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Correlation between SOX2 and Survivin clinical features in patients with salivary adenoid cystic carcinoma

Yejiao Luo [a], Tong Liu [a], Wei Fei [b,*], Xiao-Guang Yue [c]

[a] Department of Stomatology, The First Affiliated Hospital of Chengdu Medical College, 610500 China
[b] Department of Stomatology, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Sichuan Provincial Key Laboratory for Human Disease Gene Study, 610072, China
[c] Rattanakosin International College of Creative Entrepreneurship, Rajamangala University of Technology Rattanakosin, Thailand

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ABSTRACT

Objective: In this study, expression of cancer stem cells (CSCs)-related factor-Sex-determining region of Y chromosome-related high-mobility-group box 2 (SOX2) and anti-apoptotic specific factor- Survivin in salivary adenoid cystic carcinoma (SACC) was detected to provide important clues for effective SACC prevention and treatment by combining clinical pathological parameters analysis.
Methods: Paraffin and fresh specimens were collected from SACC patients who underwent surgery at the Oral and Maxillofacial Surgery Department of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. The experimental group was designed as SACC tissue, and the control group normal paracancerous normal gland tissue. (1) SOX2 and Survivin expression were detected using immunohistochemistry and analyzed by comnining clinical pathological parameter analysis. (2) mRNA and protein expression levels of SOX2 and Survivin were detected using RT-PCR, Western Blot.
Results: 1. Immunohistochemistry: (1) SOX2 was mainly expressed on the nucleus. The SOX2 positive rate was 28.57% in clinical stage I–II, and 76.92% in stage III–IV. (2) Survivin was mainly expressed in the cytoplasm. The Survivin positive rate was 61.90% in clinical stage I–II, and 76.92% in stage III–IV. (3) There was a clear correlation between SOX2 and Survivin. 2. RT-PCR and Western Blot: The mRNA and protein expression levels of SOX2 and Survivin were significantly higher in the experimental group than in the control group ($P < 0.01$).
Conclusion: (1) The mRNA and protein expression level of SOX2 and Survivin was significantly higher in SACC tissues than in paracancerous normal salivary gland tissues, indicating that both of the two are tissue-specific and may become SACC oncogenes. (2) SOX2 and Survivin are significantly correlated in expression, which may coorinatively participate in SACC incidence and development.

Introduction

SOX2, an important set of transcription factors in the sex-determining genome, is located on the 3q26.33 chromosomal locus and encodes a transcription factor containing a high mobility group (HMG) DNA junction region [1]. It is usually considered as a necessary condition for maintaining multi-directional differentiation potential of embryonic stem cells (ESCs) and self-renewal of tissue-specific adult stem cells [2]. Currently, SOX2 is believed to maintain the anti-apoptotic properties of tumor cells including stem cells and CSCs by participation in regulation of the complex transcription factor network [3–5]. However, the role and mechanism of SOX2 in the anti-apoptotic properties of salivary adenoid cystic carcinoma (SACC) remains yet unclear.

Survivin, also known as baculoviral inhibitor of apoptosis repeat-containing 5 (BIRC5), is located on the 17q25 human chromosomal locus [6] as the most potent member of the family of inhibitors of apoptosis proteins (IAPs). Survivin can directly inhibit the activity of Caspase-3 and Caspase-7 apoptotic terminal effectors via the classical caspases pathway and prevent cell number reduction by regulating the cell cycle to promote cell proliferation [7–9]. Study on neural stem cells in vitro found that SOX2 can directly upregulate Survivin expression and inhibit mitochondrial-dependent apoptotic pathways. Conversely, inhibition of SOX2 will lead to decreased Survivin expression, and trigger mitochondrial apoptosis pathways, inducing programmed cell death. It suggests

* Corresponding author.
E-mail address: 463445624@qq.com (W. Fei).

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Table 1
Clinical datas.

Type	Case number	parotid gland	salivary gland	sublingual gland	palatine gland	I–II	III-IV
Sieve/tubular type	26	10	3	9	4	19	7
Solid type	8	4	0	3	1	2	6
Total	34	14	3	12	5	21	13

that Survivin exerts its anti-apoptosis effect as downstream signal molecule of SOX2. However, the role of SOX2/Survivin pathway in SACC is yet unclear. This study intends to preliminarily discuss the role of SOX2 and Survivin in SACC incidence and development by detecting their expression in SACC tissue.

Materials and methods

Experimental material

Experimental grouping design

In this experiment, the subjects were divided into experimental group and control group, with SACC tissue in the former, and paracancerous normal gland tissue in the latter.

