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Connection between *SOX7* Expression and Breast Cancer Prognosis

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Background: *SOX7* exerts a repressing effect against tumors and imposes vital influences on malignancies. Our research discussed the importance of *SOX7* in breast cancer prognoses.

Material/Methods: *SOX7* mRNA expression in breast cancer tissues samples and matched adjacent normal controls of breast cancer patients was measured by quantitative real-time-polymerase chain reaction (qRT-PCR). The relationship of *SOX7* with clinicopathological characteristics were analyzed via chi-square test. The association of *SOX7* levels with clinical outcomes was evaluated adopting the Kaplan-Meier method and multivariate Cox proportional hazards regression model.

Results: *SOX7* mRNA degree of expression exhibited a declining tendency in breast cancer tissue compared to paired bordering normal tissue specimens ($P<0.001$). In addition, the reduced *SOX7* degree of expression had a strong correlation to larger cancer mass dimension ($P=0.006$) and lymph node metastasis ($P=0.001$). Survival analysis revealed that the overall survival (OS) time was much shorter among cases harboring low *SOX7* degree of expression compared to high degree of expression ($P=0.005$). Moreover, *SOX7* expression alone could predict OS among breast cancer patients (hazard ratio=3.956, 95% confidence interval=1.330–11.772, $P=0.013$).

Conclusions: *SOX7* expression was downregulated in breast cancer tissues, and it could function as a useful prognostic marker in breast cancer.

MeSH Keywords: Biomarkers, Pharmacological • BRCA1 Protein • Prognosis • SOXF Transcription Factors

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Background

Breast cancer (BC) is the most prevalent tumor among women worldwide and makes up around 23% of all cancers and 14% of cancer-related deaths [1]. The majority of BC cases are hormone-dependent [2]. BC is molecularly classified into different phenotypes, such as luminal-A, luminal-B, HER2-overexpressing (HER2+) or triple-negative breast cancer (TNBC) [3,4]. During the past 2 decades, despite of the advancement in early detection, prevention and treatment options, the disease still has poor incidence and mortality rates [1,5]. Moreover, most of the BC deaths can be attributed to distant metastasis or other related conditions [6,7]. Currently, although a number of biomarkers have been proposed to evaluate BC prognoses, non-invasive and available biomarkers to accurately predict the prognosis of BC patients are still needed.

The SOX gene family belongs to the high mobility group (HMG) super family, and it reportedly plays a role in regulating embryonic, gut, B cell, muscle, nervous system and cardiovascular system development, as well as sex differentiation [8–11]. As a member of subfamily SOXF, *SOX7* is located in a region of chromosome 8p23.1 and is approximately 7.7 kilobase pairs in length [12]. *SOX7* has been strongly associated with not only the target genes, but also the Wnt/β-catenin signaling pathway. Moreover, it has also been reported that *SOX7* serves as a developmental regulator in various developmental processes, such as hematopoiesis, vasculogenesis, myogenesis, and cardiogenesis [13]. *SOX7* expression has been reported to be abnormal in different human cancers. Reportedly, *SOX7* exhibits an upward tendency in pancreatic and gastric cancers [14]. On the contrary, *SOX7* has also been shown to display a declining trend in prostate, colorectal, ovarian, and lung cancers [15–17]. One study demonstrated that *SOX7* expression was downregulated among breast cancer tissues in comparison to cancer-free tissues [18]. Nevertheless, the importance of *SOX7* in the prognosis of BC patients has not previously reported.

In our research, we aimed to further confirm the abnormal degree of expression of *SOX7* in BC, and its clinical significance in BC advancement.

Material and Methods

Cases and specimens

There were 100 BC tissues specimens and matched adjacent normal samples acquired from patients at Weihai Municipal Hospital. The diagnosis of BC was made by 2 pathologists, and none of patients had received any treatments (radiotherapy or chemotherapy) prior to their surgical operation. In addition, all tissue specimens were resected and frozen at −80°C for later use. This research was authorization by the Ethical Committee of the hospital, and all individuals were informed of the purpose of the study and had given written informed consent. All patients were followed from 3 to 80 months to determine the survival outcome. Clinical information of all patients is summarized in Table 1.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from the clinical tissue specimens using TRIzol reagent (Invitrogen), and reverse transcription was implemented employing transcriptase cDNA synthesis kit as per the manufacturer's instructions. The purity of total RNA was gauged by applying a NanoDrop 1000 Spectrophotometer. Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted by SYBR Premix Ex Taq™ (TaKaRa) on the Applied Biosystems 7500 Fast Real-Time PCR System. *GAPDH* represented endogenous reference. PCR primer sequences contained: *SOX7*, 5'-ACCAACGGGTCCCACAGA-3' (sense) and 5'-CCACTCAAGGCACA AGAAGG-3' (antisense); *GAPDH*, 5'-TCGTGCGTGACATTAAGGAG-3' (sense) and 5'-ATGCCAGGGTACATGGTGGT-3' (antisense). Relative expression level of *SOX7* was normalized to *GAPDH* and determined by the ΔΔCT method.

