Clinicopathological significance of Sox10 expression in triple-negative breast carcinoma

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Abstract

Purpose The present study aimed to investigate the Sox10 expression in the pathological diagnosis of triple-negative breast cancer (TNBC). Furthermore, its correlation with the clinicopathological characteristics and disease-free survival rate in patients with TNBC was also evaluated to identify the diagnostic utility of Sox10 as a reliable biomarker for diagnosis and prognosis of TNBC.

Methods Using immunohistochemistry, we identified the expression of Sox10, GATA-3, FOXA1, GCDFP15 and MGB in 376 cases of primary invasive breast cancer, and 77 cases of metastatic breast cancer. The expression of Sox10 in different molecular subtypes of primary invasive breast cancer and metastatic breast cancer were also compared. Furthermore, the correlation between Sox10 expression and clinicopathological parameters and disease-free survival (DFS) of patients with primary TNBC were also analyzed.

Results Expression of Sox10 was only detected in the myoepithelial cells of normal breast, but not in any other types of cells, including luminal cell and fibroblasts. The positive rate of Sox10 in primary and metastatic TNBC was significantly higher than that in the other two types ($P < 0.001$, $P < 0.001$, respectively). The sensitivity and specificity of Sox10 expression in primary TNBC and metastatic TNBC were significantly lower than GATA-3, significantly higher than FOXA1, GCDFP15, and MGB ($P < 0.001$, $P = 0.0004$, $P = 0.0064$, $P = 0.0229$, respectively). In 71 cases of primary TNBC, a higher expression rate of Sox10 was significantly associated with high-grade tumors, late-stage tumors, and tumors with involvement of four or more lymph node metastases ($P = 0.0145$, $P = 0.0105$, $P = 0.0249$, respectively).

Conclusion Sox10 may be used as a novel reliable putative marker for the diagnosis of TNBC. Notably, Sox10 combined with GATA-3 expression may serve as a supplementary differential diagnostic biomarker for primary and metastatic TNBC. Besides, Sox10 may be a good predictor of the prognosis of primary and metastatic TNBC. This study also highlights the significance of targeting Sox10 as a promising potential therapeutic target gene for TNBC therapy.

1. Introduction

Breast carcinoma represents the most prevalent malignancy affecting women worldwide. Triple-

negative breast cancer (TNBC), a specific molecular subtype of breast cancer, is predominantly characterized by the lack of expression of targeted biomarkers including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Accumulating evidence has shown that patients with TNBC account for 10–15% of all breast carcinoma cases; however, Hispanic and African-American women paradoxically represent the majority of cases [1]. Notably, in China, nearly 12% − 20% of all the breast carcinoma diagnoses are TNBC, which is considerably higher compared to Western countries [2]. TNBC is highly aggressive and poorly differentiated tumors that exhibit higher recurrence and metastatic rates and a worse overall prognosis than women with other subtypes of breast carcinoma. Thus, TNBC has become the major focus of breast carcinoma research [1]. More recently, it has been established that there is considerable heterogeneity in natural history and prognosis within TNBC; thus, exhibiting different clinicopathological characteristics. Because TNBC is prone to distant metastasis and does not express ER and PR, therefore, it presents a formidable challenge to the pathologist to differentiate it from metastatic adenocarcinomas originating from other sites. Moreover, in clinical practice, it can be difficult to establish the origin of a putative breast metastasis if the primary tumor is either triple-negative for breast biomarkers (ER/PR/HER2) or if there is a loss of biomarker expression in the metastasis. Therefore, novel molecular target with high sensitivity and specificity for TNBC are highly desirable to facilitate timely and accurate diagnosis of primary or metastatic TNBC and assist to appropriately classify breast carcinoma.

Immunohistochemistry (IHC) as an ancillary technique has become an indispensable tool in anatomic pathology. Tissue-specific IHC markers used in conjunction with histologic morphology are particularly beneficial in identifying tumors of unknown origin. Till date, numerous immunohistochemical markers have been suggested to be beneficial in assisting the histopathological diagnosis of the breast carcinoma, and many biomarkers have gained acceptance and translated into routine use. Among them, gross cystic disease fluid protein (GCDFP-15) [3] and mammaglobin (MGB) serve as most commonly applied breast-specific immunohistochemical markers in the diagnosis of breast carcinoma or metastatic breast carcinoma [3]. GCDFP-15 has been recognized as a major constituent protein in

breast cyst fluid and may be expressed in cells with characteristics similar to sweat gland, including acinar and salivary gland, skin, and vulva. Besides breast carcinoma, GCDFP-15 expression has been well documented in only a few cancers including prostate cancer and skin adnexal cancers [4]. Predominantly, GCDFP-15 exhibit high sensitivity and specificity for breast carcinoma and is often used as an effective immunohistochemical marker to determine the potential source of breast metastases in carcinoma with unknown primary sites.

