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Hsp27 expression in invasive ductal breast carcinoma

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Abstract: The aim of this study was to determine the intensity of Hsp27 protein expression in fibrocystic breast changes (FC) and invasive ductal breast carcinoma (IDC) and to examine its impact on patients' clinico-pathological characteristics and overall survival. Immunohistochemical reactions were conducted on archival samples of 20 cases of FC and 101 cases of IDC treated in 1999–2002. Nuclear-cytoplasmic Hsp27 expression was observed in 92 (92.1%) of the examined cases of IDC, and all the cases of FC. Significantly higher Hsp27 expression was observed in G2 (p < 0.01) and G3 cases (p < 0.0001) compared to FC. HER-2 positive cases had higher Hsp27 expression (p = 0.0153), than HER-2 negative cases. Our research showed that Hsp27 could have an impact on tumor malignancy. Moreover, a positive correlation between the expression of Hsp27 and HER-2 positive cases was demonstrated. (Folia Histochemica et Cytobiologica 2012, Vol. 50, No. 4, 527–533)

Key words: breast cancer, Hsp27, heat shock protein

Introduction

Breast cancer is the most frequent female cancer in terms of diagnosis (in Europe, in 2006, 429,900 cases were diagnosed which accounted for 13.5% of all cancer cases), and one of the three most frequent reasons for mortality from among all types of cancer (131,900 cases) [1]. In the United States in 2010, approximately 207,000 new cases of breast cancer were diagnosed and around 40,000 died from the disease [2]. Although the diagnostic process of breast cancer has been improved, this disease poses a great health problem, because of the lack of reliable molecular

Correspondence address: P. Dziegiel, Department of Histology and Embryology, Medical University in Wroclaw, Chalubinskiego St. 6a, 50–368 Wroclaw, Poland; tel.: + 48 71 784 13 54, fax: + 48 71 784 00 82; e-mail: piotr.dziegiel@am.wroc.pl prognostic factors. However, the development of molecular biology has made it possible to define new potential prognostic factors which may have clinical significance. One of these could be heat shock protein 27 (Hsp27).

Heat shock proteins (Hsps) are highly conserved proteins of which the expression in the cell increases in stress conditions [3]. They have many functional activities such as control of new protein formation, facilitation of transport through the cell's membrane, or repair of stress factor-induced damage [3, 4]. Hsp27 belongs to the family of small Hsps and is expressed in the cytoplasm of a mammal's cells such as myocardial and skeletal muscle cells [5, 6]. It protects cells from apoptotic factors like cytokines, cytostatic drugs, and ionic radiation by increasing the activation of the NF- κ B signaling pathway and by influencing the cellular inflammatory response [7, 8]. Increased level of glutathione and modulation of microfilament stability have been found to correlate with a high level of

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528 J Grzegrzolka et al.

Hsp27 expression [9]. Hsp27 has been noted to be overexpressed in breast, ovarian, esophageal, liver and lung cancer [10]. It is significantly associated with estrogen receptor expression in endometrial and female breast carcinoma [10]. High expression of Hsp27 has been found to correlate with resistance to cisplatin, doxorubicin and other anticancer drugs used in breast cancer chemotherapy [11]. The expression of Hsp27 decreases the metastatic potential by increasing the expression of E-cadherin and decreasing the expression of MCAM/MUC18 (MCAM, melanoma cell adhesion molecule) in melanoma cells [12].

In this research, we determine the expression of Hsp27 protein in fibrocystic breast changes (FC) and human invasive ductal breast carcinoma (IDC) using immunohistochemical methods with regards to the patients' clinicopathological data.

Material and methods

Patients. The study was performed on archival paraffin blocks of 20 cases of FC and 101 cases of IDC from patients treated in the Lower Silesian Oncology Center in Wroclaw during the years 1999–2002. Most of the patients were treated surgically with radical mastectomy or conservative quadrantectomy followed by axillary lymph node resection. In cases of disease stage 3 and above, neoadjuvant therapy was administered. All tissue specimens used in this study were collected before the beginning of the treatment. The clinical and pathological data were obtained from the archives of the Lower Silesian Oncology Center (Table 1). Patients were followed for 52.77 (range 1–118) months.

