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HtrA1 loss is related to aggressive behavior parameters in sentinel node positive breast cancer

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Summary. Aim: HtrA1, a member of the High Temperature Requirement Factor A family of oxidative stress-response proteases seems to play a role as a tumor suppressor, being down-regulated in a series of human cancers during their progression. Particularly, low HtrA1 mRNA levels have been observed in breast cancer patients with more aggressive clinical features. These have been shown to relate to a longer disease free and overall survival, with more pronounced effects in axillary nodes positive patients. Subjects and Methods: We have analyzed for immunohistochemical HtrA1 expression a series of 66 sentinel node positive breast cancers through Tissue Micro Array technology. Results: HtrA1 was absent to low in 29 cases, medium in 19 cases and high in 18 cases. Our data revealed a positive significant relation between HtrA1 expression level and estrogen (p=0,002) and progestinic receptor expression (p=0.003) and a negative correlation with histological grading (p=0.028), proliferation index (p=0.05), common BC histotypes (p=0.040), luminal A and B subtypes (p=0.001), metastasis development (p<0.0001) and local relapse (p<0.0001). Finally, no correlation was recorded between HtrA1 expression level and breast cancer histology type and metastasis to non sentinel nodes. Interestingly HtrA1 loss in SLN metastasis was able to predict positive non sentinel nodes (p=0.001). Conclusions: Low HtrA1 expression is significantly related to breast cancer poor prognosis parameters, and HtrA1 loss in sentinel nodes is related to metastasis of non sentinel nodes, offering a further marker useful for BC prognostic stratification.

Key words: Breast Cancer, HtrA1, Tumor suppressor, Tissue MicroArray

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Introduction

Breast cancer still presents as the most frequent cancer in women, recording nearly a million new cases worldwide and about 400,000 deaths each year (Parkin et al., 2005). Breast cancer is a heterogeneous disease because of its different clinical, histopathological and molecular features. Hence, a wide variation of breast cancer progression is commonly observed in cases with comparable clinic-pathological features. In the past the discovery of molecular markers with a prognostic and predictive value, such as estrogen, progesteron and HER2 receptors, has facilitated the prognostic stratification of patients and the discovery of new cancer therapies. Indeed, hormonal receptor expression shows a more favourable prognosis while HER2/neu over-expression significantly worsens the prognosis. However, both estrogen receptors and HER2/neu represent specific markers of target therapy. More recently, DNA-microarray-expression-profiling studies have identified 5 major subtypes of breast cancers: the luminal A and B, the HER2+, the normal breast-like and

the basal-like (basaloid) subgroups (Sorlie et al., 2001; van de Vijver et al., 2002). Through expression-profiling studies the risk stratification of BC patients is greatly improved. In addition, a simple immunohistochemical panel has been used for the definition of breast cancer subtypes similar to those previously defined using gene expression analyses. However, the stratification of patients and their selection for appropriate adjuvant therapies still encounters difficulties (Goldhirsch et al., 2009). Therefore, novel diagnostic, prognostic and predictive markers should be identified to better categorize BC patients, mainly for the correct selection of adjuvant therapies and possibly for the proposal of novel perspectives for more efficient therapeutic strategies. Currently the surgical treatment of BC is addressed at the clinical stage. Sentinel lymph node (SLN) biopsy is standard care for early stage BC patients with no clinical evidence of axillary neoplastici. However metastasis in SLN is not always associated to metastasis in axillary non sentinel lymphnode (NSLN) (Reynolds et al., 1999; Takei et al., 2007), thus the need for factors predicting NSLN metastasis, i.e. primary outcome, is constantly increasing, in order to send to axillary surgery only BC patients that could obtain a real benefit (Gur et al., 2009).

Most BC-growth and progression are estrogen-dependent. Through epidemiological and clinical studies it is evident that estrogens play a relevant role in induction, promotion and progression of a variety of human cancers, mainly BC and endometrial cancer, but also ovary, prostate, liver, brain and lymphoid tissues neoplasms (Burns and Korach, 2012). In addition, some animal models support this data, as chronic administration of estrogens is responsible for a high frequency of mammary cancers in rat strains and malignant kidney tumours in Syriam hamster (*Mesocricetus auratus*) (Li and Li, 1990; Kong et al., 1999). Moreover, in Syriam Hamster it has been demonstrated that initial estrogen administration induces an increased expression of HtrA1 RNA and protein through oxidative stress, but during prolonged estrogen treatment cancer develops with a significant reduction of both HtrA1 RNA and protein (Zurawa-Janicka et al., 2008).