Immunohistochemistry (IHC)

BC tissue and adjacent normal tissue samples were fixed by formalin and embedding into paraffin. Sections were dewaxed using xylene and then they were rehydrated applying different concentration alcohols and ddH_2O. Then 3% hydrogen peroxide was used for inactivating endogenous peroxidases in phosphate buffer saline (PBS), and the samples were incubated with the primary antibody at 4°C for overnight. The secondary antibody was added at room temperature for 1-hour incubation.

Then, the tissue samples were detected with a streptavidin-peroxidase compound. We obtained the scores through the ratio and intensity of positive staining cells: 0 score was 0–5%; 1 score was 6–35%; 2 score was 36–75%; 3 score was more than 75%. Tissue samples with score 0–1 were considered as low expression of *SOX7*, otherwise, tissue samples were defined as high *SOX7* expression with scores 2 or 3.

Statistical analysis

Data synthesis was accomplished using SPSS 18.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 5 software. Quantitative data were compared using *t*-test, and chi-square test

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Table 1. Relationship between *SOX7* expression and clinicopathological characteristics.

Characteristic	N (N=100)	*SOX7* expression		*P*
		High (N=59)	Low (N=41)	
Age				0.313
≥50	52	24	28	
<50	48	17	31	
Tumor size (cm)				0.006
≥2	61	18	43	
<2	39	23	16	
Lymph node metastasis				0.001
Positive	68	20	48	
Negative	32	21	11	
ER				0.104
Positive	53	26	27	
Negative	47	15	32	
PR				0.194
Positive	33	17	16	
Negative	67	24	43	
Histological type				1.000
Ductal	64	26	38	
Lobular	36	15	21	

ER – estrogen receptor; PR – progesterone receptor.

appraised potential link for *SOX7* degree of expression to clinicopathologic categorical variables. Kaplan-Meier survival curves with log-rank statistics assessed overall survival (OS) for BC sufferers. In addition, multivariate Cox analysis detected *SOX7* importance for BC patients' prognoses. *P*-value less than 0.05 was considered statistically significant.

Results

SOX7 expression was downregulated in BC

To characterize the role of *SOX7* in BC, we analyzed its mRNA degree of expression among BC tissue specimens and bordering cancer-free samples by qRT-PCR. Accordingly, the expression of *SOX7* exhibited a declining tendency among malignant specimens in comparison to normal controls (mean±standard deviation: 2.39±0.72 versus 3.57±0.87, Figure 1). Moreover, we also analyzed the protein expression difference of SOX7

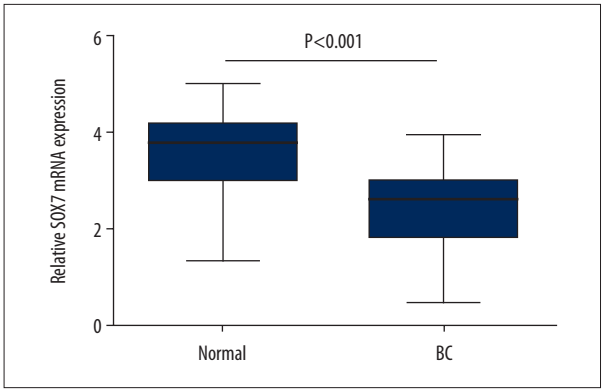

Figure 1. Relative SOX7 mRNA expression. The expression of SOX7 expression in breast cancer tissues was significantly lower than in normal control tissues (P<0.001).

using immunohistochemistry (IHC) analysis between BC tissues and adjacent normal tissues. The results showed that the positive rate of SOX7 in BC tissues was 27% (27 out of 100) and the percentage was as high as 83% (83 out of 100) in adjacent normal tissues, the difference was significant (*P*<0.001, Figure 2). Furthermore, based on these findings, we evaluated the association between *SOX7* levels and clinical parameters of BC patients. The patients were classified into high expression and low expression groups according to the average expression level of *SOX7*. The results unveiled *SOX7* degree of expression held strong relation to tumor size (*P*=0.006) and lymph node metastasis (*P*=0.001; Table 1), but not with other clinical characteristics, including age, estrogen receptor (ER), progesterone receptor (PR), and histological type (all, *P*>0.05). All these results suggested that *SOX7* expression might play a role as a tumor suppressor in the development of BC.

