Cell Stress and Chaperones
DOI 10.1007/s12192-013-0475-2

ORIGINAL PAPER

Prognostic implication of HSPA (HSP70) in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy

Silvina B. Nadin · Mayra L. Sottile · Maria M. Montt-Guevara · Gisel V. Gauna · Pedro Daguerre · Marcela Leuzzi · Francisco E. Gago · Jorge Ibarra · F. Darío Cuello-Carrión · Daniel R. Ciocca · Laura M. Vargas-Roig

Received: 7 August 2013 / Revised: 24 October 2013 / Accepted: 29 October 2013

Abstract Neoadjuvant chemotherapy is used in patients with locally advanced breast cancer to reduce tumor size before surgery. Unfortunately, resistance to chemotherapy may arise from a variety of mechanisms. Heat shock proteins (HSPs), which are highly expressed in mammary tumor cells, have been implicated in anticancer drug resistance. In spite of the widely described value of HSPs as molecular markers in cancer, their implications in breast tumors treated with anthracycline-based neoadjuvant chemotherapy has been poorly explored. In this study, we have evaluated, by immunohistochemistry, the expression of HSP27 (HSPB1) and HSP70 (HSPA) in serial biopsies from locally advanced breast cancer patients ($n=60$) treated with doxorubicin (DOX)- or epirubicin (EPI)-based monochemotherapy. Serial biopsies were taken at days 1, 3, 7, and 21, and compared with prechemotherapy and surgical biopsies. After surgery, the patients received additional chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil. High nuclear HSPB1 and HSPA expressions were found in invasive cells after DOX/EPI administration ($P<0.001$), but the drug did not affect the cytoplasmic expression of the HSPs. Infiltrating lymphocytes showed high nuclear HSPA ($P<0.01$) levels at postchemotherapy. No correlations were found between HSPs expression and the clinical and pathological response to neoadjuvant therapy. However, in postchemotherapy biopsies, high nuclear (>31 % of the cells) and cytoplasmic HSPA expressions (>11 % of the tumor cells) were associated with better DFS ($P=0.0348$ and $P=0.0118$, respectively). We conclude that HSPA expression may be a useful prognostic marker in breast cancer patients treated with neoadjuvant DOX/EPI chemotherapy indicating the need to change the administered drugs after surgery for overcoming drug resistance.

Keywords Breast cancer · Neoadjuvant chemotherapy · Anthracyclines · Heat shock proteins · Prognostic factors

Abbreviations

HSPs	Heat shock proteins
DOX	Doxorubicin
EPI	Epirubicin
FAC	5-Fluorouracil adryamicin, cyclophosphamide
5-FU	5-Fluorouracil
C	Cyclophosphamide
M	Methotrexate
CMF	Cyclophosphamide, methotrexate, 5-fluorouracil
FEC	5-Fluorouracil, epirubicin, cyclophosphamide
ER	Estrogen receptor
PgR	Progesterone receptor
TP	Tumor progression

Ciocca DR and Vargas-Roig LM contributed equally to this work.

S. B. Nadin · M. L. Sottile · M. M. Montt-Guevara · G. V. Gauna · L. M. Vargas-Roig
Tumor Biology Laboratory, Institute of Medicine and Experimental Biology of Cuyo, National Scientific and Technical Research Council, Mendoza, Argentina

P. Daguerre · M. Leuzzi · J. Ibarra
Luis Lagomaggiore Hospital, Mendoza, Argentina

F. E. Gago · L. M. Vargas-Roig
Medical School, National University of Cuyo, Mendoza, Argentina

F. D. Cuello-Carrión · D. R. Ciocca
Oncology Laboratory, Institute of Medicine and Experimental Biology of Cuyo, National Research Council of Argentine, Mendoza, Argentina

S. B. Nadin (✉)
Institute of Medicine and Experimental Biology of Cuyo, C.C. 855 (5500), Mendoza, Argentina
e-mail: snadin@mendoza-conicet.gob.ar

Published online: 05 December 2013

SD	Stable disease
PR	Partial response
CR	Complete response
pCR	Pathological complete response
MiR	Microscopic residual disease
MaR	Macroscopic residual disease
DFS	Disease-free survival
OS	Overall survival

Introduction

Over the last years, many efforts have been made in searching molecular markers of drug sensitivity/resistance in cancer patients. Heat shock proteins (HSPs) were primary involved in resistance to hyperthermia in all living cells and have also been implicated in anticancer drug resistance (Ciocca et al. 1992; Ciocca and Vargas-Roig 1997; Khalil et al. 2011). HSPs have been classified by their molecular weight in the following families: HSP110 (HSPH), HSP90 (HSPC), HSP70 (HSPA), HSP60 (HSPD), and small HSPs (e.g., HSPB1; Kampinga et al. 2009). The principal members of the HSPA family are HSPA8 (HSC70) constitutively formed, HSPA5 (glucose-regulated protein GRP78) localized in the endoplasmic reticulum, the mitochondrial HSPA9 (mtHSP70), and HSPA1A (HSP72) inducible formed (Goloudina et al. 2012). Acting as molecular chaperones, HSPs participate in the refolding of misfolded proteins as well as in the degradation of proteins through the proteasome pathway (Lindquist and Craig 1988; Ellis 2007). Malignant cells including mammary carcinoma cells express high levels of HSPs. Some of these proteins are able to promote cell survival by inhibiting apoptosis and senescence, and have been proposed as prognostic markers in cancer (Ciocca and Calderwood 2005; Calderwood et al. 2006; Sherman 2010; Ciocca et al. 2013). In rodent tumor models, HSP27 (HSPB1) or HSP70 (HSPA) overexpression have been implicated in increased tumor growth and metastatic potential (Jäättelä 1995; Garrido et al. 1998). HSPA synthesis can be induced by physical or chemical conditions, through activation of the HER-2/neu pathway (Khaleque et al. 2005). In addition, HSPA may regulate the cell death program affecting extrinsic and intrinsic pathways (Goloudina et al. 2012).

In cancer, the best studied members of the small HSPs family are HSPB1 (HSP27), HSPB4 (αA-crystallin), and HSPB5 (αB-crystallin; Kampinga et al. 2009). HSPB1 mediates its molecular activities through phosphorylation-dependent changes involved in its protein folding and cell regulatory functions (Calderwood et al. 2010). In breast tumors, HSPB1 promotes malignant transformation by inhibiting apoptosis and senescence (Garrido et al. 2006; Sherman et al. 2007). In addition, HSPB1 seems to interact and modulate the PTEN levels in MCF-7 cells (Cayado-Gutiérrez et al. 2013).

Anthracyclines, doxorubicin (DOX) and epirubicin (EPI) are among the most effective anticancer drugs for breast cancer. They are also commonly used in the treatment of diverse malignant tumors, including acute lymphoblastic and myeloid leukemia, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, soft tissue sarcomas, osteosarcoma, lung, ovarian, gastric and thyroid carcinoma, among others (Kizek et al. 2012). Anthracyclines, as topoisomerase II poisons, are characterized by their ability to induce DNA double-strand breakage through the stabilization of covalent complexes between topoisomerase II and DNA (Binaschi et al. 2001; Minotti et al. 2004). Topoisomerase II-DNA damage is followed by cell cycle arrest and cell death. Resistance to these agents may occur, and significantly limits their efficacy in clinical oncology (Chien and Moasser 2008). One of the major mechanisms of anthracyclines resistance is through the drug efflux, mediated by P-glycoprotein (P-gp or p170), a 170 kDa membrane protein encoded by the *MDR1* gene, that pumps substrates from tumor cells (Coley 2008). Other molecules implicated with anthracycline resistance are HSPs. In vitro studies have shown that elevated HSPB1 and HSPA expression levels in human MDA-MB-231 and MCF-7 breast cancer cells were associated with DOX resistance (Ciocca et al. 1992). Moreover, MDA-MB-231 breast cancer cells, which normally express low levels of HSPB1, were transfected with a full-length HSPB1 construct and resulted in threefold more resistant to DOX (Oesterreich et al. 1993). The overexpression of HSPB1 protected MDA-MB-231 cells from apoptosis induced by DOX, which was associated with an altered topoisomerase II expression (Hansen et al. 1999).

