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Immunohistochemical evaluation of mitogenic activity in breast cancers by TFF1/PS2 protein and HER2 oncoprotein expression in western Algeria

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Abstract
Breast carcinoma is a disease with a tremendous heterogeneity in its clinical behavior. Newer prognostic factors and predictors of response to therapy are needed. The purpose of the current study was to investigate TFF1 expression in breast cancer, and its relation with tumor malignancy and proliferation measured by HER2 status. This is a prospective study conducted on 538 women with primary ductal/lobular invasive breast carcinomas in the department of pathology at the regional military university hospital of Oran (Algeria). The mitogenic activity was evaluated by a conventional immunohistochemical (IHC) approach, validated as a replacement technique for microarray analysis by labeling the antigens TFF1, HER2, ERα, and PgR. Correlations between the different parameters were carried out. TFF1 was correlated positively with hormonal receptors (HR) *(P*<0.0001) and negatively with HER2 *(P*<0.0001) and histological grade but with marginal significance *(P*<0.1). According to mitogenic activity, patients were individualized into two subgroups: low proliferation tumors (Luminal A) representing 51.3% of cases and high proliferation ones representing 48.7% of cases (Luminal B, Basal, HER2, Claudin-Low). Mitogenic activity majored by HER2 overexpression correlates with aggressiveness parameters such as high histological SBR grade, larger tumor size, young age at presentation, and negative TFF1/HR status. IHC methods are less expensive and more cost-effective for the establishment of molecular subclasses.

Keywords: Breast cancer, HER2, mitogenic activity, TFF1

INTRODUCTION

According to data from our registry 11000 new cases of breast carcinoma are recorded each year, it is the most common malignant neoplasm affecting Algerian women. Patients with breast carcinoma in Algeria are significantly younger than those in the West; it may suggest that this neoplasm has some unique biological features that need to be explored.

The kinetics of tumor proliferation can be explored by several elements: the modified SBR grade [1] and the mitotic index [2-3] seem to be a precise and reproducible factor. These two elements of proliferation and negativity of the hormonal receptors are the best tools for predicting the effectiveness of chemotherapy [4-5]. However, the mitotic count may be difficult to assess because of cellular and nuclear alterations that are certainly related to sampling, fixation conditions, tumor volume, and tumor heterogeneity [6].

Trefoil factor 1 (TFF1; previously named pS2) is abnormally expressed in about 50% of human breast tumors [7], it is expressed predominantly in ER^+ tumors. Previous studies have shown that TFF1 protein expression correlated with prognostic factors and endocrine response [8]. HER2, otherwise known as neu or c-erbB-2, is the product of an oncogene amplified and overexpressed in breast carcinomas [9]. It has been reported that 10-34% of breast carcinomas overexpress the HER2 receptor. This characteristic is associated with more aggressive tumor behavior [10].

The aim of the present study was to investigate the immunohistochemical expression of TFF1 protein in breast cancer cases, and its relation with tumor malignancy and proliferation measured by HER2 oncoprotein expression.

MATERIALS and METHODS

538 patients from the west Algerian region with a mean age of 50.3 years (aged 25 to 90 years) with primary ductal/lobular invasive breast carcinomas were studied after informed constent. Medical records of patients were taken at random. Patients were treated between September 2009 and January 2016. Clinicopathological data were collected from pathological reports. Tumor grading was performed according to modified Bloom and Richardson methods [1].

IHC staining for TFF1, ERα, PgR and HER2 was carried out in representative 2µm sections of paraffin-embedded tissues, following manufacturer's instructions. Sections attached on silanized slides were de-waxed in xylene and rehydrated in graded ethanol. Detection of TFF1 was done using Dako Rabbit Polyclonal Antibody Code No. (HPA003425; SIGMA-ALDRICH). Detection of ERα was done using Dako, primary antibody, anti-ERα clone, Code No. EP1 (M3643; Dako; Glostrup; Denmark). Detection of PgR was done using Dako primary antibody, anti-PgR

Code No. (PgR 636 Dako; Glostrup; Denmark). Detection of HER2 receptors was done using Dako antihuman c-erb B-2 oncoprotein HercepTest Kit, Code No. (K5204; Dako; Glostrup; Denmark.

