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Molecular biomarkers for prediction of response to treatment and survival in triple negative breast cancer patients from Egypt

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ABSTRACT

Background: Triple negative breast cancer (TNBC) is an aggressive phenotype of breast cancer with reduced survival and poor prognosis. Increased VEGF-A, IGF-I, IGF-IR and TGF-β1 expressions were detected in breast cancer. However, little is known about their prognostic and predictive roles in TNBC.
Aim: We assessed the possible prognostic and predictive values of VEGF-A, IGF-I/IGF-IR and TGF-β1 in TNBC cases by measuring their protein and mRNA expression in TNBC and non-TNBC cases.
Methods: VEGF-A, *IGF-I*, *IGF-IR* and *TGF-β1* RNA and their corresponding proteins were assessed in 43 TNBCs, 53 non-TNBCs and 30 normal breast tissues (NBT) by real time PCR (qPCR) and immunohistochemistry (IHC); respectively. Results were related to clinico-pathological factors, response to treatment and survival rates.
Results: Increased mRNA expression of *VEGF-A*, *IGF-I*, and *IGF-IR* was significantly higher in TNBC (65.1%, 65.1%, and 72.1%) than non-TNBC (28.1%, 33.96% and 28.3%) and NBT (0.00%) ($P < 0.001$). Similarly, TNBC patients were significantly associated with high expression of VEGF-A, IGF-I, and IGF-IR proteins (67.44%, 62.79% and 83.72%) than non-TNBC (20.75%, 35.86% and 20.75%) and NBT (0.00%) ($P < 0.001$). Protein and RNA expression levels of all studied markers showed high concordance in all investigated patients (correlation coefficient exceeding 0.5 and 0.4, respectively). In the TNBC group, metastasis and poor response to treatment were significantly associated with VEGF-A ($P < 0.001$, $P = 0.007$, respectively), IGF-I ($P < 0.001$, $P < 0.001$, respectively), IGF-IR ($P = 0.001$, $P = 0.015$, respectively) and TGF-β1 ($P < 0.001$, $P = 0.007$, respectively) protein levels. Multivariate logistic regression showed that IGF-I was the only independent prognostic factor for reduced OS ($P = 0.034$) and DFS ($P = 0.026$) in the TNBC patients.
Conclusions: VEGF-A, IGF-I and IGF-IR play an important role in the development and progression of TNBC compared to non-TNBC. Therefore, they could be used as prognostic and predictive biomarkers as well as candidates for targeted therapy. However, only IGF-I can predict survival in those patients.

1. Introduction

Breast cancer is a heterogeneous disease that shows conflicting clinical behavior, outcome and response to therapy (Abd El-Rehim et al., 2005; Mattie et al., 2006). The triple-negative breast cancer (TNBC), which is characterized by the lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor (HER-2/neu) expression accounts for 10–17% of all breast cancer cases (Carey et al., 2007), depending on the thresholds used to define these receptors and the methods for HER-2/neu assessment. The triple-negative tumors are usually larger than the non-triple-negative with higher grade and higher rates of node positivity (Cleator et al., 2007; Kurebayashi, 2009). In addition, patients with TNBC respond poorly to hormonal therapy and no effective and specific targeted therapy is available yet (Ismail-Khan and Bui, 2010). This emphasizes the need to develop new therapeutic approaches and biologically-based targeted therapies for the TNBC patients (McCarthy et al., 2012), which could only be achieved by understanding the complexity of this heterogeneous group of tumors and their genetic profiles (Carey et al., 2007). The identification of sensitive and reliable biomarkers could also help to predict response to treatment in this group of patients (Koo et al., 2009).

The transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor-A (VEGF-A) facilitate tumor metastasis signaling pathways through promoting angiogenesis (Dumont et al., 2003).

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Table 1
Clinicopathological features of triple-negative and non-triple negative breast cancer patients.

Characteristics	Total no.	Tumor subtype		Statistics
		TNBC (43) (%)	Non-TNBC (53) (%)	
Age at diagnosis				
≤35	8	4 (9.3%)	4 (7.55%)	$\chi^2 = 0.32$
36–49	34	14 (32.56%)	20 (37.74%)	P = 0.85
≥50	54	25 (58.14%)	29 (54.71%)	
Tumor size (cm)				
<4	52	19 (44.2%)	33 (62.3%)	$\chi^2 = 3.13$
≥4	44	24 (55.8%)	20 (37.7%)	P = 0.077
Menopausal status				
Pre-menopausal	44	19 (44.19%)	25 (47.17%)	$\chi^2 = 0.085$
Post-menopausal	52	24 (55.81%)	28 (52.83%)	P = 0.71
Stage				
Early (I–II)	55	26 (60.5%)	29 (54.7%)	$\chi^2 = 0.5$
Late (III)	41	17 (39.5%)	24 (45.3%)	P = 0.48
Grade				
1	2	0 (0.00%)	2 (3.8%)	$\chi^2 = 3.13$
2	79	34 (79.07%)	45 (84.9%)	P = 0.21
3	15	9 (20.9%)	6 (11.3%)	
LN metastases				
Negative	26	3 (6.98%)	23 (43.4%)	$\chi^2 = 15.9$
Positive	70	40 (93.02%)	30 (56.7%)	P < 0.001*
Metastasis				
M0	51	12/39 (30.77%)	39/51 (76.47%)	$\chi^2 = 18.8$
M1	39	27/39 (69.23%)	12/51 (23.53%)	P < 0.001*
Metastatic sites				
Single	19	10/27 (37.07%)	9/12 (75%)	$\chi^2 = 4.79$
Multiple	20	17/27 (62.96%)	3/12 (25%)	P = 0.029*

TNBC: triple negative breast cancer; LN: lymph node; M0: distant metastasis absent; M1: distant metastasis present.
* Significantly different at P < 0.05.

