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Clinicopathological significance of SOX4 and epithelial–mesenchymal transition markers in patients with laryngeal squamous cell carcinoma

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Abstract

Background and aim: Sex-determining region-Y-related high-mobility-group box 4 (SOX4) is associated with the metastasis and prognosis of many cancer types. However, studies on the role of SOX4 in laryngeal squamous cell carcinoma (LSCC) are few, and hence the mechanism is unclear. Epithelial–mesenchymal transition (EMT) allows neoplastic cells to gain the plasticity and motility required for tumor progression and metastasis. This study aimed to analyze the relationship between SOX4 and EMT, and their relationship with clinicopathological factors and related prognosis.

Methods: Immunohistochemical staining was used to detect the positive expression of SOX4 protein, EMT-related transcription factor protein, and related marker protein in 127 LSCC tissue samples. At the same time, data on various parameters of clinical pathology and postoperative survival were collected.

Results: The positive expression rate of SOX4 and Slug in LSCC was related to pathological differentiation, lymphatic invasion, and pathological tumor node metastasis (TNM) of a tumor. The expression rates of ZEB1, Twist, E-cadherin, N-cadherin, and β-catenin in LSCC correlated with lymphatic invasion and pathological tumor node metastasis. The expression of SOX4, combined expression of SOX4 and ZEB1, and lymphatic invasion were independent prognostic factors for the total survival time of patients with LSCC.

Conclusions: In summary, SOX4 was vital in the LSCC EMT process, which might be mediated by transcription factor ZEB1. SOX4 and ZEB1 might serve as potential biomarkers of metastasis and prognosis, as well as promising therapeutic targets of LSCC.

Abbreviations: EMT = epithelial–mesenchymal transition, LNM = lymph node metastasis, LSCC = laryngeal squamous cell carcinoma, OS = overall survival, pTNM = pathological tumor node metastasis.

Keywords: EMT, laryngeal squamous cell carcinoma, SOX4, ZEB1

1. Introduction

Malignant tumors of the larynx are common in the head and neck, and squamous cell carcinoma is the most common pathological type. Laryngeal squamous cell carcinoma (LSCC) ranks second in the mortality rate of malignant tumors in the respiratory system. At present, the most important clinical treatment is surgery. The injury to normal anatomical structure during surgery inevitably has a negative effect on swallowing, vocalization, and breathing functions of patients after surgery, thus reducing the quality of life of patients. Although chemotherapy, radiotherapy, and biotherapy have made some progress after surgery, the long-term curative effect is not ideal.[2] Local recurrence and metastasis are still the biggest problems for patients with laryngeal cancer, and the long-term survival rate is still not high.[3] Therefore, identifying molecular markers that may predict the metastasis and prognosis of LSCC can provide new ideas for clinical treatment and is vital in improving the therapeutic effect of patients.

Sex-determining region-Y-related high-mobility-group box 4 (SOX4) is a sex-determining gene located on human chromosome 6p22.3. It encodes a protein with a molecular weight of 47 kDa. The SOX4 protein has 2 functional domains. The first is the highly conserved HMG box region at the N-terminal. The HMG box can combine with a specific region of DNA to fold it 90°, thus enhancing the binding of transcription factors to change its conformation and regulate its functional activity. Another functional domain of the SOX4 protein is located in the transactivation domain at the C-terminal, which can directly combine with the promoter region of DNA to regulate gene expression.[4] The SOX4 protein is involved in regulating physiological processes, such as embryonic differentiation, nervous system development, and sex determination.[5] At the same time, SOX4 is involved in the progression of various malignant tumors.[6] Most studies have shown an increase in the

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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expression of SOX4 in various malignant tumors, including breast cancer,[7] bladder cancer,[8] stomach cancer,[9] colon cancer,[10] prostate cancer,[11] and cervical cancer.[12] SOX4 is related to poor prognosis and disease progression. However, some studies have shown that increased expression of SOX4 promotes apoptosis and inhibits tumor metastasis.[13–15] In addition, studies on the role of SOX4 in laryngeal squamous cell carcinoma compared with other tumors are few. Only 2 studies used RT-qPCR to analyze the increased expression of SOX4 mRNA in 86[16] and 36[17] patients with laryngeal cancer. However, the relationship between SOX4 protein expression and related clinicopathological factors and prognosis has not been fully explored.

Epithelial–mesenchymal transition (EMT) refers to the phenomenon in which epithelial cells transform into mesenchymal cells under specific physiological or pathological conditions, which is mainly manifested in the phenotypic change and polarity disappearance of epithelial cells, the disappearance of intercellular adhesion structure, and the enhancement of migration and mobility. The expression of E-cadherin, an epithelial marker on the cell surface, is downregulated, while the expression of N-cadherin, vimentin, and fibronectin is upregulated during EMT. These changes simultaneously activate transcription factors such as Snail, Slug, Twist, and zinc finger E-box-binding homeobox, among which E-cadherin is a key molecule during EMT.[18] E-cadherin is connected with the cytoskeleton through β-catenin to maintain a stable connection between epithelial cells. When EMT occurs, β-catenin enters the nucleus through the cytoplasm and activates the transcription of the EMT gene.[19] Previous studies proved that EMT was involved in the progression of LSCC and closely related to tumor metastasis and invasion.[15]

Taken together, SOX4 is closely related to the metastasis and development of malignant tumors. However, related studies in LSCC are few, and hence the mechanism is unclear. This study was novel in exploring the relationship between SOX4 and clinicopathological factors in LSCC in detail. In this study, the expression levels of SOX4 protein; EMT-related transcription factor proteins Slug, Twist, and ZEB1; and their related marker proteins E-cadherin, N-cadherin, and β-catenin were examined, and their relationship with clinicopathological factors was discussed. This study showed that SOX4 in LSCC was closely related to EMT and participated in tumor progression.