MGB is a member of the secretoglobin family of protein with a predicted molecular weight of 10 kD cloned from breast cancer epithelial cells[5]. The protein has been suggested as a tumor-associated marker for the diagnosis of breast carcinoma [6]. GCDFP-15 and mammaglobin are positively expressed in approximately 50% and 75% of breast carcinomas, respectively; but their sensitivity remains poor, particularly in high-grade breast cancer such as triple-negative breast carcinoma [3–6].

GATA binding protein 3 (GATA-3) belongs to the GATA family of zinc finger protein transcription factors. It plays crucial role in mammary development and luminal epithelial cell differentiation [7] as well as mediates the development, proliferation and differentiation of a wide variety of other tissue types including kidney [8], nervous system [9], T cells [10], and hair follicles [11]. GATA-3 is highly expressed in the luminal A subtype of breast cancer [12]. There is increasing evidence that GATA-3 is a potential tool for differentiating primary and metastatic breast carcinoma [13–17].

Fork box protein A1 (FOXA1) is a member of the forkhead box gene family of winged-helix transcription factors. It plays a pivotal role in the regulation of organogenesis of many organs and is closely associated with the pathogenesis of a number of cancer types, including breast carcinoma [18]. FOXA1 expression is associated with molecular subtypes and may be indicative of good prognosis in breast carcinoma [18]. Moreover, it also regulates the expression level of ERa in breast carcinoma [19].

Sox10 is a member of Sox transcription factor family and mediates the differentiation of neural crest cells into melanocytes, oligodendrocyte, and glia and promotes a mesenchymal transition in the mammary cell. Sox10 is expressed in many different cells and tissues including Schwann cells of peripheral nerves, epidermal melanocytes, oligodendrocytes of the cerebral cortex, mast cells,

myoepithelial cells of submucosal bronchial glands, and acinar of mammary and corresponding tumors. Sox10 immunohistochemical expression is also present in melanoma, peripheral nerve sheath tumors, and salivary gland myoepithelioma [20]. Recently, it has been reported that Sox10 is highly expressed in primary breast carcinoma, predominantly by basal-like breast carcinoma and unclassified TNBC [21, 22].

Based on the findings of the literature review, we hypothesized that since the expression rate of Sox10 in TNBC is considerably higher than in other types of breast carcinoma. It could possibly be used as a putative biomarker for TNBC. Therefore, this study aimed to evaluate the expression of Sox10 in TNBC to assist to classify breast carcinoma, particularly metastatic TNBC for routine surgical and pathological diagnosis and compare the specificity and sensitivity of Sox10 with GATA-3, FOXA1, GCDFP15, and MGB in TNBC. Furthermore, the correlation between Sox10 protein expression and clinicopathological characteristics of primary TNBC was also determined to evaluate its prognostic significance in TNBC.

2. Materials And Methods

2.1. Specimens

The present study included tumor specimens from primary invasive breast carcinomas (only ductal and lobular cancers; n = 376), metastatic breast carcinomas (only ductal and lobular cancers; n = 77) and 20 normal breast tissue specimens were also collected. Tumor tissues of primary invasive breast carcinomas were collected from biopsy and surgical resection from the Department of Pathology, Affiliated Hospital of Jiangnan University, between January 2010-June 2013. However, tumor tissues from metastatic breast carcinomas and normal breast tissues were obtained between January 2016 and September 2018. None of the patients with primary invasive breast carcinomas received preoperative chemotherapy or radiotherapy. Hematoxylin and eosin (H&E)-stained tissue section and immunohistochemical (IHC) staining of all specimens were reviewed by two experienced and qualified pathologists in our hospital. All the cases included had complete clinicopathological data. According to the emerging St., Galen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2017 [23], molecular analysis is difficult to be widely applied in the clinic due to the high