Immunohistochemistry (IHC). Tissue samples were fixed in 10% buffered formalin and embedded in paraffin. For immunohistochemical staining, 4-µm-thick paraffin sections were cut. Deparaffinization and antigen retrieval were performed in Target Retrieval Solution, pH 9 (97°C, 20 min) and PT Link platform (Dako, Glostrup, Denmark). Then the sections were washed in TBS and stained (4°C, overnight) with primary rabbit polyclonal antibody directed against p-Hsp27 (sc-101700, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and with primary antibody against Ki-67 (MIB-1; 1:100; RT, 20 min; Dako) using the Autostainer Link 48 (Dako). Then the slides were washed in TBS and visualization of the studied antibodies was performed using the EnVision FLEX system (Dako) according to the manufacturer's instructions. All slides were counterstained with Mayer's hematoxylin (Dako).

For all sections, a staining for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) was performed as previously described [13]. Briefly, 4- μ m-thick paraffin sections were cut, dewaxed, gradually rehydrated and boiled in Antigen

Table 1. Patient and tumor characteristics

Mean age in years (range)	57 ± 11 (35–83)		
Parameters	Number	%	
Age			
≤ 50	31	30.09	
> 50	70	69.91	
Menopausal status			
Pre	68	67.33	
Post	33	32.67	
Grade of malignancy			
G1	9	8.91	
G2	52	51.49	
G3	40	39.60	
pT			
T1	55	54.46	
T2	35	34.65	
T3	8	7.92	
T4	3	2.97	
pN			
pN0	52	51.49	
pN1, pN2, pN3	46	45.54	
pNx	3	2.97	
ER			
Positive	81	80.20	
Negative	20	19.80	
PR			
Positive	70	69.03	
Negative	31	31.97	
HER-2 by IHC			
Positive	16	15.84	
Negative	85	84.16	
Ki-67			
< 25%	72	71.29	
≥ 25%	29	28.71	

Retrieval Solution (pH 6; Dako). The activity of endogenous peroxidase was blocked with 3% H₂O₂. Slides were then incubated overnight at 4°C with primary antibodies (clone 1D5 at 1:100 dilution for ER, clone PgR 636 at 1:100 dilution for PR; both Dako). Following incubation with secondary biotinylated antibodies (Biotinylated Link), reactions with the streptavidin-biotinylated peroxidase complex (LSAB+ System-HRP; Dako) were performed. The peroxidase substrate of 3,3'-diaminobenzidine (DAB+ Chromogen) was used as a chromogen. All the sections were counterstained with hematoxylin. Expression of HER-2 was examined using a HercepTest™ (Dako) kit, according to the procedure recommended by the manufacturer.

3 pts — 51–80% cells with positive reaction

4 pts - > 80% cells with positive reaction

the product of these parameters (A×B)		
A	В	
0 pts — no cells with positive reaction	0 pts — no staining	
1 pt — to 10% cells with positive reaction	1 pt — low intensity of staining	
2 pts — 11–50% cells with positive reaction	2 pts — moderate intensity of staining	

Table 2. Remmele scale: percentage of positive cells (A) and the intensity of color reaction (B). The final score represents the product of these parameters $(A \times B)$

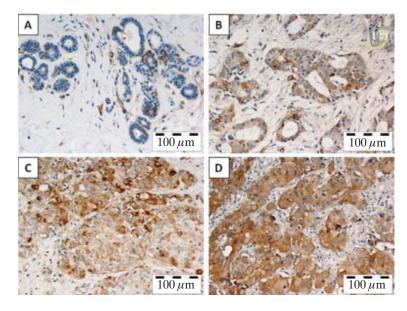


Figure 1. Hsp27 expression in breast duct cells (A) and IDC cases of G1 (B), G2 (C) and G3 (D). Magnification × 200

Histopathological examination and analysis of IHC. H&E sections were evaluated by two independent pathologists (WA, DP) to confirm the diagnosis and assess the grade of malignancy and presence of necrosis. Sections were regarded as positive when the extent of the necrotic area was greater than 10%.

All immunostainings were evaluated by two pathologists using a BX-41 light microscope (Olympus, Tokyo, Japan). For the Hsp27 evaluation, immunoreactive scale of Remmele was applied [14] (Table 2). The Ki-67 antigen was evaluated according to tumor cell positivity and encoded as follows: 0 (0% cells stained), 1 (1–10% cells stained), 2 (11–25% cells stained), 3 (26–50% cells stained), and 4 (51–100% cells stained).

