

Serological Detection of Heat Shock Protein hsp27 in Normal and Breast Cancer Patients¹

Mariel A. Fanelli, F. Darío Cuello Carrión,
Judith Dekker, Joop Schoemaker, and Daniel R. Ciocca²

Laboratory of Reproduction and Lactation, Regional Center for Scientific and Technological Research, Mendoza 5500, Argentina [M. A. F., D. C. C., D. R. C.], and The Institute for Endocrinology, Reproduction and Metabolism, Medical Centre of the Vrije Universiteit, Amsterdam, the Netherlands [J. D., J. S.]

Abstract

Heat shock protein M_r 27,000 (hsp27) is found in many human breast cancer cells and tissues; its expression is associated with the presence of estrogen receptors, lower cell proliferation, and resistance to certain chemotherapies. The purpose of this study was to assess whether hsp27 may be present in sera from women with primary breast cancer and to know whether autoantibodies to hsp27 may be found in these patients. The study was performed by Western blot analyzing sera from 42 normal premenopausal women, 20 normal postmenopausal women, and 36 breast cancer patients. hsp27 was clearly detected in sera by immunoblotting but only after immunoprecipitation. The mean hsp27 levels in cancer patients were higher than in the control patients; however, 66% of the breast cancer patients showed hsp27 within the normal range, indicating low sensitivity. Moreover, cancer patients with metastatic disease did not show significantly higher hsp27 levels than cancer patients without metastases. Serum hsp27 levels did not correlate with the hsp27 levels in tumor tissues detected by immunohistochemistry. Elevated CA 15-3 levels were not associated with high hsp27 values. Autoantibodies against hsp27 were not detected by immunoblotting in normal sera and in sera from breast cancer patients. As a consequence, serological determination of this biomarker is unlikely to be of utility in the detection and follow-up of breast cancer patients.

Introduction

Several biomarkers have been evaluated in serum from breast cancer patients. The primary use is in following the clinical course of the disease (detecting metastatic progression) and monitoring response to therapy (1–5). CA 15-3 is one of the

most efficient tumor markers; it is defined by two monoclonal antibodies (DF3 and 115D8), and its specificity is about 95%, whereas its sensitivity is about 65% (6). To increase the sensitivity, a combination of tumor markers has been tested without significant results (5, 6). At present, there are not adequate tumor markers to detect early stages of breast cancer; therefore, they are not applicable for screening. There is still a need for additional serological biomarkers useful in breast cancer patients.

hsps³ belong to a family of proteins induced by heat shock and others stressful situations (*e.g.*, hypoxia and anoxia, oxidant injury, and accumulation of damaged proteins). Many of the hsps are also constitutively expressed, playing important roles as molecular chaperones in normal cell function (7–9). We are particularly interested in one of the small hsps, hsp27, because this protein is estrogen-regulated and appears with increased frequency in human breast cancer tissues (10). The expression of hsp27 in breast cancer biopsies has been controversially associated with the clinical outcome of the disease (11–17). In a recent definitive study evaluating a large number of axillary lymph node-negative breast cancer patients, hsp27 did not appear as a clinically useful prognostic marker (18). The expression of this protein has not been associated with a response to tamoxifen in patients with advanced disease (19). Of interest is the *in vitro* and clinical data supporting the association of hsp27 with drug resistance in breast cancer cells and tissues (20–22). In addition, controversial associations of hsp27 expression with prognosis have been reported in several other malignant diseases (23–28).

hsp27 seems to function as a molecular chaperone and is involved in signal transduction pathways of cell regulators and in the development of resistance to stressful conditions. Most of hsp27 is found in the cytoplasm of breast tumor cells (29); however, in MCF-7 cells grown in the ascites fluid of nude mice, hsp27 is found in the apical cytoplasm of the cells, sometimes appearing in granules, suggesting secretion (30). In the present study, we have investigated whether hsp27 can be detected in serum samples from healthy women and from women with different stages of breast cancer to know whether quantitative detection of this protein could be a serviceable serological biomarker.

