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Original Article

The role of SOX2 in angiogenesis in breast cancer

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Abstract: Objective: SOX2 belongs to the SOX gene family of high-mobility transcription factors indispensably involved in gene regulation in pluripotent stem cells and neural differentiation. Herein, we investigate the relationship and significance of SOX2 expression and angiogenesis in breast cancer. Methods: Immunohistochemical SP method was applied to detect the expression of SOX2 and to label microvessels with CD105 in 90 cases of breast carcinoma. Results: The positive rates of SOX2 and MVD ($CD105^+$) were 84.62% and 11.51±2.11 in group of lymph node metastasis, which were much higher than those in the group of non-lymph-node-metastasis, which were 44.00% and 10.00±1.63, respectively. There was a significant difference between the two groups ($P<0.01$). The positive rate and MVD were 82.24% and 11.40±2.13 in group of grade (II+III), which were much higher than those of the grade I group, which were 45.45% and 10.14±1.67, respectively ($P<0.01$, $P<0.05$). MVD showed statistically significant differences between the SOX2-positive group (11.62±2.05) and SOX2-negative group (9.63±1.41) ($P<0.01$). Conclusion: Expression of SOX2 indicates highly malignant tumor. SOX2 may facilitate lymph node metastasis in breast carcinoma by promoting angiogenesis.

Keywords: SOX2, CDI05, angiogenesis, breast cancer

Introduction

The SRY-related HMG-box (SOX) family is a group of related transcription factors that have demonstrated importance in developmental and stem cell biology [1]. SOX2 is a gene that encodes for a transcription factor belonging to the SOX gene family and contains a high-mobility group (HMG) domain, which permits highly specific DNA binding. It was discovered and characterized in humans in 1994 [2]. The most important function of SOX2 is as a critical regulator of embryogenesis and it is necessary for cellular reprogramming, but some studies have shown SOX2 to be amplified in various cancer types and to affect cancer cell physiology via involvement in complicated cell signaling and protein-protein interactions [3]. In our study, we investigated the expression of SOX2 in breast cancer tissues, and estimated their effects on tumor progression by analyzing the correlation between SOX2 expression and various clinicopathological characteristics.

Endoglin, also known as CD105, is an accessory receptor for transforming growth factor beta (TGF-β) and its expression is upregulated in actively proliferating endothelial cells [4]. CD105 has been suggested as an appropriate marker for tumor-related angiogenesis and neovascularization [5]. Microvascular density (MVD) was assessed by CD105-positive tumor vessels. Several studies demonstrate the potential of CD105 in tumor diagnosis, prognosis, and therapy [6]. In this investigation, the expression of CD105 in breast cancer tissues was determined in order to analyze the correlation between SOX2 expression and clinicopathological features.

Patients and methods

Patients

The study included 90 invasive breast cancer patients treated in the Third Affiliated Hospital of Soochow University from 2013 to 2014. All samples were harvested from surgically resected specimens. Ethical approval was given by the biomedical ethics committee of the Third Affiliated Hospital of Soochow University. Histological grade was assessed using the crite-

Table 1. Correlation between SOX2, MVD expression, and clinicopathological features

Clinicopathological features	n	SOX2		Positive rate (%)	χ^2	P	MVD ($CD105^+$)	t	P
		-	+						
Age									
<50 years	28	10	18	64.3	1.7	P>0.05	11.43±1.93	1.04	P>0.05
≥50 years	62	14	48	77.4			10.94±2.16		
Size									
≥3 cm	52	12	40	76.9	0.81	P>0.05	11.40±2.10	1.69	P>0.05
<3 cm	38	12	26	68.4			10.66±2.03		
Lymph node metastasis									
Yes	65	10	55	84.6	15.23	P<0.01	11.51±2.11	3.22	P<0.01
No	25	14	11	44			10.00±1.63		
Staging									
I	22	12	10	45.5	11.57	P<0.01	10.14±1.67	2.53	P<0.05
II-III	68	12	56	82.4			11.40±2.13		