requirement for specimens and high-cost of detection. Therefore, the diagnosis based on the findings of IHC staining remains the gold standard in diagnostics and prognostics of breast carcinoma. IHC staining has been extensively applied for the detection of ER and PR, Ki67, together with in situ hybridization analysis for Her2 overexpression or amplification. However, detection of Ki67 index through IHC staining remains controversial, as it is difficult to define an applicable Ki67 cutoff. The cutoff of Ki67 in our laboratory was defined as 15%. Thus, the collected tissue from primary invasive breast cancer groups and metastatic breast carcinomas were all classified into 3 groups: Luminal (A and B) (n = 240), HER2 overexpressed (n = 65) and triple-negative (n = 71); Luminal (A and B) (n = 44), HER2 overexpressed (n = 11), triple-negative (n = 22). 71 cases of primary TNBC were followed-up from the beginning of surgery. As of October 2018, the follow-up period was of 5 years. 25 cases of primary TNBC exhibited disease-free survival and 46 cases had recurred or metastasized (including 12 cases of simple liver metastasis, 13 cases of simple lung metastasis, 6 cases of simple bone metastasis, 7 cases of simple brain metastasis, 2 cases of soft tissue metastasis and 6 cases of multiple metastases). Of these, 23 cases died due to metastasis.

The clinical staging criteria for primary breast carcinoma were based on the clinical staging criteria recommended by the Union for International Cancer Control (UICC) and the United States Joint Commission for Cancer (AJCC). Tumor histological classification and differentiation grades were determined based on the Nottingham Combined Histology Grade.

2.2. Immunohistochemistry

For immunohistochemical analysis, tissue specimens were fixed in 10% formalin, embedded in paraffin and dehydrated in an ethanol gradient, and serially sectioned into a 4-μm thickness. H&E staining was performed to verify the pathological diagnosis by experienced pathologists. Immunostaining was performed on sections with a standard immunostaining protocol using a Venta Benchmark XT Automated Stainer (Roche, Basel, Switzerland). Briefly, sections were dewaxed in xylene and hydrated in an ethanol gradient. Antigen retrieval was performed by heating the tissue sections at 100 °C for 30 minutes in citrate buffer. Moreover, endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 10 minutes. Subsequently, the sections were

incubated in 5% bovine serum albumin (BSA) at room temperature for 30 minutes. Primary antibodies ER, PR, ki-67, GATA-3, FOXA1, Sox10, GCDFP15, MGB monoclonal antibodies (Shanghai, China) and HER2 monoclonal antibody (Roche, Basel, Switzerland), respectively were added drop-wise followed by overnight incubation at 4 °C. The slides were washed and incubated with a horseradish peroxidase-conjugated secondary antibody for 30 min. The immunostaining was carried out by staining with 3, 3’-diaminobenzidine tetrahydrochloride solution (GTVisionII Immunohistochemistry Detection Kit for Rabbit/Mouse, Gene Tech, Shanghai, China) counter-stained with hematoxylin, dehydrated and mounted and the sections were examined under a microscope. Pertinent information concerning the primary antibodies is summarized in Table 1. Simultaneously, sections recognized to stain positively were used as a positive control. For the negative control, the primary antibody was replaced with phosphate-buffered saline. Fluorescence in situ hybridization (FISH) was evaluated using the PathVysion HER2 DNA Probe Kit (Abbott-Vysis, Chicago, USA).

Table 1
Primary antibody information

Antibody	Source	Clonality	Clone	Species	Dilution
ER	Ventana	Monoclonal	SP1	Mouse	1/500
PR	Ventana	Monoclonal	SP2	Mouse	1/500
HER2	Ventana	Monoclonal	4B5	Rabbit	1/300
ki-67	Ventana	Monoclonal	MIB1	Mouse	1/500
GATA3	Ventana	Monoclonal	L50-823	Mouse	1/300
FOXA1	Ventana	Monoclonal	2F83	Mouse	1/200
Sox10	Ventana	Monoclonal	EP268	Rabbit	1/500
GCDFP15	Ventana	Monoclonal	23A3	Mouse	1/300
MGB	Ventana	Monoclonal	304-1A5	Mouse	1/800

2.3. Decision criteria

ER, PR, ki67, GATA-3, FOXA1, Sox10 were localized in the nucleus, Her2 in membrane and GCDFP15 and MGB in the cytoplasm. The positive staining signal was brown and yellow. The HE-stained and immunostained sections were reviewed independently by two pathologists to obtain a consensual score. Five visual fields were selected in each section. The immunostaining for ER, PR, GATA-3, FOXA1, Sox10, GCDFP15, and MGB was considered positive when more than 1% of tumor cells were stained positive. While the guidelines for immunohistochemical testing of HER2 were adopted by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) in 2018.

2.4. Statistical analysis

Statistical analyses were performed using the SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Fisher

2-tailed exact tests were performed to compare the differences between groups. Pearson’s correlation was used to analyze the association between IHC markers and the clinicopathological data. Disease-free survival (DFS) were plotted using the Kaplan-Meier method and was compared using the log-rank test. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Expression of Sox10 in normal breast tissue

The positive rate of Sox10 in myoepithelium of 20 normal breast tissues was 100% (20/20). Sox10 expression was not observed in breast luminal cells and stromal fibroblasts (Fig. 1).