HtrA1 (also referred to as Prss11, or IGFBP-5) is one of the 4 members of the High Temperature Requirement Factor A (HtrA) family of oxidative stress-response proteases identified in human normal fibroblasts (Zumbrunn and Trueb, 1996). It is ubiquitously expressed in normal human tissues as well as in mature layers of the epidermis, in secretory breast epithelium, in the liver, and in tubules of the renal cortex (De Luca et al., 2003). HtrA1 has been correlated to the pathogenesis of a series of human diseases such as osteoarthritis (Hu et al., 1998), Alzheimer's disease (Grau et al., 2006), macular degeneration and appears to play a role in some neuromuscular diseases (Yang et al., 2006), in particular muscular dystrophy (Bakay et al., 2002).

In addition, HtrA1 seems to play a role as a tumor suppressor. In fact, it has been reported to be absent or down-regulated in a series of human cancers such as gastric, breast, ovarian (Chien et al., 2004), endometrial (Mullany et al., 2011), hepatocellular carcinomas (Zhu et al., 2010), mesothelioma (Baldi et al., 2008), melanoma (Baldi et al., 2002), and lung cancer during their progression (Esposito et al., 2006). Its role as suppressor is played out in different plans.In fact HtrA1 down-regulation promoted cell anchorage independent in growth ovarian cancer cell line SKOV3 and over-expression of HtrA1 inhibited melanoma cell proliferation *in vivo* in a mouse model (Baldi et al., 2002; Chien et al., 2006). It has also been implicated in chemotherapeutic responsiveness. Indeed, HtrA1 expression enhanced sensitivity to cisplatin and paclitaxel (Chien et al., 2006), thus, the response rate to cisplatin based treatment regimens in patients with ovarian or gastric cancers is significantly correlated to the level of expression of HtrA1 (Chien et al., 2004; Catalano et al., 2011). Finally, HtrA1 has been identified together with two other genes, MTSS1, CLPTM1, as an indicator of doxorubicin responsive patients from non-responsive breast cancers in 95% of samples (Folgueira et al., 2005)

HtrA1 expression has not been widely studied in breast cancer, but a reduction has been described in BC cells with respect to normal ductal and acini cells (Wang et al., 2012). In addition, MCF cells with induced low expression of HtrA1 levels showed epithelial-to mesenchymal transition with acquisition of mesenchymal phenotypic characteristics and a concomitant reduction of response to DNA damage. These effects are prevented in the case of induced HtrA1 overexpression (Wang et al., 2012). Recently, a low level of HtrA1 mRNA in a series of breast cancers has been significantly related to shorter disease free and overall survival, particularly in the group of patients with more than 4 positive axillary nodes (Lehner et al., 2013).

Here we examined the expression of HtrA1 expression in a series of positive SLN pT1 BC cancer, in order to evaluate its association to the primary outcome and the main clinic-pathological parameters.

Material and methods

Selection of patients

Sixty-six cases of BC samples were collected from the Pathology Unit of the National Cancer Institute "Giovanni Pascale" of Naples, Italy. The study was approved by the Internal Review Board of the INT Fondazione Pascale (Naples, Italy) (CEI 556/10 of 12/3/2010). The cases, diagnosed between 1999 and 2006, were included in this study on the basis of the availability of diagnostic paraffin blocks that were thick enough to provide a minimum of 50 sections and of clinical information of more than 4 years. All cases were reviewed by AB and RF, according to WHO

classification criteria, using standard tissue sections and immunohistochemical slides. Criteria of selection included small tumors (pT1) with positive SLN.

Medical records for all cases were reviewed for clinical information and pathological parameters were evaluated for each tumor included in the study: patient age at initial diagnosis; tumor size; histologic subtype; histologic grade; nuclear grade; metastasis in NSLN, defined as primary outcome; tumor stage; tumor recurrence or distant metastasis; and type of surgery (for tumor removal); estrogen and progesteron receptors, HER2 and proliferation index (ki67). Finally, histotype subgroups have been defined, where possible, according to the Prat method (Table 1), considering several immunohistochemical subtypes: luminal A, Luminal B, HER2- enriched, basal-like and a normal breast-like group, with significant differences in incidence, survival and response to therapy (Voduc et al., 2010).