HSPB1 protein expression has been related to poor prognosis in some tumor types (osteosarcoma, ovarian, liver, gastric, and prostate cancer) and with good prognosis in others (endometrial and esophageal; Ciocca et al. 1992; Zoubeidi and Gleave 2012). On the other hand, HSPA expression has been correlated with poor prognosis (breast cancer, endometrial cancer, uterine cervical cancer, and transitional cell carcinoma of the bladder), with good prognosis (esophageal cancer, pancreatic cancer, renal cancer, and melanoma) and showed no correlation with prognosis in ovarian, oral, head and neck, gastric and prostate cancer, and leukemia (Ciocca and Calderwood 2005; Ciocca et al. 2013). In patients with primary breast cancer, it has been reported that a lower HSPA1A expression correlates with relapse and metastasis (Torronteguy et al. 2006). In a previous study, using peripheral blood lymphocytes from cisplatin-treated cancer patients, we found that high nuclear/cytoplasmic ratio of HSPB1 was related to longer disease-free survival (DFS) and overall survival (OS; Nadin et al. 2007). In addition, our group has reported that a high nuclear proportion of HSPA correlated significantly with drug resistance in biopsies of breast cancer patients treated with induction chemotherapy with 5-fluorouracil, adryamicin, and cyclophosphamide (FAC). We

have also observed that patients whose tumors expressed nuclear or high cytoplasmic proportion of HSPB1 had shorter DFS and that the combination of high levels of expression of HSPB1 and HSPA showed a strong correlation with DFS (Vargas-Roig et al. 1998). In tumor biopsies from breast cancer patients before the initiation of DOX chemotherapy, the expression of GRP78 was associated with shorter time to recurrence (Lee et al. 2006). The expression of HSPBP1 (a co-chaperone that binds to and regulates HSPA) was lower in patients with a higher incidence of metastasis (Souza et al. 2009). At present, HSPs are becoming important therapeutic targets, specially the use of HSPs inhibitors, principally against HSPC (HSP90), which have shown interesting effects in some clinical and experimental trials (Almeida et al. 2011).

On the other hand, a number of studies have shown that infiltrating lymphocytes in tumor tissue are associated with improved survival in breast cancer patients (Schmidt et al. 2008; Mohammed et al. 2012). Denkert et al. have reported that tumor-associated lymphocytes are independent predictor factors of good response to anthracycline/taxane neoadjuvant chemotherapy in breast cancer patients (Denkert et al. 2010). In addition, tumor-infiltrating lymphocytes (TILs) have been found to be mainly T-lymphocytes, expressing a $CD8^+$ phenotype (Leong et al. 2006; Liu et al. 2012; Mahmoud et al. 2011). Recent evidences have shown that anthracyclines antitumor activity depends on the host intact immune system (Apetoh et al. 2008a). Two post-transcriptional events are necessary for anthracyclines immunogenicity: the translocation of calreticulin to the tumor cell membrane and the release of high-mobility-group box 1 (HMGB1) from the tumor cell (Obeid et al. 2007; Apetoh et al. 2007). In the light of these facts, the combination of anthracyclines with immunomodulators is being assessed in clinical trials (Zitvogel et al. 2008; Apetoh et al. 2008b).

The conditions of biological markers before chemotherapy treatment, together with changes induced by treatment, might be useful to predict sensitivity or resistance to neoadjuvant therapy and provide an opportunity for understanding the mechanism of action of therapies. Therefore, the objective of this research was to determine if HSPB1 and HSPA expression is affected by anthracycline treatment, and if these molecular markers predict anthracycline responsiveness and are related to survival after completion of treatments.

Materials and methods

Patients

From November 1996 to December 2005, 60 patients with locally advanced breast tumors (stages II and III) were considered eligible and thus included for this study. Patients were required to have histological proof of invasive carcinoma, to be at least 18 years of age, have a performance status of 90 % by the Karnofsky scale, have a serum billirubin level <0.5 mg/dl, serum creatinine level of <1.5 mg/dl, and have a normal cardiac function. The patients were assessed to be metastasis free at the time of diagnosis by careful clinical evaluation, by X-ray of the chest, bone scintigraphy, and liver ultrasound. None of the patients had previously received any treatment for the disease. The main clinical and pathological characteristics of the patients are shown in Table 1. All indications were discussed in a multidisciplinary oncology meeting and the informed signed consent was obtained from the patients according to our research ethics requirements approved by the Ethic Committee of the Lagomaggiore Hospital of Mendoza in accordance with the precepts established by the Helsinki Declaration.

Neoadjuvant chemotherapy

Patients received monochemotherapy consisting of 75 mg/m^2 DOX or 120 mg/m^2 EPI for four cycles before surgery (Bonadonna et al. 1995). Initial diagnosis was made by core biopsy. The serial biopsies were taken at days 1, 3, 7, 21, and at surgery. After neoadjuvant chemotherapy, surgical excision of the tumor was performed in all patients even if they presented a complete clinical response (in this case, the marked

Table 1 Main characteristics of the patients entered into the study

Characteristic	Number
Age (years)	
Range	29–71
Mean	51
Hormonal status	
Premenopausical	29 (48.3 %)
Postmenopausical	31 (51.7 %)
Clinical stage	
II	28 (46.7 %)
III	32 (53.3 %)
Tumor size	
T2 (>20 and ≤50 mm)	19 (31.7 %)
T3 (>50 mm)	36 (60.0 %)
T4	5 (8.3 %)
Histological type	
Infiltrating ductal	56 (93.3 %)
Infiltrating lobular	4 (6.7 %)
Clinical response	
CR	2 (3.3 %)
PR	15 (25.0 %)
SD	43 (71.7 %)
Pathological response	
MiR	4 (6.7 %)
MaR	56 (93.3 %)

SD stable disease, *PR* partial response, *CR* complete response, *MiR* microscopic residual disease, *MaR* macroscopic residual disease

area of the tumor was excised). After surgery, these patients received six cycles of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF). They also received standard radiotherapy and hormonotherapy according to the medical doctor judgment evaluating estrogen receptor alpha and progesterone receptor (PgR) status.

The clinical response was assessed by measuring the tumor changes in the product of the two largest diameters recorded at baseline and at the end of chemotherapy (before surgery). Tumor progression (TP) was defined as an increase of at least 25 % of tumor size, stable disease (SD) as tumor size increase less than 25 % or reduction less than or equal to 50 %, partial response (PR) as tumor shrinkage greater than 50 %, and complete response (CR) as a total disappearance of all clinical signs of the disease. Lumpectomy or mastectomy was performed approximately 3 weeks after the last cycle of neoadjuvant chemotherapy. Consolidative radiation therapy was applied (40–50 Gy to the chest wall and 50 Gy to the lymph nodes). The specimens from surgery were carefully evaluated for the presence of residual disease by the pathologist. Pathological complete response (pCR) was defined as the absence of invasive carcinoma in the breast, microscopic residual disease (MiR) was defined as <1 mm of invasive carcinoma, and macroscopic residual disease (MaR) was defined as ≥1 mm or multiple foci of invasive carcinoma throughout the specimen.

The presence of TIL was evaluated considering their localization (intratumoral or peritumoral) and density (weak, <20 lymphocytes; moderate, 20–50 lymphocytes; and dense, >50 lymphocytes) (Yamaguchi et al. 2012).

Follow-up

The follow-up of the patients was periodically performed clinically and by the diagnostic tools mentioned above (X-rays, etc.). The mean time of follow-up (defined as time to death or to the last follow-up visit) was 72.6 months (range, 14–168 months). DFS was calculated as the period from diagnosis until the date of the first distant recurrence or the last follow-up. OS was calculated as the period from diagnosis until the date of death.

Immunohistochemistry

Pre- and postchemotherapy samples were immediately fixed in 10 % buffered formalin and embedded in paraffin. Serial 5 μm-thick sections were mounted onto 3-aminopropyltrietoxysilane (Sigma, St. Louis, MO, USA)-coated slides for subsequent analysis.

The primary antibodies used were rabbit polyclonal antibody against HSPB1 (HSP25/27) kindly provided by Dr. M. Gaestel (Max-Delbrück Center for Molecular Medicine, Berlin, Germany) used at 1:2,000 dilution; mouse monoclonal antibody BRM-22 against the inducible and constitutive forms of HSPA (Sigma-Aldrich, USA) used at 1:1,000 dilution; and mouse monoclonal antibody against HSPA1A (SPA-810 Stressgen) used at 1:500 dilution. Tissue sections were incubated with the primary antibodies overnight at 4 °C in humidity chambers. We used, as second antibody, anti-rabbit and anti-mouse IgG (whole molecule) biotin conjugated (Dako Corp, Carpinteria, CA, USA) at 1:50 dilution (45 min). Diaminobenzidine (0.5 mg/ml)/hydrogen peroxide (0.01 %) was used as chromogen substrate. Slides were lightly counterstained with hematoxylin and observed with a Nikon Eclipse E200 microscope. Sections from the serial biopsies (pre- and postchemotherapy) were always processed together. Nonspecific mouse IgG1 antibody and purified rabbit pre-immune serum (Dako, Kingsgrove, NSW, Australia) were used as isotype-negative controls. All of the slides were reviewed and scored separately by two observers who were blinded to the clinical outcome of the patients; discordant cases were re-evaluated and resolved by consensus.

The samples were evaluated in intensity and proportion of cells with positive immunoreactions using a scoring system reported previously (Gago et al. 1998). Briefly, the intensity score used was no staining=0, weak staining=1, moderate staining=2 and strong staining=3; the proportion score used was <1 %=0, 1–10 %=1, 11–30 %=2, 31–66 %=3, and >66 %=4.

