Original Research

Role of transforming growth factor-β1 in triple negative breast cancer patients

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HIGHLIGHTS

- This two-centered retrospective study highlights the high expression of cytoplasmic TGF-β1 in TNBC is associated with higher histologic grade and lymph node status, as well as reduced DFS.
- Our observation that the prognostic role of TGF-β1 in TNBC suggests the potential rationale for using therapeutic strategies based on targeting TGF-β1 in advanced tumors.

ARTICLE INFO

ABSTRACT

Background: We aimed to demonstrate the prognostic value of TGF-β1 in triple negative breast cancer (TNBC) and its association with clinicopathological characteristics of TNBC.
Materials and methods: A total of 180 women were randomly selected from non-metastatic invasive TNBC patients diagnosed at two hospitals between 2003 and 2012. Lmmunohistochemistry was performed to semi-quantify the expression of TGF-β1. Relationship between TGF-β1 expression and clinicopathological features was performed by Chi-square test. Univariate and multivariate survival analyses were performed to identify the prognostic role of TGF-β1 expression on survival outcomes.
Results: Of the 180 women included in this study, 67 (37.2%) patients expressed high level of TGF-β1. High expression of cytoplasmic TGF-β1 was correlated with higher histologic tumor grade ($P < 0.001$) and lymph node status ($P < 0.001$), and more axillary lymph node dissection ($P = 0.029$). High cytoplasmic TGF-β1 expression was associated with reduced disease-free survival (DFS) and overall survival (OS) by log-rank test ($P_{DFS} < 0.001$, $P_{OS} = 0.045$). However, multivariate survival analyses showed that high TGF-β1 was marginally correlated with unfavorable DFS (hazard ratio (HR) 1.796, 95% CI 0.995—3.242, $P = 0.052$), while it was not significantly associated with OS (HR 0.747, 95% CI 0.367—1.522, $P = 0.422$).
Conclusions: This multi-centered retrospective study highlights the high expression of cytoplasmic TGF-β1 in TNBC is associated with higher histologic grade and lymph node status, more axillary lymph node dissection, as well as reduced DFS. Our observation that the prognostic role of TGF-β1 in TNBC suggests potential rationale for using therapeutic strategies based on targeting TGF-β1 in advanced tumors.

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1. Introduction

Triple negative breast cancer (TNBC), which is immunohistochemically defined by the absence of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), accounts for 10—17% of all breast

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cancer cases [1–3]. Patients with TNBC have a more aggressive phenotype and a worse prognosis compared to those with other subtypes of breast cancer due to the high propensity for metastatic progression and lack of specific targeted treatments [4]. Because of the loss of target receptors, TNBC do not benefit from hormonal or anti-HER2 targeted therapies. Although TNBC shows high sensitivity to chemotherapy, the 10-year overall survival remains low [3,5]. Therefore, understanding the molecular pathways associated with TNBC is critical for developing more effective targeted therapies for this life-threatening breast cancer subtype.

Transforming growth factor-β (TGF-β) is an important regulator of normal mammary gland development and function, as well as of the development and progression of breast tumors. TGF-β has been suggested to play a dual role in breast cancer [6]. In mammary carcinogenesis, TGF-β initially acts as a tumor suppressor. Throughout breast cancer development, however, cells become increasingly resistant to the anti-proliferative effects of TGF-β, and TGF-β then functions as a tumor promoter. In later stage, cancer cells lose their responsibility to the growth inhibition effect of TGF-β, and TGF-β is believed to promote tumor progression, in part by enhancing tumor cell motility and invasiveness and the capacity to form metastases [7–9]. TGF-β signaling is mainly mediated through two transmembrane receptors, TGF-β type I receptor (TβRI), and TGF-β type II receptor (TβRII). The ligand binding then leads to phosphorylation and activation of SMAD2 and SMAD3 [10].

Although the role of TGF-β in breast cancer progression and metastasis has been studied intensively in preclinical studies, little is known about its clinical prognostic role in breast cancer, particularly in TNBC. Some studies found that lack or low expression of TGF-β or TβRII was correlated with higher grade TNBC and more advanced tumor stages [11,12]. Whereas others showed that high tumor or serum TGF-β1 expression was associated with reduced disease-free survival (DFS) rate of TNBC [13,14]. Interestingly, another report from Canada investigated that expressions of TβRI and TβRII in TNBC had no correlation with patient outcomes [15]. Given the complex role of TGF-β on prognosis and clinical features of TNBC, we aimed to better demonstrate the prognostic value of TGF-β1 in TNBC and its association with clinicopathological characteristics of TNBC in this multi-centered retrospective cohort study.