PR and ER analysis was evaluated according to the percentage of positive cancer cells in whole tissue sections on a four-grade scale as follows: 0% - 0, 1-10% - 1, 11-50% - 2; and 51-100% - 3. Sections scoring 1 and above were regarded as positive. HER-2 sections scoring 3 were regarded as positive [15].

Statistical analysis. Statistical analysis was performed by Prism 5.0 (GraphPad, CA, USA). Correlations between expression of Hsp27 and Ki-67 were analyzed by Spearman's correlation test. The correlations between expression of Hsp27 and clinicopathological parameters were analyzed by: Kruskal-Wallis, Mann-Whitney tests (tumor grade) and Fischer exact test. The Mantel Cox test was used to compare the differences in patient survival. Results were considered statistically significant with p < 0.05 in all analyses.

3 pts — intense staining

Results

Hsp27 cytoplasmic expression was noted in breast duct cells of FC (2.50 ± 0.76) and in myofibroblasts of breast acini and vessels of breast stroma (Figure 1A). Hsp27 expression was noted in 92 (92.1%) examined cases of IDC (4.97 ± 3.10 ; Figures 1B–D). In some cases, additional expression was observed in cancer cell nuclei. Significant difference in Hsp27

J Grzegrzolka et al.

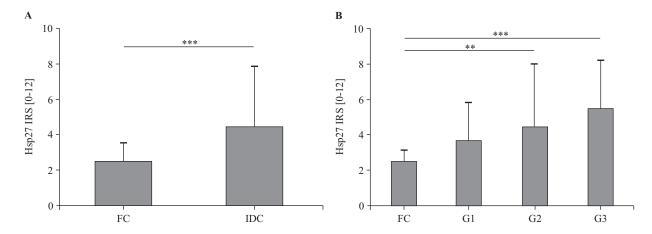


Figure 2. Statistical analysis revealed significantly higher Hsp27 expression in IDC compared to breast duct cells of FC (p < 0.0001) (A). When the relationship between its expression in mastopathies and in different malignancy grades was analyzed, significantly higher Hsp27 expression was observed in G2 (p = 0.0047) and G3 cases (p < 0.0001) (B). **p < 0.005, ***p < 0.0001

expression was observed between FC and IDC (Figure 2A). Moreover, when this relationship was analyzed regarding the grade of malignancy, a positive trend of Hsp27 expression was observed in IDC, although it was not significant (Figure 2B). Almost significant higher expression of Hsp27 was noted in G3 cancers compared to cases of G1 (p = 0.0524).

Hsp27 expression was associated with HER-2 positivity (p = 0.0153), and cases which were HER-2 positive tended to have higher levels of Hsp27 (p = 0.072; Table 3, Figure 3). Our study revealed no correlation of Hsp27 expression with tumor cell proliferation measured by the expression of Ki-67 antigen. Univariate analysis showed no impact of Hsp27 on patients' clinical outcome and event free survival.

Discussion

The expression of Hsp27 in malignant cells has been observed in many studies. To date, its expression has been detected in breast, endometrial, ovarian, lung cancer, melanoma and others [10, 16–24]. In ovarian, prostate and gastric cancer, a high level of Hsp27 expression was associated with a poor clinical outcome [10]. Some studies have shown that Hsp27 is not a useful prognostic factor, and therefore on the basis of results obtained by others we analyzed its expression in a different group of patients with IDC [25].

Our study showed significant differences between the expression of Hsp27 in the group of IDC cases compared to FC cases. Although our study did not show any statistical difference between Hsp27 expression in cases of FC and G1 cases of IDC, significantly higher Hsp27 expression was noted in FC in relation to G2 and G3 IDC cases. Hsp27 expression was almost significantly higher in G3 than in G1 cases. There was also a trend of higher expression of Hsp27 in G2 compared to G1. This indicates that Hsp27 could influence IDC malignancy. However, our results did not reveal any positive correlation between level of Hsp27 and Ki-67.