Tissue microarray building

Following conventional protocols we used a tissue-microarray device (Galileo TMA) to construct a single-tissue-microarray block of 150 cores, including two cores for each one tumoral case and 18 normal breast tissue as controls. All tumours and controls were reviewed by 2 experienced pathologists (MDB, RF). The tumor-cell-rich areas were marked in the paraffin blocks. Two selected 0.6-mm cores from different areas were included, along with ten normal breast tissue samples.

Immunohistochemistry analysis

Immunohistochemical staining was performed on TMA slides from formalin-fixed, paraffin embedded tissues, to evaluate the expression of HtrA1, ER, PR, c-erbB-2, Ki-67, EGFR and CK 5/6. In addition, in the same series 15 SLN metastasis with quite representative tissue were selected for HtrA1 immunostaining. Then, paraffin slides were deparaffinised in xylene and rehydrated through graded alcohols.

Antigen retrieval was performed with slides heated in a bath for 20 minutes at 97°C using a 0.01 M citrate buffer (pH 6.0 for HtA1, PR, c-erbB-2, Ki-67) or Tris-EDTA (pH 9 for ER and CK5/6).

After antigen retrieval, the slides were allowed to cool. They were then rinsed with TBS and the endogenous peroxidase was inactivated with 3% hydrogen peroxide. After protein block (BSA 5% in PBS 1x), the slides were incubated with primary antibodies (Table 2). The sections were rinsed in TBS and incubated for 20 minutes with Novocastra Biotinylated Secondary Antibody (RE7103), a biotin-conjugated secondary antibody formulation that recognized mouse and rabbit immunoglobulins. Next the sections were rinsed in TBS and incubated for 20 minutes with Novocastra Streptavidin- HRP (RE7104) and then peroxidase reactivity was visualized using a 3,3'-diaminobenzidine (DAB). Finally, the sections were counterstained with hematoxylin and mounted.

Antigen expression was evaluated independently by three pathologists (RF, MDB and AB) using light microscopy. The pathologists were unaware of the clinical outcome. For each sample at least 5 fields (inside the tumor and in the area exhibiting tumor invasion) and >500 cells were analyzed. Using a semiquantitative scoring system microscopically and referring to each antigen scoring method in other studies, an observer evaluated the intensity, extent and sub-cellular distribution of ER, PR, c-erbB-2, and Ki-67.

The cut-off used to distinguish "positive" from "negative" cases was ≥1% ER/PR positive tumor cells. Immunohistochemical analyses of c-erbB-2 expression describe the intensity and staining pattern of tumor cells. Only membrane staining intensity and pattern were evaluated using the 0 to 3+ score as illustrated in the HercepTest kit (DAKO) scoring guidelines. Scores of 0 or 1+ were considered negative for *HER2/neu* expression, 2+ was uncertain, and 3+ was positive. Cases with score 2+ underwent fluorescence *in situ* hybridization analysis. For the proliferative index Ki-67 was defined as the percentage of immunoreactive tumour cells out of the total number of cells. The percentage of positive cells per case was scored according to 2 different groups: group 1: ≤14% (low proliferative activity); group 2: >14% (high proliferative activity).

In scoring HtrA1 protein expression the immunopositivity of the cytoplasm was considered, essentially as already described (Mullany et al., 2011), and evaluated as absent to low (less than 1% of positive cells); medium (from 1% to 20% of positive cells) and high expression (more than 20% of positive cells). This

Table 1. Schematic immunohistochemical prophile of breast cancer subtypes.

	Luminal A	Luminal B	Her2-enriched	Basal-like (TN)
ER	+	+	-	-
PR	+	+	-	-
cErbB2	-	+/-	+	-
EGFR	+/-	+/-	+/-	+
CK 5/6	+/-	+/-	+/-	+

Table 2. Antibodies characteristics.

Antibodies	clone		
HtrA1		1:50	Baldi et al., 2008
ER	Clone ID5,	1:35	DAKO, Ely, UK
PgR	PR Clone 636	1:50	DAKO, Ely, UK
Ki67	MIB1	1:75	DAKO, Ely, UK
Her2	Policlonal	1:75	DAKO, Ely, UK
EGFR	EGFR pharmDx™ Code K1492 test		DAKO, Ely, UK
Ck 5/6	5/6 Ab-2 Clone D5/16 B4	1:10	Thermoscientific

protocol of quantitation for HtrA1 has been set up and successfully used by our research group in several scientific investigations (Baldi et al., 2008). Validation of TMA sections was performed comparing the immunohistochemical data from TMA sections and whole sections for routine diagnosis, for ER, PgR, Her2 and Ki67. A significant correspondence of these antigens in the sections was observed.