Materials and Methods

Study Population. The study involved 14 healthy premenopausal women, 20 postmenopausal women, and 30 women with breast cancer (6 premenopausal and 24 postmenopausal). Because hsp27 is estrogen-regulated during the menstrual cycle in normal human endometrium (31), three blood samples were taken from each of the healthy women at different stages of the menstrual cycle: menstrual phase (day 2), end of follicular

Received 1/30/98; revised 6/3/98; accepted 6/15/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by Grant BID 802/OC-AR PID 101 from the National Agency for Scientific and Technological Promotion of Argentina and by the Vrije Universiteit of Amsterdam.

² To whom requests for reprints should be addressed, at Laboratory of Reproduction and Lactation, Casilla de Correo 855, 5500 Mendoza, Argentina. Phone: 54-61-286112; Fax: 54-61-287370; E-mail: cricyt@lanet.losandes.com.ar.

³ The abbreviations used are: hsp, heat shock protein; ER, estrogen receptor.

Table 1 Main characteristics of the study population

	Cases	Age ($\bar{x} \pm SD$)	Disease stage		Tumor type ^a		ER content ^b		
			I-II	IV	IDC	MUC	+	-	NT
Controls									
Premenopausal	14	34 \pm 4							
Postmenopausal	20	59 \pm 9							
Breast cancer patients									
Premenopausal	6	39 \pm 8	5	1	6			4	2
Postmenopausal	24	63 \pm 11	15	9	23	1	14	6	4

^a IDC, infiltrating ductal carcinoma; MUC, mucinous carcinoma.

^b NT, not tested.

phase (days 13–14), and mid-luteal phase (days 18–22). Therefore, a total of 42 samples from healthy premenopausal women were analyzed. We also analyzed sera from 20 normal postmenopausal women. From the 30 women with breast cancer entering into the study, we studied 36 blood samples (in five cases, two or three samples were taken at different times). In addition, 11 (36.6%) of the women with breast cancer were suffering distant metastases, whereas the other patients had no evidence of recurrent disease or new breast cancer since completion of initial local treatment. In eight breast cancer patients, tumor samples were collected for comparison of hsp27 expression levels in blood and tumor tissue (blood and tumor samples were obtained the same day). Table 1 shows the main characteristics of the patients entering into the study.

Serum Measurements. Blood samples were collected by venipuncture during the mornings. After collection, blood was allowed to clot, and serum samples were recovered after refrigerated centrifugation at $2300 \times g$ at 4°C for 10 min. Serum samples were then stored at -70°C until analyses.

To have a stringent immunological analysis to determine the specificity and quantity of hsp27 in the serum, we decided to study the protein by immunoblotting procedures rather than by ELISA. Serum proteins were separated by SDS-PAGE followed by Western blotting as described elsewhere (32). One lane was loaded with molecular weight markers (Rainbow Marker; Amersham, Buckinghamshire, UK): lysozyme (M_r 14,300); trypsin inhibitor (M_r 21,500); carbonic anhydrase (M_r 30,000); ovalbumin (M_r 46,400); BSA (M_r 69,000); and phosphorylase B (M_r 97,000). Another lane was loaded with a positive control, which was also used to calibrate band densities for interassay variations. The positive control (60 μg of cytosol protein loaded on the stacking gel) was obtained from a human breast cancer tumor sample expressing hsp27. Because almost no specific hsp27 protein band was detected by Western blot in the serum samples directly loaded on the gel (25 μl of serum diluted 1:100 in sample buffer), we decided to enrich hsp27 by immunoprecipitation before electrophoresis. Immunoprecipitation was carried out using an antibody against hsp27 attached to magnetic iron oxide particles (BioMag; Advanced Magnetics, Inc., Cambridge, MA). Coupling of the mouse monoclonal antibody C11 against hsp27 (33) to the magnetic particles was performed according to manufacturer's instructions. Then, 25 μl of serum or of the cytosol from the positive control were incubated with 50 μl of the magnetic particles attached with the antibody, and the final volume of 1 ml was completed with buffer (0.01 M Tris, 0.1% NaN_3 , 0.1% w/v BSA, 0.15 M NaCl, and 0.001 M EDTA, pH 7.4). After overnight incubation (with shaking), the particles were magnetically separated and then washed three times with the buffer. After the last washing, 250

μl of sample electrophoresis buffer were added to the particles, the obtained solution was boiled for 3 min, and then 25 μl were loaded on the gel.