Scorings of biomarkers were performed according to criteria as follows: TFF1 was defined as positive when stained cells ≥10%; Score 0: negative (-); Score 1: positive (+) [11]. A semi-quantitative score was used to record results of ERα and PgR staining [12]. The DAKO scoring system for HER2/neu used was defined as negative for scores of 0, 1+, or 2+ and positive for score of 3+.

Correlations between variables were determined by Pearson's correlation coefficient using SPSS Inc.software V20.0. The significance level was set at $P<0.05$.

RESULTS

The characteristics of the patients are listed in Table 1. Infiltrating ductal carcinoma (IDC) was the largest group, accounting for 68.2%. Women aged 40 to 49 years were the most affected with a frequency of 31.8% (171 cases).

Table 1. Clinicopathological characteristics of patients

Item	N (%)
Total	538
Age (years)	
<50	296 (55.0)
≥50	242 (45.0)
Grade	
I	19 (3.5)
II	335 (62.3)
III	184 (34.2)
Size (cm)	
T1 : ≤2	109 (20.3)
T2: 2< - ≤5	179 (33.3)
T3: >5	250 (46.5)
Nodal status	
0	149 (27.7)
1-3	141 (26.2)
4-9	102 (19.0)
10^+	146 (27.1)
Metastasis	
Present	140 (26.0)
Absent	398 (74.0)
Histological type	
IDC	367(68.2)
ILC	171(31.8)
Menopausal status	
Premenopausal	292 (54.3)
Postmenopausal	246 (45.7)
pS2 expression	
Negative	155 (28.8)
Positive	383 (71.2)
ERα expression	
Negative	180 (33.5)
Positive	358 (66.5)
PgR expression	
Negative	241 (44.8)
Positive	297 (55.2)
HER2 status	
0,1+,2+	447 (83.1)
3+	91(16.9)

Evaluation of mitogenic activity by TFF1 and HER2 status

A negative correlation was found between TFF1 and HER2 expressions ($p=0.001$). The dominant subtype was [$TFF1^+/HER2^-$] (62.3%). $TFF1^-$ tumors overexpressed HER2 in 08.0% of cases, while overexpression of both markers was found in only 8.9% of cases (Table 2). The fraction of $TFF1^+$ tumors consisted mainly of $HER2^-$ cases that of $HER2^+$ ones (87.5% vs 12.5%).

Table 2. Frequencies of [TFF1/HER2] subtypes

Subtypes	N (%)
[$TFF1^-/HER2^-$]	112(20.8)
[$TFF1^-/HER2^+$]	43 (08.0)
[$TFF1^+/HER2^+$]	48 (8.9)
[$TFF1^+/HER2^-$]	335 (62.3)

Similarly, a significant negative correlation was observed between ERα expression and HER2 ($p=0.001$). In the fraction of $HER2^-$ cases, only 29.1% were $ER\alpha^-$ whereas 70.9% were $ER\alpha^+$.

Expression profiles of TFF1/ERα/PgR and HER2

The results (Table 3) showed that hormone receptor positive tumors with an overexpression of TFF1 were more likely to be $HER2^-$ than $HER2^+$ (44.6% vs. 5.4%). Similarly, tumors with negative hormone receptors without overexpression of TFF1 were more likely to be $HER2^+$ than $HER2^-$ (16.7% vs. 6.9%). It was then found that $TFF1^+$ tumors were generally hormone receptors positive and $HER2^-$ rather than $HER2^+$. This subtype [$TFF1^+/ER\alpha^+/PgR^+/HER2^-$] represented in this class a frequency of 89.2% (240 patients) compared to the subtype [$TFF1^+/ER\alpha^+/PgR^+/HER2^+$] which represented only 10.8% (29 patients).