This experiment has passed the ëthics review of scientific research project by Ethics Committee of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital.

Paraffin specimens

From July 2013 to November 2016, a total of 44 paraffin specimens were collected after surgical resection by the Oral and Maxillofacial Surgery Department, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. The 34 cases in the experimental group were confirmed by postoperative pathology as SACC; the 10 cases in the control group were confirmed by postoperative pathology asparacancerous normal gland tissue. Inclusion criteria: aged 18–80 years old; no treatment before surgery; no other malignant tumors in the whole body; complete surgical paraffin specimens confirmed by postoperative pathology as SACC.

Fresh in vitro tissue samples

From August 2015 to November 2016, fresh in vitro tissue samples were collectd after surgical resection by Oral and Maxillofacial Surgery Department, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. The 14 cases in the experimental group were diagnosed as SACC by clinical and pathological examinations, and the 14 cases in the control group were diagnosed as SACC infiltration-free paracancerous normal gland tissue by intraoperative frozen pathology. The specimens were placed in cryovials immediately after treatment with RNase enzyme inhibitors, and stored at a −80 °C freezer after liquid nitrogen shock cooling.

Clinical data

The 44 specimens were followed up for 26–66 months, including 24 males and 20 females; age: 30–78 years, median age: 49.5 ± 5.2 years; site: 18 cases of parotid gland, 5 cases of submandibular gland, 15 cases of sublingual gland, 6 cases of palatine gland; clinical stage of the experimental group: 21 cases of I–II stage, 13 cases of III–IV stage; microscopic parting of the experimental group: 26 cases of sieve/tubular type, 8 cases of solid type (Table 1).

Main experimental reagents

1) SOX2 rabbit anti-human polyclonal antibody, working concentration 1:100 (Bioworld, USA);
2) BIRC5 rabbit anti-human polyclonal antibody, working concentration 1:100 (Bioworld, USA);

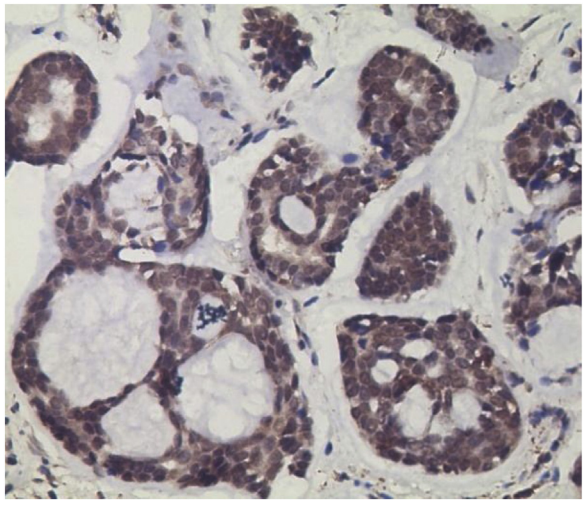


Fig. 1. Positive expression of SOX2 in tubular, cribriform histological subtype (×200).

3) concentrated DAB colorant (zhongshan jinqiao biological Co., LTD., Beijing);
4) PBS phosphate buffer (zhongshan jinqiao biological Co., LTD., Beijing);
5) citrate buffer (zhongshan jinqiao biological Co., LTD., Beijing);
6) AR class hematoxylin (balinway technology Co., LTD., Beijing).

Experimental methods

SOX2 and Survivin expression in paraffin sections were detected using immunohistochemistry and analyzed with clinical data. Meanwhile, mRNA and protein expression in fresh tissues was detected using RT-PCR and Western Blot.

Statistical analysis

IBM SPSS Statistics 20 software was used for statistical analysis of the data, and the correlation between SOX2 and Survivin expressions was tested by fisher's exact probability method. The correlation between SOX2 and Survivin expression was analyzed by Spearman correlation.

Result

In this study, SOX2 and Survivin expression was detected in the experimental group, with negative expression in the control group (Figs. 1–10). SOX2 was nuclear positive, positive expression was found in 16 of the 34 cases in the experimental group, with a positive rate of 47.06%. Low expression was found in one of the 10 cases in the control group. The difference between the experimental group and the control group was statistically significant ($P < 0.05$). Survivin was qualitatively positive, positive expression was found in 23 of the 34 cases in the experimental group, with a positive rate of 67.65%. Low expression was found in 2 of the 10 cases in the control group. The difference between the experimental group and the control group was statistically significant