2. Methods

2.1. Patients and clinical samples

The wax blocks of laryngeal squamous cell carcinoma in the First Affiliated Hospital of Bengbu Medical College were collected. All the selected cases were confirmed by pathological diagnosis and had complete clinical history and follow-up data. The time of study was from January 2010 to December 2012, and the follow-up duration was 100 months. The exclusion criteria were as follows: patients with other tumors; patients with diseases of important organs such as heart, liver, kidney, and so on; and patients with autoimmune diseases and psychosis. Finally, 127 patients were included in the study. Meanwhile, 127 adjacent nontumor tissues corresponding to the same patients were selected as controls. This study was approved by the institutional ethical review board of First Affiliated Hospital of Bengbu Medical College. The institute's ethical regulations on research conducted on human tissues were followed up to his or her death date or December 2019 (average survival time: 52.67 months; scope: 6–100 months), and the total survival time (overall survival [OS]) was calculated with the obtained data. The clinicopathological data were statistically analyzed according to the World Health Organization standard to evaluate the grade of tumor and according to the Eighth Edition of tumor node metastasis (TNM) Head and Neck Cancer Classification Review[20] to evaluate tumor-LNM (TNM) stage and LNM stage.

2.2. Immunohistochemical analysis

Immunohistochemistry was conducted following the Elivision-Plus detection kit instructions (Lab Vision, USA). The antibodies used are listed in Table 1.

2.3. Evaluation of staining

The immunohistochemical staining results were interpreted by 2 experienced pathologists using an independent double-blind method. The SOX-positive cells were located mainly in the nucleus and cytoplasm,[12] The E-cadherin-positive cells were located mainly on the cell membrane, the N-cadherin- and β-catenin-positive cells were located mainly in the cytoplasm and nucleus,[21] and the Slug-, ZEB1-, and Twist-positive cells were located mainly in the nucleus.[22,23] The areas with positive staining were observed in at least 10 representative high-power visual fields (magnified 400 times) under the microscope. The results were evaluated by measuring both the staining intensity and the number of positively stained cells. The intensity of the positive reaction was scored as negative (0), weak (1), moderate (2), and intense (3). The reactivity was assessed by the percentage of positively stained cells as 0% to 5% (0), 6% to 25% (1), 26% to 50% (2), 51% to 75% (3), and 75% to 100 (4). The scores for the intensity and the percentage of positive cells were multiplied to obtain a weighted score for each patient, giving a minimum-to-maximal score of 0 to 12. The

Table 1

Antibody information.

Antigen	Clone	Source	Company	Dilution
SOX4	ab236557	Mouse monoclonal	Abcam, Cambridge, UK	1:100
Slug	ab85936	rabbit polyclonal	Abcam, Cambridge, UK	1:150
ZEB1	ab180905	Mouse monoclonal	Abcam, Cambridge, UK	1:100
Twist	ab50581	rabbit polyclonal	Abcam, Cambridge, UK	1:100
E-cadherin	NCH-38	Mouse Monoclonal	Dako, Glostrup, Denmark	1:200
N-cadherin	6G11	Mouse Monoclonal	Dako, Glostrup, Denmark	1:100
β-catenin	sc-59737	Mouse Monoclonal	Santa Cruz, CA, USA	1:150

SOX4 = sex-determining region-Y-related high-mobility-group box 4.

expression levels of proteins were dichotomized to low (0–6) and high (7–12) values for outcome analyses.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 22.0 software package. The comparison of the expression of SOX4 between LSCC and noncancerous tissues was analyzed using the *t* test. The Fisher exact or Pearson χ^2 (2) test was used to analyze the correlation between protein expression and clinicopathological indices. Spearman correlation analysis was used to evaluate the correlations between the expression levels of these factors. The Kaplan–Meier method was used to establish the survival curves, and the log-rank test was used for comparison. Univariate and multivariate analyses were performed to analyze the influence of various factors on OS using a Cox's proportional hazards regression model. The hazard ratio and 95% confidence interval were used for analysis. A P value $< .05$ was considered statistically significant.

3. Results

3.1. Correlations between the expression of SOX4, ZEB1, slug, and Twist and clinicopathological variables

Among the selected 127 LSCC cases, 62 (62/127, 48.8%) stained positive for SOX4 (Fig. 1A), and 13 (13/127, 10.2%) stained positive for corresponding nontumor tissues. Further, 54 cases (54/127, 42.5%) stained positive for ZEB1 (Fig. 1B) and 9 (9/127, 7.1%) stained positive in corresponding nontumor tissues. Also, 49 cases (49/127, 38.6%) stained positive for Slug (Fig. 1C) and 8 (8/127, 6.3%) stained positive in corresponding nontumor tissues. Moreover, 65 cases (65/127, 51.2%) stained positive for Twist (Fig. 1D) and 11 (11/127, 8.7%) stained positive in corresponding nontumor tissues. The differences were statistically significant ($P < .05$). The expression of SOX4 and Slug in LSCC correlated with pathological differentiation, lymphatic invasion, and pathological tumor node metastasis (pTNM) ($P < .05$), but not with age, sex, tumor location, and smoking.

