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Original Article

Clinical significance of SOX2 and snail expression in esophageal squamous cell carcinoma

Na Miao[1*], Zhiqiang Wang[2*], Liping Su[1], Jinlian Ge[3], Wenmei Ma[1], Junzhi Li[1], Ming Liu[1]

Departments of [1]Pathology, [3]Clinical Laboratory, The First Affiliated Hospital of Xinjiang Medical University, Xinjiang 830054, Urumqi, China; [2]Armed Police Hospital of Xinjiang, Xinjiang 830054, Urumqi, China. [*]Equal contributors and co-first authors.

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Abstract: Objective: To study the SOX2 and Snail expression in esophageal cancer and their relationships with tumor biological behavior. Methods: Immunohistochemical method was used to study the SOX2 and Snail1 expression in 60 cases of esophageal squamous cell carcinoma patients with lymph node metastasis. Statistical analysis was done based on the immunohistochemistry data and the disease profile. Results: SOX2 expression level is positively correlated with Snail expression level in esophageal squamous cell carcinoma patients with lymph node metastasis ($r = 0.583$, $P < 0.05$). However, SOX2 or Snail expression levels are not correlated with the patients' age, tumor differentiation grade, TNM stage, or clinical pathology data ($P > 0.05$), in the same patient cohort. SOX2 expression level alone is not correlated with the patients' age, tumor differentiation grade or TNM stage ($P > 0.05$); However, SOX2 and Snail are both positively expressed in one case of esophageal squamous cell carcinoma with lymph node metastasis. Conclusion: Our study demonstrates SOX2 and Snail levels are upregulated in esophageal squamous cell carcinoma tissue with lymph node metastasis, and their expression levels are positively correlated. Our data suggests SOX2 and Snail are playing a role in carcinogenesis of esophageal squamous cell carcinomas.

Keywords: ESCC, IHC, SOX2, snail

Introduction

Esophageal carcinoma is a type of malignant tumor originated from esophageal squamous epithelium and columnar epithelial malignancies. It is one of the six fatal cancer types in the world, and also China's fourth lethal malignancies [1, 2]. Histology wise, esophageal carcinoma has two major types: squamous cell carcinoma of the esophagus (or esophageal squamous cell carcinoma, ESCC) and esophageal adenocarcinoma (EAC). In western countries, esophageal adenocarcinoma (EAC) is the major histological type, but in China, Japan and other Asian countries, ESCC is the major pathological type of esophageal cancer [3]. Esophageal disease research is related to environment, diet, nutrition, viruses, drinking water and food nitrosamines, mycotoxins. In recent years, more and more publications are related to proteomics and genetics. But there is still no clear tumorigenesis mechanism of esophageal cancer. Although in recent years, there has been progresses in the treatment of esophageal cancer including novel drugs and surgery, the postoperative survival rate has not been greatly improved, which only showed 10-20% increase of survival rate [4]. Therefore, it is of great practical significance to explore the pathogenesis, and search for potential prognostic indicators and effective drug targets for esophageal carcinoma.

SOX2 is a transcription factor that is located on chromosome 3. It is characterized by a highly conserved group of high-mobility group (HMG). SOX2 family proteins (including SOX2) could activate or inhibit the target gene expression through their HMG domain binding to the specific DNA sequence [5]. SOX2 is playing a key role in stem cell research, early embryonic development, organ formation, neural differentiation, and other important events which has drawn increasing attentions in the field. Studies have shown SOX2 is involved in the tumor development of esophageal cancer [6], gastric can-

cer [7], colon cancer [8], lung cancer [9] breast cancer [10] and other tumor types. Currently, the role of SOX2 in esophageal cancer is still unclear. In recent years, it has become a hot spot to research the regulation and expression of esophageal cancer related genes. Information statistics analysis has shown there are multiple Snail binding sites in the SOX2 promoter region. Therefore, we hypothesized that SOX2 can promote the occurrence and development of esophageal squamous cell carcinoma through its interaction with Snail.