Statistical analyses

The Wilcoxon signed-rank nonparametric test was used to determine whether differences found in the pre- and postchemotherapy biopsies were significant. Fisher's exact test was used to determine whether the expression of the markers studied correlated with the clinical and pathological response and outcome of the patients. Analyses of DFS and OS were performed by the Kaplan–Meier method. The difference between curves was evaluated with the log-rank test for censored survival or event observations. Cox proportional hazard regression models were used to examine the association between DFS, OS, nuclear HSP70, and other prognostic markers (positive lymph nodes, ER, PgR, HSP27, HER-2/neu and P170, and hormonal status). Statistical analyses were performed using the PRISM computer program (Graph Pad Software, San Diego, CA, USA) and MedCalc Version 11.6.1.0; a $P<0.05$ was considered statistically significant.

Results

DOX-based neoadjuvant monochemotherapy increased nuclear HSPB1 and HSPA expression in breast tumor biopsies

We analyzed matched serial biopsies from 54 patients (six cases did not have enough material to perform

immunohistochemistry), comparing the prechemotherapy biopsy with the biopsies taken at days 1, 3, 7, and 21 after the first monochemotherapy course and with the surgical biopsy taken at the end of neoadjuvant chemotherapy. We omitted the day 3 results because this biopsy could not be obtained in all patients. HSPB1 and HSPA were observed in the cytoplasm and nucleus of invasive tumor cells (Fig. 1). The semiquantitative evaluation of the immunoreactions showed that nuclear HSPB1 and HSPA protein levels increased after DOX administration ($P<0.001$; Fig. 2). However, the cytoplasmic expression of the HSPs did not change after DOX treatment (Fig. 2). In invasive and in in situ tumor cells, we also observed cell membrane expression of HSPB1 and HSPA before and after DOX administration in some patients (Fig. 2e, f).

In order to verify if the increased nuclear HSPA expression observed in surgical specimens resulted from the inducible form of the protein (the BRM22 antibody used by us detects both HSPA1A and HSPA8), we have performed an immunohistochemistry using a specific antibody against HSPA1A in the available samples. We observed that 62.5 % of the samples that expressed nuclear HSPA also contained HSPA1A suggesting that both the constitutive and inducible forms of the protein were present in the nuclei.

HSPB1 and HSPA protein expressions were also evaluated in normal mammary glands (when present, 103 of 271 biopsies). We did not observe significant changes in HSPs cytoplasmic expression after DOX administration; in surgical specimens, we verified that nuclear HSPA expression significantly increased ($P<0.05$) (Fig. 3). In some cases, HSPB1 and HSPA were expressed in the membrane of the tumor cells and in the apical surface of normal interlobular ducts.

DOX increased tumor infiltrating lymphocytes and their nuclear HSPA expression

We have also evaluated TIL as described in M&M. TIL increased with DOX administration in a function of time, reaching statistically significant differences between prechemotherapy biopsies and surgical specimens; these cells appeared inside the tumor and within the peritumoral stroma (Fig. 4).

Interestingly, nuclear HSPA expression was elevated ($P<0.01$) in TIL from surgical biopsies (Fig. 5a–c). The cytoplasmic expression of HSPA was increased in the biopsies taken at day 7 after chemotherapy ($P<0.05$). HSPB1 was absent in the nucleus of the lymphocytes while a very low content was noted in the cytoplasm (data not shown). We also evaluated HSPB1 and HSPA expression in the connective tissue. No significant differences were found in the cytoplasmic and nuclear expression of HSPB1 and HSPA in the fibroblasts before, during, and after DOX treatment (Fig. 5d–f).

HSPB1 and HSPA did not correlate with clinical and pathological response to DOX

The clinical response of the breast cancer patients included in this study was evaluated at the end of the DOX monochemotherapy. CR was noted in 2 patients (3.7 %), PR in 12 patients (22.2 %), and SD in 40 patients (74.1 %). No correlation with clinical response was found between nuclear or cytoplasmic HSPs expression before and after DOX administration.

Almost all of the tumors (93.3 %) had MaR; four (6.7 %) presented MiR. None of the specimens showed pCR. In this context, the pathological response did not correlate with the expression of the HSPs or with the survival of the patients. In addition, we did not find correlations between TIL and clinical/pathological response (data not shown).

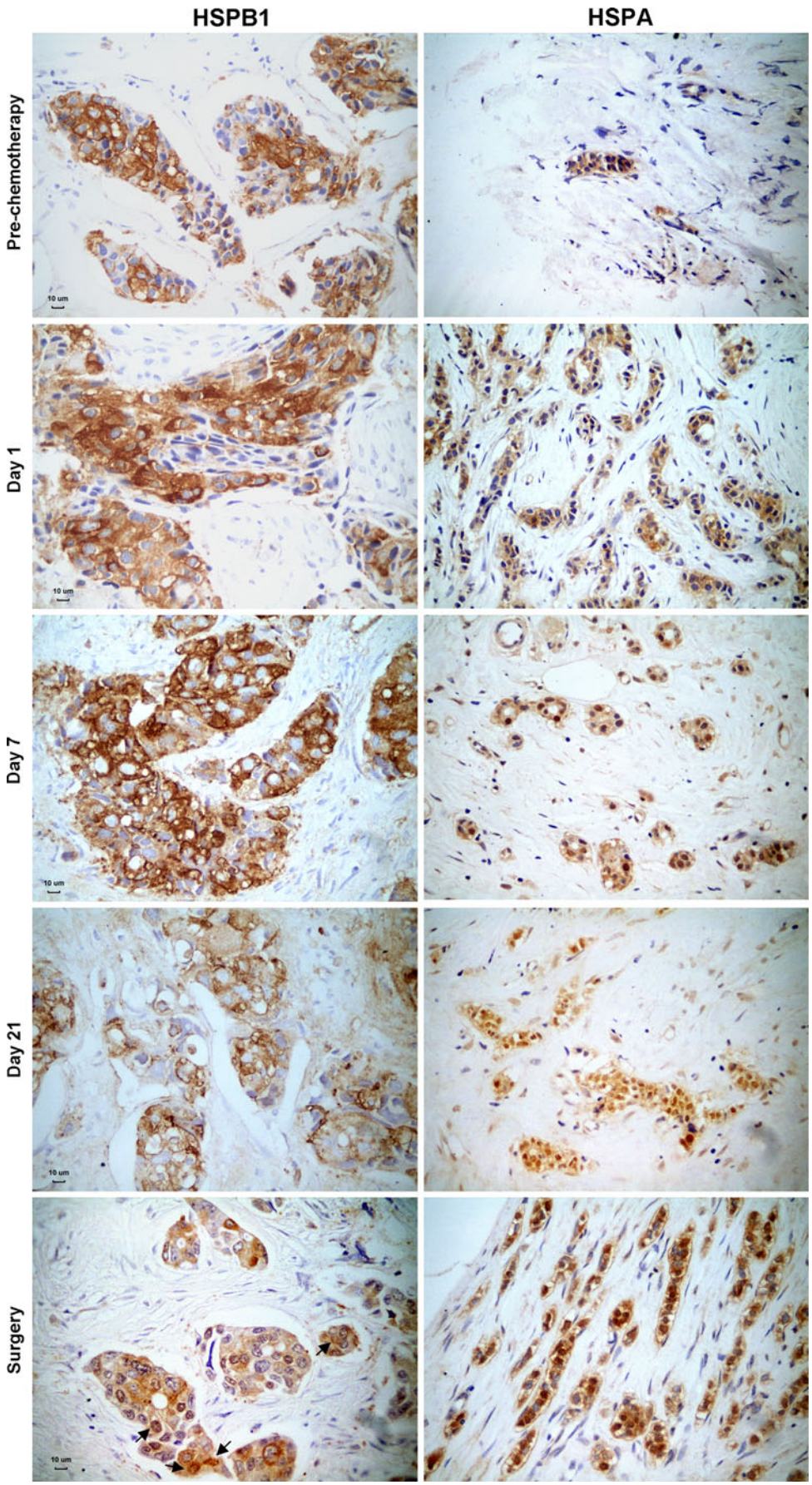
Nuclear and cytoplasmic HSPA expression correlated with the prognosis of breast cancer patients

The expression of HSPB1 and HSPA was correlated with DFS and OS using Kaplan–Meier survival curves. Of the total number of patients, 24 (40 %) died, 7 (11.7 %) experienced disease progression, and 29 (48.3 %) were disease free. HSPs expression in the biopsies taken before DOX administration did not correlate with DFS or OS. However, a high nuclear HSPA expression (>31 % of the cells; proportion score, $P\geq3$) in the postchemotherapy biopsies was associated with better DFS (Fig. 6a). In addition, the expression of HSPA in the cytoplasm of tumor cells from surgical specimens (>11 %; $P\geq2$) correlated with better DFS (Fig. 6b). Cytoplasmic HSPB1 expression after DOX administration did not correlate with disease prognosis while nuclear HSPA1A expression showed a tendency to correlate with patient prognosis, but without reaching statistical significance. We also evaluated the association of TIL with clinical outcome; no statistically significant differences were found (data not shown).