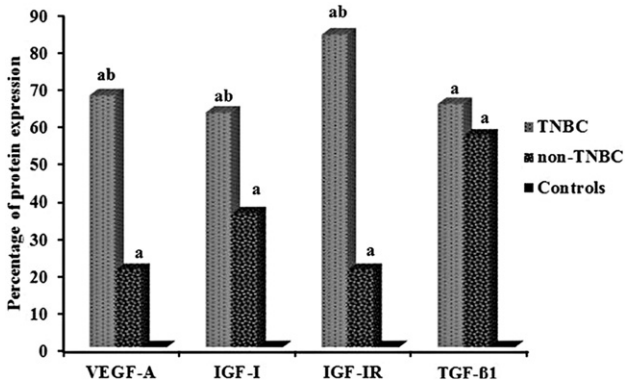

Fig. 2. The protein expression of VEGF-A, IGF-I, IGF-IR and TGF-β1 among different investigated groups. [a] Significantly different from controls at P < 0.05, [b]significantly different from non-TNBC at P < 0.05.

Therefore, repression of TGF-β1 signaling strongly prevents the development of lung and bone metastases in TNBC patients, possibly through inhibition of angiogenesis or reversal of the mesenchymal, invasive phenotypes characteristic of the basal-like TNBC cells (Tan et al., 2009). Similarly, the Insulin like growth factor receptor 1 (IGF-IR) promotes angiogenesis/lymphangiogenesis and enhances alterations in the integrins and cell adhesion complexes leading to increased breast cancer cell metastasis (Guerra et al., 1996; Hankinson, 2008). Since, TNBCs are highly proliferative neoplasms which require constant angiogenesis throughout all phases of their development, invasion and metastasis, increased expressions of VEGF-A, IGF-I and TGF-β1 are expected (Crown et al., 2012). We have previously shown that VEGF-A, IGF-I and IGF-IR expressions were significantly increased in sera of TNBC patients (Bahhnassy et al., 2015). This has prompted us to assess their protein and RNA expressions in the neoplastic tissues obtained from TNBC and non-TNBC patients in relation to standard prognostic factors, response to treatment and survival rates.

2. Methods

2.1. Patients and sample selection

The study included 96 primary invasive breast carcinoma patients who were diagnosed and treated at the National Cancer Institute, Cairo University during the period from September 2009 to January 2013. All relevant clinicopathological features of the patients are illustrated in Table 1. Normal breast tissue samples (30) obtained from

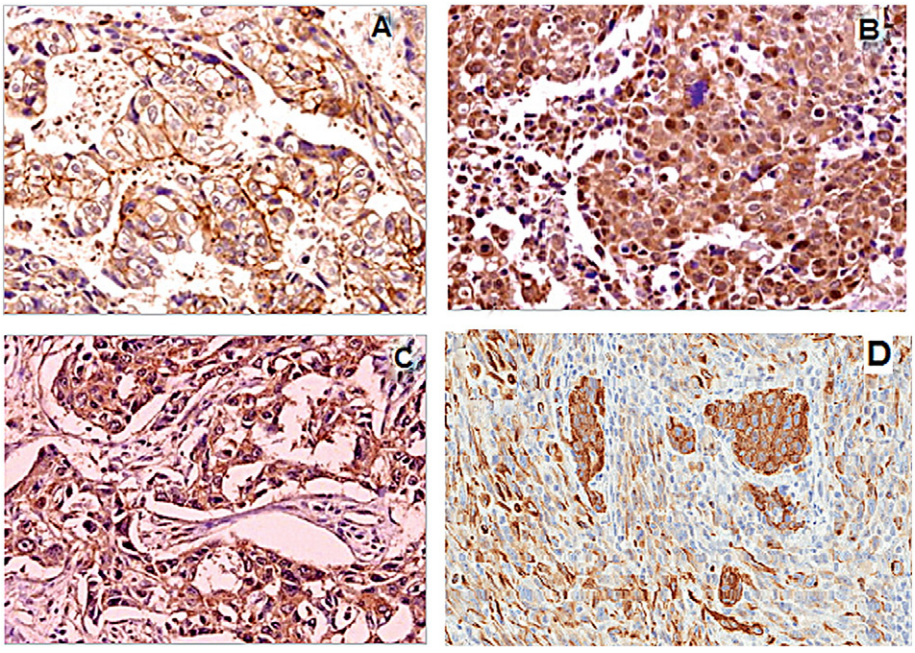

Fig. 1. Immunohistochemical detection of A) VEGF-A, B) IGF-I, C) IGF-IR and D) TGF-β1 in TNBC.