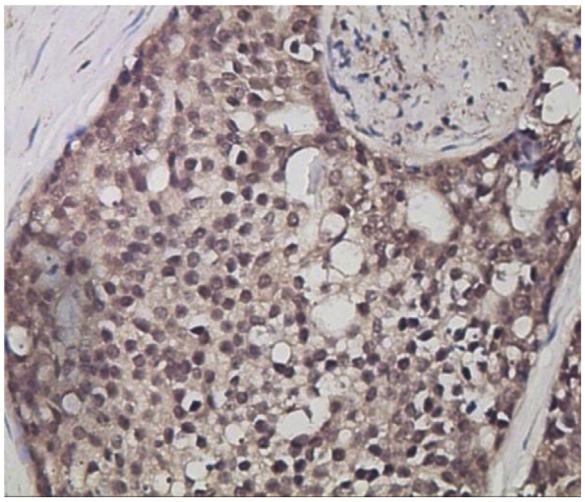


Fig. 2. Positive expression of SOX2 in solid histological subtype (×200).

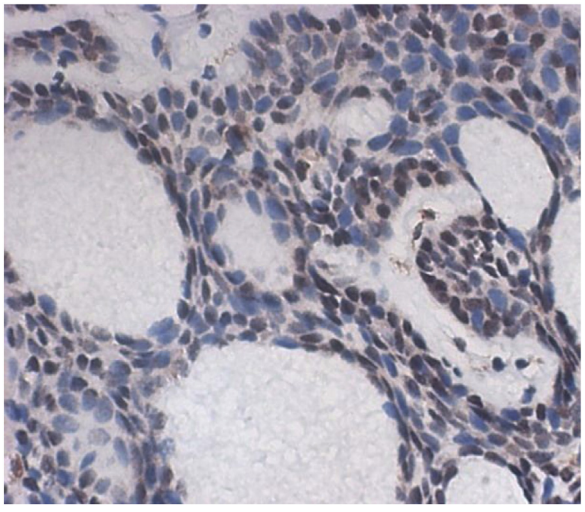


Fig. 3. Low expression of SOX2 in tubular, cribriform histological subtype (×200).

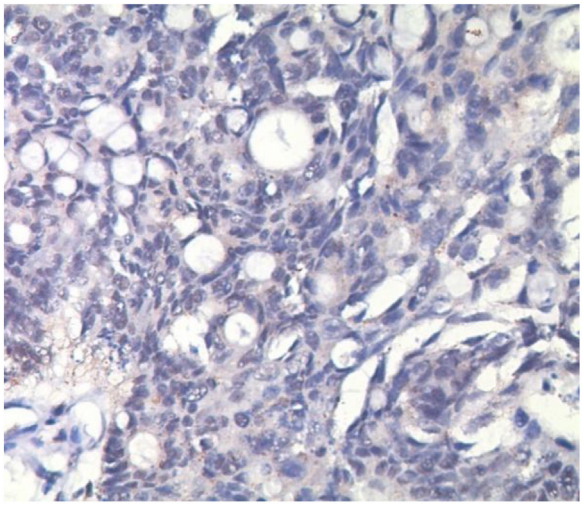


Fig. 4. Low expression of SOX2 in tubular, cribriform histological subtype (×200).

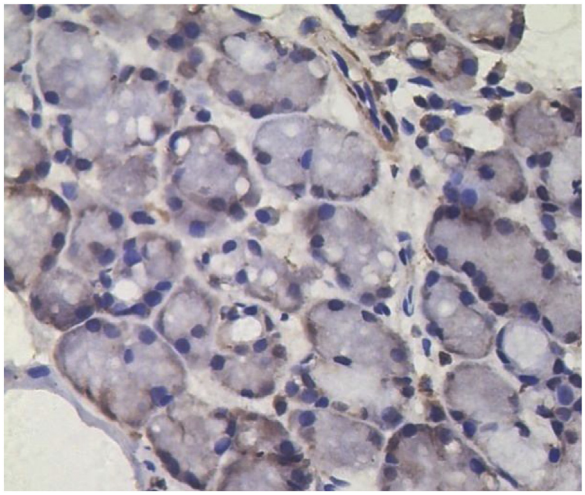


Fig. 5. Negative expression of SOX2 in adjacent normal glandular (×200).