Association of *SOX7* expression with prognosis of BC patients

To further explore the relationship between *SOX7* and prognosis of BC patients, we evaluated the prognostic significance of *SOX7* in BC patients. Kaplan-Meier curves analysis revealed that cases harboring a low *SOX7* degree of expression exhibited shorter survival time compared to high degree of expression (Log rank test, *P*=0.005; Figure 3). Moreover, multivariate analysis with Cox proportional hazard model suggested that *SOX7* could be an independent predictor (hazard ratio [HR] = 3.956, 95% confidence interval [CI]=1.330–11.772, *P*=0.013) for OS, along with lymph node metastasis (HR=8.817, 95% CI=2.338–33.252, *P*=0.001; Table 2) of BC patients.

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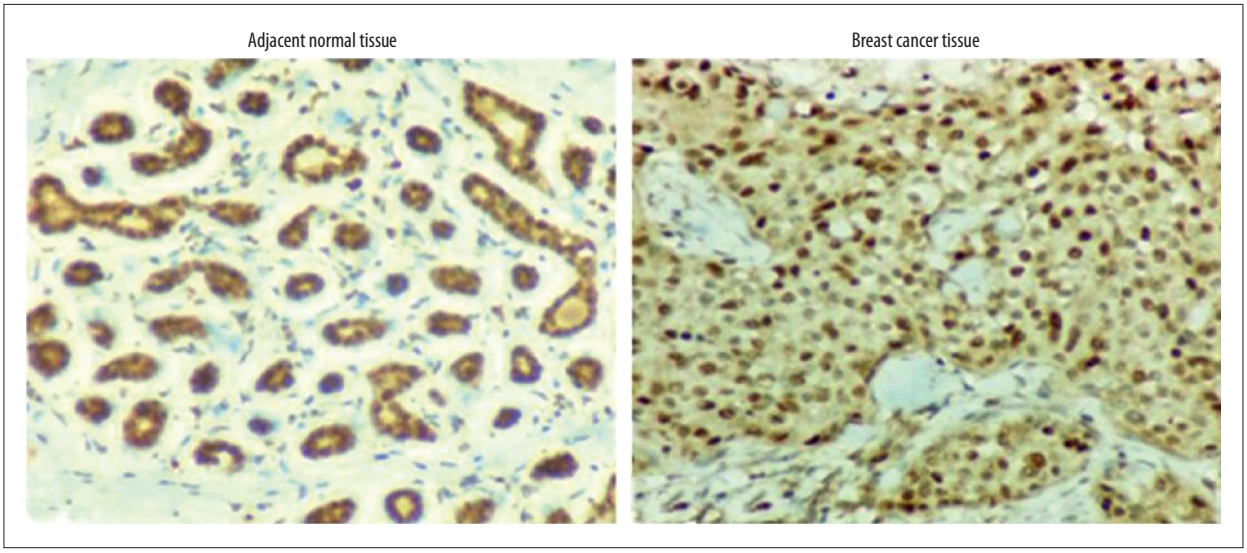

Figure 2. Protein expression difference of SOX7 using immunohistochemistry (IHC) analysis between breast cancer tissues and adjacent normal tissues (*P*<0.001).

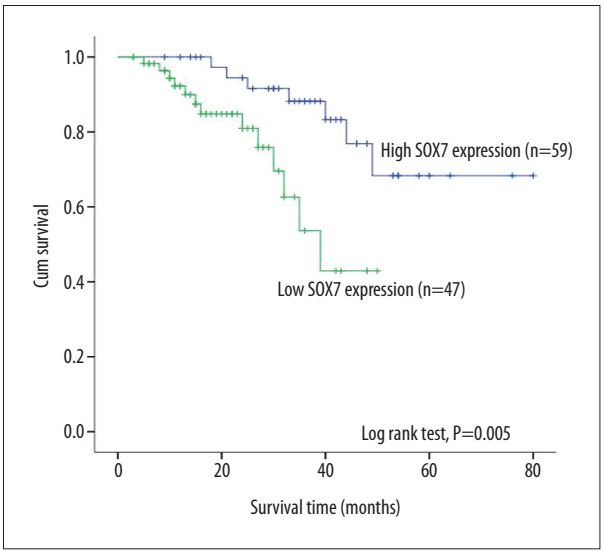

Figure 3. Kaplan-Meier analysis of the overall survival for patients with breast cancer. Patients harboring low *SOX7* degree of expression faced worsened overall survival compared to high degree of expression (Log rank test, *P*=0.005).

