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Original contribution

Expression of epithelial-mesenchymal transition–related markers in triple-negative breast cancer: ZEB1 as a potential biomarker for poor clinical outcome☆,☆☆

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Summary Triple-negative breast cancer (TNBC) is a heterogeneous group of disease with a well-known association with epithelial-mesenchymal transition (EMT) and breast cancer stem cell phenotype. Recent studies have shown that TNBC can be classified into 6 subtypes, including basal-like, mesenchymal-like, and mesenchymal stem–like subtypes. However, clinical significance of the EMT in TNBC remains unclear. We analyzed immunohistochemical expression of EMT-related markers, including EMT markers (expression of vimentin, smooth muscle actin, osteonectin, and N-cadherin; loss of E-cadherin), EMT inducers (ZEB1 and CD146), and breast cancer stem cell markers ($CD44^+/CD24^-$ and aldehyde dehydrogenase 1) in 173 TNBCs and correlated their expression with clinicopathological features of the tumors, including clinical outcome. Expressions of vimentin, $CD44^+/CD24^-$, and CD146 were more frequent in basal-like TNBCs than non–basal-like TNBCs. Whereas CD146 expression was closely associated with the expression of various EMT markers and $CD44^+/CD24^-$ phenotype, ZEB1 expression correlated only with the expression of smooth muscle actin. Expressions of vimentin, smooth muscle actin, osteonectin, and ZEB1 and loss of E-cadherin were more frequently found in metaplastic carcinomas than in other histologic subtypes. In survival analyses, EMT markers were not associated with patients' clinical outcomes. However, ZEB1 expression was found to be an independent prognostic factor for poor disease-free survival. These findings indicate that expression of EMT-related markers in TNBCs can be a signature of a certain subgroup of TNBC, which is associated with metaplastic carcinoma, and ZEB1 expression can serve as a potential biomarker to define a subgroup of TNBC associated with poor clinical outcomes.

1. Introduction

Triple-negative breast cancer (TNBC) is defined as a breast cancer subtype with absence of estrogen receptor and progesterone receptor expression and lack of human epidermal growth factor receptor 2 (HER2) amplification and/or overexpression, which accounts for 10% to 20% of all

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breast cancers [1]. Characteristically, TNBCs show aggressive behaviors and poor clinical outcomes [1]. TNBC encompasses a heterogeneous group of diseases rather than a single disease entity [2,3]. Whereas most TNBCs are classified as invasive carcinoma of no special type in histologic subtype, the rest belong to rare histologic subtypes, such as medullary carcinoma, metaplastic carcinoma, and adenoid cystic carcinoma [4]. In terms of chemoresponse, a certain subset of TNBCs is more sensitive to primary systemic therapy (PST), shows complete remission after PST, and has better prognosis, whereas others are resistant to PST and show poor clinical outcomes [3,5]. Thus, such heterogeneity of TNBCs has been an obstacle to characterize this group of breast cancer and to develop specific therapeutic targets.

Many attempts have been made to classify TNBC by gene expression signature or individual biomarkers. Recently, Lehmann et al [6] identified 6 molecular subtypes of TNBC, including basal-like 1, basal-like 2, immunomodulatory, mesenchymal-like (ML), mesenchymal stem–like (MSL), and luminal androgen receptor with distinct gene expression profiles and canonic pathways. Among them, tumors of ML and MSL subtype showed high expression of genes associated with epithelial-mesenchymal transition (EMT).

Epithelial-mesenchymal transition is an important mechanism of tumor progression, contributing to tumor invasion and metastatic dissemination [7,8]. It is also associated with the breast cancer stem cell (BCSC) phenotype [9,10]. Mani et al [11] have identified that induction of EMT in immortalized mammary epithelial cells generated $CD44^+/CD24^-$ BCSC-like cells. Some studies have also shown that mesenchymal EMT marker expression paralleled the expression of stem cell markers in breast cancers [10,12]. Among the existing breast cancer subtypes, EMT markers are more frequently expressed in TNBC or basal-like breast cancer compared with the other subtypes [13,14]; in a previous study, we showed that EMT marker expression was more frequent in basal-like breast cancers and that their expression correlated with the expression of BCSC markers ($CD44^+/24^-$ and aldehyde dehydrogenase 1 [ALDH1]) [15].

Considering the effect of EMT on tumor invasion and metastasis, it could be used as a signature of a certain subset of TNBCs with aggressive behavior. Moreover, it can also be a prognostic marker or therapeutic target in TNBCs. However, clinical significance of EMT in TNBC has been rarely studied. Moreover, comprehensive studies examining the immunohistochemical expression of EMT-related markers and their clinicopathological significance in TNBCs are still lacking.

In this study, we analyzed immunohistochemical expression of EMT-related markers, including EMT markers (expression of vimentin, smooth muscle actin [SMA], osteonectin, and N-cadherin; loss of E-cadherin) and EMT inducers (ZEB1 and CD146) using a relatively large number of TNBC samples, and we correlated their expression with clinicopathological features of the tumors, including clinical outcome. In addition, we also explored the immunohistochemical expression of BCSC markers ($CD44^+/CD24^-$ and ALDH1) in TNBCs and evaluated the relationship with EMT-related markers and clinicopathological features.

2. Materials and methods

2.1. Patient and tissue specimens

We examined the records of the Department of Pathology of Seoul National University Bundang Hospital from 2003 to 2011 and searched for cases of invasive TNBC using immunohistochemical data for standard biomarkers. Estrogen receptor and progesterone receptor were regarded as negative if there were less than 1% of positive tumor nuclei [16]. Expression of HER2 was scored according to the American Society of Clinical Oncoloy/College of American Pathologists guidelines [17], and immunohistochemical scores of 0 or 1+ were regarded as negative. For the equivocal (2+) cases, HER2-negative status was confirmed by fluorescence in situ hybridization. A total of 173 TNBCs were selected for this study. Of the 173 cases, 151 had been included in our previous study [18]. Clinicopathological characteristics of the tumors were obtained by reviewing the medical records and hematoxylin and eosin–stained slides. Baseline characteristics of 173 TNBCs are presented in Table 1. This study was approved by the institutional review board of Seoul National University Bundang Hospital.

2.2. Immunohistochemistry and scoring

Immunohistochemical staining was performed on tissue microarrays (2-mm diameter, 3 representative tissue cores), some of which were from a previous study. Four-micrometer-thick tissue sections were cut, dried, deparaffinized, and rehydrated following standard procedures. All sections were subjected to heat-induced antigen retrieval process. Staining was optimized using positive and negative controls, and EMT markers (vimentin, SMA, osteonectin, N-cadherin, and E-cadherin), EMT inducers (ZEB1 and CD146), and BCSC markers ($CD44^+/CD24^-$ and ALDH1) were used for this study. Immunohistochemical staining was carried out using an ultraView detection kit (Ventana Medical Systems, Tucson, AZ) in a BenchMark XT autostainer (Ventana Medical Systems) or manually with a Dako-Envision detection kit (Dako, Carpinteria, CA). Double immunostaining to detect $CD44^+/CD24^-$ cells was performed with EnVision G|2 Doublestain System Rabbit/Mouse (DAB+/Permanent Red; Dako) according to the manufacturer's instructions.

The expression of the markers with the exception of E-cadherin was scored as the percentage of positive tumor cells as follows: 0, no staining or staining in less than 1% of the tumor cells; 1, staining in 1% to 10% of the cells; 2, staining in 10% to 25% of the cells; 3, staining in 25% to 50% of the cells; 4, staining in 50% to 75% of the cells; and 5, staining in more than 75% of tumor cells. For E-cadherin