JBUON 2014; 19(1): 203-206
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Expression of Sox2 in cervical squamous cell carcinoma

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Summary

Purpose: *Sox2, one of the genes that maintains self-renewal of embryonic stem cells and relates to the differentiation potential of these cells, is abnormaly expressed in various human tumors. We investigated the expression Sox2 in normal cervix and cervical squamous cell carcinoma (SCC), and we also assessed the prognostic significance of Sox2 expression in FIGO stage I–II cervical SCC.*

Methods: *Immunohistochemistry was performed to define the expression of Sox2 in 20 normal cervical tissue samples and 55 samples of cervical SCC. Correlations with clinicopathological characteristics were determined by chi-square test. The prognostic impact of Sox2 expression with regard to overall disease-free survival (DFS) was determined by the Kaplan–Meier method.*

Results: *The positive expression rate in cervical SCC was 74.5% (41/55), while in normal cervix it was 20.0% (4/20; p=0.000. In addition, the expression of Sox2 did not correlate with clinical factors (p>0.05). The overall DFS rates with negative and positive expressions of Sox2 were 35.7 and 29.3%, respectively (p=0.360).*

Conclusions: *Our results show that Sox2 was overexpressed in FIGO stage I–II cervical SCC, indicating that overexpressed Sox2 may play an important role in the carcinogenesis of cervical SCC. Besides, we found that the expression of Sox2 had no relation to clinical factors and prognosis.*

Key words: *cervical squamous cell carcinoma, immunohistochemistry, prognosis, Sox2*

Introduction

In women cervical cancer is the second most common gynecologic malignancy after breast cancer. This malignancy is one of the most important causes of mortality, with 250000 deaths each year worldwide [1]. Although Pap test has contributed greatly to early diagnosis of cervical SCC, its accuracy is still in question. Kanjanavirojkul et al. [2] have recently reported that Pap test showed high diagnostic performance for HSIL and SCC cases but its performance for LSIL cases was moderate.

Lack of accurate early diagnosis and immediate treatment is the main cause of the high mortality of this disease. Therefore, it seems reasonable to develop a novel marker for early diagnosis of cervical cancer. This novel marker could be based on the theory that within a tumor there exists a small number of tumor stem cells that are responsible for tumor survival and progression [3]. Sox2 is one of the genes which maintain self-renewal of embryonic stem cells and is related to their differentiation potential [4]. So the detection of the expression of Sox2 is of particular importance concerning tumor biology. Several recent studies have demonstrated that the transcription factor Sox2 was related to several human malignant tumors, such as ovarian cancer [5], breast cancer [6], pancreatic cancer [7], lung squamous cell carcinoma [8], and gastrointestinal tumors [9]. However, few studies have reported the expression of Sox2 in cervical SCC, especially in FIGO stages I–II. Therefore, we set out to investigate the expression of Sox2 in normal cervix and cervical SCC, to find out whether the expression of Sox2 correlates with clinicopathological characteristics. Our study also evaluated the prognostic significance of Sox2 expression in the aforementioned FIGO stages.

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Received: 28/09/2013; Accepted: 28/10/2013

Table 1. Sox2 expression in relation to clinicopathological characteristics

Characteristics	*Patients N*	*Sox2 positive expression N (%)*	*Sox2 negative expression N (%)*	x^2, *p*
Age (years)				0.589
≤42	27	21 (77.8)	6 (22.2)	
> 42	28	20 (71.4)	8 (28.6)	
FIGO stage				0.519
I	39	30 (76.9)	9 (23.1)	
II	16	11 (68.8)	5 (31.2)	
Grade of differentiation				0.594
Good	19	13 (68.4)	6 (31.6)	
Moderate	30	24 (80.0)	6 (20.0)	
Poor	6	4 (66.7)	2 (33.3)	
Tumor size (cm)				0.493
<4	40	31 (77.5)	9 (22.5)	
≥4	15	10 (66.7)	5 (33.3)	
Depth of myometrial invasion				0.423
Superficial	45	32 (71.1)	13 (28.9)	
Deep	10	9 (90.0)	1 (10.0)	
Vascular invasion				0.592
Without	50	38 (76.0)	12 (24.0)	
With	5	3 (60.0)	2 (40.0)	
Parametrial invasion				0.313
Without	41	32 (78.0)	9 (22.0)	
With	14	9 (64.3)	5 (35.7)	
Lymph node metastasis				0.181
Without	47	37 (78.8)	10 (21.3)	
With	8	4 (50.0)	4 (50.0)	

Methods

Approval for this study was obtained from the local ethics committee together with written informed consent from each patient.