Multivariate analyses using Cox regression models were also done. A trend towards significant direct association between DFS and nuclear HSPA expression at surgery was found ($P=0.0521$). No significant association between DFS and OS were noted when other prognostic variables were analyzed: positive lymph nodes, ER, PgR, HSPB1, HER-2/neu, P170, and hormonal status.

Discussion

This is the first time that two HSPs (HSPB1 and HSPA) were evaluated in serial biopsies of locally advanced breast cancer patients treated with relatively high doses of anthracycline neoadjuvant monochemotherapy; we have found that both proteins suffered important modifications since they clearly


HSPB1
HSPA
Pre-chemotherapy
Day 1
Day 7
Day 21
Surgery
10 um

◀ **Fig. 1** Expression of HSPB1 and HSPA in invasive tumor cells before, during (days 1, 7, and 21), and after neoadjuvant chemotherapy. HSPB1 was expressed mainly in the cytoplasm of tumor cells. Observe some HSPB1 positive nuclei in the surgery specimen (*arrows*). Note the cytoplasmic expression of HSPA before DOX administration and the predominant nuclear expression shortly after chemotherapy. The positive immunoreactivity appears as brown deposits, and the slides were lightly counterstained with hematoxylin to reveal nuclei. A *scale bar* (10 μm) was included in the *left microphotographs*

appeared in the nucleus of cancer cells after drug treatment. The location of a given protein is very important to understand its cellular role. Several examples can be found in the literature in which a same protein has different functions according to its cellular location (Park et al. 2005; Moncalero et al. 2011; Tomar et al. 2012; Davis et al. 2013). Some reports indicate that HSPA can translocate to the nucleus and accumulate there under hyperthermia effect or other stress conditions (Szekely et al. 1995; Chughtai et al. 2001; Lepock et al. 2001; Nadin et al. 2012). The anthracyclines DOX and EPI are commonly used in the treatment of a number of diverse malignant tumors. DOX, as a topoisomerase II poison, originates DNA breaks, and also produces reactive oxygen species (ROS) (Kizek et al. 2012). Our findings are the first reported in biopsies from patients and are consistent with previous in vitro results. In this context, we have reported that DOX increased the nuclear expression of HSPA in peripheral blood lymphocytes form healthy subjects and that high nuclear expression of HSPA correlated with increased DNA repair (Nadin et al. 2003).

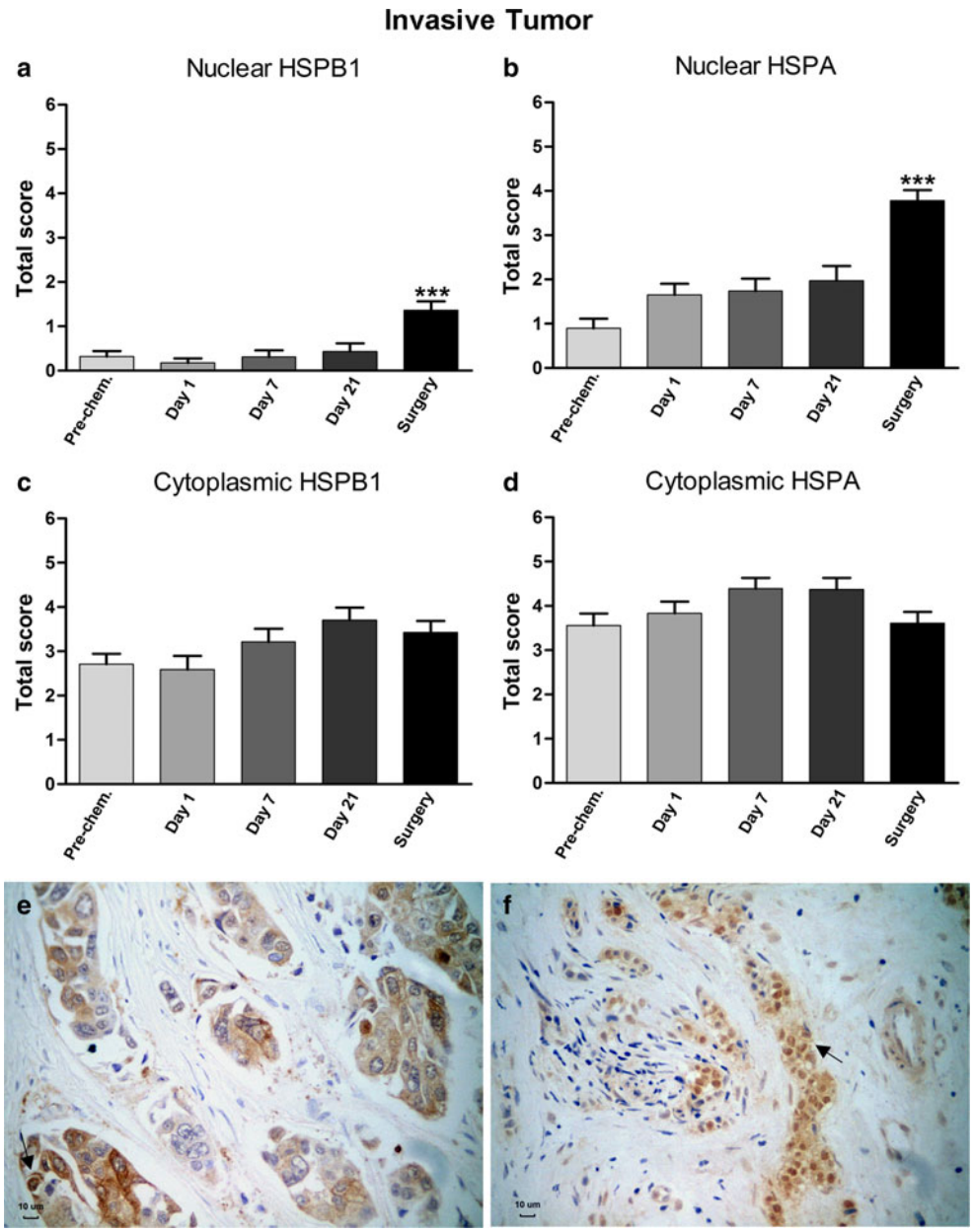


Fig. 2 HSPB1 and HSPA expression in invasive carcinomas before and after chemotherapy. **a**, **b** Nuclear HSPB1 and HSPA expression, respectively. **c**, **d** Cytoplasmic HSPB1 and HSPA expression, respectively. **e**, **f** Samples showing HSPB1 and HSPA, respectively. Note the nuclear and cytoplasmic expression of both HSPs and cell membrane immunoreactions (*arrows*). A 10 μm scale bar was included in the microphotographs. Total score represents the intensity and proportion of the positive immunostainings (see M&M section). *Bars* represent mean± SEM. ***$P<0.001$

Other authors have reported that DOX induced HSPA accumulation, probably increasing the production of ROS, which are known to induce the expression of HSPs (Huber 1992; Omar and Pappolla 1993). Previous studies have suggested a relationship between nuclear HSPs levels and DNA damage (Nadin et al. 2012; Calini et al. 2003; Niu et al. 2006); the participation of HSPB1 and HSPA in the correction of the DNA damage by the base excision repair mechanism has been demonstrated (Mendez et al. 2003). Moreover, we have reported that hyperthermia, before DOX treatment, induced the nuclear accumulation of HSPB1 and HSPA in cultured peripheral blood lymphocytes from healthy persons correlating with an increased MMR proteins expression (hMLH1 and hMSH2) and DNA repair capacity (Nadin et al. 2007). Recently, Kose et al. have identified a nuclear import carrier for HSP70 named "Hikeshi", encoded by chromosome 11 open reading frame 73, which does not belong to the importin β family. Hikeshi binds to the ATP form of HSPA for its nuclear import and dissociates from ADP form of HSPA. The nuclear transport of HSPA seems important to protect cells from heat shock-induced damage or damages induced by chemotherapeutic agents (Kose et al. 2012; Nadin et al. 2007).

