy are less responsive to treatment (Ciocca et al., 1992; Hahn and Li, 1990). Under such conditions, the presence of HSPs is not desired in cancer patients, and remedies that enable the reduction of HSP production are targeted. These elevations of HSP27 and

HSP70 expression in breast cancer have been reported by many investigators (Ciocca and Calderwood, 2005). HSP70 is often called the major stress-inducible HSP70 because its expression is rapidly induced by a variety of physical and chemical stresses, and it expressed only at low or undetectable levels in most unstressed normal cells and tissues (Leung et al., 1990). Understanding of the link between cancer and HSPs may offer insight into the ability to regulate the activities of molecular chaperones in the cell. HSPs are expressed in many

types of cancer and act as the immunodominant antigen (Kiang and Tsokos, 1998). Therefore, the ability to down regulate HSPs may be of therapeutic use for treating patients with cancer. These features of the HSPs constitute a rationale for exploring the relationship between the expression level of these proteins and other molecular markers, and clinical parameters of breast cancer. Several reports have correlated the expression of HSPs with hormonal status. In particular, HSP27 and HSP70 which are under active investigation as promising prognostic factors in breast cancer. Their value has been the subject of many studies with contradictory results. In an *in vitro* assay it has been shown that HSP27 can be induced by estrogen in estrogen-dependent cell lines (Edwards et al., 1980). In fine needle aspiration smears HSP27 expression was correlated with ER, PR content. Expression of HSP27 protein in FNA smears can be used to differentiate benign and malignant breast lesions (Mlynarczyk-Liszka et al., 2009).

In the current study HSP27 and HSP70 expression were also correlated with the expression of ER and PR receptors. We found that HSP27 expression was not correlated with the expression of ER receptors. This is not in accordance with the findings of other investigators who confirmed that the expression of HSP27 is closely associated with ER expression, and that its regulated expression occurs early along the mammary oncogenic pathway (Caroline and Morimoto, 2000). Also, expression of HSP27 has no correlation with expression of PR in our study which is in agreement with the finding of other studies (Ioachim et al., 2003; Takahashi et al., 1995; O'Neill et al., 2004). A strong association was found in this study between HSP70 expression and ER receptors content. This is in agreement with previous reports (JKalogeraki et al., 2007). We also found that HSP70 expression correlated with tumors histological grade unlike HSP 27. Other investigator has found that Hsp 27 overexpression in breast cancer is associated with early clinical stage disease. Conversely, in more advanced cancer stages where patients have axillary lymph node metastases, Hsp 27 expression was negative in most cases (Laguens et al., 2001). No consistent correlation was found between levels of ER or PR expression and tumor histological grade.

Further studies on a larger number of cases is needed to elucidate the biological significance of high HSPs expression which will assist in clarifying the prognostic role and the possible mechanism of action of HSPs and its relation to steroid receptors in breast cancer.

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