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Original

Expression and localization of *hsp70* and *hsp27* in human breast tumors

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RESUMEN

Planteamiento: Se ha estudiado la inducción de las proteínas de choque térmico (hsps) 70 y 27 en 35 pacientes con cáncer de mama comparando con tumores no malignos. Material y métodos: Se han hecho estudios de inmunohistoquímica sobre cortes de parafina de los 35 pacientes con cáncer de mama empleando anticuerpos frente a las proteínas hsp70 y hsp27. Al mismo tiempo se han analizado los tumores mediante técnicas de Western blot con los mismos anticuerpos. Resultados: Se ha encontrado expresión de hsp70 en todos los tumores, pero no sucede lo mismo con hsp27. La expresión de hsp70 es alta en todos los casos, con más del 60% de células teñidas por campo en cada tumor. La expresión de hsp27 es menor en los casos donde hay reacción positiva. La expresión de hsp70 parece estar relacionada con los procesos de proliferación en tejido mamario, mientras que en tumores malignos hay una localización nuclear de hsp70. Conclusiones: Se puede decir que los resultados del trabajo apoyan el empleo de hsp70 como marcador de malignidad en el cáncer de mama, dada su expresión aumentada y translocación nuclear relacionada con el cáncer. **Rev Esp Patol 2001; 34(1): 9-17.**

Palabras clave: hsp70 - hsp27 - Cáncer de mama - Malignidad - Proliferación celular

SUMMARY

Background: The expression of heat shock proteins (hsps) 70 and 27 was studied in 35 breast cancer patients. Nonmalignant tumors were used as controls. Materials and methods: Immunohistochemistry and Western blot techniques were applied for each of 35 malignant tumors using specific hsp70 and hsp27 antibodies. Results: A positive expression of hsp70 was found in all cases, but this was not the case for hsp27. The expression of hsp70 was found in more than 60% of cells per field in each tumor, whereas hsp27, when expressed, was present in 31% of the cells at the most. The expression of hsp70 appeared to be related to the processes of proliferation in breast tissue, whereas in malignant tumors hsp70 was localized in the nuclei. Conclusions: The results support the use of hsp70 as a marker of malignancy in breast cancer, due to its cancer-related induction and localization in the nucleus. **Rev Esp Patol 2001; 34(1): 9-17.**

Key words: hsp70 - hsp27 - Breast cancer - Malignancy - Cellular proliferation

INTRODUCTION

Heat shock, other cellular stress inducers such as anoxia, heavy metal ions, drugs, and some clinical diseases trigger the synthesis of a family of proteins, evolutionarily well preserved and known as heat shock proteins *(hsps)* (1, 2). The normal counterparts of *hsps* are constitutively synthesized by the cell under nonstressful conditions, depending on the cell cycle, hormonal status, and differentiation stage. These proteins are the heat shock cognate proteins (Hsc) (1). Since all living organisms, from bacteria to humans have *hsps/hscs*, it is reasonable to think that they play fundamental roles within cells. The activity of *hsps* in clinical pathology deserves special interest. In human cancer, *hsps* have been found expressed in the ovaries, the endometrium, the liver, the blood and the breast (3-6). The physiopathological activity of *hsps* in tumor processes is not yet known, but it is related to cell growth and differentiation, and/or may interact with oncogenic products such as *p53* and retinoblastoma (7, 8). A positive correlation between *hsp70* expression and the estrogen receptor levels has been found in human breast cancers, whereas the correlation is negative between *hsp70* and the tumor suppressor gene *p53* (9). Recent studies have investigated cell proliferation in relation to *hsps* and have shown a positive correlation between proliferation factors (proliferating cell nuclear antigen (PCNA)) and the expression of *hsp70* (10).

In the present study, we examined in detail the expression of two major stress proteins, *hsp70* and *hsp27*, in 35 malignant breast cancer samples from two different populations in Spain and Chile. The results were evaluated by immunohistochemistry of paraffin-embedded sections incubated with the specific *hsp70* and *hsp27* antibodies and quantified by counting the positive cells per field. To confirm the immunohistochemistry data, the antigen was identified by Western blot. The research was aimed at finding a differential relationship between *hsps* and malignant breast cancer processes. Our working hypothesis was that cancer cells, unlike normal tissue, trigger stress signals which induce *hsps*, and would possibly translocate *hsp70*, as occurs in the case of heat shock. For this purpose, samples of nonmalignant breast growth processes were also studied in parallel to find qualitative differences in the staining pattern with respect to malignant samples. The results of this study represent a step forward to the validation of the use of *hsp70/hsp27* as markers in breast cancer.