Material and methods

Patients and tissue samples

60 cases of esophageal samples of esophageal squamous cell carcinoma patients underwent transthoracicsubtotal esophagectomy and 40 lymph node samples from patients with metastasis were collected from the First Affiliated Hospital of Xinjiang Medical University (2011 to 2014) and First People's Hospital of Aksu Prefecture (2004 to 2014). Clinical data were collected by reviewing medical records and pathologic data. All patients enrolled in the study have consent forms signed and the study was approved by the medical ethics committee of Xinjiang Medical University. All the surgical specimens resected were fixed in 10% formalin and embedded in paraffin. Tissue sections (3 μm) were prepared in serials and stained for SOX2 and Snail.

Immunohistochemistry

Tissue sections were processed with xylene (3×10 min) and alcohol gradient for deparaffinization and hydration, after which sections were heated in 0.01 M citrate buffer solution (pH 9.0) for 20 minutes for antigen retrieval. The sections were cooled to room temperature (RT) before being treated with 3% H_2O_2 in methanol for 15 minutes to block endogenous peroxidase activities. After incubation overnight at 4°C with the primary antibodies against SOX2 (1:100, rabbit monoclonal antibody, Cell Signaling) or Snail (1:200, rabbit monoclonal antibody, Bioworld), the sections went through incubation one more time for 30 minutes with the secondary antibodies (ZSGB, China) at 37°C and stained with DAB solution. After counterstaining with hematoxylin, the sections were sealed after passing through ethanol gradient.

The results of immunohistochemical processing for SOX2 (nucleic staining) and Snail (nucleocytoplasmic staining) were analyzed by two independent experienced pathologists blindly. Ten areas were selected randomly and the rating was done according to the methods reported previously.

Staining density was graded as follow: 1 (≤ 25% staining); 2 (26%-75% staining) and 3 (≥ 76% staining). Staining intensity evaluated: 1 for no positive staining; 2 for mild staining; and 3 for intense staining. The two grades of each specimen were then multiplied and categorized into negative (-) for scores < 3 and positive (+) ≥ 3.

Statistical analysis

Using SPSS 17.0 statistical software for statistical analysis results, respectively using Wilcoxon Signed Ranks, Test method to compare SOX2 in esophageal squamous carcinoma and its corresponding adjacent tissues, SOX2 and Snail in esophageal squamous carcinoma and its corresponding lymph node metastasis carcinoma expression difference. compare SOX2 and Snail expression level of grading and the relationship between clinical pathological parameters between the two groups with Mann-Whitney U test, use Kruskal - between multiple sets of Wallis test. SOX2 and Snail in esophageal squamous carcinoma and its corresponding lymph node metastasis in the organization express correlation with Pearson correlation analysis test. Differences were considered to be statistically significant when P value < 0.05, and all P values are two-tailed in all analyses. The TNM staging was following esophageal squamous cell carcinoma TNM staging criteria (UICC, 2009 specific staging criteria for phase 0: Tis N0 M0; phase I: T1 N0 M0; phase II: T2 N0 M0; phase III: T3 N0 M0; T1 N1, M0; T2 N1, M0; T3, N1, M0; phase IV: T4; for any N, M0; any T, N, M1.

Results

60 cases of esophageal squamous cell carcinoma and 40 cases which have lymph node metastasis corresponding to primary tumor tissue were IHC stained for SOX2 and Snail.