The prognosis of the disease is important to individualize cancer therapies and to plan the patient's follow-up (Ciocca and Calderwood 2005). We found that HSPB1 and HSPA expression had no predictive value, but HSPA showed a prognostic value in breast cancer patients treated with DOX/EPI neoadjuvant chemotherapy followed by CMF. In spite of

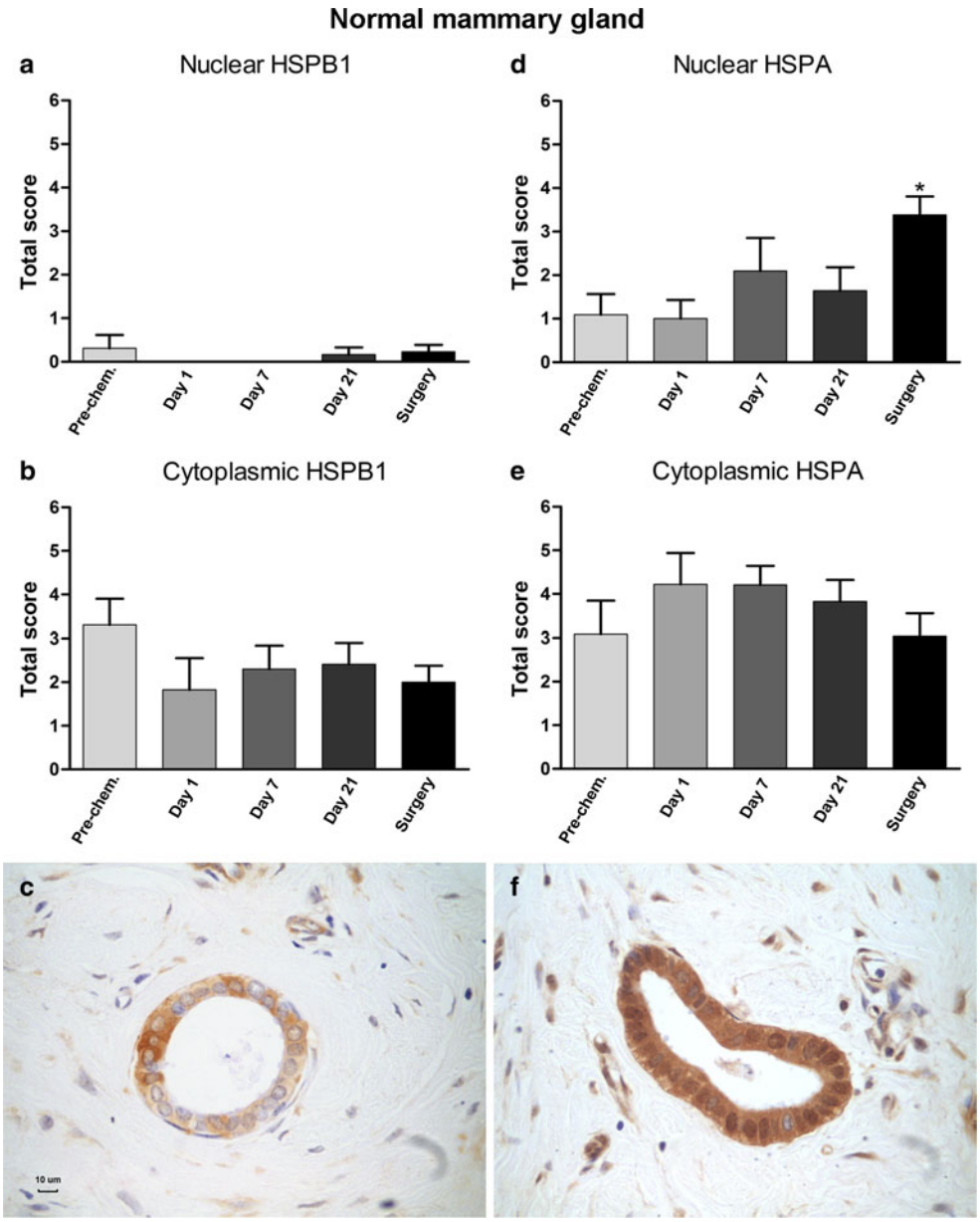


Fig. 3 Expressions of HSPB1 and HSPA in normal mammary tissue before and after neoadjuvant chemotherapy. **a**, **d** Nuclear HSPB1 and HSPA expressions, respectively. **b**, **e** Cytoplasmic HSPB1and HSPA expressions, respectively. **c**, **f** Images of normal breast ducts from postchemotherapy biopsies with positive staining for HSPB1 and HSPA, respectively. Note the accumulation of HSPA in the nucleus of the ductal epithelial cells. *Bars* represent mean±SEM. A 10 μm *scale bar* was included in one of the microphotographs. Total score represents the intensity and proportion of the positive immunostaining.*$P <$ 0.05

their suggested functions as markers of bad prognosis, we observed that nuclear and cytoplasmic expression of HSPA but not HSPB1, significantly correlated with better DFS after DOX monochemotherapy. We have also studied HSPA1A expression (HSPA inducible form) in surgical specimens. HSPA1A showed a tendency to correlate with patient prognosis, but it was not statistically significant (probably to the low number of samples).

In our study, infiltrating lymphocytes showed high nuclear HSPA proportion after chemotherapy, but did not correlate with prognosis. It has been reported that a lower HSPA expression in tumor cells was associated with relapse and metastatic disease in breast cancer patients (Torronteguy et al. 2006). However, Thanner et al. have reported that node-negative breast cancer patients with cytoplasmic expression of HSPA showed a significantly diminished survival after first recurrence (Thanner et al. 2003). Is HSPA accomplishing a function as a damage-associated molecular patterns (DAMP) molecule? In the middle of the 1990s, Polly Matzinger proposed the "danger theory", which express that the immune system may distinguish among dangerous and innocuous endogenous signals (Matzinger 1994). Dying, stressed, or injured cells release or expose molecules that function as danger signals for the immune system. These signals were called DAMPs. DAMPs such as HMGB1, surface-exposed calreticulin, secreted ATP, HSPA/1A, HSPC are essentials for the immunogenic cell death (ICD) in cancer cells (Krysko et al. 2012; Wheeler et al. 2009; van Eden et al. 2012). Among the agents that can induce ICD are DOX, oxaliplatin, γ-irradiation, mitoxantrone, and cyclophosphamide (Krysko et al. 2012). DOX may cause the release of surface-exposed HSPA, HSPC, reticulin, and secreted ATP, inducing ICD in tumor cells (Obeid et al. 2007). Dying tumor cells release HMGB1 that interacts with Toll-like receptor 4 on dendritic cells promoting tumor-specific T-cell responses (Apetoh et al. 2008b). Based on these observations, in the present study, we have also evaluated the presence of TIL. After chemotherapy, we observed a significant increase of intratumoral and peritumoral TIL. The predictive/prognostic implications of TIL was also evaluated in pretherapeutic and posttherapeutic biopsies, but no correlations were found. Many authors have reported the relation between TIL with patients outcome in a larger number studies with some controversies (Fox et al. 2011; Mohammed et al. 2012). Galon et al. have reported that the adaptive immune response plays a role in preventing tumor recurrence once human colorectal cancer become clinically detectable. Intratumoral T cells could modify tumor cells in ways that attenuate its metastatic potential increasing overall survival of the patients (Galon et al. 2006). It is necessary to conduct more studies with homogeneous groups of patients and treatments with consensus in the criteria of evaluation to validate the use of TIL as predictive markers. If HSPA acts as a DAMP, the presence of the inflammatory reaction in the tumor could be indicative of the ICD and good prognosis for the patient. In the near future, we hope to identify TIL subtypes in surgical specimens and correlate them with chemotherapy responsiveness and survival of cancer patients.

On the other hand, many factors influence the ability of drugs to kill tumor cells, including drug pharmacokinetics and metabolism, changes in the extracellular microenvironment,

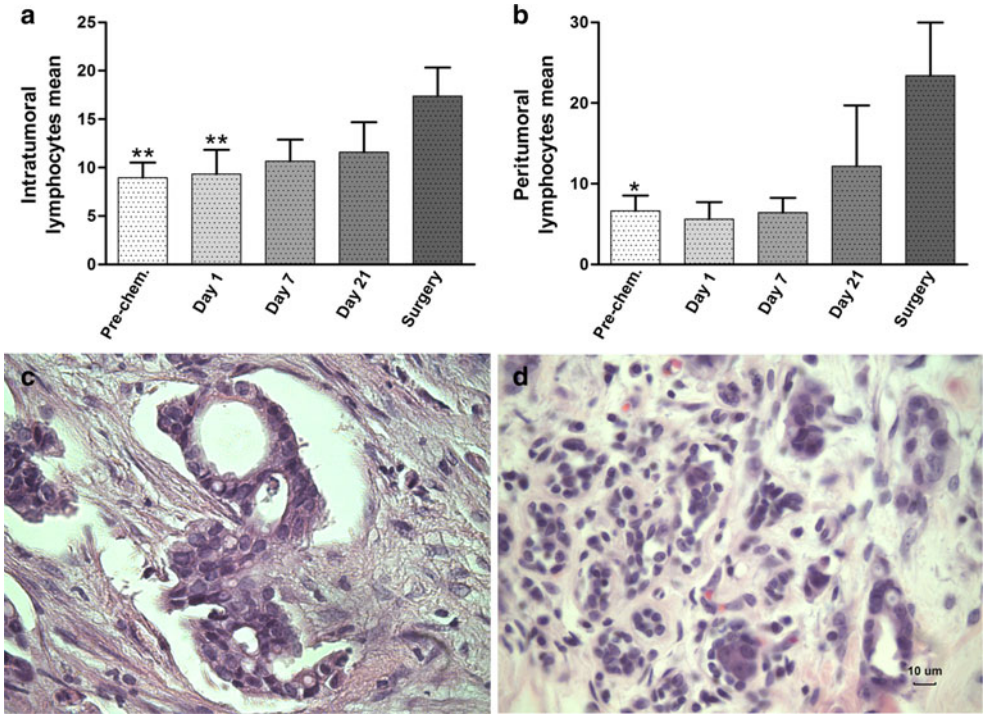


Fig. 4 Tumor-infiltrating lymphocytes before, during (days 7 and 21), and after neoadjuvant anthracycline-based chemotherapy. **a** Intratumoral lymphocytes mean ± SEM. **b** Peritumoral lymphocytes mean ± SEM. **c**, **d** Photomicrographs taken from prechemotherapy biopsy and surgery specimen from one breast cancer patient, respectively. A 10 μm *scale bar* was included in one of the microphotographs. **P*<0.05; ***P*<0.01