Tissue sample collection

Formalin-fixed and paraffin-embedded postoperative tissue samples from January 2007 to December 2008 were obtained from the archives of the Department of Pathology of the Second Affiliated Hospital of Soochow University. Fifty-five samples were from patients with FIGO stages I–II cervical SCC and 20 samples were from normal cervix tissue. None of the patients had received radiotherapy or chemotherapy before surgery. The main clinicopathological characteristics of the 55 patients are summarized in Table 1. The patient age ranged between 20 to 68 years (median 42). Thirty-nine (70.91%) patients had FIGO stage I and 16 (29.09%) stage II. Low, intermediate, and high grade disease was seen in 19,30 and 6 cases, respectively. Forty patients (72.73%) had tumors < 4cm and 15 (27.27%) had tumors >4cm. Forty-one (74.55%) patients had no parametrial invasion and 47 (85.45%) had no lymph node metastasis.

Immunohistochemistry

Two serial slides, each 3-μm thick, were cut from paraffin-embedded tissue. One slide was used for hematoxylin & eosin (H&E) staining and the other one for immunohistochemical staining. Anti-human Sox2 mouse monoclonal antibody (AM2048a; Abcam, Cambridge, MA; 1:100 dilution) was used. After deparaffinization and hydration, the slides were subjected to antigen retrieval by pressure-cooking for 30 min. Endogenous peroxidase activity was neutr alized using peroxide block placement on the slides for 15 min at room temperature. The slides were then incubated with anti-Sox2 monoclonal antibody for 30 min at 4°C. This was followed by incubation with peroxidase-conjugated polymer (ChemMate EnVision/HRP;Gene Tech,Shanghai,China) for 30 min at room temperature. Chromogen reaction was developed after exposure of the material to 3,3'-diaminobenzidine tetrahydrochloride (Gene Tech, Shanghai, China) for 10 min. Finally, hematoxylin was used as a light nuclear counterstain.

Assessment of Sox2 expression (Figures 1, 2)

All slides were evaluated independently by two experienced pathologists. The biological intelligence image navigator (Olympus FSX100, Japan) was used for image acquisition of Sox2 with brownish yellow positive cytoplasmic staining. All slides were rated by both the intensity of the staining and the ratio of positive cells. The intensity of the staining was classified as strong (3), medium (2), weak (1), and negative (0) with the ratio of positive cells <10% scoring 0, 10-25% scoring 1, 25-50% scoring 2, 51-75% scoring 3, and > 75% scoring 4. The histological score was defined as the ratio of positive cells score multiplied by the intensity of the staining score. According to the histological score, we divided all slides into 0 (-) negative,1-4 (+) weakly positive,5-8 (++) positive,9-12 (+++) strongly positive, (-) and (+)negative expression, (++) and (+ + +) positive expression [10].

Statistics

The correlations with clinicopathologic characteristics were determined by Chi-square test. The prognostic impact of Sox2 expression with regard to overall DFS was determined by Kaplan–Meier method. For all tests, a two-sided p <0.05 was considered significant.

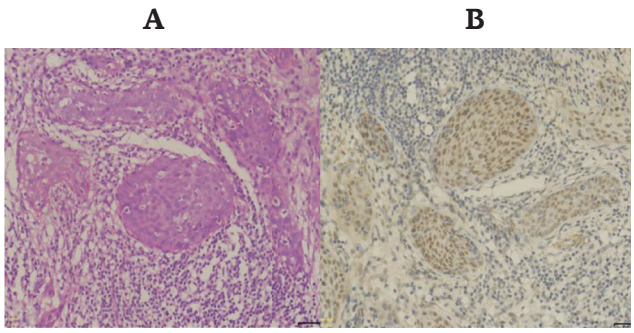

Figure 1. A: Cervical squamous cell carcinoma (H&E); **B:** Expression of Sox2 in cervical squamous cell carcinoma (immunohistochemistry).

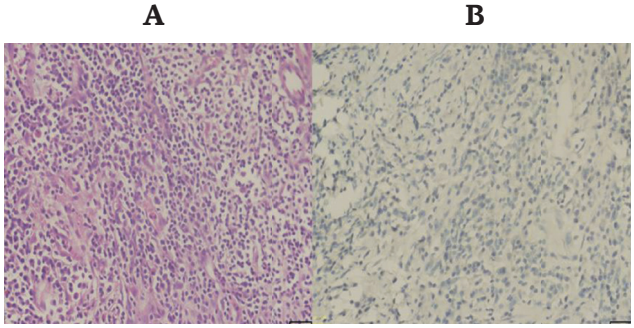

Figure 2. A: Normal cervix staining (H&E); **B:** Expression of Sox2 in normal cervix (immunohistochemistry).

Table 2. Expression of Sox2 in cervical squamous cell carcinoma and normal cervix

Tissue type	*Patients N*	*Sox2 positive expression N (%)*	*Sox2 negative expression N (%)*	*x^2, p*
Normal cervix	20	4 (20.0)	16 (80.0)	
Cervical SCC	55	41(74.5)	14 (25.5)	0.000

SCC: squamous cell carcinoma

Results

Overexpression of Sox2 in cervical SCC

Immunohistochemistry showed that the positive expression rate of Sox2 in normal cervix and cervical SCC was 20.0% (4/20) and 74.5% (41/55), respectively (p=0.000; Table 2).