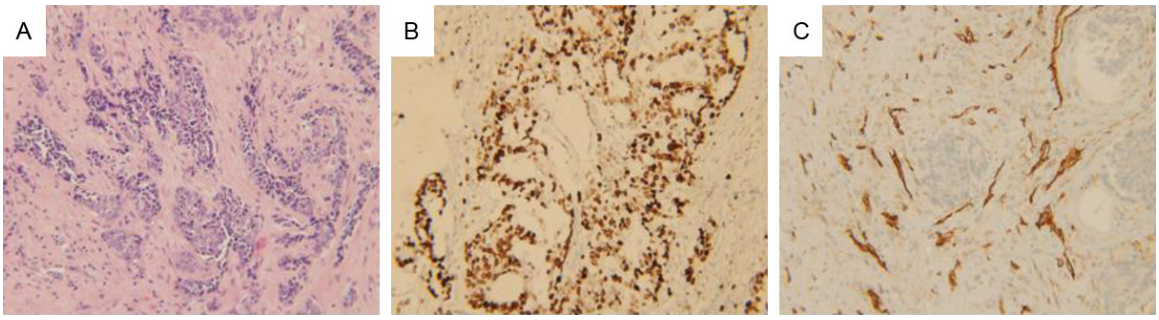

Figure 1. A: Invasive breast cancer; B: Positive expression of SOX2 in invasive breast cancer; C: Positive expression of CD105 in invasive breast cancer.

Table 2. Correlation between SOX2 and MVD expression in gastric cancer tissues

Spearman correlation analysis (n=90)	SOX2	MVD
SOX2		
r	1	0.67
p	-	0.003
MVD ($CD105^+$)		
r	0.67	1
p	0.003	-

ria developed by SBR (Scarff-Bloom-Richardson). All specimens were grade III.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed using the ElivsionTM method. The following primary antibodies were used in our study: SOX2 (clone 169545, Abcam, USA) and CD105 (clone AF6440, Abcam, USA). The secondary antibody and DAB solution were provided by Maxim Company (Fuzhou, China). All samples were fixed in formalin solution and embedded in paraffin. Sections (3-4 μm) were dewaxed in xylene, dehydrated in ethanol, and incubated in 3% H_2O_2 for 15 min to destroy the activity of endogenous peroxidase. After incubation in 10% normal bovine serum for 10 min, each slide was incubated with the primary antibodies at 4°C overnight. Biotin-labeled mouse-rabbit immunoglobulin was chosen as the second antibody. Staining steps were carried out according to kit instructions, and we chose positive breast cancer histologic section as positive control, with non-immune animal serum IgG replacing the antibody as negative control.

Evaluation of immunostaining

Immunostaining was scored by two pathologists. The intensity (I) of staining was graded on a scale of 0-3+, with 0 representing no detect-

Table 3. Univariate and multivariate analysis of the clinicopathological and molecular features for overall survival

Factor	Univariate analysis		Multivariate analysis	
	HR (95.0% CI)	*P* value	HR (95.0% CI)	*P* value
Age				
>50 years vs. ≤50 years	0.95 (0.81-1.26)	0.576	-	-
Size				
≤3 cm vs. >3 cm	1.08 (0.54-1.65)	0.315	1.42 (0.57-3.36)	0.22
T pathological staging				
T1 vs. T2+T3	2.98 (2.13-4.08)	0.006*	1.79 (1.33-2.65)	0.015*
Staging				
I vs. II+III	2.33 (1.58-2.84)	0.005**	3.08 (2.68-3.59)	0.563
Lymph node metastasis				
Positive vs. negative	1.55 (1.06-2.63)	0.048*	2.02 (1.68-2.39)	0.256
SOX2				
Positive vs. negative	1.51 (1.12-2.09)	0.039*	1.46 (0.81-2.49)	0.023*

*P<0.05, **P<0.01.

able staining and 3+ representing the strongest staining. Four strongest staining regions were randomly scored under a 40× field. In each of the four regions, the rate of positive cell staining (R) under a 400× field was calculated. R was defined as: 0, no staining; 1, ≤10% tumor cells staining; 2, 11-50% tumor cells staining; 3, 51-75% tumor cells staining; and 4, >75% tumor cells staining. Samples with scores <3 were considered as negative and with scores ≥3 were considered as positive. Histochemistry score =I×R.

Microvascular density (MVD) was based on CD105 positive tumor vessels. The distribution of $CD105^+$ blood vessels was observed at low magnification first, and then five regions were selected randomly to calculate the rate of positive cell staining under a 400× field. All $CD105^+$ single endothelial cells and/or endothelial cell clusters were regarded as blood vessels. A vessel lumen was not a criterion, but there had to be a clear demarcation between the vessels.