The discrepancy in the interpretation was resolved through joint analysis of each case by all pathologists involved in the evaluation in order to revise and define a more appropriate percentage of positive cells (Franco et al., 2011).

Statistical analysis

Descriptive analysis was made using median values and 95% Confidence Interval (CI). Correlations between different variables were performed using the chi-square test or the Spearman correlation test when appropriate. SPSS software (version 17.00, SPSS, Chicago) was used for statistical analysis. A P value of less than 0.05 was considered to indicate statistical significance.

Results

Patients clinical features

Main clinical-pathological features are reported in Table 3.

Our series includes 66 patients, with a mean age of 57 yrs (range 32-80 years). All patients with a tumor smaller than 2 cm were addressed to breast wide excision and SLN biopsy. In 26 cases neoplasia involved the left breast, in 40 cases the right breast. All selected patients showed positive SLN. Thus all were addressed to axillary surgery, being positive 27 cases (41%). All patients were treated with radiotherapy, as adjuvant therapy after breast wide excision. Selection covers several years, during which chemotherapic protocols changed. Briefly in 58 cases adjuvant chemiotherapy and ormonotherapy were also administered; in 6 cases only hormonotherapy. In the course of follow-up local relapse was observed in 5 cases, in a mean period of 34.6 months (range 25-49 months). In 23 cases metastasis was recorded in a mean period of 3.5 months (range 11-60 months). 3 patients were dead in a mean period of 41.3 months (range 2-86 months).

Patients pathological features

The main pathological data of our series are reported in table 3. Our series includes 42 cases of infiltrative ductal carcinomas (IDC) (12 with moderate grade, 30 with high grade), 16 cases of infiltrative lobular carcinomas (ILC) (4 of moderate grade and 12 of high grade), 4 cases of infiltrative duct-lobular carcinomas (3 of moderate grade, 1 of high grade), 1 cases of infiltrative tubular-lobular carcinomas of high grade; 1 case of tubular carcinoma of moderate grade, 1 case of micro-papillary carcinoma of high grade; 1 case of infiltrative papillary carcinoma of moderate grade. In addition *in situ* carcinomas have been found in 9 cases.

Correlations between immunohistochemical and pathological parameters

Table 4 summarizes the results from immunohistochemical analysis of the 66 valid breast cancer specimens. HtrA1 staining was always cytoplasmic while c-ErbB2 staining was at the membrane level. Moreover Ki67, estrogen and progestinic receptors were always nuclear. Figure 1 presents some typical immunohistochemical staining for HtrA1.

By Pearson's χ^2 test, a positive statistically significant correlation was recorded between HtrA1 expression level and estrogen and progestinic receptor

Table 3. Main Clinic-pathological data.

Number of patients	66
Median age (range)	57 (32-77) years
N status	
SLN+ and NSLN-	39 (59%)
SLN+ and NSLN+	27 (41%)
M development	
M0	43 (65%)
M1	23 (35%)
Local Relapse	
Absent	61 (93%)
Present	5 (7%)
Grading	
G1	6 (9%)
G2	36 (55%)
G3	24 (36%)
HtrA1 score in primitive tumor	
Absent to low	29 (44%)
Medium	19 (29%)
High	18 (27%)
HtrA1 score in SLN	
Absent to low	8/15 (53%)
Medium	0
High	7/15 (47%)
ki67 score	
Low	17 (26%)
High	47 (64%)
Estrogen receptor	
Negative	13 (20,3%)
Positive	51 (79,7%)
Progesterone receptor	
Negative	14 (22,6%)
Positive	48 (77,4%)
c-ErbB2 status	
0	46 (70,8%)
1+	4 (6,2%)
2+	6 (9,2%)
3+	9 (13,8%)
Histological subgroup	
Luminal A	14 (21,9%)
Luminal B	38 (59,4%)
HER3+	10 (15,6%)
Basal like	2 (3,1%)

expression. A negative correlation was identified between HtrA1 expression and histological grading, proliferation index, M status, and relapse. The relation analysis between HtrA1 and HER2 expression showed a negative trend of statistical correlation. Moreover, a correlation was shown between HtrA1 and common breast cancer histology type and the immunohistochemical subtypes. In particular, HtrA1 appeared to