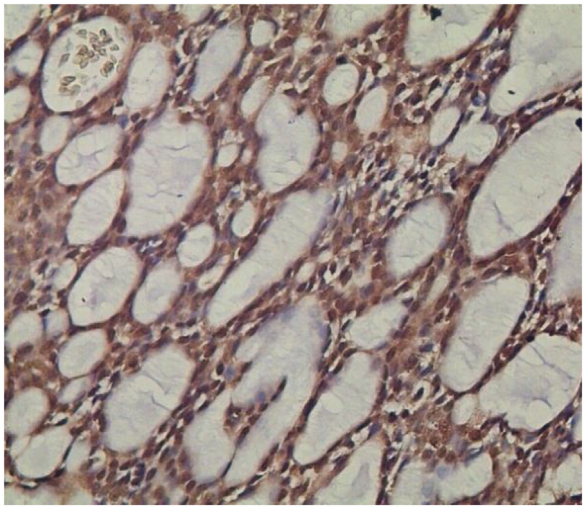


Fig. 6. Positive expression of Survivin in tubular, cribriform histological subtype (×200).

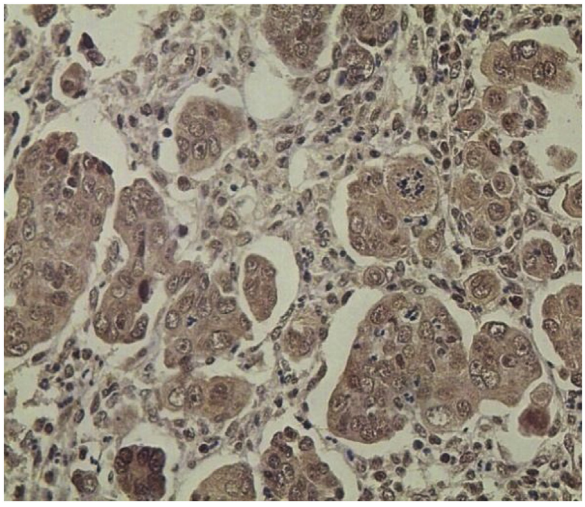


Fig. 7. Positive expression of Survivin in solid histological subtype (×200).

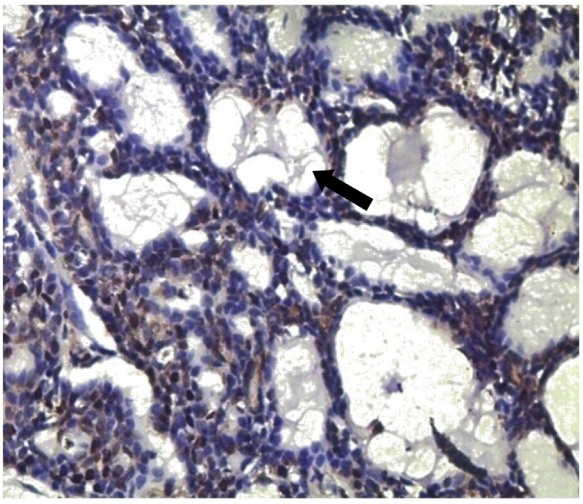


Fig. 8. Low expression of Survivin in tubular, cribriform histological subtype (×200).

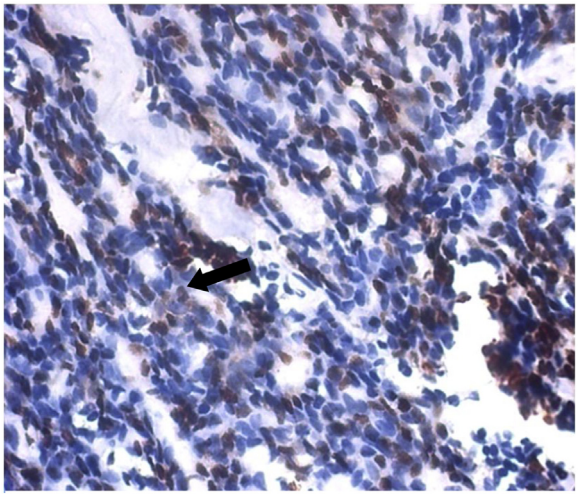


Fig. 9. Low expression of Survivin in tubular, cribriform histological subtype (×200).

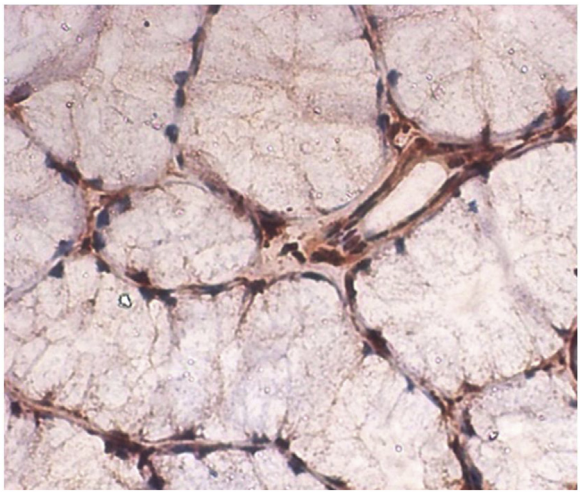


Fig. 10. Negative expression of Survivin in adjacent normal glandular (×200).