Detection of the specific hsp27 band on the nitrocellulose paper was performed using a rabbit polyclonal antibody generated against a chimeric hybrid protein containing the NH_2 -terminal of murine hsp25 and the COOH-terminal of human hsp27 (34) in a dilution of 1:2000 (with dilution buffer containing 4.4 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 37 mM NaCl, 0.6 mM thimerosal, and 1% BSA, pH 7.4). After overnight incubation at 4°C with shaking, the membranes were washed and incubated with a swine antibody to rabbit immunoglobulins biotinylated (Dako, Glostrup, Denmark; 1:4000) for 60 min. After washing, the membranes were incubated with peroxidase-labeled streptavidin biotin complex (1:6000) for 45 min. Washed membranes were then incubated with chemiluminescence reagents (Dupont NEN, Boston, MA) following manufacturer's instructions. The light was captured on autoradiography film (Sigma Chemical Co., St. Louis, MO). The bands were screened and quantitated using a computer/image analysis system (NIH Image ver. 1.6).

Because autoantibodies against hsps have been reported in some autoimmune diseases (35, 36), we also explored whether normal and breast cancer patients might have autoantibodies against hsp27. This was carried out incubating nitrocellulose membranes containing hsp27 (positive breast cancer cytosol) with serum from breast cancer and normal patients (1:50 dilution, overnight, at 4°C). After washing, the membranes were incubated with goat anti-human polyvalent antibody, biotinylated (Sigma; diluted 1:1000), and band detection was performed as described above. This protocol was performed according to that used for the detection of p53 autoantibodies in breast cancer patients (37).

CA 15-3 levels in serum samples from breast cancer patients were determined using an enzyme immunoassay kit (CIS Bio International, Gif-Sur-Yvette Cedex, France), according to the kit's instructions.

Tissue Measurements. The presence of hsp27 and ER in the tumor biopsy samples were evaluated by immunohistochemistry as described elsewhere (29, 38) using routinely processed, formalin-fixed, paraffin-embedded tumor tissues. Specific hsp27 and ER immunostaining on tumor cells was semiquantitatively evaluated, taking into account the intensity of staining (0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining) and the proportion of stained cells (0, no staining; 1, <10%; 2, 10–33%; 3, 34–66%; 4, >66%). This scoring system was applied for the cytoplasmic and nuclear immunostainings, and it has been validated on several studies (38, 39).

Statistical Analysis. A computer program (GraphPad PRISM, San Diego, CA) for the PC was used for statistical analysis. Data from serum samples of normal and breast cancer patients were subjected to ANOVA (one-way ANOVA) with post test: Newman Keuls and unpaired t tests with Welch's correction to determine whether their differences in the extent of hsp27 content were significant; significance was accepted with a probability value of $P \leq 0.05$.

Results

On immunoblot analysis using sera from breast cancer patients and normal sera, none of the sera tested showed a clear and distinct band with a M_r of 27,000 daltons. Therefore, to increase the sensitivity of the detection technique, immunoblot analysis was performed on sera after immunoprecipitation with a monoclonal antibody against hsp27. Under these conditions, a spe-

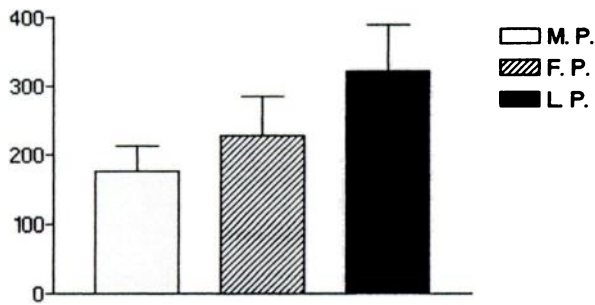


Fig. 1. hsp27 levels expressed in absorbance for each phase of the menstrual cycle are seen. □, M. P., menstrual phase, 176 ± 37 ; ▨, F. P., follicular phase, 228 ± 56 ; ■, L. P., luteal phase, 321 ± 68 . No statistically significant differences were found. Data shown are means; bars, SE.