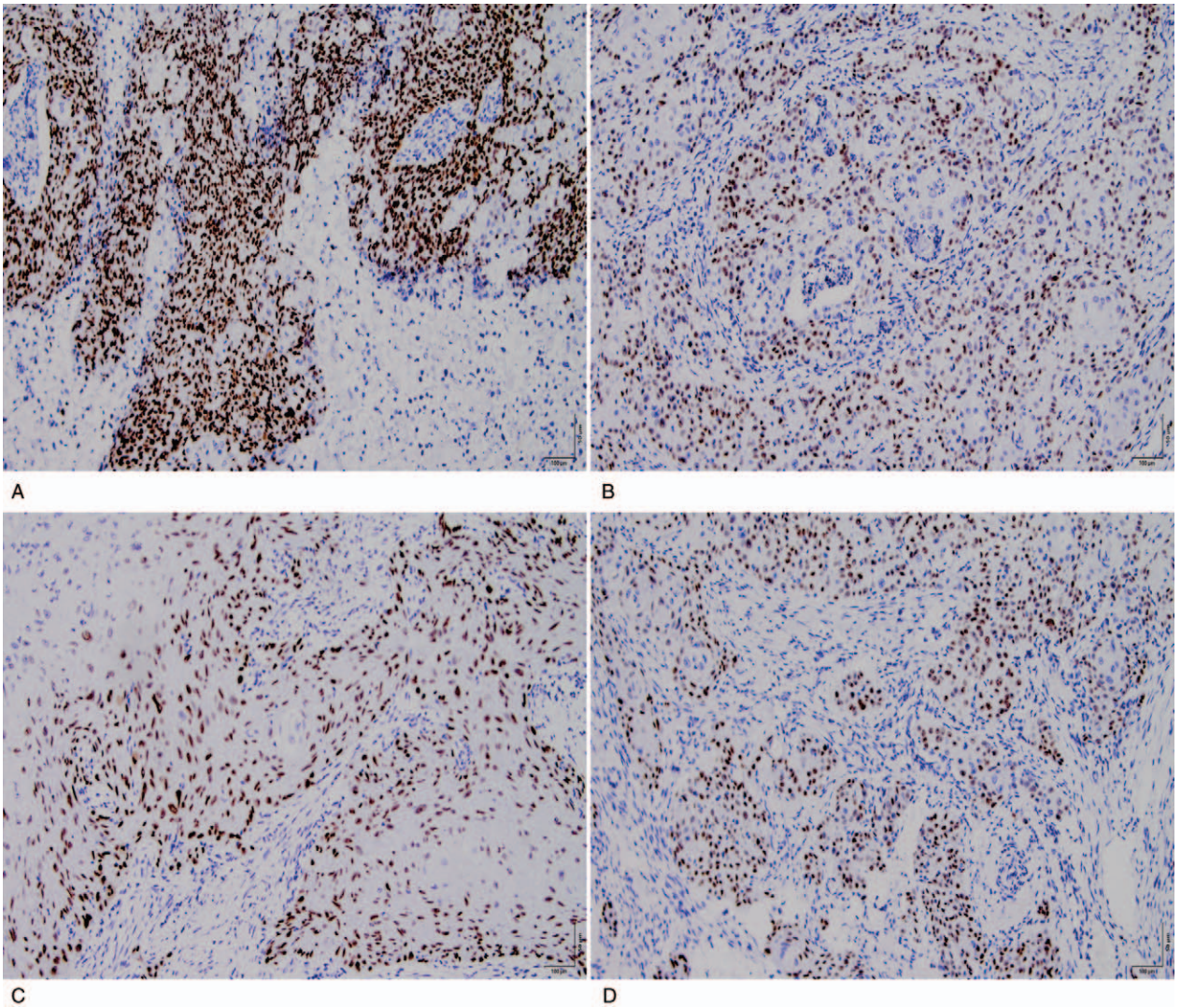

Figure 1. Immunostaining of SOX4, or ZEB1 or Slug and their related marker proteins E-cadherin, N-cadherin, β-catenin in LSCC. A, Positive staining of SOX4 in LSCC (100 magnification); scale bar,100 μm. B, Positive staining of ZEB1 in LSCC (100 magnification). C, Positive staining of Slug in LSCC (100 magnification). D, Positive staining of Twist in LSCC (100 magnification). E, Positive staining of E-cadherin in the LSCC (100 magnification). F, Positive staining of N-cadherin in LSCC (100 magnification). G, Positive staining of β-catenin in LSCC (100 magnification). LSCC = laryngeal squamous cell carcinoma.