3.2. Comparison of Sox10 expression among different molecular subtypes of primary breast cancer and metastatic breast carcinoma

The positive rates of Sox10 in luminal A and B, HER2 overexpressing, and TNBC were 0%, 3.1%, and 67.6%, respectively. The positive rates of Sox10 in metastatic breast cancer in luminal A and B, HER2 overexpressing, and TNBC were 0%, 9.10%, and 68.18%, respectively. The positive rate of Sox10 expression in primary and metastatic TNBC was significantly higher than that in other molecular subtypes ($P < 0.001$, $P < 0.001$) (Table 2, Table 3).

Table 2

Comparison of Sox10 expression among different molecular subtypes of primary breast cancer

	+	-	n
Luminal(A,B)	0(0%)	240(100%)	240
HER2	2(3.1%)	63(96.9%)	65
TNBC	48(67.6%)	23(32.4%)	71
n	50	110	376
X2	359.047		
P	< 0.001		

Table 3

Comparison of Sox10 expression among different molecular subtypes of metastatic breast carcinoma

	+	-	n
Luminal(A,B)	0(0%)	44(100%)	44
HER2	1(9.10%)	10(90.90%)	11
TNBC	15(68.18%)	7(31.82%)	22
n	16	61	
X^2	71.263		
P	< 0.001		

3.3. Sensitivity and specificity of Sox10 and GATA-3, FOXA1, GCDFP15, MGB in primary TNBC and metastatic TNBC

The sensitivity and specificity of Sox10 and GATA-3 in primary TNBC were 67.60% and 87.32%, and 31.58% and 40.79%, respectively;The sensitivity and specificity of Sox10 and GATA-3 in metastatic TNBC were 68.18% and 86.36%, and 32.61% and 41.3%, respectively. Compared with other markers, the sensitivity and specificity of Sox10 in primary and metastatic TNBC were only significantly lower

than GATA-3; however, significantly higher than FOXA1, GCDFP15, and MGB ($P < 0.001$, $P = 0.0004$, $P = 0.0064$, $P = 0.0229$, respectively) (Table 4, Table 5).

Table 4

Sensitivity and specificity of Sox10 and GATA-3, FOXA1, GCDFP15, MGB in primary TNBC

	+	-	sensitivity	specificity	
Sox10	48(67.60%)	23(32.40%)	67.60%(48/71)	31.58%(48/152)	
FOXA1	21(29.58%)	50(70.42%)	29.58%(21/71)	13.82%(21/152)	
GATA-3	62(87.32%)	9(12.68%)	87.32%(62/71)	40.79%(62/152)	
GCDFP15	7(9.86%)	64(90.14%)	9.86%(7/71)	4.60%(7/152)	
MGB	14(19.72%)	57(80.28%)	19.72%(14/71)	9.21%(14/152)	
n	152	203			
X^2			19.06	12.67	
P			< 0.001	0.0004	

Table 5

Sensitivity and specificity of Sox10 and GATA-3, FOXA1, GCDFP15, MGB in metastatic TNBC

	+	-	Sensitivity	specificity
Sox10	15(68.18%)	7(31.82%)	68.18%	32.61%
FOXA1	4(18.18%)	18(81/82%)	18.18%	8.70%
GATA-3	19(86.36%)	3(13.64%)	86.36%	41.30%
GCDFP15	3(13.64%)	19(86.36%)	13.64%	6.52%
MGB	5(22.73%)	17(77.27%)	22.73%	10.87%
n	46	64		
X^2			7.42	5.17
P			0.0064	0.0229

3.4. Correlation between the expression of Sox10 and clinicopathological characteristics of primary triple-negative breast carcinoma

For primary TNBC, the expression of Sox10 was significantly correlated with the pathological grade of tumors. The positive rate of Sox10 in high-grade tumors was significantly higher than that in low- and middle-grade tumors ($P = 0.0145$). The expression of Sox10 was also significantly associated with clinical stages. The positive rate of Sox10 in late-stage tumors was higher than that in early- and middle-stage tumors ($P = 0.0105$). Moreover, Sox10 expression was also significantly correlated to lymph node metastasis. The positive rate of Sox10 in tumors with four or more lymph node metastases was higher than that in tumors without lymph node metastasis and with 1–3 lymph node metastases ($P = 0.0249$). However, there was no significant correlation with other clinicopathological characteristics of primary TNBC including the age of onset, tumor diameter, and pathological type (Table 6).