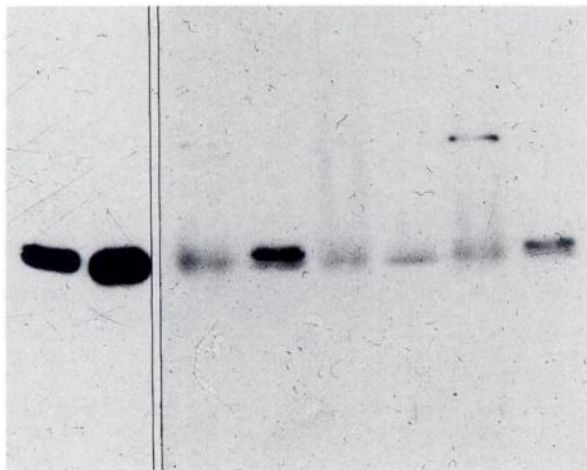


Fig. 2. Immunoblotting for hsp27 of samples obtained from a breast tumor tissue (positive control, left side of the panel) and from sera of breast cancer patients ($n = 3$). Two lanes for each sample were run, one directly loading the cytosol and sera, and the other loading the samples after immunoprecipitation. Note the increase of intensity of the hsp27 band after immunoprecipitation with a monoclonal antibody against hsp27 attached to magnetic particles.

cific hsp27 band could be identified both in normal sera and in sera from breast cancer patients. Fig. 1 shows that hsp27 levels were higher in normal women during the mid-luteal phase of the menstrual cycle but without reaching statistically significant differences when compared with the other stages of the menstrual cycle examined.

hsp27 could also be identified in the serum samples from breast cancer patients after immunoprecipitation (Fig. 2). The hsp27 levels in normal women and in cancer patients are shown in Fig. 3. The statistical analysis showed that the mean hsp27 serum levels in postmenopausal breast cancer patients were higher than those observed in postmenopausal controls ($P = 0.0077$, unpaired t test with Welch's correction). However, 66% of the breast cancer patients showed hsp27 levels within the normal range. Other comparisons did not reach significant differences. It is important to note that cancer patients with metastatic disease did not show significantly higher hsp27 levels than cancer patients without metastases ($P = 0.051$, unpaired t test with Welch's correction). The 400 absorbance hsp27 value was then defined as cut-point, taken into account the values obtained in the normal controls (mean \pm SD: premenopausal, 241 ± 210 ; postmenopausal, 187 ± 142).

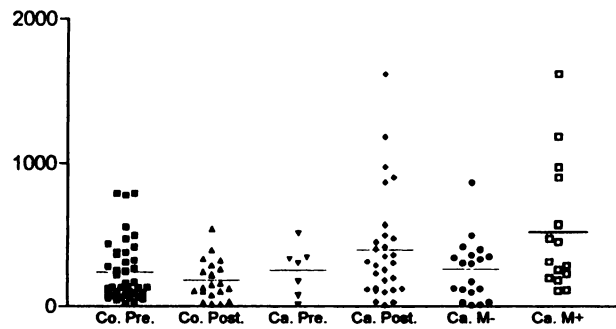


Fig. 3. hsp27 levels in normal women and breast cancer patients. Bars, mean for each group. Co. Pre., control premenopausal women (■; $n = 242$); Co. Post., control postmenopausal women (▲; $n = 187$); Ca. Pre., cancer premenopausal patients (▼; $n = 256$); Ca. Post., cancer postmenopausal patients (◆; $n = 404$); Ca. M-, cancer patients without metastases (●; $n = 263$); Ca. M+, cancer patients with metastases (□; $n = 501$).