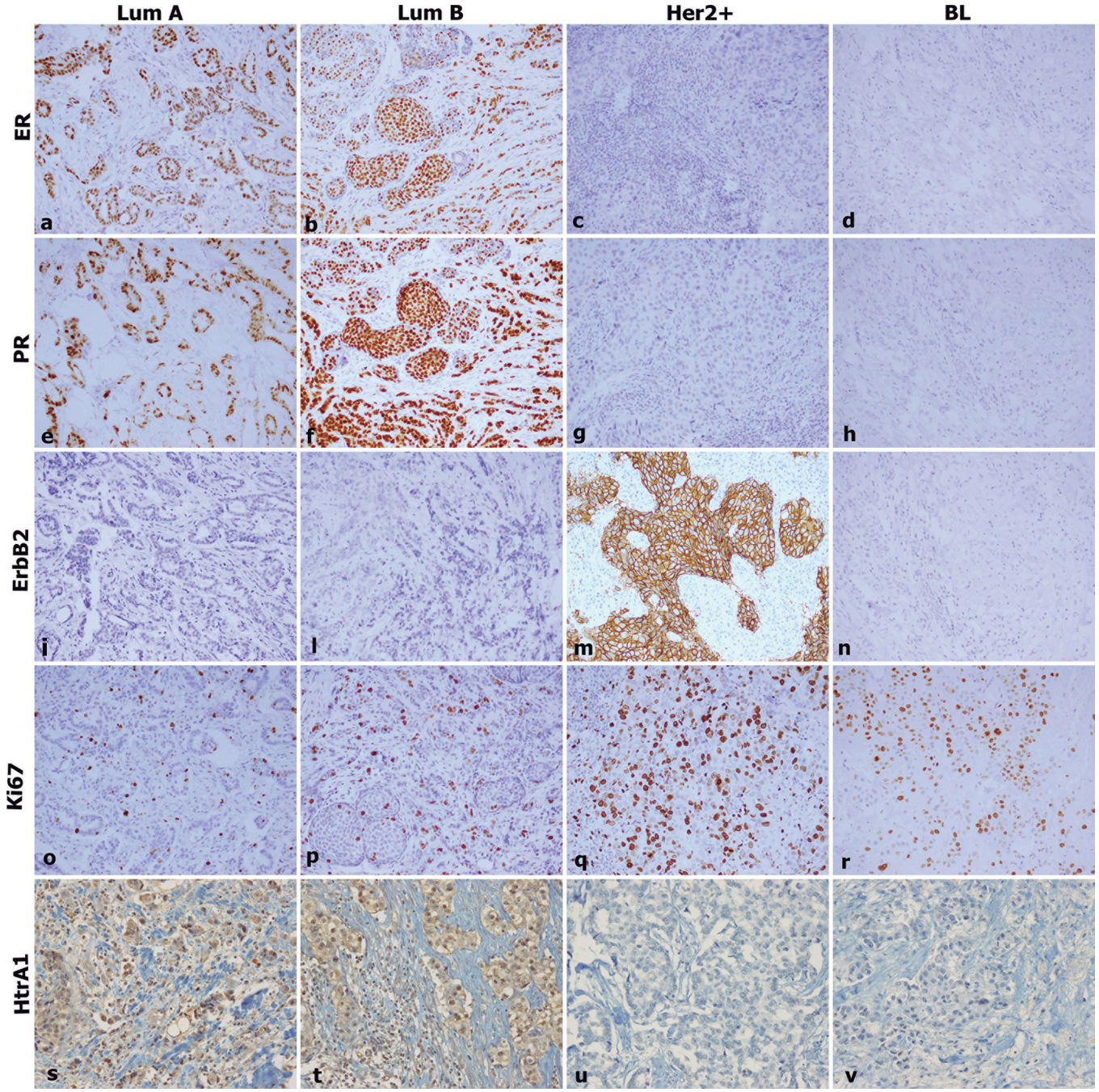

Fig. 1. ER, PR, ErbB2,Ki67 and HtrA1 expressions in Luminal A, Luminal B, HER2 enriched and Basal-like moleculars subtypes. **a.** Immunopositivity for ER in Lum A. **b.** Immunopositivity for ER in Lum B. **c.** Immunonegativity for ER in HER2+. **d.** Immunonegativity for ER in BL. **e.** Immunopositivity for PR in Lum A. **f.** Immunopositivity for PR in Lum B. **g.** Immunonegativity for PR in HER2+. **h.** Immunonegativity for PR in BL. **i.** Immunonegativity for ErbB2 in Lum A. **l.** Immunonegativity for ErbB2 in Lum B. **m.** Immunopositivity for ErbB2 in HER2+. **n.** Immunonegativity for ER in BL. **o.** Ki67<14% in Lum A. **p.** Ki67>14% in Lum B. **q.** Ki67 high in HER2+. **r.** Ki67 high in BL. **s.** HtrA1 high cytoplasmatic expression in Lum A. **t.** HtrA1 high cytoplasmatic expression in Lum B. **u.** Immunonegativity for HtrA1 in HER2+. **v.** Immunonegativity for HtrA1 in BL. x 20

Table 4. Pearson's χ^2 test (and statistical significance) between HtrA1 and morphological and clinical markers in breast cancer.

	Histology	Grading	Estrogen receptor	Progestinic receptor	Ki67	HER2	N status	Histological subgroup	M status	Relapse
Htra1	21.785	9.089	14.405	13.642	7.788	14.946	NS	29.220	44.482	19.051
primitive BC	0,040	0.028	0.002	0.003	0.051	0.092		0,001	<0.0001	<0.0001
HtrA1 SLN	NS	NS	NS	NS	NS	NS	0.001	NS	NS	NS

be highly expressed in the luminal subtypes and absent or with low expression in Her2 enriched subtype. In addition HtrA1 expression in primary BC and relative SLN were significantly associated (p=.001). Finally, in BC primary tumors no correlation was found between HtrA1 expression and N status of NSLN, defined as primary outcome. Interestingly, HtrA1 loss in SNL was significantly related to metastasis in other locations different from non sentinel nodes (p=.001). These results are summarized in Table 4.

Discussion