The relationships between clinicopathological factors and the expression level of Sox2 demonstrated that there was no significant association of Sox2 expression with any of the following: age, FIGO stage, grade of differentiation, size of the tumor, depth of myometrial invasion ,vascular invasion , parametrial invasion or lymph node metastasis.

Sox2 expression and disease-free survival

DFS rate of patients with positive expression of Sox2 was 29.3%, and with negative expression it was 35.7% (x^2=0.837, p=0.360; Figure 3).

Discussion

Sox2, located in chromosome 3q26.3, is a member of the Sox (SRY-related high mobility group box) family, which all contain a high mobility group (HMG) domain very similar to that in the sex-determining gene SRY [11]. So far, more than 20 members of the SOX gene family have been identified and proved to play an important role in stem cell biology, the regulation of organ development, and cell type specification [12]. Several recent studies have demonstrated that the transcription factor Sox2 is related to many human malignant tumors. Maier et al. [13] reported that the expression of

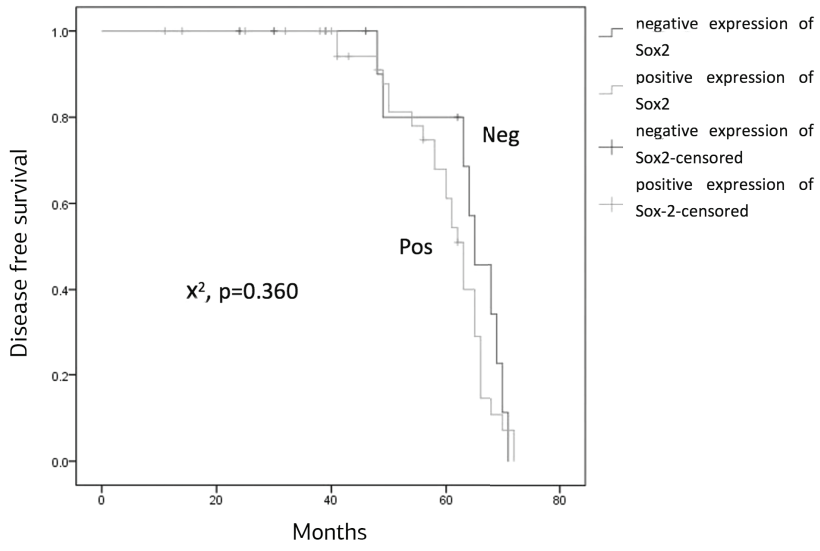

Figure 3. Kaplan–Meier curves of disease free survival according to Sox2 expression in patients with FIGO stage I–II cervical squamous cell carcinoma.

Sox2 was amplified in squamous cell carcinomas of different organ sites, such as squamous cell carcinomas of the lung, esophagus, oral cavity, cervix uteri, penis , and skin. Jing and Zheng [14] reported that using immunohistochemistry, the positive expression rate of Sox2 in normal cervix, CINIII and cervical cancer were 25.0, 83.3 and 77.7%, respectively, the differences being statistically significant; these findings are consistent with our study.

Research encompassing many human malignant tumors confirmed that the expression of Sox2 was related to tumor stage. For example , Schoenhals et al. [15] reported that the expression of Sox2 and the tumor stage were positively correlated in bladder cancer. However, our study found no correlations in cervical SCC, which was consistent with the Jing and Zheng study [14]. These authors also found that Sox2 was expressed in poorly differentiated cervical cancer cells while no expression was found in differentiated cervical cancer cells. This suggests that Sox2 might play an important role in the inhibition of tumor cells' differentiation. However, in our study we didn't find such correlation between the expression of Sox2 and the grade of differentiation, in contrast to the Jing and Zheng study, possibly because our tumor samples were from FIGO stage I–II cases of cervical SCC. These authors reported that the expression of Sox2 was not correlated with other clinicopathological characteristics, such as age, FIGO stage, parametrial invasion or lymph node metastasis. We not only demonstrated them, but also found that the expression of Sox2 had no relationship with the size of the tumor, the depth of myometrial invasion or vascular invasion.

Sholl et al. [16] reported that high expression of Sox2 in lung adenocarcinoma was an independent predictor of poor prognosis. But in cervical SCC we found no obvious correlation between them.

In conclusion, we found that the expression of Sox2 was significantly higher in cervical SCC compared to normal cervix. The expression of Sox2 had no correlations with clinicopathological characteristics such as age, FIGO stage, tumor size, depth of myometrial invasion, vascular invasion, grade of differentiation, or lymph node metastasis. What's more, the expression level of Sox2 had no apparent relation with prognosis in early-stage cervical SCC. However, the results of this retrospective study are potentially limited by the relatively small number of patients, and may not give the most representative picture of this malignancy. Therefore, more studies with larger numbers of patients are required.

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