Table 2 CA15-3 and hsp27 levels in breast cancer patients

Stage	Patients	CA 15-3 % > 50 units/ml	hsp27 % > 400 A
I-II	20	5	20
IV	10	100	60

Table 3 hsp27 values in serum and tumor tissues from breast cancer patients

Patient no.	hsp27 in serum (A) ^a	ER	hsp27 in tissue ^a		
			Cytoplasm	Nuclear	Total score
1	409	+	3 + 3	0	6
2	202	+	2 + 2	1 + 1	6
3	357	NT	2 + 2	0	4
4	350	+	2 + 4	0	6
5	131	+	2 + 3	0	5
6	320	+	2 + 4	1 + 1	8
7	122	+	3 + 2	1 + 1	7
8	80	-	2 + 3	0	5

^a Serum hsp27 levels were evaluated in A, whereas in the tissues, hsp27 and ER levels were evaluated according to intensity of staining and proportion of stained cells (see scores in "Materials and Methods").

hsp27 serum values were then compared with CA 15-3 serum levels; in our study, all of the patients with high CA 15-3 values (≥ 50 units/ml) had advanced disease with distant metastases. As shown in Table 2, only 60% of the patients with distant metastases showed high hsp27 values (> 400 A). Moreover, high hsp27 values were seen in 20% of the breast cancer patients without distant metastases (5% for CA 15-3 levels).

In eight of the breast cancer samples tested, we studied the paraffin blocks to correlate the hsp27 levels in the sera with the hsp27 expression levels in the tumor tissues (Table 3). No correlation was found in hsp27 expression levels in the sera and matched tumor tissues. High hsp27 levels were observed in 55% of ER+ patients and in 45% of ER- patients (data not shown).

Finally, no anti-hsp27 antibodies were detected on immunoblot analysis in the serum from the breast cancer patients examined ($n = 11$) and from normal controls ($n = 5$); however, other bands at M_r 32,800, 54,000, 61,800, and 67,000 were observed (sera from breast cancer patients).

Discussion

The hsp27 status of breast cancer patients has been investigated previously in cancer tissues, and the data have shown: (a) correlation between the presence of hsp27 and estrogen receptor (39, 40); (b) lack of prognostic significance in lymph node-negative patients (18); (c) an inverse correlation of hsp27 overexpression with cell proliferation (29); and (d) association of hsp27 with resistance to chemotherapy (20–22). However, there are no studies evaluating the presence of hsp27 in serum samples or the production of autoantibodies to hsp27 in breast cancer patients. hsp27 together with other hsps are molecular chaperones; they may be escorting proteins targeted to different cell compartments. hsps are mainly present in the cytoplasm but sometimes may be located in the nucleus and on the cell surface (7–10, 41). For example, hsp72 has been found expressed only on the cell surface of human tumor cells after a nonlethal heat stress, but not on normal cells (42). We can then speculate that under certain circumstances, hsps might be released to the circulation actively or in the clearance of cell contents that are liberated during cell replacement/renewal, and that they might also induce autoantibodies.

In the present study, we have not found autoantibodies against hsp27 in normal or in breast cancer patients; however, our observations are compatible with the presence of hsp27 in serum in human normal women and in women with breast cancer. In previous studies, autoantibodies against certain hsps (*i.e.*, hsp60, hsp72, and hsp90) have been found, but not against hsp27 (35, 36). Moreover, to our knowledge, no autoantibodies against hsps have been detected in cancer diseases.

The quantity of hsp27 present in the serum is very low because the protein could be clearly detected only after enrichment by immunoprecipitation. hsp27 is found in many tissues, but higher expression is seen in estrogen-target tissues (10). We then asked whether the protein is in part released from these tissues to the blood with more predominance at certain stages of the menstrual cycle; in fact, more hsp27 content has been noted in the endometrium during the luteal phase of the menstrual cycle (31). Our study shows that hsp27 levels were rising from menstrual days to proliferative phase to luteal phase; however, these changes did not reach statistical significance.