MATERIAL AND METHODS

Materials

A total of 35 samples from malignant tumors were analyzed. The samples belonged to two different populations: 23 from Spain, and 12 from Chile. The criteria for selection, excepting the malignancy of the tumors, were based on the reports of the respective pathology departments, and the general diagnosis markers for the patients are given in Tables 1 and 2, respectively. In parallel, a total of 10 different nonmalignant breast growth processes, including breast adenoma and fibrocystic diseases were also included in the study. All the samples for this study were obtained after primary diagnosis and therefore patients had not been subjected to previous therapy. For this reason the clinical responses to therapy are not yet available. Follow-up studies should include these data in future papers.

Processing of tumor samples

Freshly extracted tumors were either fixed in 70% ethanol (for protein extraction purposes) or, more usually, fixed in 10% buffered formalin at room temperature for 24 to 48 h, dehydrated and embedded in paraffin blocks. Serial sections, 4 µm thick, were mounted onto 3-aminopropyltriethoxy saline-coated slides (Sigma). For histological studies, paraffin blocks prepared as described above, were sectioned, deparaffinized and processed for immunohistochemical analysis, and then counterstained with hematoxylin-eosin. Different sections of the same tumor were used for *hsp70* and *hsp27* antibodies. For immunoblotting, deparaffinized pieces of each tumor, corresponding to 100 mg, were frozen in liquid nitrogen, ground with a mortar and pestle, and homogenized subsequently in 1 × Laemmli PAGE-SDS sample buffer.

Immunoblotting procedures

For SDS-electrophoresis analysis, approximately 20 µg of total protein was loaded in 10% PAGE-SDS minigels

Table 1. Diagnostic markers of breast tumors in a population from Arica (Chile).

Tumor	PCNA (%)	Ki67 (%)	p53 (%)	C-erbA (%)	STR-R (%)	PROG-R (%)
1	24	18	2	1	45	60
2	65	39	6	2	3	6
3	25	15	5	1	55	18
4	22	18	28	4	37	49
5	78	8	17	88	17	8
6	25	12	15	57	66	75
7	63	49	12	33	28	79
8	29	12	7	14	12	8
9	16	18	12	16	18	12
10	22	14	16	8	8	7
11	45	8	78	12	15	6
12	22	6	15	8	78	45

(Mini Protean II, Bio Rad Laboratories, Madrid, Spain) with stacking gels of 4%. Prestained molecular size markers (Amersham, Ibérica, Madrid, Spain) and rainbow marker, broad range, were run in parallel. After electrophoretic separation, gels were blotted onto nitrocellulose membrane sheets (ECL membranes, Amersham) in 10% methanol, Tris-glycine buffer, at 25 V overnight. Blots were blocked in phosphate buffered

Table 2. Diagnostic markers of breast tumors in a population from Cartagena (Spain).

T (age)	Type	Metastasis	PCNA (%)	Ki67 (%)	p53 (%)	c-erbB2 (%)	STR-R (%)	PROG (%)
1 (48)	Ductal	–	52	99	39	19	68	90
2 (53)	Ductal	–	65	29	38	26	14	9
3 (47)	Ductal	Yes	39	22	17	24	22	18
4 (41)	Ductal	–	46	36	20	19	33	32
5 (39)	Ductal	–	39	12	33	49	41	39
6 (55)	Lobular	–	62	39	19	18	18	40
7 (53)	Ductal	–	58	42	25	19	40	18
8 (49)	Ductal	–	37	18	32	7	29	30
9 (47)	Ductal	–	52	32	14	38	39	19
10 (34)	Ductal	–	29	13	18	16	51	25
11 (48)	Ductal	Yes	34	16	19	14	59	48
12 (48)	Ductal	–	48	29	22	56	60	59
13 (52)	Ductal	Yes	38	29	21	19	18	19
14 (51)	Ductal	–	52	36	19	23	29	33
15 (59)	Ductal	Yes	36	18	13	24	30	18
16 (82)	Ductal	–	37	18	29	39	37	17
17 (36)	Lobul.	–	42	29	31	41	34	7
18 (48)	Ductal	–	29	15	18	33	50	20
19 (48)	Ductal	–	52	48	7	19	51	33
20 (47)	Ductal	Yes	62	39	14	18	19	14
21 (52)	Ductal	Yes	19	34	29	24	22	19
22 (51)	Ductal	–	20	19	13	23	24	20
23 (49)	Ductal	–	31	18	12	22	33	21