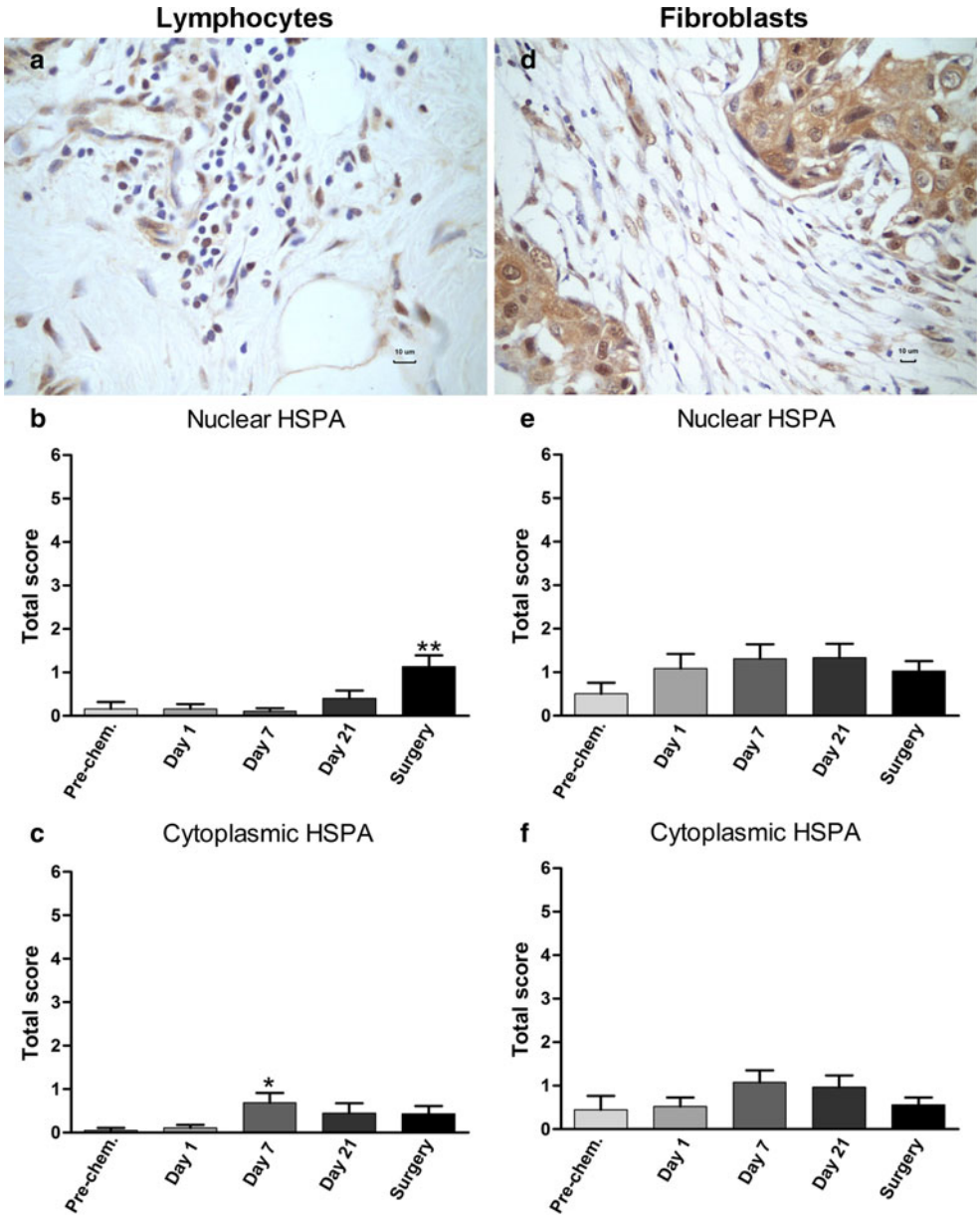


Fig. 5 HSPA expression in lymphocytes and fibroblasts in breast biopsies before and after neoadjuvant chemotherapy. **a**, **d** Images taken from a postchemotherapy biopsy. A 10 μm *scale bar* was included in one of the microphotographs. Note the positive HSPA nuclear staining. **b**, **e** Nuclear HSPA expression in lymphocytes and fibroblasts, respectively. **c**, **f** Cytoplasmic HSPA expression in lymphocytes and fibroblasts, respectively. Total score represents the intensity and proportion of the positive immunostaining. *Bars* represent mean ± SEM. *$P<0.05$; **$P<0.01$

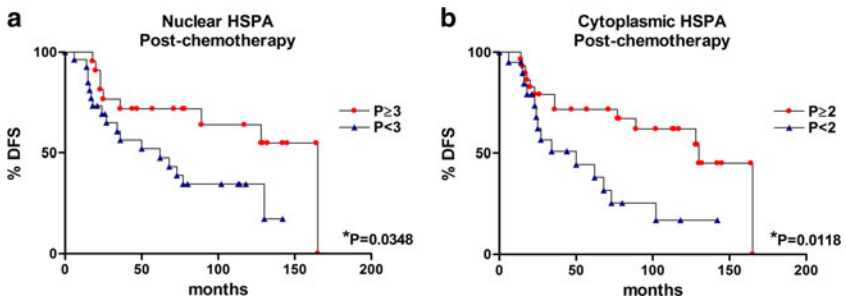


Fig. 6 Disease-free survival (DFS) curves in function of nuclear and cytoplasmic HSPA expression. High HSPA nuclear expression (proportion ≥3) and high HSPA cytoplasmic expression (proportion ≥2) at postchemotherapy correlated with DFS (**a** and **b**, respectively). The exact *P* value is indicated in each figure. *$P<0.05$

changes in ability to repair DNA after drug-induced damage, alterations in apoptotic signaling pathways, and changes in the expression of proteins and enzymes associated with tumor resistance (Coley 2008). Human breast cancer cells expressing high HSPB1 and HSPA expression after hyperthermia resulted more resistant to DOX treatment. However, heat shock does not confer cross-resistance to other anticancer agents, such as 5-fluorouracil (5-FU; Ciocca et al. 1992). In a previous study, we have included breast cancer patients treated with FAC or 5-FU, EPI, cyclophosphamide (FEC) induction chemotherapy, reporting that a combination with high nuclear or cytoplasmic expression of HSPB1 and high nuclear expression of HSPA correlated with shorter DFS (Vargas-Roig et al. 1998). We believe that the apparent discrepancy with our present study is due to the election of a different therapeutic strategy: polychemotherapy vs monochemotherapy before surgery (FAC/FEC vs DOX) and to the addition of a different scheme of chemotherapy after surgery (the patients from our present study received six cycles of CMF; in our previous study, breast cancer patients were treated with FAC/FEC—the same drugs used before surgery). We can assume that tumor cells from surgical specimens expressing high nuclear HSPB1 and HSPA levels are those resistant to the administered drug. Then, HSPs acting as DAMPs may induce signals to generate an ICD. The patients of the present study received CMF after surgery and the resistance against DOX was probably overcome and therefore the prognosis of the patients was significantly improved. In our previous study, patients received the same drugs after surgery and DOX resistance was not overcome. In addition, DOX and cyclophosphamide are ICD inducers, as they combined the action of ROS and endoplasmic reticulum stress to activate danger signals that collaborate to carry DAMPS toward the extracellular space (Obeid et al. 2007; Panaretakis et al. 2009).