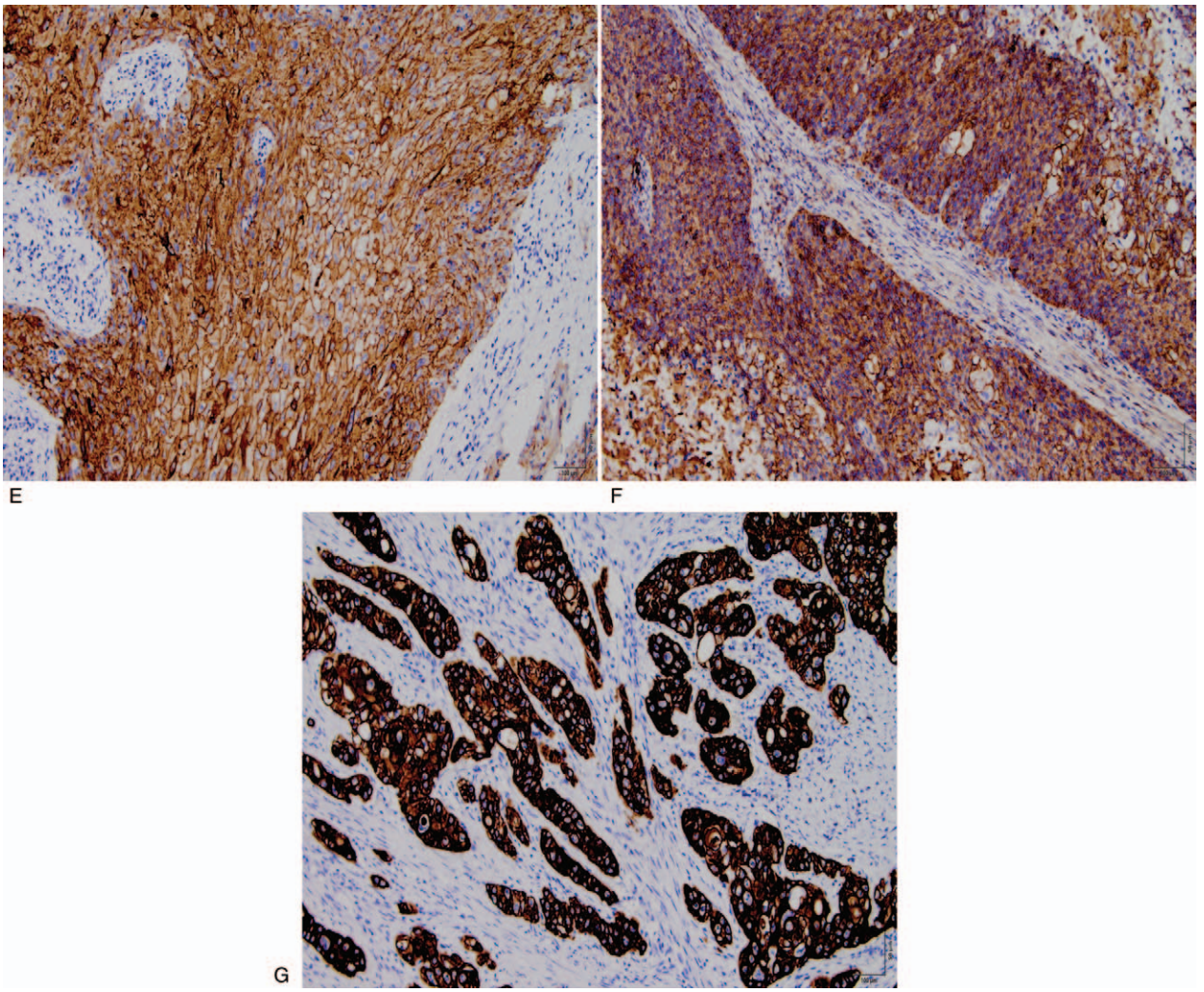

Figure 1. (Continued).

The positive expression of ZEB1 and Twist in LSCC correlated with lymphatic invasion and pTNM of the tumor ($P < .05$), but not with age, sex, location of the tumor, and smoking. Detailed statistical results are shown in Table 2.

3.2. Correlations between the expression of E-cadherin, N-cadherin, and β-catenin and clinicopathological variables

Among the 127 selected LSCC cases, 44 (44/127, 34.6%) were negative for E-cadherin immunohistochemical staining (Fig. 1E), and 112 (112/127, 88.2%) were positive in corresponding nontumor tissues. Also, 56 cases (56/127, 44.1%) were positive for N-cadherin immunohistochemical staining (Fig. 1F) and 91 (91/127, 71.7%) were positive in corresponding nontumor tissues. Further, 55 cases (55/127, 43.3%) were positive for β-catenin immunohistochemical staining (Fig. 1G) and 94 (94/127, 74.0%) were positive in corresponding nontumor tissues. The differences were statistically significant ($P < .05$). The expression of E-cadherin, N-cadherin, and β-catenin in LSCC correlated with lymphatic invasion and pTNM of tumors ($P < .05$), as shown in Table 3, but had no correlation with age, sex, tumor location, and smoking (data not shown).

3.3. Univariate and multivariate analyses

The univariate analysis by the Kaplan–Meier method showed that the survival time of patients with positive expression of SOX4 was significantly shorter than that of patients with negative expression of SOX4 (log-rank = 16.671, $P < .001$), and the difference was statistically significant (Fig. 2A). The survival time of patients with positive expression of ZEB1 was significantly shorter than that of patients with negative expression of ZEB1 (log-rank = 7.478, $P < .05$), and the difference was statistically significant (Fig. 2B). No significant difference in survival time was found between patients with positive expression of Slug and those with negative expression of Slug (log-rank = 2.766, $P = .096$), and the difference was not statistically significant (Fig. 2C). No significant difference in survival time was observed between patients with positive and negative expression of Twist (log-rank = 1.485, $P = .223$), and the difference was not statistically significant (Fig. 2D). The multivariate analysis showed that the positive expression of SOX4 and lymphatic invasion were independent prognostic factors for the total survival time of patients with LSCC after the surgery. The influence of clinicopathological factors on the postoperative survival time of these patients is shown in Table 4

Table 2

Correlations between SOX4, ZEB1, Slug, Twist, and clinicopathological variables in LSCC.

