EXPRESSION OF SOX9 IN ESOPHAGOGASTRIC JUNCTION ADENOCARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL CHARACTERISTICS AND PROGNOSIS

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ABSTRACT

Objective*: We aim to explore the expression of SOX9 in adenocarcinoma tissue at the esophagogastric junction and its correlation with clinicopathological features and prognosis.*

Methods: *From January 2013 to May 2014, 114 samples of AEG tissues and 72 samples of normal paracancerous tissues at the surgical margin from the Department of Pathology at our hospital were collected. Using immunohistochemistry, we examined the esophagogastric junction adenocarcinoma (AEG) samples and compared their expression levels of human sex-determining region Y box protein 9 (SOX9) to SOX9 expression levels in adjacent cancer tissues. We further analyzed the correlation between SOX9 expression levels and clinicopathological characteristics and prognosis.*

Results: *The high expression rate of SOX9 in AEG tissues was 46.49% (53/114), which was significantly higher than the low expression rate of SOX9 in adjacent tissues—8.33% (6/72). The expression of SOX9 was correlated with the Lauren classification, tumor invasion depth, lymph node metastasis, distant metastasis, and the TNM stage in patients with AEG (P<0.05). It was not related to the patients' sex, age, tumor location, or tumor diameter tissue differentiation (P>0.05). According to Kaplan-Meier analysis, the 5-year survival rate of patients with high SOX expression was 30.19% (16/53), which was significantly lower than the 5-year survival rate of patients with low SOX expression (54.10%; 33/61). COX multivariate analysis revealed that the degree of tissue differentiation, depth of infiltration, and distant metastasis were independent risk factors affecting the prognosis of patients with AEG.*

Conclusion: *SOX9 is highly expressed in AEG tissues. Its expression level is related to the occurrence, development, invasion, and metastasis of AE. While it affects the prognosis, SOX9 cannot be used as an independent risk factor for AEG patients.*

Keywords: *SOX9, esophagogastric junction adenocarcinoma, clinicopathological features, prognosis, correlation.*

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Introduction

In recent years, the incidence of adenocarcinomas of the esophagogastric junction (AEG) in the esophagus and stomach has gradually increased globally, accounting for approximately 50% of all gastric cancers. This may be related to the development of population screening and the application of endoscopic technology[(1)]. However, the incidence of AEG in China is unclear. Epidemiological statistics from areas with a high incidence of esophageal cancer in China have found that the morbidity and mortality of esophageal cancer decrease with the increasing incidence and mortality of gastric cancer (primarily cardia cancer onset), indicating that the incidence of AEG in China is on the rise[(2)]. According to the relationship between the tumor body and the dentate line, AEG can be classified according to three classification systems: Siewert, WHO, and Liverpool. The Siewert system is recognized both in China and internationally[(3)]. The pathogenesis of AEG is not clear. Gastroesophageal reflux disease, esophageal hiatus, long-term chronic gastric acid, and Barrett's esophageal cancer are related to the occurrence of Siewert type I, while cardia metaplasia and Helicobacter pylori infection are related to Siewert type II[(4)]. Clinical studies have shown that obesity, smoking, and certain drugs can cause AEG, while eating more lutein, cellulose, and vitamin B6 can significantly reduce the risk of AEG[(5)]. Current-

ly, the treatment of AEG both in China and internationally is based primarily on surgery. The goal is to completely remove the primary focus of the tumor and thoroughly dissect the local lymph nodes[6]. AEG has various disease characteristics that differ from esophageal and gastric cancers: it has generated more controversy regarding approaches, methods, prognostic factors, and adjuvant treatment with various types of surgery and about 50% of patients have recurrence after AEG radical surgery[7]. Early AEG screening remains technically unfeasible, so the detection of tumor markers can be an indicator for early SEG screening[8]. Human sex-determining region Y-box protein 9 (SOX9) belongs to the E subfamily of the SOX gene family; it participates in gonadal formation and cartilage differentiation[9].

Several studies have shown that SOX9 is highly expressed in a variety of tumor tissues such as lung adenocarcinoma and breast cancer; its high expression is predictive of poor prognosis[10]. This study aims to investigate the expression of SOX9 in AEG tissues and its correlation with clinicopathological features and prognosis.