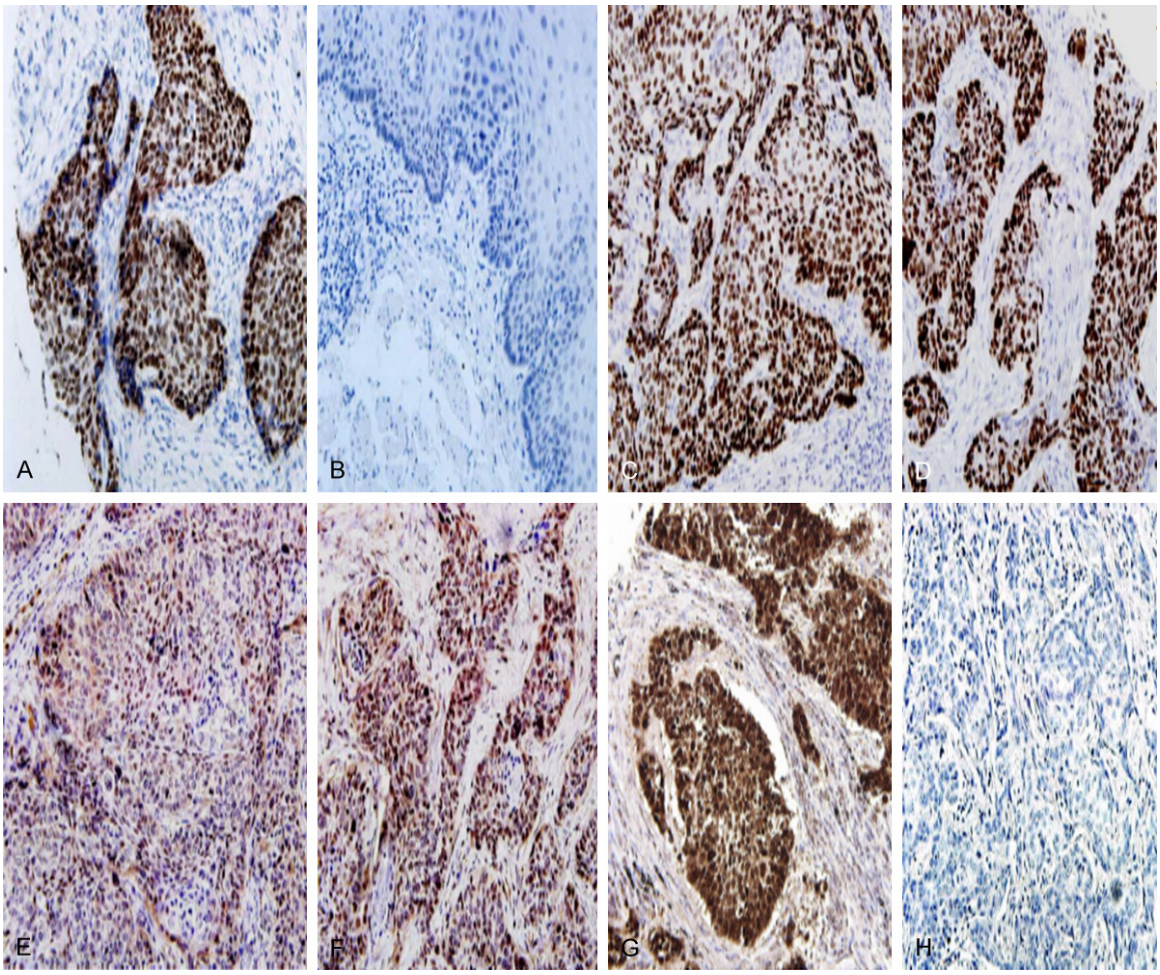

Figure 1. A. SOX2 expression in esophageal squamous carcinoma tissues (×200). B. SOX2 expression in tumor adjacent tissues (×200). C. SOX2 expression in esophageal squamous carcinoma tissues (×200). D. SOX2 expression in lymph node metastasis carcinoma tissues(×200). E. The expression of Snail in esophageal squamous carcinoma tissues (×200). F. Snail expression in lymph node metastasis carcinoma tissues (×200). G. Positive control of SOX2 in lung squamous cell carcinoma (×200). H. Negative control of esophageal squamous cell carcinoma.

Table 1. SOX2 expression in esophageal squamous cell carcinoma and its corresponding tumor adjacent tissues

Histological type	Cases number	Positive number	Negative number	*P* Value
Esophageal carcinoma	57	29	28	0.0001
Peritumoral tissue	57	9	48	

The expression of SOX2 in esophageal squamous cell carcinoma, tumor adjacent tissue and metastatic lymph nodes

The expression of SOX2 in esophageal squamous cell carcinoma tissue was localized in the nucleus (**Figure 1**). The positive expression in esophageal squamous cell carcinoma (ESCC) rate was 51% (29/57), while the positive expression rate in tumor adjacent tissue was only 16% (9/57). The results showed that SOX2 expression in esophageal squamous cell carcinoma was higher than that in the tumor adjacent tissues ($P < 0.05$), suggesting that SOX2 expression was upregulated in esophageal squamous cell carcinoma (**Figure 1**; **Table 1**). Combined with clinical and pathological data, analysis showed that the expression of SOX2 was irrelevant with age, tumor differentiation, or TNM stage ($P > 0.05$) (**Table 2**). In the 40 cases of esophageal squamous cell carcinoma with lymph node metastasis cohort, the expression of SOX2 was detected, and the positive expression rate of SOX2 was 45% in esoph-