Down-regulation of the serine protease HTRA1 has been demonstrated in several cancers. Proteases are generally involved in protein catabolism, but through cleaving specific substrates, they could also influence cell survival and death (Turk, 2006; Radisky and Bissell, 2006).However, the treatment of patients based on metalloproteinase inhibitors did not show the desired effects on cancer progression, even producing an enhancement of tumor growth (Coussens et al., 2002; Overall and Lopez-Ortin, 2002). This implies that some extracellular proteases might actually have anti-tumor properties, such as serine proteases PRSS3 (also known as trypsinogen IV), PRSS8 (prostasin), and PRSS21 (testisin) (Radisky et al., 2006). In this context HtrA1 could also be included. Although its physiological role has not been completely clarified, its ability to reduce tumoral cell motility, growth and invasiveness has led to its consideration as a tumor suppressor (Baldi et al., 2002; Chien et al., 2004, 2009; Mullany et al., 2011). Many HtrA1 extracellular and intracellular target proteins have been recognized (Baldi et al., 2002; Chien et al., 2009; He et al., 2010, 2012). Particularly, it has been demonstrated for HtrA1 a role in inducing apoptosis and in the control of cytoskesleton stability (Chien et al., 2009; He et al., 2012). Interestingly, epigenetic mechanisms have also been described for HtrA1 down-regulation, such as promoter methylation or histone-deacetylation (Wang et al., 2012; Lehner et al., 2013). Reduced HTRA1 expression has been shown in over 50% of cases in both ovarian and endometrial cancer (Chien et al., 2004; Bowden et al., 2006; Mullany et al., 2011). In these tumors a relation to more aggressive behavior has also been shown. In melanoma, decreased HtrA1 is associated with higher-grade tumor, with lower HtrA1 expression in node metastasis compared with respective primitive tumors (Baldi et al., 2002). In addition, low HtrA1 expression has been related to poor prognosis in mesothelioma and hepatocellular carcinomas (Baldi et al., 2008; Zhu et al., 2010). Finally low response to cytotoxic agents related to decreased HtrA1 expression has been observed in gastric and ovarian cancer (Chien et al., 2006; Catalano et al., 2011). Few studies have investigated the role of HtrA1 expression in breast cancer. *In situ* and invasive breast cancer has previously shown a significant reduction or loss of HtrA1 expression (Wang et al., 2012).