Table 3. Expression profiles of TFF1 in relation to hormonal receptors and HER2

Subtypes	N (%)
[$ER\alpha^-/PgR^-/TFF1^-/HER2^-$]	37 (6.9)
[$ER\alpha^-/PgR^-/TFF1^-/HER2^+$]	90 (16.7)
[$ER\alpha^-/PgR^-/TFF1^+/HER2^-$]	28 (5.2)
[$ER\alpha^-/PgR^-/TFF1^+/HER2^+$]	7 (1.3)
[$ER\alpha^-/PgR^+/TFF1^-/HER2^-$]	2 (0.4)
[$ER\alpha^-/PgR^+/TFF1^-/HER2^+$]	2 (0.4)
[$ER\alpha^-/PgR^+/TFF1^+/HER2^-$]	10 (1.9)
[$ER\alpha^-/PgR^+/TFF1^+/HER2^+$]	4 (0.7)
[$ER\alpha^+/PgR^-/TFF1^-/HER2^-$]	12 (2.2)
[$ER\alpha^+/PgR^-/TFF1^-/HER2^+$]	2 (0.4)
[$ER\alpha^+/PgR^-/TFF1^+/HER2^-$]	57 (10.6)
[$ER\alpha^+/PgR^-/TFF1^+/HER2^+$]	8 (1.5)
[$ER\alpha^+/PgR^+/TFF1^-/HER2^-$]	8 (1.5)
[$ER\alpha^+/PgR^+/TFF1^-/HER2^+$]	2 (0.4)
[$ER\alpha^+/PgR^+/TFF1^+/HER2^-$]	240 (44.6)
[$ER\alpha^+/PgR^+/TFF1^+/HER2^+$]	29 (5.4)

In addition, the statistical study found a positive correlation between HER2 expression and SBR grade ($p=0.001$), and a marginal negative correlation between both TFF1 expression ($p=0.09$), ERα expression ($p=0.084$), PgR expression ($p=0.054$) and SBR grade.

So, two subgroups of breast cancer cases can be distinguished: a group that was TFF1$^+$, with low mitogenic potential and therefore low malignancy, and another group that was TFF1$^-$, with high mitogenic potential and therefore high malignancy.

The [HER2$^-$/TFF1$^+$] subtype presented a relatively smaller mean tumor size (3.4 cm) and a more advanced mean age at diagnosis (56.23 years). On the other hand, the subtype [HER2$^+$/TFF1$^-$] presented a larger mean tumor size (4.9 cm) and a younger mean age at diagnosis (44.51 years). The difference between these 2 subtypes was statistically very significant (Table 4).

It was also found that HER2$^+$ cases tended to be of greater tumor size, reflecting significant mitogenic activity within these tumors. Of the 16.9% HER2$^+$ cases (8.20%) were size T3.

Table 4. Association of [HER2/TFF1] subtypes with patient's age and tumor size

Parameters	[HER2$^-$/ TFF1$^+$]	[HER2$^+$/ TFF1$^-$]	*p*-value
Patients N° (%)	335 (62.3)	43 (08.0)	-
Mean age (year)	56.23	44.51	0.0003
Mean tumor size (cm)	3.4	4.7	0.042

Evaluation of mitogenic activity on the basis of molecular classification

Results allowed to classify patients into 8 subtypes based on the overexpression of ERα, PgR and HER2 (Table 5). These tumors were subsequently divided into 5 molecular subtypes (Table 6), allowing subdividing them into low proliferation tumors (Luminal A) representing 51.3% of cases and high proliferation ones representing 48.7% of cases (Luminal B, Basal, HER2, Claudin-Low).

Table 5. Expression profiles of ERα, PgR and HER2

Subtypes	N (%)
[ERα$^-$/PgR$^-$/HER2$^-$]	44 (8.2)
[ERα$^-$/PgR$^-$/HER2$^+$]	118 (21.9)
[ERα$^-$/PgR$^+$/HER2$^-$]	12 (2.2)
[ERα$^-$/PgR$^+$/HER2$^+$]	6 (1.1)
[ERα$^+$/PgR$^-$/HER2$^-$]	69 (12.8)
[ERα$^+$/PgR$^-$/HER2$^+$]	10 (1.9)
[ERα$^+$/PgR$^+$/HER2$^-$]	248 (46.1)
[ERα$^+$/PgR$^+$/HER2$^+$]	31 (5.8)

Table 6. Classification of tumors into molecular subtypes

Molecular subtypes	N (%)	Proliferation
Luminal A	276 (51.3)	Low
Luminal B	69 (12.8)	
Basal	44 (8.2)	High
HER2	118 (21.9)	
Claudin-Low	31 (5.8)	

DISCUSSION

Numerous data have suggested a beneficial role of TFF1 expression in human breast cancers. *TFF1* is a classical estrogen-regulated gene possessing a canonical estrogen response element on its promoter [13].