(P < 0.05). The following results are obtained from analysis of the above data and clinical material:

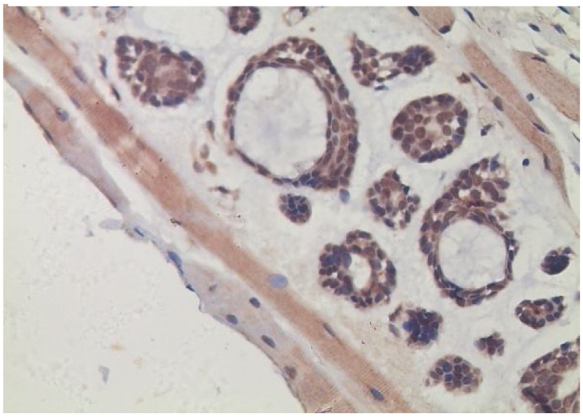


Fig. 11. Positive expression of SOX2 in SACC located in invasion muscle (×200).

Clinical stage

In the experimental group, there are 21 cases in clinical stage I–II, 6 cases (28.57%) showing positive SOX2 expression; 13 cases in stage III–IV, 10 cases (76.92%) showing positive SOX2 expression. The Fisher exact probability method is used to compare the two groups, and the difference is statistically significant (P < 0.05).

In the experimental group, there are 21 cases in clinical stage I–II, 13 cases (61.90%) showing positive Survivin expression; 13 cases in stage III–IV, 10 cases (76.92%) showing positive Survivin expression. The fisher exact probability method is used to compare the two groups, and the difference is not statistically significant (P > 0.05) (Table 2).

Degree of histological differentiation

In the experimental group, there are 26 cases of Sieve/tubular type, 9 cases (34.61%) showing positive SOX2 expression; 8 cases of solid type, 7 cases (87.50%) showing positive SOX2 expression. The Fisher exact probability method is used to compare the two groups, and the difference is statistically significant (P < 0.05).

In the experimental group, there are 26 cases of Sieve/tubular type, 15 cases (57.69%) showing positive Survivin expression; 8 cases of solid type, 8 cases showing positive Survivin expression (100%). The fisher exact probability method is used to compare the two groups, and the difference is statistically significant (P < 0.05) (Table 3).

Age, gender, site

SOX2 and Survivin expression in SACC shows no significant correlation with the patient's age, gender, and tumor site. The difference is not statistically significant (P > 0.05).

Correlation between SOX2 and survivin

Spearman rank correlation analysis of SOX2 and Survivin expression in the experimental group (Table 4) shows significant correlation between the two (r = 0.482, P = 0.004).

RT-PCR experiment results

The mRNA expression of SOX2 and Survivin is higher in the experimental group than in the control group. One-way ANOVA of paired data shows statistically significant difference between the experimental group and the control group in mRNA expression of SOX2 and Survivin (P < 0.01) (Tables 5 and 6, Figs. 12–15).

Table 2
SOX2 and Survivin expression data with clinical stages.

Stage	Case number	SOX2 positive	SOX2 negative	P value	Survivin positive	Sruvivin negative	P value
I–II	21	6	15	0.012	13	8	0.465
III–IV	13	10	3		10	3	

Table 3
SOX2 and Survivin expression data with histological subtypes.

Stage	Case number	SOX2 positive	SOX2 negative	P value	Survivin positive	Sruvivin negative	P value
Sieve/tubular type	26	9	17	0.014	15	11	0.034
Solid type	8	7	1		8	0	

Table 4
The relationship between SOX2 and Survivin expression in SACC (n = 34).

Variable	SOX2		r	P
	P	N		
Survivin			0.482	P = 0.004
P	7	16		
N	9	2		

Abbreviation: N=negative; P=positive.

Table 5
The relative mRNA expression value of SOX2 and Survivin.

	sox2 amplification multiple = $2^{-\Delta\Delta}$CT	P value	Survivin amplification multiple = $2^{-\Delta\Delta}$CT	P value
Experimental group	13.95 ± 14.15	0.002	11.07 ± 7.63	0.000
Control group	1.21 ± 0.69		1.17 ± 0.76	

Note: mean ± standard deviation.

Table 6
The relative mRNA expression value of SOX2 and Survivin at P_{50}, P_{75} and P_{25}.