Materials and methods

General information

From January 2013 to May 2014, we collected 114 AEG tissues and 72 normal cancerous tissues at the surgical margin from our pathology department.

We applied the following inclusion criteria:

- Aged 18 to 65 years;
- The case was clearly AEG before surgery;
- No chemoradiotherapy was performed before surgery;
- The patient agreed and provided informed consent;
- Participation was approved by the hospital ethics committee.

There were 60 males and 54 females, with an average age of 40.32±15.65 years. All patients were followed for more than 5 years. Survival was counted from the date of surgery to the end of follow-up or the date of death.

Primary reagents and instruments

Reagents

EnVision detection kit (Shanghai Kelei Biotechnology Co., Ltd.), SOX9 antibody (Beijing Biolab Technology Co., Ltd.), p-21 antibody, CyciinE antibody (Shanghai Univ Biotechnology Co., Ltd.)

Instruments

Fluorescence microscope (Olympus), real-time PCR instrument (Nanjing Vedeng Medical Co., Ltd.), low-temperature high-speed centrifuge (Yancheng Anxin Experimental Instrument Co., Ltd.), CO_2 cell incubator (Panasonic Health Medical Equipment Co., Ltd.), thermostatic shaker (Dongguan Kezheng Instrument Co., Ltd.), inverted microscope (Shanghai Zhengye Instrument Equipment Co., Ltd.), protein electrophoresis tank (Shanghai Shujun Instrument Equipment Co., Ltd.), Ultra-pure water machine (Shenzhen Kerui Environmental Protection Equipment Co., Ltd.), Protein Nucleic Acid Analyzer (Shanghai Farun Scientific Instrument Co., Ltd.).

Methods

Immunohistochemical staining

Tissues were fixed with neutral formaldehyde after resection, dehydrated with gradient alcohol, and embedded in paraffin. We performed 5μm serial sections, strictly following the operation steps of the immunohistochemistry kit.

The specimens were baked at 60oC, dewaxed, and dehydrated. They were soaked in citrate buffer (PH7.4) for antigen retrieval for 30 minutes, placed in 3% H_2O_2 for 10 minutes, and left at room temperature with goat serum for 30 minutes. Primary antibodies were added and the specimens were incubated overnight. They were rinsed 3 times with PBS solution (5min/time). Secondary antibodies were added and the specimens were incubated for 1h at room temperature and rinsed 3 times with PBS solution (5min/time). PBS replaced the primary antibody as a blank control.

Evaluation criteria for immunohistochemistry

Analysis by immunohistochemical results revealed that SOX9 is primarily expressed in the cytoplasm, though the nucleus was also visible. The brown-yellow particles appeared in the cytoplasm as the standard. We analyzed the degree of staining and the percentage of stained cells. The staining intensity score was 0 for no staining, 1 for weak staining, 2 for medium-intensity staining, and 3 for strong staining. We scored cells based on the percentage of the total number of cells: 0 points for ≤5%, 1 point for 6-25%, 2 points for 26-50%, and 3 points for ≥50%. We used the H-score method for scoring: ≤2 indicates low expression and >2 indicates high expression.

Statistical methods

We performed data analysis using SPSS 23.0. Differences between groups were assessed with the χ^2 test. Survival rates were analyzed based on the Kaplan-Meier method. Survival differences were tested according to the Log-Rank method.

The factors affecting the prognosis of patients were evaluated with the Cox proportional hazards regression model; P<0.05 was considered statistically significant.

Results

Expression of SOX9 in AEG and paracancerous tissues

The high expression rate of SOX9 in AEG tissue was 46.49% (53/114), which was significantly higher than the low expression rate of SOX9 in paracancerous tissues (8.33%; 6/72), as seen in Figure 1.

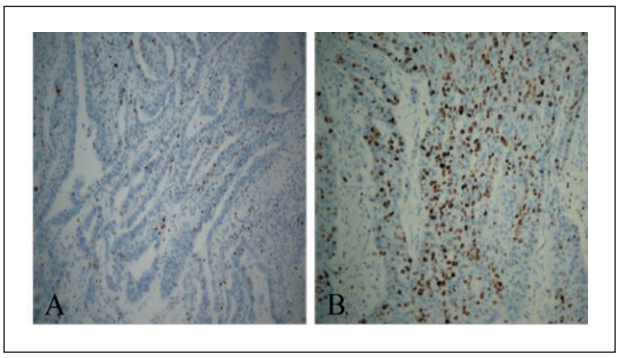

Figure 1: SOX9 expression in AEG and paracancerous tissues A: SOX9 negatively expressed in paracancerous tissues; B: SOX9 positively expressed in AEG tissues.

