RESEARCH ARTICLE

# Clinical and Prognostic Significance of SOX11 in Breast Cancer

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## Abstract

**Recently, the transcription factor SOX11 has gained extensive attention as a diagnostic marker in a series of cancers. However, to date, the possible roles of SOX11 in breast cancer has not been investigated. In this study, immunohistochemical staining for SOX11 was performed for 116 cases of breast cancer. Nuclear SOX11 was observed in 42 (36.2%) and cytoplasmic SOX11 in 52 (44.8%) of breast cancer samples. Moreover, high expression of cytoplasmic and nuclear SOX11 was associated with clinicopathological factors, including earlier tumor grade, absence of lymph node metastasis and smaller tumor size. Kaplan-Meier survival curves demonstrated high nuclear SOX11 expression to be associated with more prolonged overall survival than those with low expression and it could be an independent predictor of survival for breast cancer patients. It is worthwhile to note that cytoplasmic SOX11 was not correlated with prognosis of breast cancer patients. These data suggest the possibility that nuclear SOX11 could be as a potential target for breast cancer therapy.**

**Keywords:** SOX11 - breast cancer - nuclear location - survival - prognosis

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## Introduction

As the most common malignancy in women in the world, the incidence of breast cancer had increased in recent years (Khan et al., 2014). The estimated lifetime risk of breast cancer with a BRCA1 or BRCA2 mutation can be as high as 65%-74% (Madjd et al., 2014). Her-2 is a well-characterized therapeutic target and it also could be a biomarker for predict prognosis of breast cancer patients (Press et al., 1993). Taking into account the limited effective methods in treatment of breast cancer, identification of more promising markers related with outcome of patients is of great importance. These biomarkers would facilitate finding more effective therapies for breast cancer patients.

The SOX gene family is a group of developmentally regulated transcription factors and it contains nearly 20 SOX genes (Lefebvre et al., 2007). Most of them have vital functions in the determination of cell fate and differentiation (Wegner., 1999; Kiefer., 2007; Lefebvre et al., 2007). SOX11 located in 2p25.3 and is critical for outgrowth and survival of neural cell (Azuma et al., 1999). Recently, the transcription factor SOX11 has gained the extensive attention as a diagnostic marker for gliomas (Weigle et al., 2005), ovarian cancer (Brennan et al., 2009) and B cell lymphoma (Ek et al., 2008; Wang et al., 2008; Mozos et al., 2009; Chen et al., 2010; Fernàndez et al., 2010). Moreover, nuclear SOX11 expression was correlated with favourable outcome in ovarian cancer. It was noted that SOX11 expression decrease the growth and invasion capacity of nasopharyngeal carcinoma cells (Zhang et al., 2013). These results suggested SOX11 could be as a tumor suppress gene. However, to date, the possible roles of SOX11 in breast cancer has not been investigated.

The aim of this study was to explore the expression pattern of SOX11 in breast cancer and its correlation with clinicopathologic factors. Of clinical interest, for the first time, we revealed that nuclear SOX11 expression was associated with better prognosis of breast cancer patients

## Materials and Methods

*Patients and tissue specimens*

Formalin-fixed, paraffin-embedded tissues from 116 patients with breast cancer were randomly selected from Department of Pathology, the first people's Hospital of Jining City Affiliated to Jining Medical University, between May 1995 and August 1996, with mean age of 54.7 years (range, 33-76 years). Clinicopathologic characteristics for these patients were detailed in table 1. The patients were followed up by interview in phone call. The period of follow-up was 47-206 months. The study was approved by the first people's Hospital of Jining City. The informed consent of samples was obtained by each patient.

*Immunohistochemical analysis*

4-μm sections were cut from the selected paraffin

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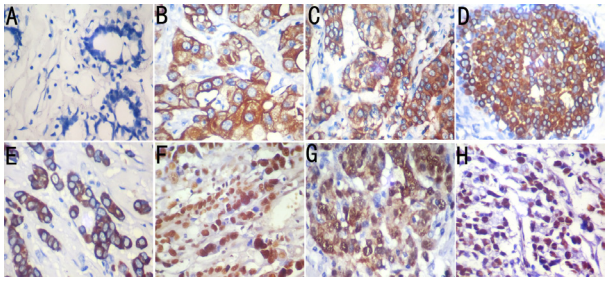


**Figure 1. Immunohistochemical staining of SOX11 in breast cancer.** A) expression of SOX11 was absent in normal breast tissue; B) C) D) E) high expression of cytoplasmic SOX11 in breast cancer tissues; F) G) H) high nuclear SOX11 expression in breast cancer tissues.