saline (PBS, 130 mM sodium chloride/10 mM disodium phosphate/10 mM monosodium phosphate, pH 7.2), 5% skimmed milk, for at least 3 h at room temperature, and developed with mouse monoclonal antibodies corresponding to the inducible form of human stress proteins *hsp70* and *hsp27* (Sigma-Aldrich Company, Alcobendas, Madrid, Spain). The secondary antibody was an HRP-coupled rabbit antimouse (Amersham). The primary and secondary antibodies were incubated for 1 h at room temperature with shaking. Dilutions of antibodies were carried out following the manufacturer's instructions in each case. After each antibody dilution, three washes in PBS, for 10 min each, were performed at room temperature. The chemiluminescence reaction (ECL) was carried out according to the manufacturer's (Amersham) instructions.

Immunohistochemistry procedures

The protocol for the sections embedded in paraffin was as follows: firstly, the sections were mounted on poly-L-lysin slides. Before incubation of the antibodies, the slides were immersed in sodium citrate 0.1 M, and preheated in a 750 W microwave oven for 7 min/M to expose antigens. As primary antibodies, monoclonal mouse, anti-human *hsp27* and *70* mouse (Menarini) were diluted 1:100 in PBS. The secondary antibody was a biotinylated anti-mouse immunoglobulin G (IgG) (used in a 1:400 dilution in PBS). Incubations of antibodies and washes were done as for Western blot. Further treatment with peroxidase-coupled avidin allowed immunostaining with diaminobenzidine. The mean percentage of cells stained in five fields of the same preparation was used as the score for each preparation, as is usual in pathology. The results were scored independently by two different pathologists. The magnification was 400x.

Statistical methods

The arithmetic mean of cell staining values in five different microscopic fields was used to quantify the immunohistochemistry results in each sample. The Spearman correlation test (11) was used to determine the correlation between the values for *hsp70* and *hsp27*.

RESULTS

Expression of hsp70 and hsp27 in breast cancer patients

Tables 3 and 4 summarize the results obtained in a population from Cartagena, Spain, and a population from Arica, Chile. The *hsp70* and *hsp27* expressions, given as the percentage of cells showing positive immunostaining with the specific antibodies, are shown. In addition, the pathological diagnosis, the patient's age, and the presence or absence of node metastasis were also recorded.

The *hsp70* immunoreaction was recorded in a semiquantitative way, taking the average percentage of stained cells in five fields as the final value. The *hsp70* immunoreaction was positive in all tumors, and the intensity of the reaction was rather high with more than 60% of stained cells per field in all the 35 breast cancers studied. This result is illustrated in Figure 1, where the immunostaining of a breast carcinoma is shown as an example. As can be seen in Figure 1A, the staining of *hsp70* includes the carcinoma area, as well as the areas of infiltration surrounding it (original ×400). At a higher magnification (original ×1000) (Fig. 1B), nuclear staining was clearly visible as was the cytoplasmic reaction. This figure illustrates the situation found in the breast cancers analyzed. The immunohistochemistry data demonstrate the presence, and localization in the nucleus, of *hsp70* in all the 35 samples and in both populations.

The expression of *hsp27* is not as high as *hsp70*, under 50% of stained cells per field (Tables 3 and 4), with an average of 31% in the population from Table 3, and of 8.7% in the population from Table 4. As the expression of *hsp27* was below 10% in two patients of the population from Chile (Table 3), and in several cases of the population from Spain (Table 4), *hsp27* may not be directly related to the presence of tumoral growth *per se*, but perhaps is rather dependent on the particular characteristics of the tumor that remain to be elucidated. Figure 2 shows the *hsp27* immunostaining reaction in breast cancer cells, where a much lower immunoreaction (weaker, with a lower percentage of positive cells) than in the case of *hsp70* is observed.