		P_{50}	P_{75}	P_{25}
SOX2	Experimental group	9.42	17.17	2.99
	Control group	1.2	1.56	0.77
Survivin	Experimental group	11.56	15.78	4.56
	Control group	1.14	1.73	0.65

Note: P_{50}: median; P_{75}: upper quartile; P_{25}: lower quartile.

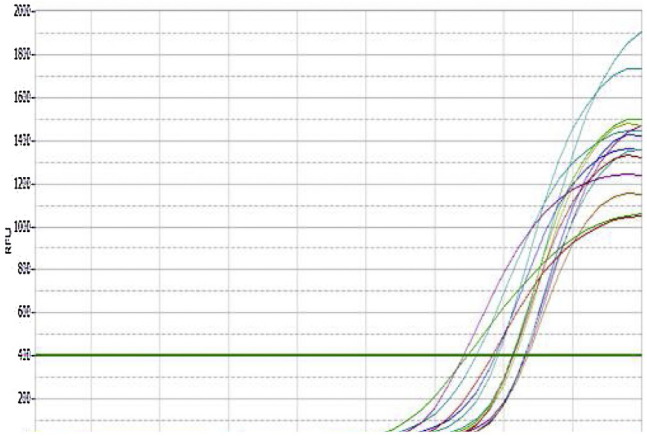


Fig. 12. SOX2 amplification plot.

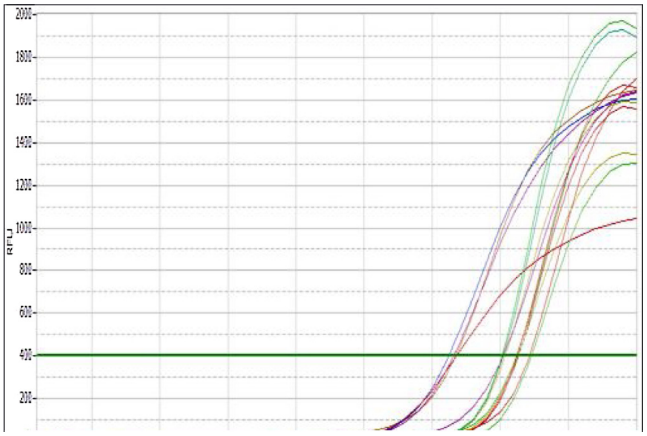


Fig. 13. Survivin amplification plot.

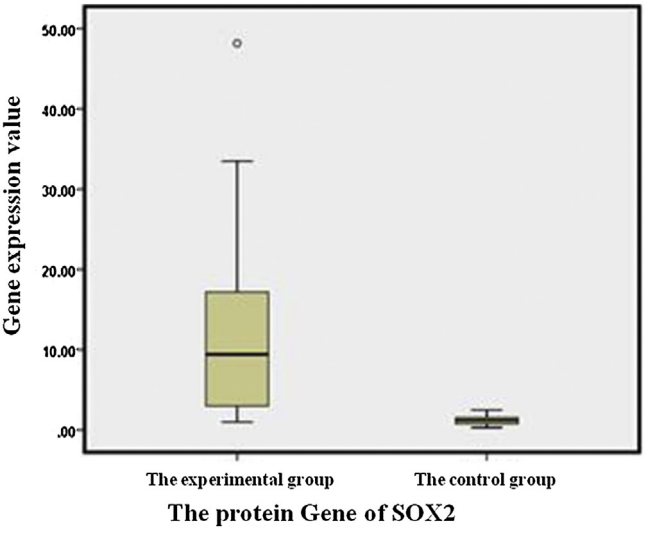


Fig. 14. mRNA of SOX2 amplification status.

Table 7
The relative protein expression value of SOX2 and Survivin.

		Mean ± standard deviation	P value
SOX2	Experimental group	0.80 ± 0.21	0.000
	Control group	0.12 ± 0.04	
Survivin	Experimental group	0.35 ± 0.06	0.000
	Control group	0.10 ± 0.04	

Western blot experiment results

The protein expression of SOX2 and Survivin is higher in the experimental group than in the control group (Tables 7 and 8, Figs. 16–18). One-way ANOVA test on paired sample data finds statistically significant difference in the relative protein expression of SOX2 and Survivin between the experimental group and the control group (P < 0.01).