These results conflict with recently published data which demonstrated that Hsp-27 expression was inversely correlated with the grade of malignancy of invasive ductal breast cancer [26]. In astrocytoma and hepatocellular cancer, Hsp27 expression was positively correlated with grade of malignancy [27–29]. However, no significant correlation between grade of malignancy and level of Hsp27 expression in ovarian cancer, squamous cell carcinoma of the tongue and esophagus, bladder carcinoma or gastric cancer was observed [30–32]. That suggests that the expression of Hsp27 has a heterogeneous character and is specific for the type of tumor; the role of this protein in carcinogenesis remains unclear.

Hsp27, which is also known as a cytoplasmic estrogen receptor associated protein (p29), selectively binds GTP and to a lesser extent ATP and plays a role in the estrogen response intracellular pathways [33, 34]. Our study did not show any correlation between Hsp27 expression and the expression of ER and PR. Our data is in agreement with similar results obtained by others [26, 35–38], although the study by Ciocca et al. described a positive correlation between Hsp27 and ER [16]. This discrepancy needs further investigation.

We have also shown that Hsp27 expression correlates with HER-2 expression. Similar results were

Characteristics	Number (%)	Hsp27 expression (number (%)		p
		IRS 0-4	IRS 6-12	
Age				
≤ 50	31 (30.09)	13 (41.93)	18 (58.07)	0.1358
> 50	70 (69.91)	41 (58.6)	29 (41.4)	
Menopausal status				
Pre	33 (32.67)	13 (39.39)	20 (60.61)	0.0587
Post	68 (67.33)	41 (60.29)	27 (39.71)	
Tumor size				
pTl	55 (54.45)	29 (52.72)	26 (47.28)	0.8437
pT2-4	46 (45.55)	25 (54.35)	21 (45.65)	
Lymph nodes				
N0	52 (51.49)	30 (57.69)	22 (42.31)	0.4178
N1-3	46 (45.54)	22 (47.82)	24 (52.18)	
ER				
Positive	81 (80.20)	43 (53.08)	38 (46.92)	1.0000
Negative	20 (19.80)	11 (55.00)	9 (45.00)	

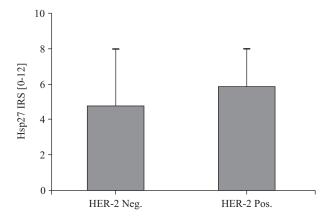
38 (54.28)

16 (51.61)

4 (25.00)

50 (58.82)

Table 3. Correlations between Hsp27 expression and clinico-pathological characteristics of studied patients. Significant p values are given in bold



70 (69.03)

31 (31.97)

16 (15.84)

85 (84.16)

PR

Positive

Negative

Negative

HER-2 Positive

Figure 3. Intensity of Hsp27 expression in HER-2 negative and positive cases of IDC

demonstrated by Liebhardt et al. [39]. The study by Meng et al. revealed that heat-shock transcription factor HSF1 has an important role in cellular transformation induced by HER-2 [40]. Cells with knockdown of HSF1 presented lower foci formation and reduced tumor growth when xenografted to mice. In HER-2 positive breast cancer cell lines, loss of HSF1 expression was associated with increase of p21 and

decrease of cells survival, Hsp27 and Hsp72 expression [40, 41]. The results obtained in this study are in agreement with the results obtained by Meng et al. in their research. Kang et al. demonstrated in breast cancer cells that the high expression of Hsp27 increases HER-2 stability, by the formation of a HER-2--Hsp27 complex therefore reducing the susceptibility to Herceptin treatment [41]. Cardoso et al. focused their work on the description of various markers in breast cancer and metastatic lymph nodes, including HER-2 and Hsp27, but the authors did not analyze the relationship between those two proteins [42]. Their results showed that Hsp27 was present in 44% of the examined primary tumors (compared to our 92.1%). These differences might be the result of using a different cut-off for the definition of positivity [42]. Moreover, in our study, all cases of diffuse cystic mastopathies were characterized by the expression of Hsp27.

32 (45.72)

15 (48.39)

12 (75.00)

35 (41.18)

0.8317

0.0153

Our results showed that Hsp27 may be involved in the early steps of carcinogenesis. Although our results did not demonstrate any correlation between Hsp27 expression and clinico-pathological factors, the correlation of Hsp27 and HER-2 may be an interesting point of research in determining carcinogenesis

J Grzegrzolka et al.

and progression of IDC. Moreover, a significantly higher Hsp27 expression in less differentiated cases of IDC may be indicative of its role in the early steps of carcinogenesis.

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