Table 6

Correlation between the expression of Sox10 and clinicopathological characteristics of primary triple-negative breast carcinoma

Clinico-pathological parameters		n	Sox10		X^2	P
Age		71	+	-		
< 59		44	28	16	0.42	0.515
≥ 59		27	20	7		
Grade						
Ⅰ-Ⅱ		33	17	16	5.98	0.0145
Ⅲ		38	31	7		
Stage						
Ⅰ-Ⅱ		28	14	14	6.54	0.0105
Ⅲ		43	34	9		
Diameter(cm)						
≤ 2		28	16	12	2.31	0.1285
> 2		43	32	11		
Lymph node metastasis						
0		46	29	17	7.39	0.0249
1 ~ 3		16	14	2		
≥ 4		9	9	0		
Morphology						
Ductal carcinoma		69	48	21	1.71	0.1915
Lobular carcinoma		2	0	2		

3.5. Correlation between the expression of Sox10 and the prognosis of primary triple-negative breast carcinoma

Kaplan-Meier survival analysis showed that the expression of Sox10 was associated with disease-free survival in patients with primary TNBC. In 71 cases of primary TNBC, the disease-free survival time of the Sox10 positive group was significantly lower than that of the negative group ($P = 0.00008$) (Fig. 2).

4. Discussion

With the substantial increase in the incidence of breast carcinoma, more and more women are suffering from breast cancer. Breast carcinoma is a highly heterogeneous disease and constitutes different molecular subtypes, which are HER2, luminal A, luminal B, claudin-low, and basal-like types. Most of TNBCs were included in the basal-like subtype. Moreover, the different molecular types of breast cancer exhibit distinct molecular mechanisms and act as biologically distinct entities that require different therapeutic management. Besides, survival analysis revealed a poor prognosis in patients diagnosed with basal-like type while the two ER + groups showed a variable outcome.

If we encounter TNBC with a high grade of tumors without ductal carcinoma in situ, particularly

metastatic TNBC, and is often difficult to diagnose. Therefore, apart from recognizing the clinical course, some specific immunohistochemical markers are needed to further confirm the diagnosis. Presently, ER, GCDFP15 and MGB are the most commonly used markers in the diagnosis of breast carcinoma. However, recently, an increasing number of studies have considered GATA-3 as a useful marker in breast carcinoma [12–17]. FOXA1 is known for molecular subtyping of breast carcinoma and its expression is associated with good clinical outcomes and is also considered as a molecular marker for breast carcinoma diagnostics [18]. More recently, Sox10 with more than 20 SOX family transcription factors has been characterized by HMG DNA binding domain sequence and recognized to play a crucial role in the development of the nervous system, immune system, and skeletal system [20].

Existing studies suggested that Sox10 plays an important role in assisting the diagnosis of melanoma, peripheral nerve-derived tumor, and salivary gland myoepithelial tumors[21]. Notably, Sox10 is a sensitive lineage marker for both primary and metastatic TNBC; however, the diagnostic value of Sox10 in TNBC remains to be elucidated. Notch signaling pathway plays a central link in maintaining stem cell characteristics and regulating cell differentiation in breast tissue [24]. Notch gene is usually activated in glandular precursor cells of breast lobules [25]. In vitro studies on mouse mammary epithelial cells revealed that Notch4-PBP mediated cell proliferation required the involvement of Sox10, however, the other roles of Sox10 in the differentiation and development of mammary epithelial cells remain elusive [26]. Studies showed that Sox10 was only expressed in myoepithelial cells of normal breast tissue by IHC method, but not in other cells [27]. In this study, we also found that Sox10 was only expressed in the myoepithelial cells around the lobules and ducts in normal breast tissue, but not in the other lumen cells. Consistently, it was also localized in the nucleus, as reported previously. Myoepithelial cells exist not only in the breast but also in many tissues, including soft tissue, salivary gland. Sox10 expression was detected in salivary gland myoepithelial tumors such as acinar cell carcinoma, adenoid cystic carcinoma, myoepithelial carcinoma, and epithelial-myoepithelial carcinoma; however, not in mucoepidermoid carcinoma and ductal carcinoma from the non-myoepithelial origin [28, 29]. Miettinen et al [30] also suggested that Sox10 was not only a

diagnostic marker for schwannoma and malignant melanoma, but also expressed in myoepithelial tumors in soft tissue. Considering the results from these studies, we can conclude that Sox10 is a good diagnostic marker for myoepithelial tumors, and the expression of Sox10 may suggest that the tumors may originate from myoepithelium.