		SOX4			Slug			ZEB1			Twist		
Variable	n	Negative	Positive	P	Negative	Positive	P	Negative	Positive	P	Negative	Positive	P
Gender													
Female	19	12	7	.257	11	8	.732	12	7	.587	8	11	.526
Male	108	53	55		67	41		61	47		54	54	
Age (yr)													
<60	56	30	26	.632	37	19	.339	31	25	.667	27	29	.904
≥60	71	35	36		41	30		42	29		35	36	
Tumor location													
Supraglottic	40	24	16	.203	22	18	.268	27	13	.109	20	20	.891
Glottic	76	34	42		47	29		38	38		36	40	
Subglottic	11	7	4		9	2		8	3		6	5	
Smoking history													
No	61	31	30	.938	34	27	.206	38	23	.291	32	29	.430
Yes	66	34	32		44	22		35	31		30	36	
T classification													
T1+T2	85	45	40	.572	56	29	.141	49	36	.957	48	37	.014*
T3+T4	42	20	22		22	20		24	18		14	28	
Pathologic differentiation													
Well	45	32	13	.003*	35	10	.017*	29	16	.338	23	22	.296
Moderate	61	23	38		33	28		31	30		32	29	
Poor	21	10	11		10	11		18	3		7	14	
Lymphatic invasion													
Negative	94	56	38	.001*	63	31	.029*	62	32	0.001*	52	42	.013*
Positive	33	9	24		15	18		11	22		10	23	
pTNM													
I–II	77	51	26	<.001*	56	21	.001*	51	26	0.013*	46	31	.002*
III–IV	50	14	36		22	28		22	28		16	34	

LSCC = laryngeal squamous cell carcinoma, pTNM = pathological tumor node metastasis, SOX4 = sex-determining region-Y-related high-mobility-group box 4.

In this study, the results on the combined expression of SOX4 and Slug, SOX4 and ZEB1, and SOX4 and Twist were analyzed by univariate and multivariate analyses. The results showed that the negative expression of the combination of SOX4 and Slug was associated with a better prognosis compared with the expression of other combinations (log-rank = 10.979, $P < .05$; Fig. 3A). The negative expression of the combination of SOX4 and ZEB1 was also associated with a better prognosis compared with the expression of other combinations (log-rank = 15.532, $P < .001$; Fig. 3B). Further, the negative expression of the combination of SOX4 and Twist was related to a better prognosis compared with the expression of other combinations (log-rank = 7.624, $P < .01$; Fig. 3C). The multivariate analysis showed that the combined expression of SOX4 and ZEB1 was an independent prognostic factor for the total survival time of patients with LSCC. Specific results are shown in Table 5.

3.4. Spearman correlation coefficient analysis

Spearman correlation coefficient analysis showed a positive correlation between the expression of SOX4 and Slug in 127 LSCC tissues ($r = 0.197$, $P < .05$). Also, a positive correlation was observed between the expression of SOX4 and ZEB1 ($r = 0.180$, $P < .05$). Further, a positive correlation was found between the expression of SOX4 and Twist ($r = 0.229$, $P < .05$). Moreover, a negative correlation was noted between the expression of SOX4 and E-cadherin ($r = -0.215$, $P < .05$). Also, a positive correlation was found between the expression of SOX4 and N-cadherin ($r = 0.275$, $P < .01$). Further, a positive correlation was observed

Table 3

Correlations between E-cadherin, N-cadherin, β-catenin and clinicopathological variables in LSCC.

		E-cadherin			N-cadherin			β-catenin		
Charistics	n	Loss	Positive	P	Negative	Positive	P	Negative	Positive	P
Lymphatic invasion										
Negative	94	56	38	.021	58	36	.026	58	36	.045
Positive	33	27	6		13	20		14	19	
pTNM										
I–II	77	41	36	<.001	49	28	.029	51	26	.007
III–IV	50	42	8		22	28		21	29	

pTNM = pathological tumor node metastasis.