Statistical analysis

The correlations between the expressions of SOX2, microvascular density (MVD) ($CD105^+$ blood vessels) and clinicopathological characteristics were analyzed by the χ^2 test. The correlations of the expression levels of SOX2 and MVD were analyzed by Spearman correlation coefficients. The MVD value was analyzed by the t test. The influence of these proteins on survival was assessed by the Cox proportional hazards model using the backward-LR method. Survival curves were plotted using the Kaplan-Meier method and the statistical difference was analyzed using the log-rank test. For all statistical analyses, SPSS 17.0 software (SPSS, Chicago, IL, USA) was used, and a significant difference was considered as $P<0.05$.

Results

Patients' characteristics

There were 90 females in the postoperative patients with an age range of 31-79 years (median 56 years), 65 patients had lymph node metastasis. According to the American Joint Committee on Cancer (AJCC) standards, all patients were staged from I to III in this study: there were 22 patients in stages I and 68 patients in stages II and III. Other clinicopathological features are shown in **Table 1**.

Expression of SOX2 and $CD105^+$ blood vessels in breast cancer

SOX2-positive staining was detected in 73.3% of breast cancers. Immunohistochemistry showed that the expression of SOX2 was localized to the nucleus (**Figure 1B**). CD105 mainly was expressed in cytoplasm of the vascular endothelial cells and/or cell clusters (**Figure 1C**).

Correlation between SOX2 or CD105 expression and clinicopathological features

The positive rates of SOX2 were significantly higher in tissues with lymph node metastasis and late AJCC stage than in tissues without lymph node metastasis or with earlystage (lymph node+, 84.6%; lymph node-, 44%; $P<0.01$. Stages II and III, 82.4%; stage I, 45.5%; $P<0.01$). The number of $CD105^+$ blood vessels was significantly higher in tissues with lymph node metastasis and late stage than in tissues without lymph node metastasis or with early AJCC stage (lymph node+, 11.51±2.11; lymph node-, 10.00±1.63; t=3.22, $P<0.01$. Stages II and III,

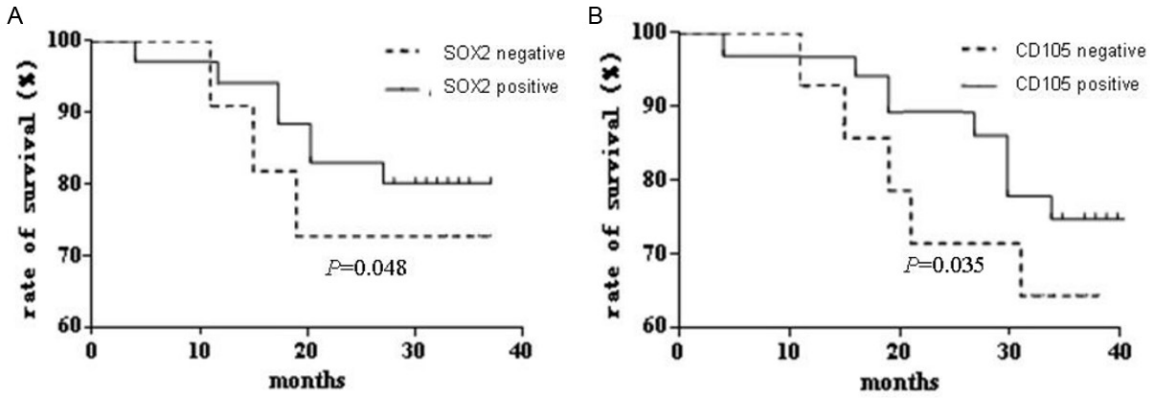

Figure 2. Survival curves for patients with different expression levels of SOX2 or CD105; A: OS of SOX2-positive patients was significantly longer than that of SOX2-negative patients (30.0 vs. 18.0 months, *P*=0.048); B: OS of CD105-positive patients was significantly longer than that of CD105-negative patients (31.0 vs. 19.0 months, *P*=0.035).

11.40±2.13; stages I, 10/14±1.67; t=2.5, *P*<-0.01). In contrast, no statistical association was seen between SOX2 and other features such as age, tumor size, as shown in **Table 1**.

Correlation between SOX2 expression and the number of MVD

A positive correlation between SOX2 expression and the number of MVD in breast cancer tissues was confirmed by Spearman correlation analysis. The correlation coefficient (r) was 0.67 (*P*=0.003) (**Table 2**).