Relationship between SOX9 expression levels and clinicopathological characteristics of AEG patients

The expression of SOX9 was correlated with the Lauren classification, tumor invasion depth, lymph node metastasis, distant metastasis, and the TNM stage in patients with AEG (P<0.05). It was not related to the patients' sex, age, tumor location, or tumor diameter tissue differentiation (P>0.05), as seen in Table 1.

Correlation between SOX expression and prognosis in patients with AEG

According to Kaplan-Meier analysis, the 5-year survival rate of patients with high SOX expression was 30.19% (16/53)-significantly lower than the 5-year survival rate of patients with low SOX expression, which was 54.10% (33/61), as seen in Figure 2.

Clinical features	n	SOX9 expression		*P*	
		Low expression (n=61)	High expression (n=53)		
Sex				0.371	0.132
Male	81	45 (73.77)	36 (67.92)		
Female	33	16 (26.23)	17 (32.08)		
Age (year)				1.712	0.235
<60	61	36 (59.02)	25 (49.02)		
≥60	53	25 (40.98)	28 (52.83)		
Tumor site				4.023	0.089
Cardiac gastric fundus	34	14 (22.95)	20 (37.74)		
Gastric body	38	25 (40.98)	13 (24.53)		
Pylorus	42	22 (36.07)	20 (37.74)		
Tumor diameter (cm)				1.623	0.235
<5cm	52	31 (50.82)	21 (39.62)		
≥5cm	62	30 (49.18)	32 (60.38)		
Differentiation Degree				2.635	0.132
Moderately/highly differentiated	49	22 (36.06)	27 (50.94)		
Poorly differentiated	65	39 (63.93)	26 (49.06)		
Lauren type				7.568	0.019
Gut type	60	25 (40.98)	35 (66.04)		
Diffused type	46	32 (52.46)	14 (26.42)		
Mixed type	8	4 (6.56)	4 (7.55)		
Infiltration depth				8.365	0.023
T1~T2	23	18 (29.51)	5 (9.43)		
T3~T4	91	43 (70.49	48 (90.57)		
Lymph node metastasis				4.465	0.014
No	34	23 (37.70)	11 (20.75)		
Yes	80	38 (62.30)	42 (79.25)		
Distant metastasis				4.172	0.041
No	97	56 (91.80)	41 (77.36)		
Yes	17	5 (8.20)	12 (22.64)		
TNM staging				5.378	0.009
I~II	40	27 (44.26)	13 (24.53)		
III~IV	74	34 (55.74)	40 (75.47)		

Table 1: Relationship between SOX9 expression levels and clinicopathological characteristics of AEG patients.

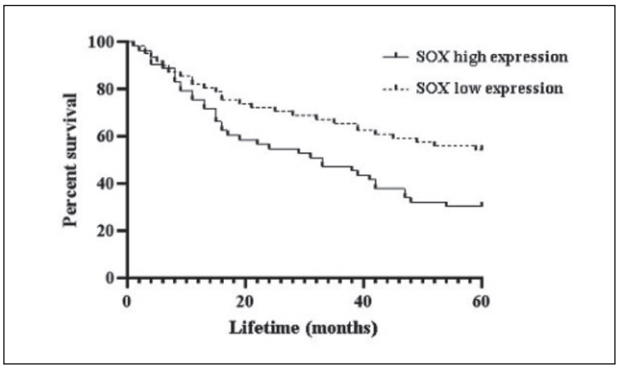

Figure 2: Correlation between SOX expression and prognosis in patients with AEG.

COX multivariate analysis in AEG patients

According to COX multivariate analysis, the degree of tissue differentiation, depth of infiltration, and distant metastasis were independent risk factors affecting the prognosis of patients with AEG. Table 2 shows the results of the analysis.