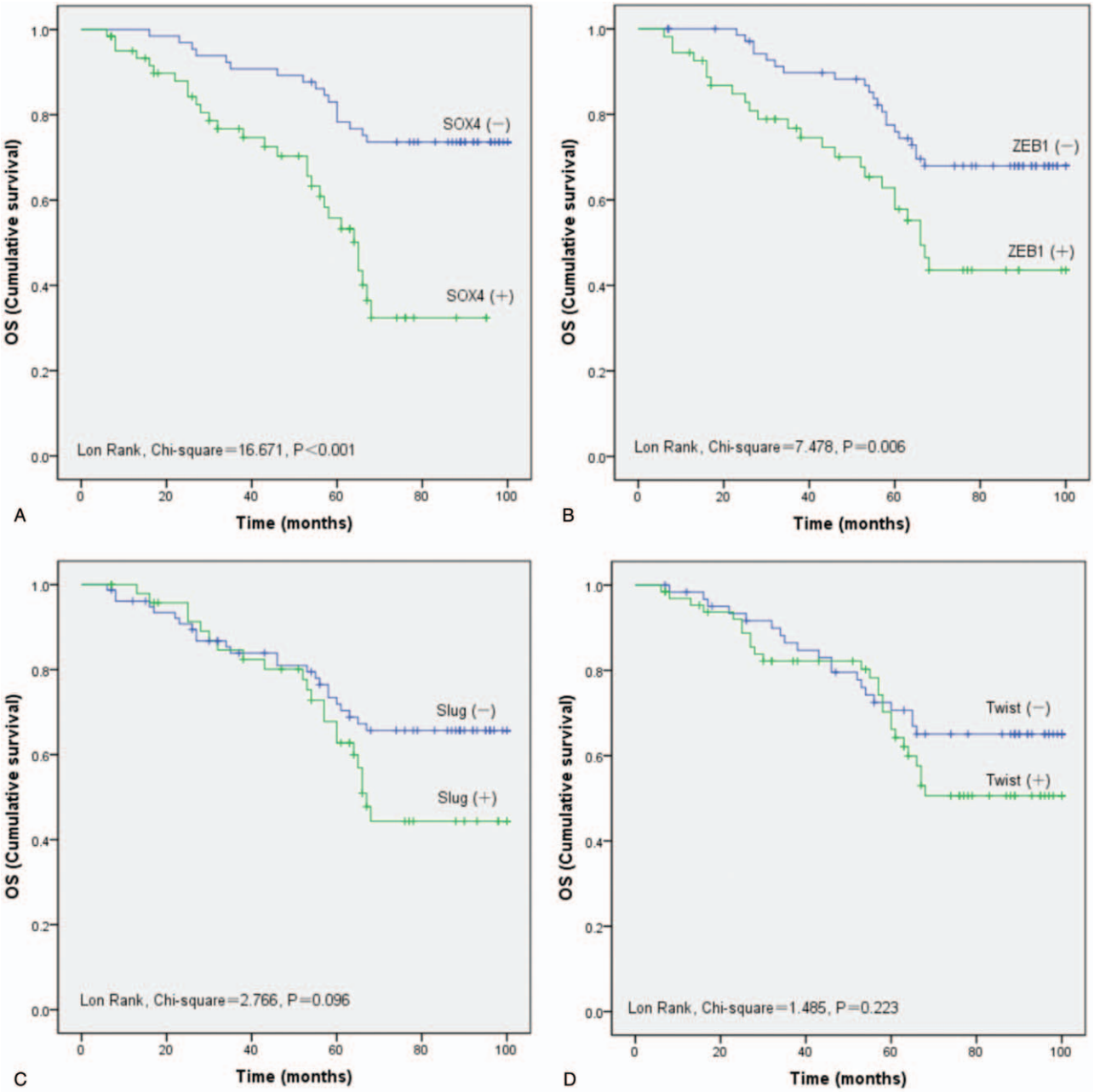

Figure 2. Kaplan–Meier analysis of the survival rate of patients with LSCC. A, Overall survival of all patients in relation to SOX4 expression (Log-rank = 16.671, $P < .001$). B, Overall survival of all patients in relation to ZEB1 expression (Log-rank = 7.478, $P < .05$). C, Overall survival of all patients in relation to Slug expression (Log-rank = 2.766, $P = .096$). D, Overall survival of all patients in relation to Twist expression (Log-rank = 1.485, $P = .223$). LSCC = laryngeal squamous cell carcinoma.

between the expression of SOX4 and β-catenin ($r = 0.259, P < .01$). The correlation among other factors is shown in Table 6.

4. Discussion

LSCC, as a common malignant tumor of the head and neck, has no obvious early symptoms, thus reducing the quality of life of patients. Local invasion and metastasis are still the main factors restricting the survival rate of patients. The development mechanism of laryngeal cancer is not very clear. Finding new targets and biomarkers for the prognosis of laryngeal cancer from the perspective of molecular biology is the focus of recent studies. Studies indicated an important role of EMT in the progression of LSCC. Also, SOX4 might participate in the EMT process of LSCC.

SOX4 is one of 64 cancer marker genes, indicating it as crucial in the occurrence and development of malignant tumors.[24] Most studies found that the expression of SOX4 increased in tumor tissues and was related to tumor progression. For example, Fang et al[9] and Lin et al[10] found a positive correlation of SOX4 with invasion depth, metastasis, and tumor stage in gastric cancer and colon cancer. In this study, SOX4 was also found to be related to

Table 4

Results of univariate analyses and multivariate analysis of overall survival (OS) time.

	Univariate analysis			Multivariate analysis		
Variable	**HR**	***P* value**	**95% CI**	**HR**	***P* value**	**95% CI**
Gender						
Female/male	0.474	.154	0.170–1.322	0.841	.763	0.273–2.589
age (yr)						
<60/≥60	1.431	.242	0.786–2.206	1.588	.194	0.790–3.194
Tumor location						
Supraglottic/glottic /subglottic	0.841	.479	0.522–1.357	0.822	.528	0.448–1.509
Smoking history						
No/yes	1.147	.647	0.638–2.064	1.010	.977	0.503–2.030
T classification						
T1+T2/T3+T4	1.345	.327	0.744–2.432	1.748	.191	0.757–4.037
pathologic differentiation						
Well/moderate/poor	1.111	.608	0.742–1.664	0.686	.156	0.407–1.154
lymphatic invasion						
Negative/positive	5.641	<.001	3.117–10.210	7.986	.001	2.449–26.043
pTNM						
I–II/III–IV	3.529	<.001	1.941–6.418	0.439	.252	0.107–1.794
E-cadherin expression						
Negative/positive	0.510	.052	0.259–1.005	0.838	.673	0.370–1.901
N-cadherin expression						
Negative/positive	1.819	.044	1.016–3.255	0.752	.432	0.369–1.532
β-catenin expression						
Negative/positive	1.650	.091	0.924–2.945	0.982	.958	0.494–1.952
SOX4 expression						
Negative/positive	3.324	<.001	1.806–6.120	3.273	.002	1.526–7.022
Slug expression						
Negative/positive	1.625	.101	0.910–2.904	1.076	.836	0.539–2.146
ZEB1 expression						
Negative/positive	2.202	.008	1.230–3.944	1.535	.226	0.767–3.069
Twist expression						
Negative/positive	1.433	.227	0.799–2.570	0.785	.520	0.375–1.642

pTNM = pathological tumor node metastasis.