Furthermore, Cimino-Mathews A [22], Ivanov SV [28] and others have found that Sox10 can be expressed in primary breast carcinoma, predominantly in basal-like breast carcinoma and unclassified TNBC. Recently, an IHC based study[31] has been demonstrated Sox10 as an additional marker in TNBC, especially for basal-like and metaplastic subtype. Similarly, we also found a significantly higher positive rate of Sox10 in primary and metastatic TNBC than in other two types ($P < 0.001$, $P < 0.001$, respectively), which was consistent with the findings of the previously reported literature. TNBC is considered to be a malignant tumor originating from breast myoepithelial cells, which explains why Sox10 is more positive in TNBC than in other types of breast carcinoma. The characteristic of TNBC is that ER, PR, and Her2 are not expressed and the heterogeneity is very high. TNBC has been characterized as exhibiting a negative profile for the three markers, therefore, treatment of this molecular sub-type remains highly challenging. Consequently, a large number of studies on TNBC mainly focused on deciphering novel therapeutic targets and strategies. Pathological diagnosis of TNBC remains the gold standard for pathological diagnosis. Since ER, PR, and Her2 are not expressed in TNBC, these three markers cannot be used to differentiate them from metastatic adenocarcinoma. In addition, through literature reviews and from this study, we demonstrated that besides GATA-3, FOXA1, GCDFP15, and MGB, which are most extensively used markers for routine diagnosis of breast carcinoma, exhibit low sensitivity, and specificity in distinguishing primary TNBC and metastatic TNBC. Thus, by comparing the sensitivity and specificity of expression of Sox10 with that of GATA-3, FOXA1, GCDFP15, MGB in TNBC, this study revealed that the sensitivity and specificity of Sox10 were significantly higher than those of the other three markers ($P < 0.001$, $P = 0.0004$, $P = 0.0064$, $P = 0.0229$, respectively) for both primary and metastatic TNBC. Therefore, this indicated that the sensitivity and specificity of Sox10 for TNBC were remarkably high. In routine immunohistopathology, expression of Sox10 combined with GATA-3 may serve as a promising putative immunohistochemical

marker for the diagnosis of TNBC, particularly, for metastatic TNBC.

Accumulating studies have suggested that Soxl0 plays an important role in the occurrence and development of various tumors, and participates in the proliferation, migration, and invasion of tumor cells [32, 33, 34]. Owing to differences in the expression level of Sox10 in a variety of tumors, its effect on cell function is also differently attributable to differences in mechanisms. Sox10 is overexpressed in nasopharyngeal carcinoma [32] and bladder cancer [33], which promotes the growth and metastasis of tumors and plays an oncogenic role. However, in many digestive tract tumors, the expression of Sox10 is low and plays the role of tumor suppressor [34]. However, presently there are limited clinical studies on the role of Sox10 in the occurrence and development of primary TNBC. By analyzing the correlation between the expression of Sox10 protein and clinicopathological characteristics in 71 cases of primary TNBC, the present study revealed that the positive rate of Sox10 protein was significantly correlated with pathological grade, clinical stage and a number of lymph node metastasis. The positive rate of Sox10 expression was higher in high-grade TNBC compared to low-grade TNBC ($P = 0.0145$), the positive rate of Sox10 expression in late-stage TNBC was remarkably higher than that of early-stage TNBC ($P = 0.0105$), and the positive rate of Sox10 expression was significantly higher in cases with less number of lymph node metastasis than that of more number of lymph node metastasis ($P = 0.0249$). Based on these findings, we speculated that Sox10 also plays an oncogenic role in the occurrence and development of TNBC. The prognosis of tumors is related to many factors, including the stage of tumors, the number of lymph node metastases, pathological grading, and tumor size. The later the stage, the worse the prognosis of the tumors; the higher the pathological grade, the worse the prognosis; similarly, a higher number of lymph node metastasis is also considerably associated with the prognosis of TNBC. There is a growing body of evidence which demonstrates that more the involvement of the lymph node, the more prone the tumors to metastasis and recurrence. Since the expression of Sox10 protein is correlated with tumor stage, number of lymph node metastasis and pathological grade of primary TNBC in this study, the present study indicated that the expression of Sox10 protein is highly associated with the adverse prognosis of primary TNBC, and may serve a novel putative biomarker for predicting the prognosis

and metastasis of primary TNBC and potential target genes for the treatment of TNBC. Further follow-up of the patients with primary TNBC revealed that the 5-year disease-free survival period of the patients with Sox10 protein-positive was significantly lower than that with the negative expression ($P = 0.00008$). This finding further confirmed our hypothesis that the high expression of Sox10 can be used as an index to predict the prognosis of patients with TNBC. Conceivably, the high expression of Sox10 was an independent predictor of TNBC. Thus, identifying the expression of Sox10 through IHC technique can be used as a supplementary diagnostic marker for risk prediction.