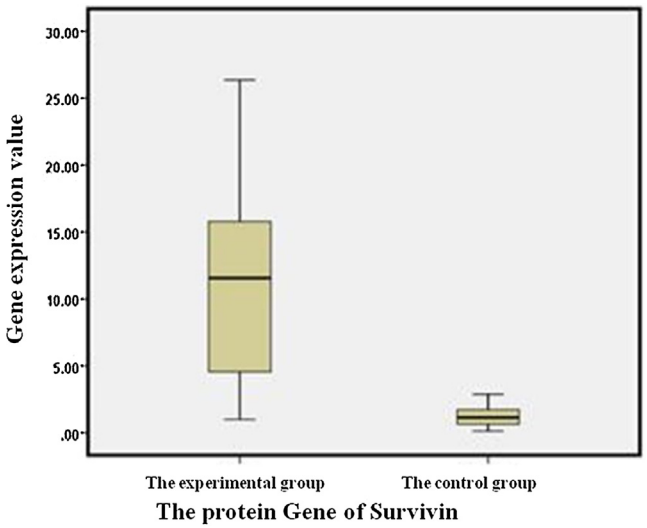


Fig. 15. mRNA of Survivin amplification status.

Table 8
Relative protein expression value of SOX2 and Survivin at P_{50}, P_{75} and P_{25}.

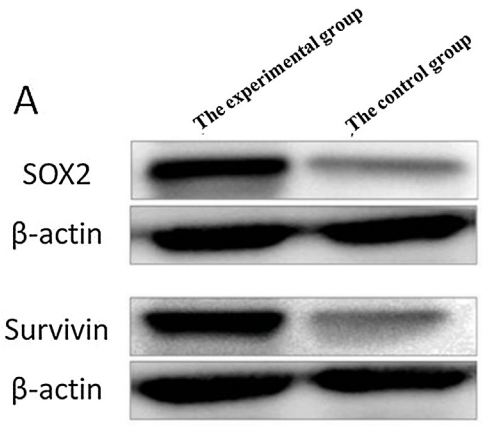
		P_{50}	P_{75}	P_{25}
SOX2	Experimental group	0.76	0.94	0.64
	Control group	0.12	0.15	0.09
Survivin	Experimental group	0.34	0.40	0.30
	Control group	0.09	0.12	0.06

Note: P_{50}: median; P_{75}: upper quartile; P_{25}: lower quartile.

Discussion

CSCs constitute a very small group of cells in tumors, which can maintain self-renewal and resistance to apoptosis as stem cells. Colnaghi and Wheatley [10] found expression of SOX2, Nanog, Oct4 and stem cell surface marker protein CD44 in further isolation of human prostate cancer stem cells. SOX2 maintains stem cell characteristics and CSCs survival by participation in regulation of complex transcription factor networks [11], and it is expression is found in a variety of malignant tumor cells [12–14]. SOX2 is also involved in the regulation of cell differentiation. Studies have confirmed that SOX2 overexpression can enhance the differentiation of human umbilical cord blood cells in vivo by naive transformation of cells [15]. Zeuschner et al. [16] found that SOX2 promotes malignant transformation of glioblastoma by regulating cell plasticity and astrocyte differentiation. SOX2 positive rate was 47.06% in this study, with different expression levels in SACCs with different differentiation degrees. It indirectly proves possible presence of CSCs in SACC. In this study, it was also found that for the 18 cases (17 cases of sieve/tubular type, 1 case of solid type) of SACC with no or low SOX2 expression, SOX2 was not expressed in the center of the tumor, but surrounding the tumor or adjacent invaded tissues such as muscle (Fig. 11). It may be possible that the peripheral tumor cells are expanding outwards; the tumor cells invading the surrounding tissues excite a signal resulting in increased SOX2 expression in this region. It may also be possible that the involvement of SOX2 high expression in this region makes tumor tissue in the region more aggressive. This suggests that SOX2 may be involved in the invasion or proliferation of tumor cells in relatively active regions. The reason for this particular phenomenon is not yet clear, which demands further in-depth study.

Survivin has no or low expression in terminally differentiated normal tissues, but is re-expressed by converting terminally differentiated cells into cell lines [17]. Thus, Survivin can be defined as a



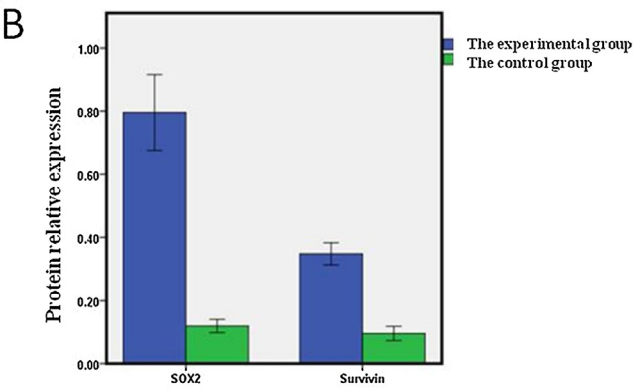


Fig. 16. The relative protein expression value of SOX2 and Survivin; A:SOX2, Survivin express in experimental group and control group through WB; B: Relative protein expression between experimental group and control group through WB ($P < 0.01$).