tumor progression, such as LNM and TNM stage. At the same time, Watanabe et al[25] found that SOX4 was related to LNM and tumor differentiation in oral squamous cell carcinoma, which was consistent with the findings of the present study. However, some studies found that the expression of SOX4 contradicted the biological behavior of laryngeal squamous cell carcinoma. For example, Li et al[16] found through in vitro cytology that the upregulation of the expression of SOX4 inhibited the expression of microRNA-625, thus enhancing the invasion and migration of laryngeal squamous cell carcinoma. However, Coskunpinar et al[17] conducted gene screening on 36 laryngeal cancer tissues and their corresponding adjacent tissues and found that the expression of SOX4 gene mRNA increased, which was not related to tumor metastasis. In addition, this study found that the overexpression of SOX4 protein negatively correlated with the prognosis of patients. Previous studies also found that the overexpression of SOX4 protein had no correlation with poor prognosis in prostate cancer,[11] nasopharyngeal carcinoma,[26] and oral squamous cell carcinoma.[27] However, the overexpression of SOX4 improved the prognosis of patients in bladder cancer[8] and hepatocellular carcinoma.[14] In a word, the mechanism of SOX4, as a gene closely related to tumor, is unclear, especially in laryngeal squamous cell carcinoma.

Local invasion and metastasis are the main factors leading to the death of patients with LSCC. Existing studies proved the involvement of EMT in the progression of LSCC.[28] Low expression of E-cadherin might lead to tumor recurrence and decrease the prognosis of patients.[29,30] Low expression of E-cadherin and high expression of β-catenin and N-cadherin had a positive correlation with tumor stage and differentiation,[21] which was consistent with the results of the present study. At the same time, the analysis of the expression of Slug, ZEB1, and Twist showed that they were all related to LSCC LNM and TNM stage; moreover, the expression of Slug was also related to the tumor pathological grade and that of Twist to the tumor stage. Previous studies also found that EMT transcription factors were closely related to the progression of squamous cell carcinoma at different sites. For instance, the overexpression of Slug in the head and neck squamous cell carcinoma changed the cell adhesion junction, causing cells to lose the function of desmosome assembly and promoting tumor metastasis.[31] ZEB1 was highly expressed in oral squamous cell carcinoma, and was related to the tumor stage, grade, LNM stage, and tumor recurrence.[23] Li et al[32] found that silencing ZEB2 in LSCC AMC-HN8 cells reduced the invasion and metastatic ability of tumor cells and promoted apoptosis and cell cycle arrest. The expression of Twist in OSCC correlated with LNM, pathologic grade, and tumor stage.[22,33] Previous studies on OSCC found that ZEB1 was related to tumor prognosis and was an independent prognostic factor,[23] the expression of Twist also negatively correlated with OSCC prognosis,[22] and the overexpression of Slug decreased LSCC disease-free survival.[29] However, the present study found that Slug and Twist were not related to the prognosis of LSCC,