Results showed that ERα, PgR and TFF1 were negatively associated with HER2, while HER2 overexpression was positively associated with SBR grade. Similar results were found by Huang *et al.,* [14].

In this work, results allowed us to individualize 2 subgroups of breast cancer according to the expression of hormonal receptors HR (ERα, PgR), TFF1 and HER2: a group that was [HR$^+$/TFF1$^+$/HER2$^-$] with low proliferative activity (grade 1-2) and therefore a low malignancy potential and another group that was [HR$^-$/TFF1$^-$/HER2$^+$] with high proliferative activity (grade 3) and therefore a high potential for malignancy. This corresponds to the positive correlation found previously between HER2 and SBR grade and the negative one found between HR, TFF1 and SBR grade. HER2$^+$ tumors were more often HR$^-$/TFF1$^-$. Similar results were found by other authors [8-14-15].

Based on the expression of TFF1 and HER2, 2 subtypes were individualized: the [HER2$^-$/TFF1$^+$] subtype characterizing patients with relatively smaller tumor size and more advanced mean age at diagnosis and [HER2$^+$/ TFF1$^-$] subtype characterizing patients with larger tumor size and younger mean age at diagnosis. The same results were found in a Jordanian population [9].

According to mitogenic activity, patients were individualized into two other subgroups: low proliferation tumors (Luminal A) representing 51.3% of cases and high proliferation ones representing 48.7% of cases (Luminal B, Basal, HER2, Claudin-Low). This corresponds with data from the literature indicating that luminal breast cancers are the most common and their epidemiology evokes the role of estrogenic exposure [16].

Our findings indicated that TFF1 has no oncogenic properties in ERα$^-$ or ERα$^+$ breast cancer cells. Similar results were found by Inaji *et al.*, [17]. As slowly proliferating tumors are generally well differentiated, these observations confirm the previously described relationships between TFF1 expression and histological grading [18]. We suggest that TFF1 is already highly expressed in malignant cells before they have acquired the property for invasion increasing with the increase of HER2 expression.

It has been reported that high concentrations of TFF1 inhibit cell proliferation and this is consistent with its putative role as a tumor suppressor gene [19]. Buache *et al*., [7] showed that in the TFF1$^-$ normal immortalized MCF10A human breast cell line, TFF1 gain-of-function did not modify anchorage-dependent or -independent cell proliferation, indicating that TFF1 is unable to induce cell cycle, or to confer oncogenic potential in normal mammary cells. Finally, transgenic mice expressing TFF1 in their mammary glands do not show increased cell proliferation or tumor formation [20].

In contrast, a recent study reports that TFF1 enhances *in-vitro* and *in-vivo* oncogenic capacity of mammary carcinoma cells. These authors concluded that TFF1 is an oncogene and that anti-TFF1 might represent a new therapeutically approach for breast cancers [21]. Interestingly, motogenic and invasive activities are required during mammary gland morphogenesis [22], suggesting that TFF1 might be involved in mammary gland ontogenesis and/or remodeling. These data indicate that TFF1 does not act as an oncogene in the mammary gland, but, conversely, exerts a beneficial function during malignant processes [7].

By acting as a motogen, TFF1 was suggested to promote cell dissemination and development of metastases, two processes associated with more aggressive tumor behavior. Potential role of TFF1 in proliferation and/or migration of breast cancer cells might be further indicated by the result

showing that none of these TFF1$^-$ patients developed distant metastases during a 3-year follow-up. This result indicates that TFF1 might significantly affect migration and/or proliferation when its concentration is high enough, i.e. at levels higher than the cut-off value [23].

CONCLUSION

Analysis of TFF1 and HER2 status in breast carcinoma is important; it provides valuable prognostic, predictive and therapeutic information. Our results showed that HER2 overexpression correlates with aggressiveness parameters such as high histological SBR grade, larger tumor size, young age at presentation, and negative TFF1/H status. Biopathologists will be the essential partners for identifying molecular subclasses, provided however that this evaluation is easily obtained using IHC techniques and not by a long and difficult micro array one.

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AUTHOR CONTRIBUTIONS

All the authors participated in data collection, data analysis and interpretation, drafting the article, revision of the article and final version of the article to be published.

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