Other explanation may be related to DNA repair mechanisms which are critical for their response to chemotherapy (Bouwman and Jonkers 2012). It is well-known that the cytotoxicity of most chemotherapy drugs depends on the induction of DNA damage. In our study, the patients received CMF after surgery. Cyclophosphamide (C) forms DNA adducts, which alters normal DNA functions. Methotrexate (M) leads to inhibition of dihydrofolate reductase, thymidylate synthase (TS), and other folate-requiring enzymes, conducing to a depletion of ATP, GTP, and TTP nucleotides pools, which affects the DNA synthesis (Kinsella et al. 1997). The active 5-FU metabolite inhibits TS, leading to depletion of deoxythymidine triphosphate, a necessary precursor for DNA synthesis. The cytotoxicity of DOX may involve multiple pathways, but the precise mechanism of their action remains obscure because of the complexity of these mechanisms: (1) intercalation into DNA, leading to inhibited synthesis of macromolecules (DNA, RNA, and proteins); (2) generation of free radicals, leading to DNA damage and/or lipid peroxidation; (3) DNA binding and alkylation; (4) DNA cross-linking; (5) interference with DNA unwinding or DNA strand separation; (6) inhibition of helicase activity; (7) direct membrane effects; and (8) inhibition of topoisomerase IIα (Minotti et al. 2004). DOX-induced DNA damages must be corrected with the participation of multiple DNA repair pathways, which may condition the efficacy of the drug in the clinic. In addition, deficiencies in DNA repair could be found in breast cancer patients, thus the identification of those breast cancer tumors with a lack of DNA-repair mechanisms is an important goal for further studies. Furthermore, HSPs have been implicated with DNA repair mechanisms. They can travel to the nucleus and, acting as molecular chaperones, they may interact with DNA repair proteins producing their stimulation and reactivation (Nadin and Ciocca 2010). We observed that DOX caused nuclear accumulation of HSPB1 and mainly HSPA. It has been shown that HSPA interacts in the nucleus of cells simultaneously with PARP1 and XRCC1. These results suggest that HSPA could play a role in DNA integrity through its association with PARP1 to create the repair protein complex (Kotoglou et al. 2009).

Overtherapy with cytotoxic drugs can be avoided in cancer patients if they are correctly identified as having good prognosis and vice versa (Ciocca and Calderwood 2005). Our study shows that high HSPA expression may be a useful prognosis marker in breast cancer patients treated with neoadjuvant DOX chemotherapy indicating the resistance to the administered drugs and the requirement to change the therapeutic scheme after surgery (CMF). However, these results need to be replicated in larger studies with other combination of drugs currently in use, for example DOX and C, to confirm the specific role of HSPA as prognostic molecular markers in breast cancer patients treated with neoadjuvant chemotherapy. The use of high doses of DOX (75 mg/m^2) for neoadjuvant chemotherapy may be considered in the clinic since after a mean follow up of 72 months, 46.3 % of the total number of the patients remained disease free at least for the local institutions which participate in our study.

Acknowledgments We want to thank Ms Maria A. Lazzaro for her technical assistance. This work was supported by the National Research Council of Argentina (PIP 5952 and PIP2428) and the National University of Cuyo (06-J285).

Conflict of interest No potential conflicts of interest were disclosed.

References

Almeida MB, do Nascimento JL, Herculano AM, Crespo-López ME (2011) Molecular chaperones: toward new therapeutic tools. Biomed Pharmacother 65:239–243

Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, Yang H, Amigorena

S, Ryffel B, Barrat FJ, Saftig P, Levi F, Lidereau R, Nogues C, Mira JP, Chompret A, Joulin V, Clavel-Chapelon F, Bourhis J, André F, Delaloge S, Tursz T, Kroemer G, Zitvogel L (2007) Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 13:1050–1059

Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Mignot G, Ullrich E, Kroemer G, Zitvogel L (2008a) Cancer is not just a disease of a tissue: it is a host disease. How to reactivate host defense against tumors using conventional therapies of cancer? Ann Endocrinol (Paris) 69:151–152

Apetoh L, Tesniere A, Ghiringhelli F, Kroemer G, Zitvogel L (2008b) Molecular interactions between dying tumor cells and the innate immune system determine the efficacy of conventional anticancer therapies. Cancer Res 68:4026–4030

Binaschi M, Bigioni M, Cipollone A, Rossi C, Goso C, Maggi CA, Capranico G, Animati F (2001) Anthracyclines: selected new developments. Curr Med Chem Anticancer Agents 1:113–130

Bonadonna G, Zambetti M, Valagussa P (1995) Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes. Ten-year results. JAMA 273:542–547

Bouwman P, Jonkers J (2012) The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Nat Rev Cancer 12:587–598

Calderwood SK, Khaleque MA, Sawyer DB, Ciocca DR (2006) Heat shock proteins in cancer: chaperones of tumorigenesis. Trends Biochem 31:164–172

Calderwood SK, Xie Y, Wang X, Khaleque MA, Chou SD, Murshid A, Prince T, Zhang Y (2010) Signal transduction pathways leading to heat shock transcription. Sign Transduct Insights 2:13–24

Calini V, Urani C, Camatini M (2003) Overexpression of Hsp70 is induced by ionizing radiation in C3H 10T1/2 cells and protects from DNA damage. Toxicol In Vitro 17:561–566

Cayado-Gutiérrez N, Moncalero VL, Rosales EM, Berón W, Salvatierra EE, Alvarez-Olmedo D, Radrizzani M, Ciocca DR (2013) Downregulation of Hsp27 (HSPB1) in MCF-7 human breast cancer cells induces upregulation of PTEN. Cell Stress Chaperones 18:243–249

Chien AJ, Moasser MM (2008) Cellular mechanisms of resistance to anthracyclines and taxanes in cancer: intrinsic and acquired. Semin Oncol 35:S1–S14

Chughtai ZS, Rassadi R, Matusiewicz N, Stochaj U (2001) Starvation promotes nuclear accumulation of the Hsp70 Ssa4p in yeast cells. J Biol Chem 276:20261–20266

Ciocca DR, Calderwood SK (2005) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cells Stress Chaperones 10:86–103

Ciocca DR, Vargas-Roig LM (1997) Heat shock proteins and drug resistance in breast cancer. In: Bernal SD (ed) Drug resistance in oncology. Dekker, New York, pp 167–190

Ciocca DR, Fuqua SA, Lock-Lim S, Toft DO, Welch WJ, McGuire WL (1992) Response of human breast cancer cells to heat shock and chemotherapeutic drugs. Cancer Res 52:3648–3654

Ciocca DR, Arrigo AP, Calderwood SK (2013) Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. Arch Toxicol 87:19–48

Coley HM (2008) Mechanisms and strategies to overcome chemotherapy resistance in metastatic breast cancer. Cancer Treat Rev 34:378–390

Davis JR, Mossalam M, Lim CS (2013) Controlled access of p53 to the nucleus regulates its proteasomal degradation by MDM2. Mol Pharm 10:1340–1349

Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C, von Törne C, Weichert W, Engels K, Solbach C, Schrader I, Dietel M, von Minckwitz G (2010) Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 28:105–113

Ellis RJ (2007) Protein misassembly: macromolecular crowding and molecular chaperones. Adv Exp Med Biol 594:1–13

Fox BA, Schendel DJ, Butterfield LH et al (2011) Defining the critical hurdles in cancer immunotherapy. J Transl Med 9:214

Gago FE, Tello OM, Diblasi AM, Ciocca DR (1998) Integration of estrogen and progesterone receptors with pathological and molecular prognostic factors in breast cancer patients. J Steroid Biochem Mol Biol 67:431–437

Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F (2006) Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313:1960–1964

Garrido C, Fromentin A, Bonnotte B, Favre N, Moutet M, Arrigo AP, Mehlen P, Solary E (1998) Heat shock protein 27 enhances the tumorigenicity of immunogenic rat colon carcinoma cell clones. Cancer Res 58:5495–5499

Garrido C, Brunet M, Didelot C, Zermati Y, Schmitt E, Kroemer G (2006) Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties. Cell Cycle 5:2592–2601

Goloudina AR, Demidov ON, Garrido C (2012) Inhibition of Hsp70: a challenging anti-cancer strategy. Cancer Lett 325:117–124

Hansen RK, Parra I, Lemieux P, Oesterreich S, Hilsenbeck SG, Fuqua SA (1999) Hsp27 overexpression inhibits doxorubicin-induced apoptosis in human breast cancer cells. Breast Cancer Res Treat 56:187–196

Huber SA (1992) Heat-shock protein induction in adriamycin and picornavirus-infected cardiocytes. Lab Invest 67:218–224

Jäättelä M (1995) Over-expression of hsp70 confers tumorigenicity to mouse fibrosarcoma cells. Int J Cancer 60:689–693

Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower LE (2009) Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 14:105–111

Khaleque MA, Bharti A, Sawyer D, Gong J, Benjamin IJ, Stevenson MA, Calderwood SK (2005) Induction of heat shock proteins by heregulin beta1 leads to protection from apoptosis and anchorage-independent growth. Oncogene 24:6564–6573