Our study shows that immunoreactive hsp27 could also be found in sera from breast cancer patients. In general, higher hsp27 levels were observed in breast cancer patients than in the controls; however, 66% of the breast cancer patients showed hsp27 levels within the normal range, indicating low sensitivity. A lack of correlation was noted between hsp27 expression in the tumor tissues and the amount of immunoreactive hsp27 in corresponding serum samples. In addition, high hsp27 levels (≥ 400 A) were seen in only 60% (6 of 10) of the patients with distant metastases, and all of these patients had pathological CA 15-3 levels (≥ 50 units/ml). Therefore, serological determination of hsp27 is unlikely to be of utility in the detection and follow-up of breast cancer patients.

References

- Berruti, A., Tampellini, M., Torta, M., Buniva, T., Gorzegno, G., and Dogliotti, L. Prognostic value in predicting overall survival of two mucinous markers: CA 15-3 and CA 125 in breast cancer patients at first relapse of disease. *Eur. J. Cancer*, 30A: 2082–2084, 1994.
- Molina, R., Agusti, C., Filella, X., Jo, J., Joseph, J., Gimenez, N., and Ballesta, A. M. Study of a new tumor marker, CYFRA 21-1, in malignant and nonmalignant diseases. *Tumour Biol.*, 15: 318–325, 1994.
- Frenette, P. S., Thirlwell, M. P., Trudeau, M., Thomson, D. M., Joseph, L., and Shuster, J. S. The diagnostic value of CA 27-29, CA 15-3, mucin-like carcinoma antigen, carcinoembryonic antigen and CA 19-9 in breast and gastrointestinal malignancies. *Tumour Biol.*, 15: 247–254, 1994.
- Cohen, A. D., Shoenfeld, Y., Gopas, J., and Cohen, Y. Immunosuppressive acidic protein serum levels in breast cancer patients in a reference to CA 15-3 levels. *Breast Cancer Res. Treat.*, 30: 197–200, 1994.
- Vankrieken, L., Heureux, F., Longueville, J., and de-Hertogh, R. Longitudinal follow-up of breast cancer patients with the tumor markers CA 549 and CA 15-3. *Int. J. Biol. Markers*, 10: 30–34, 1995.
- Vizcarra, E., Lluch, A., Cibrián, R., Jarque, F., Alberola, V., Belloch, V., and García-Conde, J. Value of CA 15-3 in breast cancer and comparison with CEA and TPA: a study of specificity in disease-free follow-up patients and sensitivity in patients at diagnosis of the first metastasis. *Breast Cancer Res. Treat.*, 37: 209–216, 1996.
- Laszlo, A. The effects of hyperthermia on mammalian cell structure and function. *Cell Prolif.*, 25: 59–87, 1992.
- Jakob, U., and Buchner, J. Assisting spontaneity: the role of Hsp90 and small hsps as molecular chaperones. *Trends Biochem. Sci.*, 19: 205–211, 1994.
- Hendrick, J. P., and Hartl, F. U. Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem.*, 62: 349–384, 1993.
- Ciocca, D. R., Oesterreich, S., Chamness, G. C., McGuire, W. L., and Fuqua, S. A. W. Biological and clinical implications of heat shock protein 27000 (Hsp27): a review. *J. Natl. Cancer Inst.*, 85: 1558–1570, 1993.
- Tandon, A. K., Clark, G. M., Chamness, G. C., Fuqua, S. A. W., Welch, W. J., Rihel, R. M., and McGuire, W. L. Heat shock stress response proteins: biological and clinical significance in breast cancer. *Proc. Am. Soc. Clin. Oncol.*, 9: 84, 1990.