Our data demonstrated that high HtrA1 expression is normally observed in ductal epithelium and is significantly related to high female steroid receptors expression in cancer cells, being high in luminal A and luminal B subtypes. In addition, the absence of expression of HtrA1 has been seen in uterine papillary serous cell lines, whilst HtrA1 expression was variable in endometrioid-type endometrial cancer cell lines. These cell lines reflect different clinical settings. Uterine papillary serous carcinoma is a highly aggressive, estrogen-independent Type II endometrial carcinoma, arising in a background of atrophic endometrium, while the more common endometrioid-type endometrial adenocarcinoma is an estrogen-dependent Type I endometrial cancer, arising from a background of endometrial hyperplasia. Of particular interest, recently, 131 breast cancer tissues were analyzed for HTRA1 transcripts through qPCR (Lehner et al., 2013). Variable mRNA levels were demonstrated, but lower HTRA1 mRNA values were observed in patients with more aggressive clinical features, such as higher tumor stage. These data have been reinforced by significant association between higher HTRA1 mRNA expression and longer OAS and DFS, also in multivariable analysis. The validation of the results has been successfully demonstrated in an independent series (Lehner et al., 2013). The limit of the study was the use of mRNA and not the effective related protein, since mRNA does not always hesitate in the protein production because of the translational mechanism of control (Lackner and Bahler, 2008). In addition, the use of mRNA is not very viable for routine purposes. Our data confirmed a significant relation between HtrA1 expression, documented through the simple immunohistochemical technique, with aggressive tumor features, such as high grade, high proliferation index, metastasis development and relapse. Moreover, our data confirmed an inverse association of HtrA1 expression with Her2 expression. In fact it was generally low in Her2 positive tumors, whilst high HtrA1 expression was generally observed in luminal A

tumors.

Besides being a prognostic biomarker, HtrA1 has been recognized as a predictive marker in many studies. Indeed, previous results in gastric and ovarian cancer support the HTRA1 proficiency to a better therapeutic responsiveness (Chien et al., 2006; Catalano et al., 2011). Particularly, in a breast cancer study, HTRA1 predicted response to doxorubicin-based chemotherapy in a panel of three biomarkers (Folgueira et al., 2005). On the other hand, low HTRA1 seems to trigger EMT in breast cancer cells (Wang et al., 2012), this phenomenon likely being related to drug resistance. The low development of metastasis described for high HTRA1 expression is probably related to the anti-migratory and proapoptotic functions (Chien et al., 2004, 2009; Ajayi et al., 2008; He et al., 2010, 2012), previously observed in this serine protease. Indeed, low HTRA1 expression seems to be associated with the metastatic phenotype of melanoma cells, whilst growth and matrix invasion of metastatic cells is repressed by high HTRA1 expression suppression (Baldi et al., 2002). Moreover, migration and invasion subsequent to HTRA1 inhibition through the siRNA technique has been demonstrated in both SKOV3 cells and immortalized breast epithelial cells (Chien et al., 2009; Wang et al., 2012). Finally, although in a small series, we have demonstrated that HtrA1 loss in SLN metastasis is related to positive NSLN. These data could provide a novel biomarker able to predict primary outcome, addressing to axillary surgery only BC patients with high risk of metastasis.

Accordingly to these results, endometrial cancer cells expressing HTRA1-siRNA injected in a mouse model are able to increase the number of micro-metastases in the lung (Mullany et al., 2011).

In conclusion, HtrA1 loss is significantly related to BC aggressiveness parameters, also in the initial disease. Thus HtrA1 immunohistochemical expression could provide further information for the clinicians in order to identify in a pT1 positive SLN patients series, cases deserving less aggressive treatment because of a more favorable behavior. On the other hand HtrA1 loss in SLNs seems to be useful to predict patients with positive NSLNs. Further studies with larger series of patients are required in order to confirm these data and to determine their real impact in clinic.

Take Home Messages

- HtrA1, a member of the High Temperature Requirement Factor A (HtrA) family of oxidative stress-response proteases, ubiquitously expressed in normal human tissues, seems to play a role as a tumor suppressor, being down-regulated in a series of human cancers during their progression.
- We have analyzed for immunohistochemical HtrA1 expression a series of 66 sentinel node positive breast cancer through Tissue MicroArray technology.
- Low HtrA1 expression is significantly related to breast cancer poor prognosis parameters, also for positive sentinel node small tumors, although with no correlation with extra-sentinel nodes involvement.
- HtrA1 loss in SLN metastasis seems to be able to predict primary outcome
- Our data provide a further marker useful for BC prognostic stratification.

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