The two populations clearly differed in the level of expression of the heat shock proteins studied. While the average expression of *hsp70* was found to be 86% in the

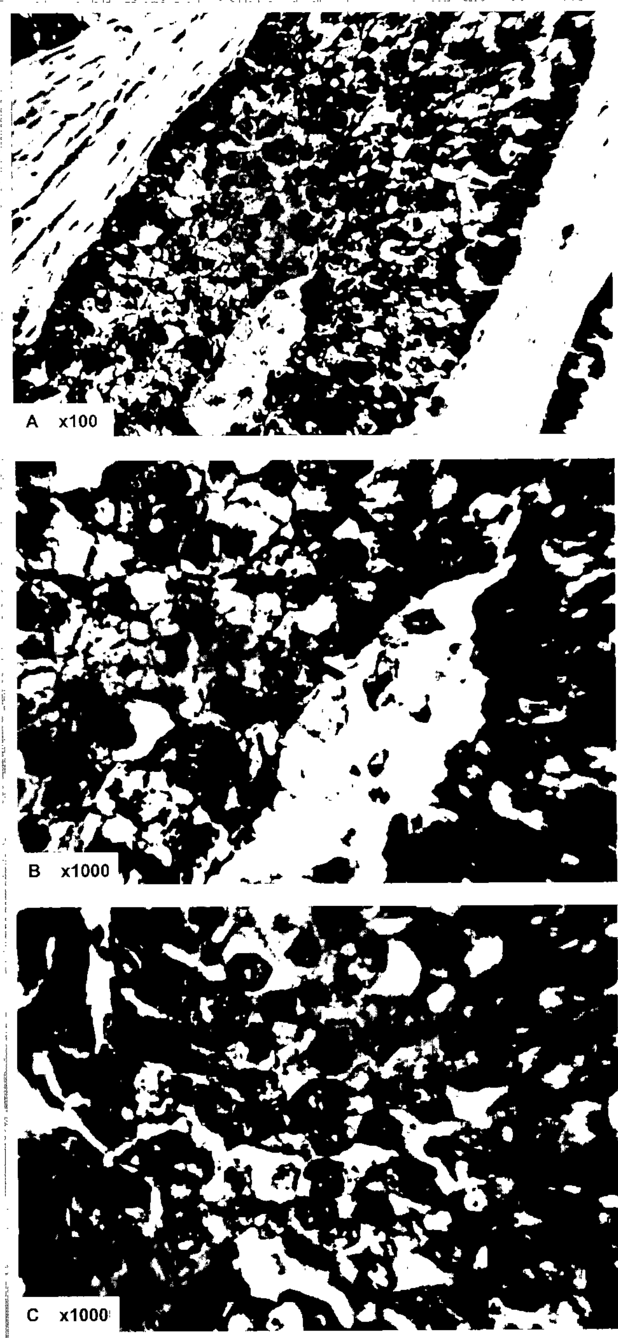

Figure 1. Expression of *hsp70* in a breast carcinoma: A) *hsp70* immunostaining in tumoral cells: positive reaction is observed all over the tumor (original, ×400); B) and C) Different areas of the tumor in A at higher magnification (original, ×1000) where stained nuclei are clearly visible in addition to cytoplasm.

population from Chile (Table 3), it was only 66% in population from Spain (Table 4). *hsp27* expression differences also tended to be the similar: 31% in the Chilean population versus 8.7% in the Spanish popula-

Table 3. Breast cancer tumors in a population from Arica (Chile).

T (age)	Type	Metastasis	*hsp70* (%)	*hsp27* (%)
1 (65)	Papillar	–	87	8
2 (62)	Ductal	–	93	5
3 (62)	Ductal	–	97	35
4 (72)	Ductal	–	94	19
5 (52)	Ductal	Yes	94	76
6 (78)	Lobular	–	89	39
7 (58)	Ductal	–	89	42
8 (65)	Lobular	–	68	22
9 (45)	Ductal	–	78	35
10 (70)	Ductal	–	62	28
11 (49)	Ductal	–	88	39
12 (62)	Ductal	–	93	26

Table 4. Breast cancer tumors in a population from Cartagena (Spain).