Table 2. The correlation of SOX2 expression and clinical/pathological data of esophageal squamous cell carcinoma

Clinicopathological parameters	Cases number n = 57	Staining intensity				*P* Value
		I	II	III	IV	
Age						
≥ 60	23	10	3	2	8	0.360
< 60	34	18	6	2	8	
Gender						
Male	40	23	7	1	9	0.057
Female	17	5	2	3	7	
Degree of differentiation						
Well	6	2	3	0	1	0.247
Moderately	26	10	3	3	10	
Poorly	25	16	3	1	5	
TNM stage						
I	2	0	1	0	1	0.563
II	49	24	7	4	14	
III	6	4	1	0	1	
IV	0	0	0	0	0	

Table 3. SOX2 expression in esophageal squamous cell carcinoma and its corresponding lymph node metastasis

Histological type	Cases number	Positive number	Negative number	*P* Value
Esophageal carcinoma	40	18	22	0038
Lymph node metastasis	40	11	29	

ageal squamous cell carcinoma (18/40), which was significantly higher than that in metastatic lymph node tissues (28%, 11/40) (**Figure 1**; **Table 3**), (P < 0.05). This data suggested that SOX2 was highly expressed in esophageal squamous cell carcinoma.

The expression of snail in esophageal squamous cell carcinoma and its corresponding lymph node metastasis

The expression of Snail in esophageal squamous cell carcinoma was located in cytoplasm and nucleus, with a major distribution in the nucleus (**Figure 1**). The positive rate in squamous cell carcinoma was 68% (27/40), while the positive rate was 65% in the corresponding lymph node metastasis (26/40) (**Figure 1**; **Table 5**). 80.6% of Snail positive expression (25/31) was located in the nucleus, and 19.4% was localized in the cytoplasm (6/31). Therefore, there was no significant difference in the expression of Snail in esophageal squamous cell carcinoma and its corresponding lymph node metastasis (P > 0.05).

The expression of SOX2 and Snail in esophageal squamous cell carcinoma and its corresponding lymph node metastasis was not significantly correlated with clinical pathological data (age, tumor differentiation, TNM staging) (P > 0.05) (**Tables 4**, **6**). But, the expressions of SOX2 and Snail in esophageal squamous cell carcinoma and its corresponding lymph node metastasis carcinoma tissue were positively correlated, r = 0.583, P < 0.05 (**Table 7**).

Discussion

SOX2 is a transcription factor located on chromosome 3. It is in the SOX gene family that is associated with the SRY gene, mainly involved in the regulation of embryonic development and cell fate. SOX2 is involved in the development and progression of multiple tumors, such as esophageal cancer, gastric cancer, colon cancer, lung cancer and breast cancer. The transcription factor Snail is the first member found in Snail super family. Snail is highly expressed in a variety of epithelial tumors, such as stomach cancer, colon cancer, breast cancer, cervical cancer and ovarian cancer. Snail is reported to participate in the epithelial-interstitial transformation, and is closely related to the invasion and metastasis of tumor cells. Sox2 expression level is correlated to the tumor pathological types, with higher expression level in squamous cell carcinoma compared to adenocarcinoma. SOX2 also showed increased expression in a variety of squamous cell carcinoma including esophagus, lung, mouth, skin, cervix, and penis [11]. Esophageal carcinoma is a common malignant tumor which showed a feature of regional distribution in China. It has higher incidence and mortality rate in Henan, Hebei, Jiangsu, Shanxi, Xinjiang and etc. Surgery and postoperative radiotherapy and chemotherapy are major therapeutic approaches in esophageal cancer therapy.