Discussion

In our research, *SOX7* degree of expression was significantly decreased in BC tissues in comparison with cancer-free controls. Moreover, *SOX7* levels displayed strong relation to cancer dimension and lymph node metastasis. In addition, our findings also revealed that *SOX7* is a potent prognostic factor for BC patients.

The pathogenesis of BC patients might be affected by many factors. Crispo et al. reported that higher body mass index (BMI) was related to significant lower disease-free survival (DFS) rate in BC [19]. An elevated plasma fibrinogen level was related to poor prognosis of BC patients [20]. High expression of carbonic anhydrase 12 (CA12) was correlated with improved prognosis of BC patients [21]. Kovac et al. concluded that high expression level of α-actinin-1 was a prognostic factor of poor outcomes in basal-like BC [22]. The study of Fu et al. reported that lower *SOX17* degree of expression alone could predict DFS and OS among BC patients [23]. Besides, *SOX1* was downregulated in breast cancer tissues and cell lines, which suggests a repressing effect against BC [24]. Furthermore, *SOX7* reportedly had a reducing trend in BC [18]. Considering the possible effect of *SOX7* on breast cancer, we investigated the relation of its degree of expression to the prognosis of BC patients.

As a member of the subfamily SOXF, *SOX7* is essential in governing embryonic development. Until now, *SOX7* has been reported to curbing the effects of a variety of human cancers, including pancreatic, glioblastoma, breast, liver, and lung cancers [17,25–27]. Enhanced *SOX7* expression may inhibit the progression and development of pancreatic and liver cancer [26]; and may suppress cell growth of oral squamous cell carcinoma and NSCLC [17,28]. Furthermore, the silencing of *SOX7* can significantly increase the multiplication, emigration, and intrusion of non-tumorigenic breast cells [25]. Meanwhile, downregulation of *SOX7* has been found in different cancers [15–17]. Consistent with these previous studies, we found *SOX7* degree of expression in BC tissues exhibited a declining tendency in comparison with bordering cancer-free control tissues.

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Table 2. Multivariate analysis of prognostic factors in breast cancer.

Parameters	HR (95%CI)	*P*
SOX7 expression	3.956 (1.330–11.772)	0.013
Lymph node metastasis	8.817 (2.338–33.252)	0.001

HR – hazard ratio; CI – confidence interval.

Meanwhile, previous studies have supported that *SOX7* might have potential usage as an independent prognostic marker in lung cancer, gastric cancer, myelodysplastic syndromes, and breast cancer [25,29,30]. Moreover, *SOX7* has also been reported to have a suppressive effect against hepatocarcinogenesis and so could function as a potential treatment [31]. Through our research, we demonstrated, for the first time, that downregulation of *SOX7* expression had a close relation to poor outcomes in BC. In addition to its prognostic value, we also observed that declined *SOX7* expression was correlated with larger tumor size and lymph node metastasis, which further supported the notion that *SOX7* might play a crucial role in BC development.

Currently, *SOX7* has been demonstrated to interact with β-catenin to compete with TCF/LEF action, or activate the expression of target genes, such as *SPRY1*, *SLIT2*, *TRIB3*, and *MTHFD2* [32,33]. Correspondingly, *SOX7* may represent a target for the p38 MAPK pathway. Inhibiting this pathway could repress *SOX7* expression [34]. However, despite the growing body of evidence for the suppressive role of *SOX7* in many cancers, the functional mechanisms of this process still need to be investigated. Limitations in this study should not be ignored. Firstly, this was a single center study and the sample size was not large enough. Secondly, the mechanism of *SOX7* impacting BC prognosis was not investigated in this study. Further studies with large sample size should be well designed and performed in the future.

Conclusions

The data from our research demonstrated that the *SOX7* degree of expression was downregulated in BC tissues. Moreover, *SOX7* degree of expression might function as a useful prognostic marker in BC. However, further research is still needed to illustrate the molecular mechanism for *SOX7* functioning in BC development.

Conflict of interests

None.

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