T (age)	Type	Metastasis	*hsp70* (%)	*hsp27* (%)
1 (48)	Ductal	–	60	10
2 (53)	Ductal	–	75	8
3 (47)	Ductal	Yes	80	0
4 41)	Ductal	–	65	3
5 (39)	Ductal	–	23	0
6 (55)	Lobular	–	55	6
7 (53)	Ductal	–	60	15
8 (49)	Ductal	–	75	12
9 (47)	Ductal	–	60	13
10 (34)	Ductal	–	61	8
11 (48)	Ductal	Yes	70	20
12 (48)	Ductal	–	68	5
13 (52)	Ductal	Yes	57	8
14 (51)	Ductal	–	65	0
15 (59)	Ductal	Yes	80	0
16 (82)	Ductal	–	75	10
17 (36)	Lobular	–	69	7
18 (48)	Ductal	–	82	6
19 (48)	Ductal	–	69	12
20 (47)	Ductal	Yes	85	17
21 (52)	Ductal	Yes	86	20
22 (51)	Ductal	–	40	15
23 (49)	Ductal	–	60	5

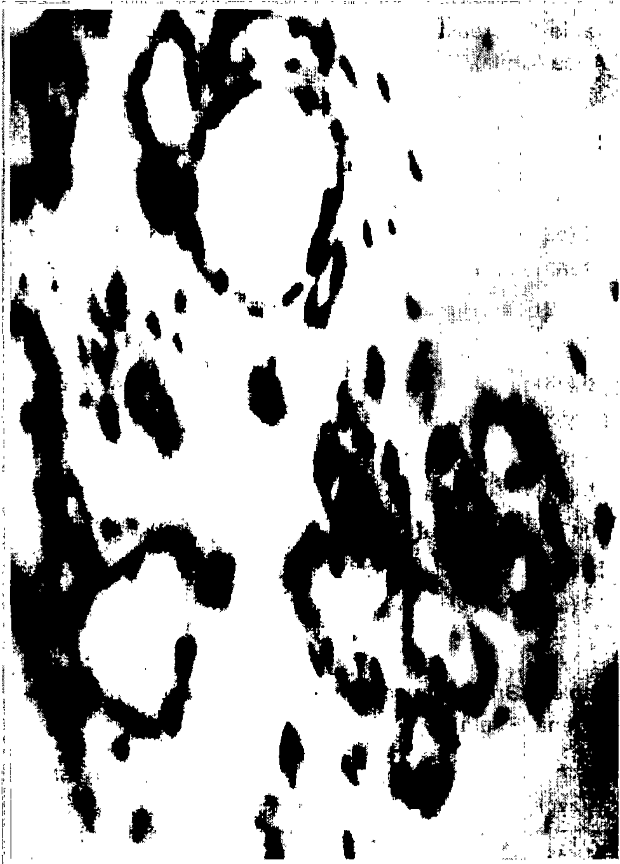

Figure 2. Expression of *hsp27* in an adenocarcinoma, always at cytoplasmatic level, and in sparsed cells (original, ×400).

tion. This difference between the level of *hsp70* and *hsp27* in both populations might be due to environment. The Chilean population lives in the Atacama desert, whereas the Spanish population is from Cartagena on the Mediterranean coast. The conditions of air pollution and diet are different. Another factor of variation may be the age of patients: the patients from the Spanish population are younger than those from the Chilean population. Finally, ethnic differences (genetic background) might play a role because the Chilean population is from an area with an abundant local Aymaran indigenous population.

The expression of *hsp70* and *hsp27* is positively correlated ($r=+0.48$, $p<0.05$) in both populations as a whole. If we consider the expression of both antigens in relation to tumoral prognosis, a high stress score (considered as *hsp70+hsp27*) seems to be related to the presence of metastasis, and hence to a less favorable prognosis (except for patient 13 in Table 3). On the other hand, a high intensity of the *hsp27* immunoreaction is also related to metastasis. In fact, the only case of metastasis found in the population in Table 3 had positive *hsp27* staining in 76% of cells, whereas in the population in Table 4, three patients with metastasis had more than 15% of *hsp27*-stained cells.

In addition to the immunohistochemistry, a confirmation of *hsp70* expression was carried out by Western blot of the samples. Positive controls for *hsp70* and *hsp27* proteins were run in parallel for the blots (data not shown). Figure 3 (A and B) shows the result, with 100% positive *hsp70* bands in total protein extracts from tumors. In this figure, weaker and irregular expression of *hsp27* (compared with *hsp70*) can also be observed. This tallies with the lower percentage of *hsp27* positive cells observed among the total cell population in the preparations.