Table 4. The correlation between SOX2 expression in esophageal squamous cell carcinoma and its corresponding lymph node metastasis and clinicopathological data

Clinicopathological parameters	Cases number n = 40	Staining intensity				*P* Value
		I	II	III	IV	
Age						
≥ 60	17	8	2	2	7	0.410
< 60	23	11	3	2	7	
Gender						
Male	21	13	4	1	3	0.063
Female	19	6	3	5	5	
Degree of differentiation						
Well	4	1	2	0	1	0.187
Moderately	17	3	7	3	4	
Poorly	19	11	2	3	3	
TNM stage						
I	1	0	1	0	0	0.436
II	25	14	3	3	5	
III	14	4	6	5	5	
IV	0	0	0	0	0	

Table 5. Snail expression in esophageal squamous cell carcinoma and its corresponding lymph node metastasis

Histological type	Cases number	Positive number	Negative number	*P* Value
Esophageal carcinoma	40	27	13	0.966
Lymph node metastasis	40	26	14	

Table 6. The correlation between Snail expression in esophageal squamous cell carcinoma and its corresponding lymph node metastasis and clinicopathological data

Clinicopathological parameters	Cases number n = 57	Staining intensity				*P* Value
		I	II	III	IV	
Age						
≥ 60	23	6	7	6	4	0.640
< 60	17	7	2	3	5	
Gender						
Male	29	9	3	9	8	0.541
Female	11	4	6	0	1	
Degree of differentiation						
Well	6	2	3	1	0	0.477
Moderately	16	3	4	4	5	
Poorly	18	8	2	4	4	
TNM stage						
I	0	0	0	0	0	0.164
II	10	3	2	4	1	
III	30	10	7	5	8	
IV	0	0	0	0	0	

The incidence of esophageal cancer is associated with risk factors including: human papillomavirus (HPV), chemical carcinogens susceptibility genes, activation of keratin and oncogenes or inactivation of tumor suppressor genes, cell cycle regulation abnormality and etc. It is a complicated process that involved multiple gene interactions [12]. Therefore, treatments targeting a single gene usually could not achieve efficient tumor growth inhibition. There are many regulations of gene expression control points in gene activation, transcription, transcriptional processing, translation, post-translational process since gene expression regulation is a multi-stage process.

Transcription factors are playing a crucial role in maintaining tumor differentiation, proliferation, metastasis and response to treatment. Therefore, more focus should be paid to the transcription factors SOX2 and Snail in esophageal squamous cell carcinoma. In this study, immunohistochemical analysis suggested the positive expression of SOX2 in esophageal squamous cell carcinoma rate was 51% (29/57), higher than the corresponding non-tumor adjacent tissue (16%, 9/57). Sox2 was positively expressed in 45% (18/40) esophageal squamous cell carcinoma and 28% (11/40) paired lymph node metastasis samples. The IHC positive rate for Snail was 68% (27/40) and 65% (26/40).

The expressions of SOX2 and Snail were positively correlated ($r = 0.583$, $P < 0.05$). SOX2 expression was localized in the esophagus squamous cell nucleus while most of Snail expression was in the nucleus.

The underlying mechanism remains to be further investigated. The tumor occurrence is a multi-step process, involving activation of a variety of genes and inactivation of tumor suppressor genes. Since the genetic material of eukaryotes was located in nucleus, mRNA transcription has to be translocated from the nucleus into cyto-

Table 7. The correlation between the expression of SOX2 and Snail in esophageal squamous cell carcinoma and its corresponding lymph node metastasis

SOX2	Snail		r	*P* value
	Negative	Positive		
Negative	27	24	0.538	0.0001
Positive	0	29		

plasm. Thus, transcription and translation process are not coupled together which may be the result of biological evolution. The sites of transcription and translation are not isolated, primary transcripts will be directly translated into protein, memory cells who have a large number of non functional proteins will affect the normal physiological activities.

There are many functional proteins which are synthesized by ribosomes in the cytoplasm: such as histones, DNA polymerase, RNA polymerase, gene regulatory proteins and etc. However, they enter into the nucleus through the pores of the nuclear membrane under the guidance of the nuclear localization signal. The expression of transcription factor is localized in the nucleus and plays a role of transcriptional regulation. The experiment results proved that SOX2 and Snail may be involved in the occurrence and development of esophageal squamous cell carcinoma. Along with the discovery of more specific markers, signaling pathways, and tumor stem cells with deeper understandings, it is of great significance to elucidate the pathogenesis of esophageal cancer, and to prevent tumor metastasis, recurrence, as well as provide new therapeutic targets for clinical treatment.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Junzhi Li and Ming Liu, Department of Pathology, The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, Xinjiang, China. E-mail: 1242738181@qq.com (JZL); zisuzi89757@163.com (ML)

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