Clinical pathological parameters	Relative Risk Value	95% CI	*P*
Sex	0.827	0.552~1.246	0.532
Age	0.891	0.876~1.025	0.140
Tumor site	1.345	0.830~1.856	0.056
Tumor diameter	1.052	0.713~1.563	0.308
Differentiation degree	0.171	0.596~1.092	0.005
Lauren classification	1.695	1.042~3.038	0.356
Infiltration depth	2.321	0.115~3.413	0.028
Lymph node metastasis	0.852	0.568~1.215	0.623
Distant metastasis	0.789	0.568~1.056	0.019
TNM staging	0.605	0.493~1.023	0.058
SOX9 protein expression	0.272	0.892~1.562	0.089

Table 2: COX multivariate analysis in AEG patients.

Discussion

While AEG has the basic characteristics of gastric cancer, it also has unique pathological characteristics and biological behavior; its special anatomical location, rapid progress, tendency to lymph node metastasis, and high recurrence rate lead to a low 5-year survival rate among patients.

Modern molecular biology technology is developing rapidly. Clinical studies have found that high expression levels of SOX9 can promote the growth, angiogenesis, and invasion of prostate cancer. The abnormal expression of SOX9 is closely related to the development of breast and colorectal cancer, but the effect with AEG is not clear[11]. Therefore, exploring the relationship between SOX9 and AEG can further elucidate the mechanism of AEG occurrence and development, thus providing new ideas for targeted therapy.

SOX9 is an important nuclear transcription factor that can be expressed in multiple human tissues and organs. Clinical studies have shown that SOX9 is most highly expressed in the testis but is not expressed in the thymus or spleen[12]. The abnormal expression of SOX9 is closely related to the occurrence of various diseases. Clinical studies have shown that when SOX9 is over-expressed in the prostate, it can promote tumor cell proliferation and regulate certain factors to promote tumor formation[13]. Studies have confirmed that the expression level of SOX9 in normal gastrointestinal metaplasia mucosa tissue is significantly higher than in precancerous intestinal metaplasia mucosa tissue. This suggests that the expression level of SOX9 is closely related to the occurrence and development of gastric cancer and can also be used as an important factor for evaluating human intestinal-type gastric cancer[14]. Wong et al.[15] found that the abnormally high expression of SOX9 in advanced gastric cancer tissue may be related to the worsening of gastric cancer, which aligns with the results of this study.

In this study, we used immunohistochemistry to detect the expression of SOX9 in AEG tissues and paracancerous tissues. The results indicate that the high expression rate of SOX9 in AEG tissues was 46.49% (53/114), significantly higher than the low expression rate of SOX9 in paracancerous tissues, which was 8.33% (6/72). Subsequently, we analyzed the correlation between the expression of SOX9 and the clinicopathological characteristics of patients, finding that the expression of SOX9 was correlated with the Lauren classification, tumor invasion depth, lymph node metastasis, distant metastasis, and the TNM stage in patients with AEG ($P<0.05$). It was not related to the patients' sex, age, tumor location, and tumor diameter tissue differentiation ($P>0.05$). Correlation analysis showed that the 5-year survival rate of patients with high SOX expression was 30.19% (16/53), which was significantly lower than the 5-year survival rate of patients with low SOX expression at 54.10% (33/61). Finally, COX multivariate analysis revealed that the degree of tissue differentiation, depth of infiltration, and distant metastasis are independent risk factors that affect the prognosis of AEG patients. The high expression rate of SOX9 in AEG tissues is 46.49% (53/114), significantly higher than the low expression rate of SOX9 in paracancerous tissues, which was 8.33% (6/72). The expression of SOX9 was correlated with Lauren classification, tumor invasion depth, lymph node metastasis, distant metastasis, and TNM stage in patients with AEG ($P<0.05$).

It was not related to the patients' sex, age, tumor location, and tumor diameter tissue differentiation ($P>0.05$). According to correlation analysis, the 5-year survival rate of patients with high SOX expression was 32% (16/53), which was significantly lower than the 5-year survival rate of patients with low SOX expression (55% (33/61)). COX multivariate analysis revealed that the degree of tissue differentiation, depth of infiltration, and distant metastasis were independent risk factors affecting the prognosis of patients with AEG.

SOX9 is highly expressed in AEG tissues. Its expression level is related to the occurrence, development, invasion, and metastasis of AEG and affects the prognosis of patients. However, SOX9 cannot be used as an independent risk factor for assessing prognosis in AEG patients. This study has several limitations. The sample size is small; therefore, additional randomized and large-sample studies are warranted.

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