hsp70 and 27 expression in malignant vs. nonmalignant breast tumors

The high *hsp70* expression in breast tumors is suggestive of *hsp70* expression related to tumor growth. However, a survey of other nonmalignant breast growth processes was considered necessary. In Figure 4 (originals, ×400 A and ×1000 B), the staining pattern of a fibroadenoma (a very common, nonmalignant disease of the breast) is shown. Although there is a percentage of cells showing *hsp70* staining (never higher than 40%), this is always confined to the cytoplasm. In Figure 1, by comparison, stained nuclei are clearly visible in different parts of the malignant tumor (B and C, original ×1000). In nonmalignant processes, *hsp70* is present in the cytoplasm, whereas in all cases of malignant processes, staining appears in the nuclei as well as in the cytoplasm (compare Figures 1B, C and 4B, original ×1000). The pattern of nuclear staining in malignant cells is similar to the nuclear localization described for heat-induced *hsp70* in yeast and *Drosophila*.

When *hsp27* is assayed in nonmalignant lesions no significant immunostaining appears (data not shown). This different behavior of both stress proteins in relation to cancer may reflect the different nature of stimuli triggering them. The nuclear localization of *hsp70* in cancer cells supports the proposal of *hsp70* as a cancer marker. To the best of our knowledge, this is the first report of nuclear localization of *hsp70* in relation to a cancer diagnosis.

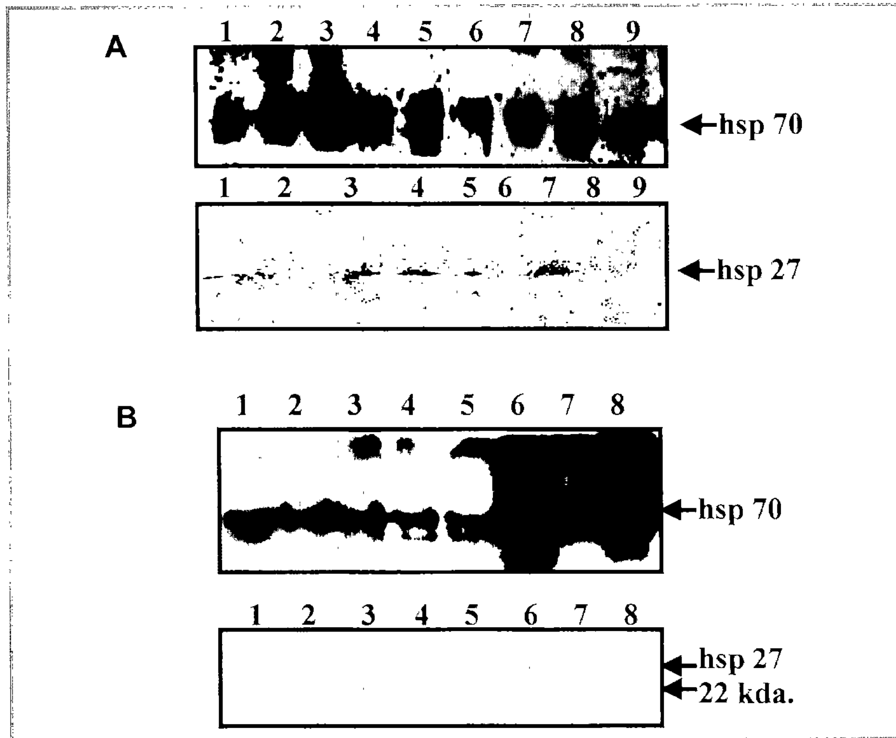

Figure 3. In A and B two examples of Western blots, after PAGE.SDS, followed by immunodeveloping by *hsp70* and *hsp27* antibodies. In all cases, Hsp 70 is strongly positive, whereas the expression is weaker, and not always present for *hsp27*.

DISCUSSION

The present study showed high levels of *hsp70* expression with nuclear translocation in 35 breast cancer patients. The expression of *hsp70* was high (>66% of cells) in all cases of malignant tumoral cells. This high expression of *hsp70* in breast tumors may be a sign of cell stress due to changes in the normal metabolism of the glandular epithelium. *hsp70* could be a stress marker which appears when the normal physiological balance of a tissue is impaired. Lazaris *et al.* (10) suggest that *hsp70* detection might be useful as a marker of the bio-