In conclusion, the findings of this preliminary study suggested that Sox10 expression is a novel prognostic biomarker for TNBC and highlight the clinical significance of targeting Sox10 as a promising potential therapeutic target gene for TNBC therapy. Further studies on larger sample size are warranted at RNA levels to establish these findings.

Declarations

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Compliance with ethical standards

Conflict of interest

We declare that We have no conflict of interest.

Ethical approval

This work was approved by the Hospital Ethical Review Committee of Jiangnan University. The committees decided that written informed consent was not required for the present study from each patient.

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Figures

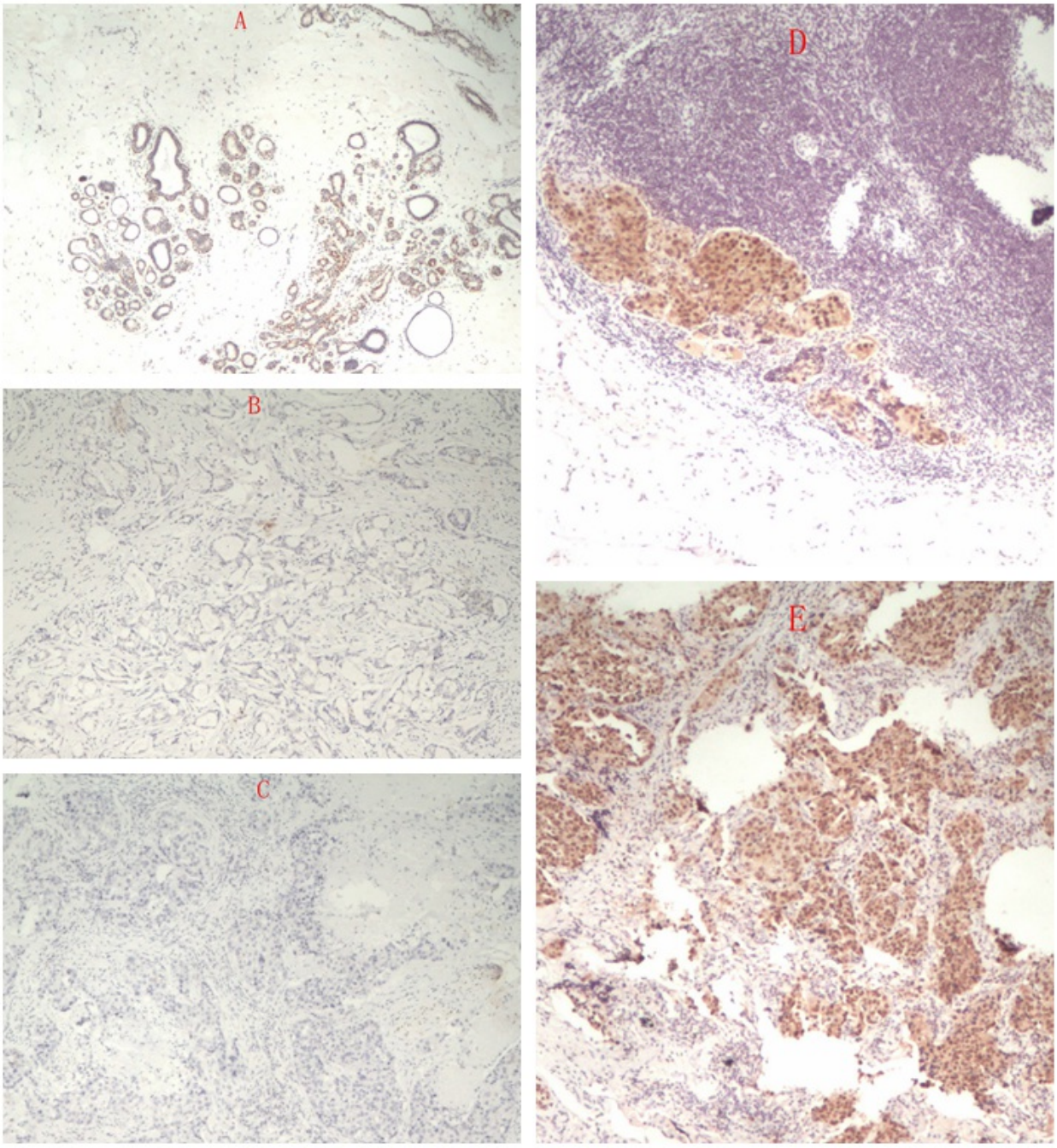

Figure 1

The luminal cells and stromal fibroblasts showing negative immunostaining for Sox10, as well as the myoepithelium showing positive immunostaining in normal breast tissues (A, 40×); Sox10-negative Luminal A and B breast carcinoma (B,C, 40×); Sox10-positive primary and metastatic TNBC (D,E, 40×).