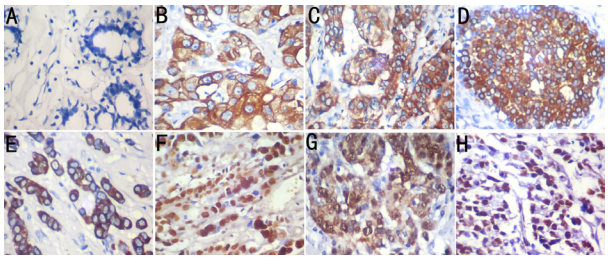


**Figure 2. Kaplan-Meier Analysis of Overall Survival Curves of Breast Cancer Patients According to SOX11 Expression.** Kaplan-Meier curves showed no statistically significant difference of overall survival in breast cancer patients according to cytoplasmic SOX11 expression. A) whereas patients with high nuclear SOX11 expression had prolonged survival times than those with low expression B)

blocks and treated with routine techniques. Then the slides were incubated with primary antibody (SOX11, Santa Cruz Biotechnology, CA, USA) and stored overnight at 4°C. Labeling was detected by adding biotinylated secondary antibodies (Maxim-Bio, Fuzhou, China), avidin-biotin complex (Maxim-Bio), and diaminobenzidine (Maxim-Bio). Sections were then counterstained with hematoxylin.

*Immunohistochemical analysis evaluation*

SOX11 immunostaining score was calculated as the intensity (0, no staining; 1,weak staining; 2, moderate staining; 3, strong staining) and the percentage (extent staining) of tumor cells that were stained (0, <5% of tumor cells stained; 1, 5-25% positive cells; 2, 26-75% positive cells; 3, more than 75% positive cells). If the product of multiplication between staining intensity and the percentage of positive cells is ≤4, it was defined low expression, while overall score >4 were defined high expression.

*Statistical analysis*

The correlation between SOX11 and clinicopathologic factors of breast cancer patients was evaluated by Fisher's exact test. Survival curves were obtained using the Kaplan-Meier method. The Cox proportional hazards regression model was performed for multivariate survival analysis. Statistical analysis was performed by using the SPSS 13.0 for windows. A significant difference was considered if the P value from a two-tailed test was less than 0.05.

**Table 1. Association Between SOX11 Expression and the Clinicopathological Features of the Breast Cancer Patients**

| Variable | N | Cytoplasmic SOX11 | | | Nuclear SOX11 | | |
|---|---|---|---|---|---|---|---|
| | | Low | High | P value | Low | High | P value |
| Age (y) | | | | | | | |
| ≤50 | 54 | 32 | 22 | 35 | 19 | | |
| >50 | 62 | 32 | 30 | 0.457 | 39 | 23 | 0.849 |
| Tumor size | | | | | | | |
| ≤2 cm | 48 | 10 | 38 | | 31 | 17 | |
| >2 cm | 68 | 54 | 14 | 0 | 43 | 25 | 0.52 |
| Tumor grade | | | | | | | |
| I and II | 70 | 53 | 17 | | 38 | 32 | |
| III | 46 | 11 | 35 | 0.254 | 36 | 10 | 0.01 |
| Histology | | | | | | | |
| IDC | 98 | 53 | 45 | | 64 | 34 | |
| ILC | 18 | 11 | 7 | 0.617 | 10 | 8 | 0.436 |
| Lymph node metastasis | | | | | | | |
| Absent | 71 | 35 | 36 | | 40 | 31 | |
| Present | 45 | 29 | 16 | 0.128 | 34 | 11 | 0.047 |

## Results

***Expression pattern of SOX11 in breast cancer tissues***

The expression of SOX11 protein in breast cancer samples and normal mammary glands adjacent to tumor were analyzed by immunohistochemistry. SOX11 was predominantly expressed in the the cytoplasm (Figure 1B-E) and nucleus of cancer cells (Figure 1F-H). Among 116 breast cancer cases, 52 (44.8%) showed high cytoplasmic expression of SOX11 and 42 (36.2%) samples exhibited high nuclear staining of SOX11. In contrast, no signal for SOX11 in non-neoplastic mammary glands tissues was found (Figure 1A).

*Correlation between SOX11 and clinicopathologic factors of breast cancer patients*

To better understand the significance of SOX11 in breast cancer, the correlation of SOX11 with the clinicopathologic variables was analyzed. As shown in Table 1, high nuclear staining of SOX11 was significantly correlated with earlier tumor grade ($p$=0.010) and absent of lymph node metastasis ($p$=0.047). For example, high levels of nuclear SOX11 showed in 32 (27.6%) patients with earlier tumor grade (I and II). However, only 10 (8.62%) patients with late stage (III) exhibited high nuclear SOX11 immunoreactivity. There was no correlation existed between nuclear SOX11 expression and variables such as age ($p$=0.849), tumor size ($p$=0.520) and histological subtype ($p$=0.436). On the other hand, a positive relationship had been found between high cytoplasmic SOX11 expression and smaller tumor size ($p$=0.000). In addition, high cytoplasmic SOX11 expression was not correlated with the other clinicopathologic factors such as age ($p$=0.457), tumor grade ($p$=0.254), histology ($p$=0.617) and lymph node metastasis ($p$=0.128).