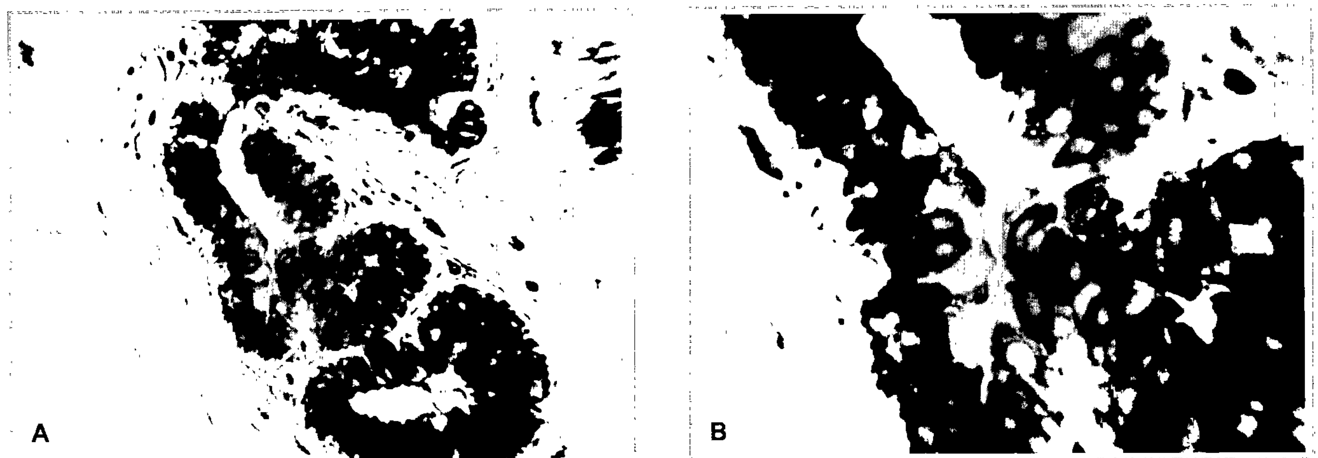

Figure 4. A) Expression of *hsp70* in a fibroadenoma (original, ×400); B) *hsp70* in the benign process, at higher magnification (original, ×1000). Staining is only restricted to cytoplasm.

logical stress experienced by cancer cells. A general relationship between *hsp70* and the proliferative activity of breast cells may be deduced from our results. Concerning *hsp70* and proliferation, a report (12) proposed *hsp70* as a cell proliferation marker. In the same context, studies by Vargas-Roig *et al.* (13) show that *hsp70* is clearly associated with the mitotic spindle and with cell proliferation, with a positive correlation among high scores of the nuclear antigen, PCNA and *hsp70* expression. Our results are in agreement with other studies (12, 13) in that *hsp70* is present in all the proliferative processes of breast tissue. Moreover, our results suggest that the presence of *hsp70* may be associated with cell proliferation in the breast as, even in benign adenomas or fibrocystic diseases, *hsp70*-positive cells are found. In these latter processes, however, nuclear staining was never observed.

The important difference shown in the present work concerns the relationship between cancer and *hsp70* nuclear localization. The specific nuclear allocation of *hsp70* found in malignant processes led us to propose *hsp 70* as a cancer diagnosis marker. *hsp70* is clearly present in most malignant breast-tumor nuclei, whereas it is absent in nuclei from nonmalignant processes. *hsp70* protein is known to be localized in the nucleus and nucleoli, shortly after heat shock. It has been reported that *hsp70* synthesis is increased in cells transfected with "cooperating" oncogenes (c-*myc*) and in association with p53 (14). In breast cancer cells, the expression of other markers, like *p53*, *c-erbB2* might trigger the *hsp70* translocation to nuclei so that these nuclear products become associated, and hence stabilized, by their putative binding to *hsp70*.

Regarding *hsp27*, in Vargas *et al.* (13) it is demonstrated that in a significant percentage of breast cancers, *hsp27* overexpression correlates inversely with cell proliferation. These results add some evidence to the idea that in human breast cancers, *hsp27* may be involved in cell growth arrest and increased differentiation, a role different from *hsp70*. Our results show the expression of *hsp27* exclusively in malignant tumors, and not in proliferative nonmalignant tumors, supporting the idea of differences between both proteins in relation to breast cancer.