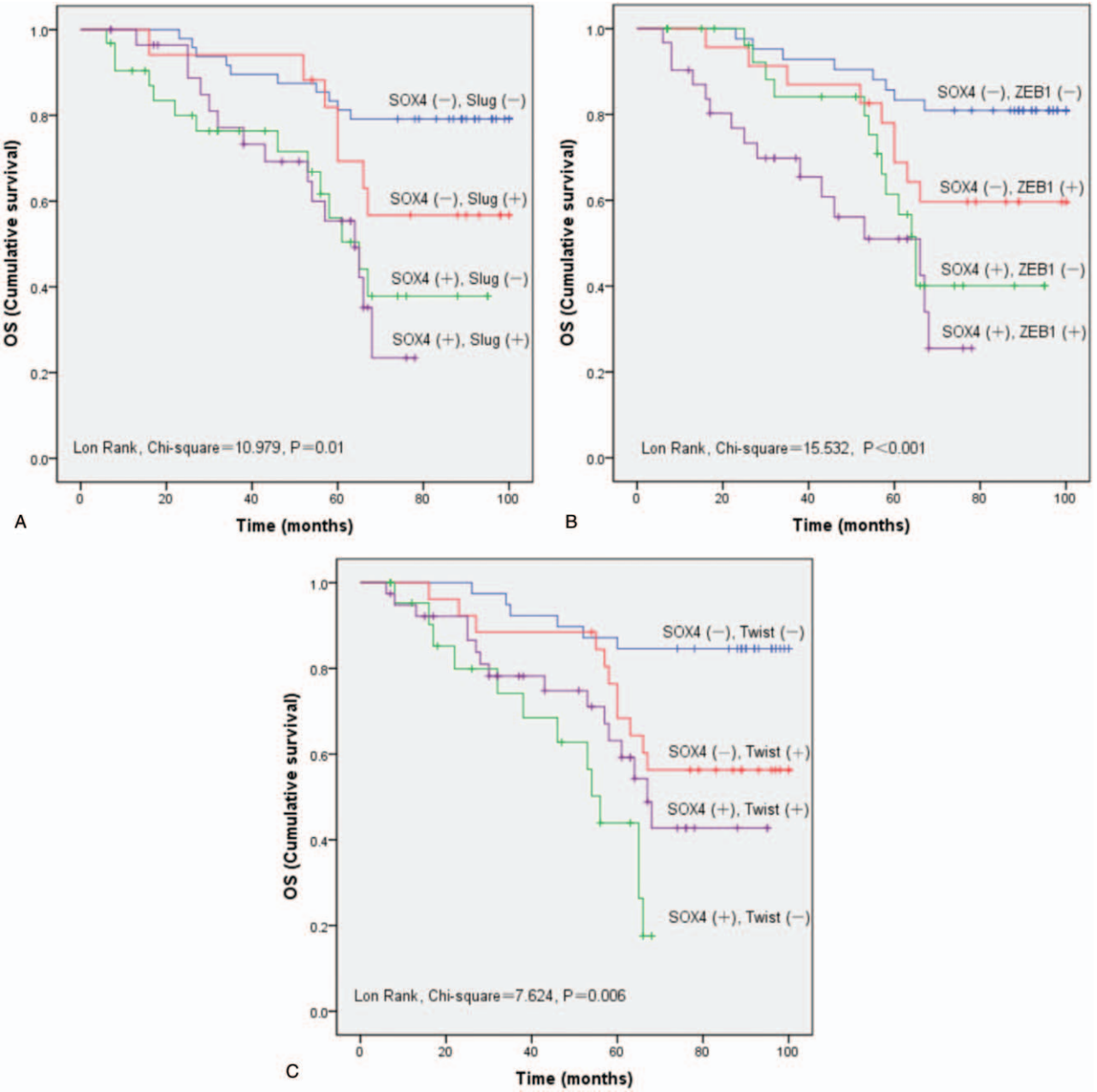

Figure 3. Kaplan–Meier analysis of the survival rate of patients with LSCC. A, Overall survival of all patients in relation to the combined expression of SOX4 and Slug (log-rank = 10.979, $P < .05$). B, Overall survival of all patients in relation to the combined expression of SOX4 and ZEB1 (log-rank = 15.532, $P < .001$). C, Overall survival of all patients in relation to the combined expression of SOX4 and Twist (log-rank = 7.624, $P < .01$). LSCC = laryngeal squamous cell carcinoma.

but ZEB1 was related to the prognosis of tumor. In a word, although EMT-related proteins had different expression levels in LSCC, they were closely related to tumor progression, and ZEB1 could be used as a factor to judge tumor prognosis.

Abnormal expression of SOX4 induced EMT in malignant tumors and enhanced the migration and invasion of tumors. The downregulated expression of SOX4 slowed down the process of EMT and even reversed it, indicating that SOX4 was the key factor in maintaining the mesenchymal state of EMT,[34] but its mechanism was still unclear. Tiwari et al[35] found that the expression of Snail2, ZEB2, and Twist decreased after eliminating the expression of SOX4 in untransformed normal murine mammary gland cells, but the expression of Snail1 and ZEB1 was not affected. Similarly, SOX4 was closely related to Wnt pathway activation; it directly acted on β-catenin, mediated downstream gene expression, and affected the EMT process of tumor.[36,37] The present study found that SOX4 was associated with the expression of EMT-related marker proteins, which was statistically significant, indicating that SOX4 might promote the progression of LSCC EMT. In addition, Slug and Twist had no correlation with LSCC prognosis, while ZEB1 correlated with LSCC prognosis. However, Slug, Twist, and ZEB1 correlated with LCSS prognosis. The analysis of SOX4 combined with ZEB1 showed that they were independent prognostic factors for LSCC, indicating that SOX4 might influence the LSCC EMT process mainly by mediating the expression of ZEB1.

Table 5

The combined expression results of univariate analyses and multivariate analysis of overall survival (OS) time.

Variables	Univariate analysis			Multivariate analysis		
	HR	*P* value	95% CI	HR	*P* value	95% CI
SOX4 and Slug expression	1.475	.001	1.163–1.871	1.106	.556	0.791–1.545
SOX4 and ZEB1 expression	1.637	<.001	1.268–2.114	2.784	.022	1.163–6.664
SOX4 and Twist expression	1.399	.007	1.095–1.786	0.927	.693	0.637–1.350

SOX4 = sex-determining region-Y-related high-mobility-group box 4.

Table 6

Results of Spearman correlation coefficient analysis.

Variables	SOX4	Slug	ZEB1	Twist	E-cadherin	N-cadherin	β-catenin
SOX4							
r	1	0.197	0.180	0.229	−0.215	0.275	0.259
P	–	.027	.043	.010	.015	.002	.003
Slug							
r		1	0.104	0.159	−0.271	0.273	0.091
P		–	.247	.074	.002	.002	.310
ZEB1							
r			1	0.012	−0.258	0.423	0.245
P			–	.898	.003	<.001	.006
Twist							
r				1	−0.183	0.201	0.218
P				–	.040	.023	.014
E-cadherin							
r					1	−0.180	−0.169
P					–	.043	.058
N-cadherin							
r						1	0.312
P						–	<.001
β-catenin							
r							1
P							–

SOX4 = sex-determining region-Y-related high-mobility-group box 4.

5. Conclusions

In summary, the present study found that SOX4 was closely related to LSCC clinicopathological factors and was an independent prognostic factor. At the same time, the combined analysis of EMT-related factors showed that SOX4 regulated the EMT process through the transcription factor ZEB1. The immunohistochemical analysis of SOX4 and ZEB1 might be useful in identifying patients at higher risk of disease metastasis after surgery, who might benefit from more active treatment. Further studies should analyze the mechanism of SOX4 regulating EMT in LSCC at the cytological and molecular levels.

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