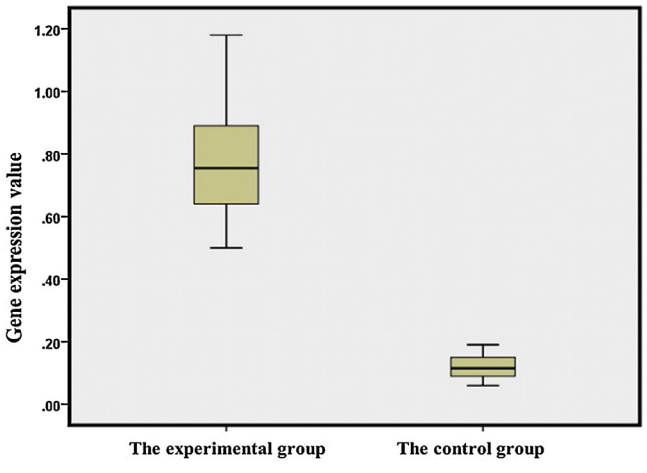


Fig. 17. Relative protein expression value of SOX2.

unique expression gene in cancer tissue, i.e. oncogene [18]. Survivin is involved in the formation of blood vessels in the body tissues. It may maintain normal proliferation of vascular endothelial cells as an anti-apoptotic protective gene [19]. Survivin may promote tumor metastasis by participation in tumor angiogenesis and anti-apoptosis effect. The positive rate of Survivin was 67.65% in this study, which was significantly higher than that of paracancerous

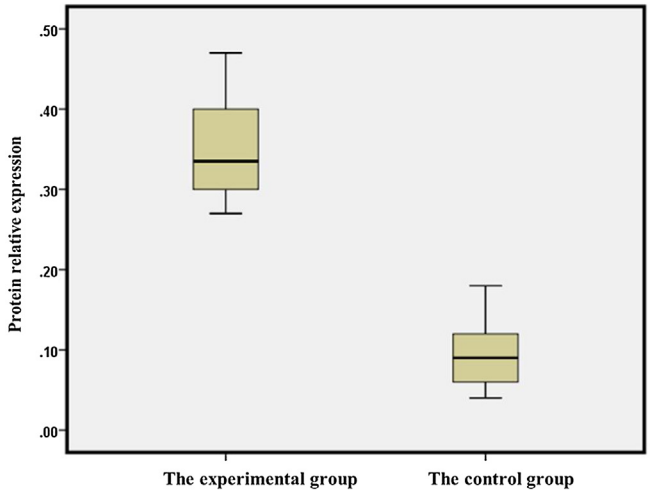


Fig. 18. Relative protein expression value of Survivin.

normal gland tissue. Meanwhile, correlation was found between abnormally high expression of Survivin and poor prognostic indicators such as metastasis, suggesting that Survivin may play an important role in SACC incidence and development as an oncogene. In most studies, Survivin overexpression has been found to be associated with local recurrence and metastasis in many malignancies [20,21]. The results of this study show that Survivin promotes SACC metastasis, which is however irrelevant with recurrence. The reason may be that SACC has properties different from other tumors. Tumors progress slowly with late timing in recurrence and metastasis. Therefore, the metastasis and recurrence are relatively hidden [22–25]. It may also be possible that the slower course of disease and the limited follow-up years results in missing metastasis and recurrence cases, thereby causing the differential result.

Some studies have found that SOX2 can upregulate the expression of Survivin and then inhibit mitochondrial dependent apoptotic pathways. Conversely, silent SOX2 can down-regulate the expression of Survivin [14]. Overexpression of SOX2 and Survivin can increase drug resistence of ovarian epithelial cancer to paclitaxel chemotherapy, causing reduced efficacy [16]. In the experiment, Spearman rank correlation analysis was made for SOX2 and Survivin expression in the experimental group, showing significant correlation between the two ($r = 0.482$, $P = 0.004$). In the experiment, the mRNA and protein expression of SOX2 and survivin was higher in the experimental group than in the control group, suggesting that Survivin may be a downstream regulatory gene of SOX2, and the possible interaction between them may give rise to the therapeutic resistance of tumor cells.

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Competing interests

None declared.

Ethical approval

Not required.

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