- Seymour, L., Bezwoda, W. R., and Meyer, K. Tumor factors predicting for prognosis in metastatic breast cancer: the presence of p24 predicts for response to treatment and duration of survival. *Cancer (Phila.)*, 66: 2390–2394, 1990.
- Thor, A., Benz, C., Moore, D. I., Goldman, E., Edgerton, S., Landry, J., Schwartz, L., Mayall, B., Hickey, E., and Weber, L. A. Stress response protein (srp-27) determination in primary human breast carcinomas: clinical, histologic, and prognostic correlations. *J. Natl. Cancer Inst.*, 83: 170–178, 1991.
- Darmstrup, L., Andersen, J., Kufe, D. W., Hayes, D. F., and Skovgaard Poulsen, H. Immunocytochemical determination of the estrogen-regulated M_r 24,000, M_r 52,000 and DF3 breast cancer associated antigen: clinical value in advanced breast cancer and correlation with estrogen receptor. *Ann. Oncol.*, 3: 71–77, 1992.
- Hurlimann, J., Gebhard, S., and Gomez, F. Oestrogen receptor, progesterone receptor, pS2, ERD5, HSP27 and cathepsin D in invasive ductal breast cancer. *Histopathology*, 23: 239–248, 1993.
- Love, S., and King, R. J. B. A 27 kDa heat shock protein that has anomalous prognostic powers in early and advanced breast cancer. *Br. J. Cancer*, 69: 743–748, 1994.
- Tetu, B., Brisson, J., Landry, J., and Huot, J. Prognostic significance of heat-shock protein-27 in node-positive breast carcinoma: an immunohistochemical study. *Breast Cancer Res. Treat.*, 36: 93–97, 1995.
- Oesterreich, S., Hilsenbeck, S. G., Ciocca, D. R., Allred, D. C., Clark, G. M., Chamness, G. C., Osborne, C. K., and Fuqua, S. A. W. The small heat shock protein HSP27 is not an independent prognostic marker in axillary lymph node-negative breast cancer patients. *Clin. Cancer Res.*, 2: 1199–1206, 1996.
- Ciocca, D. R., Green, S., Elledge, R. M., Clark, G. M., Pugh, R., Ravdin, P., Lew, D., Martino, S., and Osborne, C. K. 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.*, 5: 1263–1266, 1998.
- Ciocca, D. R., Fuqua, S. A. W., Lock-Lim, S., Toft, D. O., Welch, W. J., and McGuire, W. L. Response of human breast cancer cells to heat shock and chemotherapeutic drugs. *Cancer Res.*, 52: 3648–3654, 1992.
- Ciocca, D. R., and Vargas-Roig, L. M. Heat shock proteins and drug resistance in breast cancer. *In: S. D. Bernal (ed.), Drug Resistance in Oncology*, pp. 167–190. New York: M. Dekker, 1997.
- Vargas-Roig, L. M., Gago, F. E., Tello, O., Aznar, J. C., and Ciocca, D. R. Heat shock protein expression and drug resistance in breast cancer patients treated with induction chemotherapy. *Int. J. Cancer*, in press, 1998.
- Harrison, J. D., Jones, J. A., and Ellis, I. O. Oestrogen receptor D5 antibody is an independent negative prognostic factor in gastric cancer. *Br. J. Surg.*, 78: 334–336, 1991.
- Tetu, B., Lacasse, B., Bouchard, H.-L., Lagace, R., Huot, J., and Landry, J. Prognostic influence of hsp-27 expression in malignant fibrous histiocytoma: a clinicopathological and immunohistochemical study. *Cancer Res.*, 52: 2325–2328, 1992.
- Storm, F. K., Mahvi, D. M., and Gilchrist, K. Hsp-27 has no diagnostic or prognostic significance in prostate or bladder cancers. *Urology*, 42: 379–382, 1993.