2. Materials and methods

2.1. Patient and tissue specimens

A total of 180 women were randomly selected from all the non-metastatic invasive TNBC patients diagnosed at two hospitals between April 2003 and April 2012. The patient cohort includes 64 women from one hospital, and 116 women from the second hospital. The patients were treated with mastectomy or breast-conserving surgery followed by anthracycline and taxane based chemotherapy. Women with more than 3 positive axillary lymph nodes or patients who were treated with breast-conserving surgery received radiotherapy. Follow-up continued until death from any cause, date of last known vital status, or end of study (December 31, 2015). This study was approved by the Institutional Review Boards from each of the two hospitals, and written informed consent was obtained from all the patients.

2.2. Immunohistochemistry

All samples of tumor were routinely stained with hematoxylin and eosin (H&E) first to note the presence of tumor morphology. TGF-β1 expression was examined by lmmunohistochemical (IHC) staining. After dewaxing and rehydration, antigen retrieval was

carried by using citric acid buffer and heating. The sections were blocked with 5% BSA in PBS for half an hour to avoid nonspecific binding, incubated overnight at 4 °C with primary monoclonal antibody to TGF-β1 (MAB240, R&D, USA) at 20 μg/ml. Sections were then incubated with peroxidase-conjugated polymer backbone secondary anti-mouse IgG (K5007, Dako, Denmark). Replacing primary antibody with normal mouse IgG and PBS were used as negative control. Sections were mounted and observed after staining, positive expression of TGF-β1 was primarily in the cytoplasm. The analysis was conducted as described previously [16]. Reaction was grouped by intensity, proportion of positive cells in 20 random fields at 400 magnification, and scores was determined as follows: intensity: absent (0), light brown (1), medium (2), and dark brown (3); proportion of positive cells in each field: no positive cell (0), <10% of cells (1), 10–50% of cells (2), 50–75% of cells (3), and >75% of cells (4). The staining index (SI) was then calculated as follows: SI = intensity*proportion of positive cells. As previously described [17], optimal cutoff point was calculated by using X-tile software (version 3.6.1, Yale University School of Medicine, New Haven, CT, USA) to classify samples into low and high expression.

2.3. Statistical analyses

The relationship between TGF-β1 expression level and clinico-pathological characteristics was performed by Chi-square test. Survival outcomes were estimated with the Kaplan–Meier analysis and compared between groups by log-rank test. Multivariate Cox proportional model was performed to identify the prognostic role of TGF-β1 expression on survivals adjusting for other clinicopathological prognostic indicators. Statistical analyses were conducted using Stata 12.0 software (StataCrop, College Station, TX). All statistical tests were two-sided, and statistical significance was defined as $P < 0.05$.

3. Results

3.1. Patient characteristics and TGF-β1 expression levels

The clinicopathological characteristics of the total 180 study cohort are shown in Table 1. The mean age of these TNBC women was 49.9 (range 29–75). 171 (95%) patients had tumor that ≤ 5 cm, and 47 (26.1%) were node negative TNBC women. 119 (66.1%) patients had high grade (grade III) breast cancers. 47 (26.1%) women were treated with sentinel lymph node biopsy (SLNB), while 133 (73.9%) patients underwent axillary lymph node dissection (ALND). All the women underwent adjuvant chemotherapy, and 105 (58.3%) patients received radiotherapy.

As described in Methods, we selected SI = 7.0 as the optimal cutoff point for TGF-β1 expression by using X-tile software. Thus, tumors with SI ≥ 7.0 were defined as high TGF-β1 expression, while those with SI < 7.0 were classified as low or negative TGF-β1 expression. Fig. 1A and B have shown the IHC staining of TGF-β1 for high expression and negative expression cases, respectively. A total of 67 (37.2%) TNBC patients expressed high level of TGF-β1 in the current study.

3.2. Associations between TGF-β1 expression and clinicopathological characteristics of patients

High expression of cytoplasmic TGF-β1 was positively correlated with histologic tumor grade ($P < 0.001$) and lymph node status ($P < 0.001$), and patients with high cytoplasmic TGF-β1 tumor were more likely to underwent axillary lymph node dissection ($P = 0.029$) (Table 2). No significant associations were observed between TGF-β1 expression level and patient age, tumor size,