At the same time, the suggestion of *hsp70* as a marker for tumoral diagnosis of breast cancer also presents prospects for a new therapy in this type of cancer. Current therapies are based on drugs such as tamoxifen, an estrogen inhibitor. It has been shown, however, that cell resistance appears after prolonged treatment. *In vitro*, acquired resistance to treatment is partly due to the induction of *hsps* by the cytotoxic drugs used. Vargas-Roig *et al.* (15) have recently shown that *hsp70* and *hsp27* are involved in drug resistance in breast cancer patients treated with chemotherapy and hormonotherapy. A new type of breast cancer therapy, based on immunological treatment, using anti-*hsp* antibodies, or based on drugs that specifically inhibit heat shock protein induction, could preferentially target cells expressing *hsps*, *i.e.*, tumoral cells, at least in breast cancer. Moreover, this could also prevent the drug-resistant effect by induction of *hsps*, so frequent after long periods of treatment. In agreement with immunological therapy, the results reported by Conroy *et al.* (16) demonstrate the natural presence of anti-*hsp27* antibodies in the sera of breast cancer patients (but not in normal controls). This presence correlates with an improved survival rate.

Studies to elucidate the correlation between different tumoral markers used in breast cancer and *hsps* is under way. Nevertheless, a study of the relationship between the tumoral differentiation stage, patient survival and *hsps* is a task that needs to be taken up in forthcoming research. *In vitro* experiments using immunological and chemotherapy treatment against *hsps* might also shed some light on the possibility of new cancer therapies.

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REFERENCES

1. Morimoto RI, Tissières AG, Georgopoulos C. The biology of heat shock proteins and molecular chaperones. Cold Spring Harbour Laboratory Press, New York 1994.

2. Welch WJ. *How cells respond to stress.* Sci Am 1993; 268(5): 56-64.
3. Ciocca DR, Oesterreich S, Chamness GC, McGuire WL, Fuqua SA. *Biological and clinical implications of heat shock protein 27000 (hsp27): A review.* J Natl Cancer Inst 1993; 85: 1558-1570.
4. Delhaye M, Gulbis B, Galand P, Mairesse N. *Expression of 27 kDa heat shock protein isoforms in human neoplastic and non-neoplastic liver tissues.* Hepatology 1992; 16(2): 382-389.
5. Mileo AM, Fanuele M, Battaglia F et al. *Selective overexpression of mRNA coding for 90kDa stress protein in human ovarian cancer.* Anticancer Res 1990; 10: 903-906.
6. Yufu Y, Nishimura J, Takahira H, Ideguchi H, Nawata H. *Down regulation of a Mr 90000 heat shock cognate protein during granulocytic differentiation in HL-60 human leukemia cells.* Cancer Res 1989; 49: 2405-2408.
7. Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. *Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life.* Mol Cell Biol 1988; 8: 531-539.
8. Nihei T, Takahashi S, Sagae S, Sato N, Kikuchi K. *Protein interaction of retinoblastoma gene product pRb110 with Mr 73000 heat shock cognate protein.* Cancer Res 1993, 53: 1702-1705.
9. Takahashi S, Toshihiko M, Yoshiki W et al. *Correlation of heat shock protein 70 expression with estrogen receptor levels in invasive human breast cancer.* Anatomic Pathology 1993; 101: 519-525.
10. Lazaris A, Chatzigianni EB, Panoussopoulos D, Tzimas GN, Davaris PS, Golematis B. *Proliferating cell nuclear antigen and heat shock protein 70 immunolocalization in invasive ductal breast cancer not otherwise specified.* Breast Cancer Res Treatment 1997; 43: 43-51.
11. Sokal RR, Rohlf FJ. Biometry. W.H. Freeman and Company, New York 1969.
12. Yano M, Naito Z, Tanaka S, Asano G. *Expression and roles of heat shock proteins in human breast cancer.* Jpn J Cancer 1996; 87: 908-915.
13. Vargas-Roig L, Fanelli M, López LA, Gago FE, Tello O, Aznar JC, Ciocca DR. *Heat shock proteins and cell proliferation in human breast cancer biopsy samples.* Cancer Detect Prev 1997; 21: 441-451.
14. Welch WJ. *The mammalian stress response: Cell physiology and biochemistry of stress proteins.* In: Morimoto RI, Tissières AG, Georgopoulos C (Eds.). Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press 1994; 223-278.
15. 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; 23: 468-475.
16. Conroy SE, Sasieni PD, Amin V et al. *Antibodies to heat-shock protein 27 are associated with improved survival in patients with breast cancer.* Br J Cancer 1998; 77: 1875-1879.