26. Ungar, D. R., Hailat, N., Strahler, J. R., Kuick, R. D., Brodeur, G. M., Seeger, R. C., Reynolds, C. P., and Hanash, S. M. Hsp27 expression in neuroblastoma: correlations with disease state. *J. Natl. Cancer Inst.*, **86**: 780–784, 1994.
27. Langdon, S. P., Rabiasz, G. J., Hirst, G. L., King, R. J. B., Hawkins, R. A., Smyth, J. F., and Miller, W. R. Expression of the heat shock protein hsp27 in human ovarian cancer. *Clin. Cancer Res.*, **1**: 1603–1609, 1995.
28. Geisler, J. P., Geisler, H. E., Tammela, J., Weimann, M. C., Zhou, Z., Miller, G. A., and Crabtree, W. A study of heat shock protein 27 in endometrial carcinoma. *Proc. Am. Assoc. Cancer Res.*, **39**: 149, 1998.
29. Vargas-Roig, L. M., Fanelli, M. A., Lopez, L. A., Gago, F. E., Tello, O., Aznar, J. C., and Ciocca, D. R. Heat shock proteins and cell proliferation in human breast cancer biopsy samples. *Cancer Detect. Prev.*, **21**: 441–451, 1997.
30. Ciocca, D. R., Adams, D. J., Edwards, D. P., Bjercke, R. J., and McGuire, W. L. Estrogen-induced 24k protein in MCF-7 breast cancer cells is localized in granules. *Breast Cancer Res. Treat.*, **4**: 261–268, 1984.
31. Ciocca, D. R., Asch, R. A., Adams, D. J., and McGuire, W. L. Evidence for modulation of a 24K protein in human endometrium during the menstrual cycle. *J. Clin. Endocrinol. Metabol.*, **57**: 496–499, 1983.
32. Ciocca, D. R., Puy, L. A., and Fasoli, L. C. Study of estrogen receptor, progesterone receptor, and the estrogen-regulated M_r 24,000 protein in patients with carcinomas of the endometrium and cervix. *Cancer Res.*, **49**: 4298–4304, 1989.
33. Ciocca, D. R., Adams, D. J., Bjercke, R. J., Sledge, G. W., Edwards, D. P., Chamness, G. C., and McGuire, W. L. Monoclonal antibody storage conditions and concentration effects on immunohistochemical specificity. *J. Histochem. Cytochem.*, **31**: 691–696, 1983.
34. Engel, K., Knauf, U., and Gaestel, M. Generation of antibodies against human hsp27 and murine hsp25 by immunization with a chimeric small heat shock protein. *Biomed. Biochim. Acta.*, **9**: 1065–1071, 1991.
35. Wakui, H., Itoh, H., Tashima, Y., Kobayashi, R., Nakamoto, Y., and Miura, A. B. Specific antibodies against the stress-inducible 72-kDa protein, a member of the heat-shock protein hsp70, in healthy human subjects. *Int. J. Biochem.*, **23**: 975–978, 1991.
36. Arora, S. K., Singh, G., and Sehgal, S. Comparative evaluation of anti-heat shock protein antibodies in SLE and healthy controls. *Scand. J. Rheumatol.*, **24**: 160–163, 1995.
37. Lubin, R., Schlichtholz, B., Teillaud, J. L., Garay, E., Bussel, A., Wild, C. P., and Soussi, T. P53 antibodies in patients with various types of cancer: assay, identification, and characterization. *Clin. Cancer Res.*, **1**: 1463–1469, 1995.
38. Fanelli, M. A., Vargas Roig, M. L., Gago, F. E., Tello, O., Lucero de Angelis, R., and Ciocca, D. R. Estrogen receptors, progesterone receptors, and cell proliferation in human breast cancer. *Breast Cancer Res. Treat.*, **37**: 217–228, 1996.
39. Adams, D. J., and McGuire, W. L. Quantitative enzyme-linked immunosorbent assay for the estrogen-regulated M_r 24,000 protein in human breast tumors: correlation with estrogen and progesterone receptors. *Cancer Res.*, **45**: 2445–2449, 1985.
40. Ciocca, D. R., Stati, A. O., and Amprino de Castro, M. M. Colocalization of estrogen and progesterone receptors with and estrogen-regulated heat shock protein in paraffin sections of human breast and endometrial cancer tissues. *Breast Cancer Res. Treat.*, **16**: 243–251, 1990.
41. Piselli, P., Vendetti, S., Vismara, D., Poccia, F., Delpino, A., Ivanyi, J., and Colizzi, V. Intracytoplasmatic and membrane expression of 28-kDa, 65-kDa, and 70-kDa heat shock proteins on lymphomonocytoid tumor cells lines. *Fund. Clin. Immunol.*, **2**: 45–51, 1994.
42. Multhoff, G., Botzler, C., Wiesnet, M., Muller, E., Meier, T., Wilmanns, W., and Issels, R. D. A stress-inducible 72-kDa heat shock protein (HSP27) is expressed on the surface of human tumor cells, but not on normal cells. *Int. J. Cancer.*, **61**: 272–279, 1995.