*Influence of the SOX11 expression on overall survival in patients with breast cancer*

To elucidate the prognostic role of SOX11 in breast cancer patients, overall survival rates were estimated estimated by Kaplan-Meier survival curves. Interestingly, the overall survival time of breast cancer patients between

**Table 2. Univariate and Multivariate Analysis of Overall Survival for Breast Cancer Patients**

| Factor | Univariate analysis | | | Multivariate analys | | |
|---|---|---|---|---|---|---|
| | Hazard ratio | 95% CI | *P* value | Hazard ratio | 95% CI | *P* value |
| Age | 1.312 | 0.897-1.013 | 0.712 | | | |
| Tumor size | 1.479 | 0.593-2.781 | 0.485 | | | |
| Tumor grade | 5.523 | 2.984-11.146 | 0.002 | 2.195 | 0.894-5.743 | 0.042 |
| Histology | 1.821 | 0.719-3.918 | 0.21 | | | |
| Lymph node metastasis | 5.896 | 2.532-18.672 | 0.001 | 3.389 | 1.235-10.096 | 0.029 |
| Nuclear SOX11 | 0.116 | 0.031-0.435 | 0.002 | 0.198 | 0.049-0.884 | 0.031 |

high cytoplasmic SOX11 expression and low expression had not significantly difference ($p>0.05$) (Figure 2A). However, patients with high nuclear SOX11 expression had prolonged overall survival period than those with low expression ($p<0.05$) (Figure 2B).

To explore whether nuclear SOX11 expression is an independent predictor of survival, univariate and multivariate analyses were performed using Cox proportional hazard model to evaluate the impacts of nuclear SOX11 expression and the other pathological factors on the prognosis of breast carcinoma patients (Table 2). As illustrated in Table 2, nuclear SOX11 expression was independently correlated with prognosis of patients ($p=0.031$), together with tumor grade ($p=0.042$) and lymph node metastasis ($p=0.029$). Similarly, previous study also found tumor grade is an independent prognostic factor affecting the survival of breast cancer patients (Mutlu et al., 2013). In all clinicopathologic factors, lymph node metastasis was the most independent features predicting prognosis ($p=0.029$).

## Discussion

SOX11, a vital protein in embryogenesis and tissue remodeling, is mainly expressed at places where epithelial-mesenchymal interactions occur (Hargrave et al., 1997) and in the nervous system (Bergsland et al., 2006). Compared with its extensive expression pattern in embryonic tissues, SOX11 immunoreactivity was absent in differentiated normal tissues.

Recently, up-regulation of SOX11 mRNA was observed in a number of cancer types, including glioma (Weigle et al., 2005), neuroblastoma (Lee et al., 2002), ovarian cancer (Brennan et al., 2009). Brennan detected SOX11 mRNA expression was increased in epithelial ovarian cancer compared to normal tissues (Brennan et al., 2009). More importantly, they revealed nuclear expression of SOX11 was correlated with a prolonged recurrence-free survival. Similarly, Wang found lack expression of nuclear SOX11 in mantle cell lymphoma was associated with impaired overall survival (Wang et al., 2008). These findings suggested SOX11 plays a critical role in prognosis of human cancers and may be a new prognostic factor for cancer patients. However, in breast cancer, protein pattern of SOX11 expression and its clinical significance still obscure.

To the best of our knowledge, this is the first study that investigated the role of SOX11 in conjunction with clinical significance of the breast cancer patients. In our study, expression of SOX11 protein in breast cancer tissues was significantly higher than their normal counterparts. Our results showed that patients with high SOX11 expression had smaller tumor size and earlier tumor grade, which indicated that SOX11 could inhibit growth and progression of breast cancer. These results were consistent with Conrotto reported that knock-down of SOX11 increase in proliferation of tumor cells *in vitro*. Furthermore, SOX11 knock-down induces more aggressive tumors. *in vivo* (Conrotto et al., 2011). In the present study, our data also revealed high nuclear SOX11 expression was associated with absent of lymph node metastasis. This finding confirmed that nuclear SOX11 had important roles in inhibiting progression of breast cancer.

With regard to the prognostic influence of nuclear SOX11 expression in cancers, it was documented that high nuclear SOX11 expression had better prognosis in patients with mantle cell lymphoma (Wang et al., 2008) and ovarian cancer (Brennan et al., 2009). In the present study, as shown by Kaplan-Meier curves and multivariate Cox regression analysis, we found high nuclear SOX11 expression was significantly correlated with prolonged overall survival. It was easy to see the role of nuclear SOX11 in prognosis and its potential mechanisms to affect outcome of breast cancer patients. For one thing, ectopic SOX11-overexpression could up-regulate expression of TGF-β, an effective anti-proliferative factor (Siegel et al., 2003; Pardali et al., 2007). For another, silenced SOX11 gene was also exhibited to have an increased engraftment potential and a more aggressive behavior *in vivo* (Conrotto et al., 2011).

In summary, in the present study, we study SOX11 expression pattern in breast cancer tissues, along with its association with clinicopathologic features. SOX11 may be involved in inhibiting progression of breast cancer. Moreover, high nuclear SOX11 expression is correlated with favorable prognosis in breast cancer. Therefore, SOX11 is a promising biomarker for the treatment of breast cancer which needs further research.

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