Survival analysis

All patients were followed up for more than 3 years. Cox regression univariate analysis showed that histologic type, T2 and T3 pathological staging, lymph node metastasis, staging, SOX2 and MVD ($CD105^+$) low-expression were negative prognostic factors for OS (*P*<0.05, **Table 3**). However, other factors such as age and size had no effect on survival of patients (*P*>0.05). Moreover, Cox regression multivariate analysis confirmed that histologic type, pathological staging, SOX2 and MVD were independent prognostic factors (hazard ratio (HR)= 1.55, 95% confidence interval (CI) =10.48-1.89, *P*=0.042; HR=1.79, 95% CI=21.33-2.65, *P*=0.015; (HR) =1.46, 95% confidence interval (CI) =0.81-2.49, *P*=0.023; (HR) =1.75, 95% confidence interval (CI) =1.22-3.69, *P*=0.026; respectively, (**Table 3**).

The Kaplan-Meier curves are shown in **Figure 2**. The median OS of patients with SOX2 -positive expression was significantly longer than that of SOX2-negative cases (30.0 vs. 18.0 month, *P*=0.048, **Figure 2A**). The median OS of patients with CD105-positive expression was also significantly longer than that of CD105-negative cases (31.0 vs. 19.0 month, *P*=0.035, **Figure 2B**).

Discussion

Sex determining region Y-box 2 (SOX2) is a member of the Sox family of transcription factors with characterized by a conserved high mobility group (HMG) DNA-binding domain [7]. SOX2 was first found in mouse embryonal carcinoma cells [8]. SOX2 is well known for its functions in embryonic stem (ES) cell pluripotency, maintenance, and self-renewal, and it is an essential factor in generating inducible pluripotent stem (iPS) cells [9]. It also plays an important role in development and adult tissue homeostasis of different tissues, especially the central nervous system [10]. It has been reported that SOX2 had high expression in many cancer tissues and was associated with invasion and metastasis, as in gastric cancer and pancreatic cancer [11].

Our research showed that the expression of SOX2 was not associated with age and tumor size, but SOX2 was highly expressed in the lymph node metastasis group contrast to no lymph node metastasis group. All of this suggested that SOX2 can promote tumor lymph node metastasis. The histologic high-grade tumors show high malignancy and high invasiveness. In our research, we found SOX2 higher expression in stages II and III than Stage I, showing that SOX2 high expression indicates

high malignancy in breast cancer. This may be because SOX2 is a stem cell transcription factor, directly involved in the genesis and development of tumors. SOX2 high expression determines a high stage of tumor development.

CD105 (Endoglin) is a member of the superfamily of transcription growth factor (TGF)-beta and its expression is upregulated in actively proliferating endothelial cells [12]. Human CD105 is a 633 amino acid, 180 kDa homodimeric disulfide-linked hypoxia-inducible transmembrane glycoprotein. It contains a large extracellular domain, a hydrophobic transmembrane domain, and a short intracellular domain [13]. The extracellular domain contains an Arg-Gly-Asp (RGD) tripeptide, four *N*-linked glycosylation sites and a region of O-linked glycosylation. The intracellular domain contains many serine and threonine residues, some of which are phosphorylation sites. Two isoforms of CD105 exist, L and S, and they differ in the length of the intracellular domain, tissue distribution, and degree of phosphorylation. L-CD105 contains 47 amino acids in the cytoplasmic tail, has a high degree of phosphorylation, and is predominantly expressed in endothelial cells, whereas S-CD105 contains only 14 amino acids. Both isoforms are constitutively phosphorylated and this is likely due to the constitutively active TGF-β receptor type II (TGF-βR2). A soluble form of CD105 has also been identified in the sera of healthy and cancer patients [14]. Elevated levels of soluble CD105 have been noted in sera of patients with diseases such as metastatic melanoma, and breast cancer patients at risk of metastasis. Vascularization is necessary for tumor growth and metastasis. CD105 has been suggested as an appropriate marker for tumor-related angiogenesis and neovascularization [15]. In our study, the $CD105^+$ MVD was not associated with age and tumor size, but associated with histological grade and lymph node metastasis, suggesting that tumor angiogenesis is increased in highly malignant tumor and promotes tumor lymph node metastasis [16].

In the previous study, the relationship between SOX2 and angiogenesis has not been reported. In our research, with the SOX2 staining strength, the $CD105^+$ MVD gradually increased, suggesting that the expression of SOX2 was related to angiogenesis. The t test showed that there was a significant difference in $CD105^+$ MVD between the SOX2 positive group and the negative group, indicating that SOX2 may promote angiogenesis by some mechanism, accelerating tumor invasion and metastasis. It is well known that the vascular endothelial growth factor family (VEGF) plays an important role in tumor angiogenesis; possibly SOX2 can regulate the secretion of VEGF, causing changes in tumor angiogenesis. The mechanism remains to be further studied.

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Disclosure of conflict of interest

None.

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