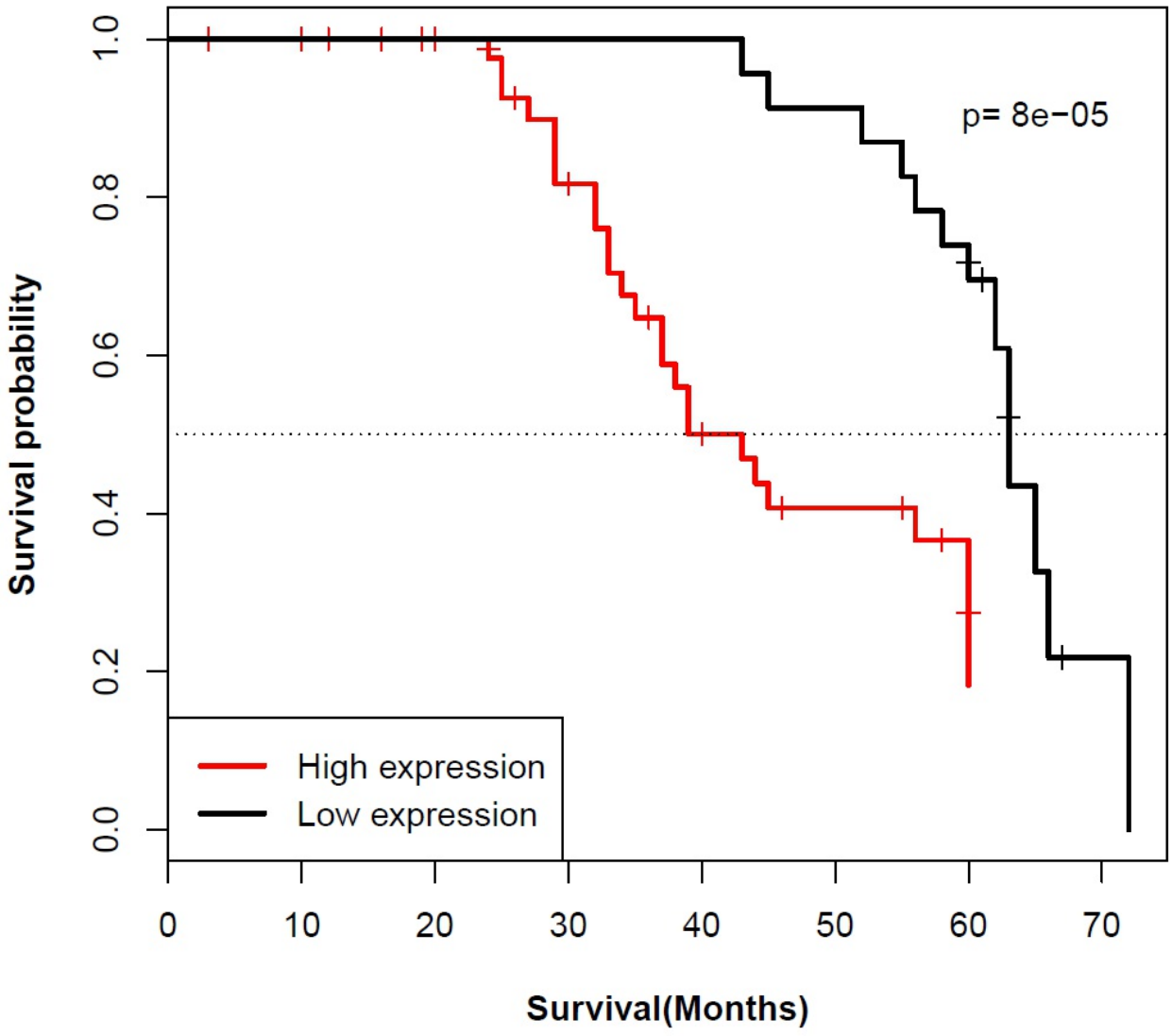

Figure 2

Correlation between the expression of Sox10 and the DFS of TNBC. P = 0.00008