Khalil AA, Kabapy NF, Deraz SF, Smith C (2011) Heat shock proteins in oncology: diagnostic biomarkers or therapeutic targets? Biochim Biophys Acta 1816:89–104

Kinsella AR, Smith D, Pickard M (1997) Resistance to chemotherapeutic antimetabolites: a function of salvage pathway involvement and cellular response to DNA damage. Br J Cancer 75:935–945

Kizek R, Adam V, Hrabeta J, Eckschlager T, Smutny S, Burda JV, Frei E, Stiborova M (2012) Anthracyclines and ellipticines as DNA-damaging anticancer drugs: recent advances. Pharmacol Ther 133: 26–39

Kose S, Furuta M, Imamoto N (2012) Hikeshi, a nuclear import carrier for Hsp70s, protects cells from heat shock-induced nuclear damage. Cell 149:578–589

Kotoglou P, Kalaitzakis A, Vezyraki P, Tzavaras T, Michalis LK, Dantzer F, Jung JU, Angelidis C (2009) Hsp70 translocates to the nuclei and nucleoli, binds to XRCC1 and PARP-1, and protects HeLa cells from single-strand DNA breaks. Cell Stress Chaperones 14:391–406

Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P (2012) Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer 12:860–875

Lee E, Nichols P, Spicer D, Groshen S, Yu MC, Lee AS (2006) GRP78 as a novel predictor of responsiveness to chemotherapy in breast cancer. Cancer Res 66:7849–7853

Leong PP, Mohammad R, Ibrahim N, Ithnin H, Abdullah M, Davis WC, Seow HF (2006) Phenotyping of lymphocytes expressing regulatory and effector markers in infiltrating ductal carcinoma of the breast. Immunol Lett 102:229–236

Lepock JR, Frey HE, Heynen ML, Senisterra GA, Warters RL (2001) The nuclear matrix is a thermolabile cellular structure. Cell Stress Chaperones 6:136–147

Lindquist S, Craig EA (1988) The heat-shock proteins. Annu Rev Genet 22:631–677

Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD, Nielsen TO (2012) CD8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res 14:R48

Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, Ellis IO, Green AR (2011) Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 29:1949–1955

Matzinger P (1994) Tolerance, danger, and the extended family. Annu Rev Immunol 12:991–1045

Mendez F, Kozin E, Bases R (2003) Heat shock protein 70 stimulation of the deoxyribonucleic acid base excision repair enzyme polymerase beta. Cell Stress Chaperones 8:153–161

Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 56:185–229

Mohammed ZM, Going JJ, Edwards J, McMillan DC (2012) The role of the tumor inflammatory cell infiltrate in predicting recurrence and survival in patients with primary operable breast cancer. Cancer Treat Rev 38:943–955

Moncalero VL, Costanzo RV, Perandones C, Radrizzani M (2011) Different conformations of phosphatase and tensin homolog, deleted on chromosome 10 (PTEN) protein within the nucleus and cytoplasm of neurons. PLoS One 6:e18857

Nadin SB, Ciocca DR (2010) Participation of heat shock proteins in DNA repair mechanisms in cancer. In: Thomas AE (ed) DNA damage repair, repair mechanisms and aging. Nova Science, New York, pp 165–186

Nadin SB, Vargas-Roig LM, Cuello-Carrión FD, Ciocca DR (2003) Deoxyribonucleic acid damage induced by doxorubicin in peripheral blood mononuclear cells: possible roles for the stress response and the deoxyribonucleic acid repair process. Cell Stress Chaperones 8:361–372

Nadin SB, Vargas-Roig LM, Drago G, Ibarra J, Ciocca DR (2007) Hsp27, Hsp70 and mismatch repair proteins hMLH1 and hMSH2 expression in peripheral blood lymphocytes from healthy subjects and cancer patients. Cancer Lett 252:131–146

Nadin SB, Cuello-Carrión FD, Sottile ML, Ciocca DR, Vargas-Roig LM (2012) Effects of hyperthermia on Hsp27 (HSPB1), Hsp72 (HSPA1A) and DNA repair proteins hMLH1 and hMSH2 in human colorectal cancer hMLH1-deficient and hMLH1-proficient cell lines. Int J Hyperthermia 28:191–201

Niu P, Liu L, Gong Z, Tan H, Wang F, Yuan J, Feng Y, Wei Q, Tanguay RM, Wu T (2006) Overexpressed heat shock protein 70 protects cells against DNA damage caused by ultraviolet C in a dose-dependent manner. Cell Stress Chaperones 11:162–169

Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, Métivier D, Larochette N, van Endert P, Ciccosanti F, Piacentini M, Zitvogel L, Kroemer G (2007) Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 13:54–61

Oesterreich S, Weng CN, Qiu M, Hilsenbeck SG, Osborne CK, Fuqua SA (1993) The small heat shock protein hsp27 is correlated with growth and drug resistance in human breast cancer cell lines. Cancer Res 1: 4443–4448

Omar R, Pappolla M (1993) Oxygen free radicals as inducers of heat shock protein synthesis in cultured human neuroblastoma cells: relevance to neurodegenerative disease. Eur Arch Psychiatry Clin Neurosci 242:262–267

Panaretakis T, Kepp O, Brockmeier U, Tesniere A, Bjorklund AC, Chapman DC, Durchschlag M, Joza N, Pierron G, van Endert P, Yuan J, Zitvogel L, Madeo F, Williams DB, Kroemer G (2009) Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. EMBO J 28:578–590

Park CH, Chang JY, Hahm ER, Park S, Kim HK, Yang CH (2005) Quercetin, a potent inhibitor against beta-catenin/Tcf signaling in SW480 colon cancer cells. Biochem Biophys Res Commun 328: 227–234

Schmidt M, Böhm D, von Törne C, Steiner E, Puhl A, Pilch H, Lehr HA, Hengstler JG, Kölbl H, Gehrmann M (2008) The humoral immune system has a key prognostic impact in node-negative breast cancer. Cancer Res 68:5405–5413

Sherman M (2010) Major heat shock protein Hsp72 controls oncogene-induced senescence. Ann N Y Acad Sci 1197:152–157

Sherman MY, Gabai V, O'Callaghan C, Yaglom J (2007) Molecular chaperones regulate p53 and suppress senescence programs. FEBS Lett 581:3711–3715

Souza AP, Albuquerque C, Torronteguy C, Frasson A, Maito F, Pereira L, Duval da Silva V, Zerwes F, Raynes D, Guerriero V, Bonorino C (2009) HspBP1 levels are elevated in breast tumor tissue and inversely related to tumor aggressiveness. Cell Stress Chaperones 14: 301–310

Szekely L, Jiang WQ, Pokrovskaja K, Wiman KG, Klein G, Ringertz N (1995) Reversible nucleolar translocation of Epstein–Barr virus-encoded EBNA-5 and Hsp70 proteins after exposure to heat shock or cell density congestion. J Gen Virol 76:2423–2432

Thanner F, Sütterlin MW, Kapp M, Rieger L, Kristen P, Dietl J, Gassel AM, Müller T (2003) Heat-shock protein 70 as a prognostic marker in node-negative breast cancer. Anticancer Res 23:1057–1062

Tomar D, Sripada L, Prajapati P, Singh R, Singh AK, Singh R (2012) Nucleo-cytoplasmic trafficking of TRIM8, a novel oncogene, is involved in positive regulation of TNF induced NF-κB pathway. PLoS One 7:e48662

Torronteguy C, Frasson A, Zerwes F, Winnikov E, da Silva VD, Ménoret A, Bonorino C (2006) Inducible heat shock protein 70 expression as a potential predictive marker of metastasis in breast tumors. Cell Stress Chaperones 11:34–43

van Eden W, Spiering R, Broere F, van der Zee R (2012) A case of mistaken identity: HSPs are no DAMPs but DAMPERs. Cell Stress Chaperones 17:281–292

Vargas-Roig LM, Gago FE, Tello O, Aznar JC, Ciocca DR (1998) Heat shock protein expression and drug resistance in breast cancer patients treated with induction chemotherapy. Int J Cancer 79:468–745

Wheeler DS, Chase MA, Senft AP, Poynter SE, Wong HR, Page K (2009) Extracellular Hsp72, an endogenous DAMP, is released by virally infected airway epithelial cells and activates neutrophils via Toll-like receptor (TLR)-4. Respir Res 10:31

Yamaguchi R, Tanaka M, Yano A, Tse GM, Yamaguchi M, Koura K, Kanomata N, Kawaguchi A, Akiba J, Naito Y, Ohshima K, Yano H (2012) Tumor-infiltrating lymphocytes are important pathologic predictors for neoadjuvant chemotherapy in patients with breast cancer. Hum Pathol 43:1688–1694

Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G (2008) Immunological aspects of cancer chemotherapy. Nat Rev Immunol 8:9–73

Zoubeidi A, Gleave M (2012) Small heat shock proteins in cancer therapy and prognosis. Int J Biochem Cell Biol 44:1646–1656