Correlation Of SOX17, MACC1, c-Met With Clinicopathological Features And Prognosis of Esophageal Squamous Cell Carcinoma

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Abstract

Aim: We mainly investigated the relationship between the expression of SOX17, MACC1 and c-Met in esophageal squamous cell carcinoma and the clinicopathological parameters of the patients and the effect on prognosis.

Methods: Expressions of SOX17, MACC1 and c-Met protein in a sample of 232 ESCC and adjacent nontumorous tissues were detected by immunohistochemistry. Their relationships and correlations with clinicopathological features and clinical prognosis were analyzed by SPSS 23.0.

Results: SOX17 was associated with Vascular invasion ($p=0.017$) and Nerve invasion ($p=0.014$). MACC1 was correlated with Tumor size ($p=0.039$) and TNM stage ($p=0.020$). c-Met was significantly associated with hematogenous metastasis ($p=0.045$). The expression of MACC1 was correlated with c-Met ($P<0.001$). c-Met expression ($p=0.021$) were associated with OS.

Conclusion: SOX17, MACC1 and c-Met may be new diagnostic targets for esophageal squamous cell carcinoma. c-Met may be a new therapeutic and prognostic target.

Introduction

Esophageal carcinoma (EC) is a malignant tumor originating from the esophageal epithelium. In the 2018 Global Cancer Statistics, EC ranks 7th in morbidity and 6th in mortality^[1]. Esophageal squamous cell carcinoma (ESCC) is the most common esophageal cancer in China with poor prognosis, and the incidence of ESCC in males is significantly higher than that in females^[2]. The causes of esophageal cancer are related to living conditions, bad dietary habits, strong carcinogens, virus infection, genetic susceptibility and so on, but the specific etiology is still unclear^[2, 3]. Esophagectomy is the main treatment for esophageal cancer, but it is highly invasive, with high morbidity and mortality^[4]. Therefore, it is of great importance and urgency to explore the molecular mechanism of the occurrence, development and metastasis of esophageal cancer and to seek new prevention and treatment targets and intervention strategies to improve the efficacy of esophageal cancer.

Recent studies have shown that the abnormal expression of some tumor stem cell factors regulates the progression of esophageal squamous cell carcinoma^[5]. Our research group has been devoted to the study of the mechanism of tumor stem cell factors (SOX family genes) in ESCC. SOX17 plays an important role in the development of human^[6], but its role in the esophagus remains to be explored.

The SOX17 gene, A member of the SOX transcription factor family^[7], is a 414-amino acid polypeptide containing a high-mobility group DNA binding domain (HMG-box) and SRY's target promoter binding element 5'-(A/T) (A/T) CAA (A/T) G-3'^[8]. SOX17 is a transcription factor that directs the regulation and development of the primary endoderm, the primary germ cells, the formalized endoderm, and subsequently the cardiovascular system and some endoderm derived organs^[9]. Studies have shown that SOX17 can inhibit MACC1, FN1 and other genes through SRY binding mediated transcriptional regulation, thus inhibiting the progression of ESCC^[10]. The expression of SOX17 in esophageal squamous cell carcinoma and its effect on prognosis need to be further studied.

MACC1 (Metastasis associated in colon cancer 1) is a newly discovered biomarker that predicts Metastasis and prognosis of patients with colorectal cancer^[11, 12]. The MACC1 gene is located on human chromosome 7p21.1 and has 7 exons, encoding a protein composed of 852 amino acid residues. A recent study reported that MACC1 was highly expressed in gastric cancer tissues. And the expression of MACC1 is related to the degree of differentiation, depth of infiltration, lymph node metastasis and stage of gastric cancer^[13]. MACC1 is a key regulator of the HGF/c-Met pathway^[11]. MACC1 can activate the HGF/c-Met signaling pathway by binding with the promoter, thereby promoting the proliferation of Osteosarcoma(OS) cells and endothelial cells, and promoting the angiogenesis of OS and the progression of OS by regulating microtubule dynamics^[14]. The expression and role of MACC1 in esophageal squamous cell carcinoma still need to be further explored.

c-Met is one of the most important receptors and pathways associated with cancer^[15]. c-Met tyrosine kinase is a cell surface receptor for hepatocyte growth factor (HGF) and is involved in the regulation of cell proliferation, apoptosis, and migration^[16]. Dysregulation of HGF/c-Met signaling has been reported as a prognostic marker for tumorigenesis, early invasion, and metastasis^[17]. Recently, it has been reported that c-Met is involved in many digestive system neoplasm, including gastric cancer, hepatocellular carcinoma, pancreatic cancer, esophageal cancer, and colon and rectal cancers^[18]. c-Met may be overexpressed in esophageal cancer, but its specific mechanism remains to be further studied.

Our aim was to study the expression of SOX17, MACC1 and c-Met in ESCC, and analyze the effects of the three on the survival and prognosis of patients with esophageal squamous cell carcinoma.

Materials And Methods

2.1 Patients

This study encompassed tissue microarray that includes a retrospective series of 232 patients identified as having ESCC diagnosed between January 2008 and June 2018 and treated at the First Affiliated Hospital of Xinjiang Medical University. The inclusion criteria are as follows: patients diagnosed as esophagus cancer from January 2008 to June 2018; squamous cell carcinoma of esophagus; no radiotherapy or chemotherapy before operation; the site of the esophagus is a primary focus; and the people of the patient include the Han and Kazakh nationality. And the exclusion criteria are as follows:

Adenocarcinoma of esophagus; Patients who have received radiotherapy or chemotherapy before surgery; Tumor metastases to the esophagus; Excluding other nations: Uyghur nationality, Mongolian, etc. In this study, 232 patients with ESCC and paracancerous nontumor tissues were randomly selected.

We collected 232 cases of ESCC paraffin-embedded tissues and adjacent noncancerous tissues. All 232 patients were treated with surgical operation and without preoperative chemoradiotherapy. Ethical approval was obtained from the ethics committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consent for the scientific use of the tissue samples and medical records were obtained from each patient. Our follow-up time ended in July 2020 through inquiring the medical records and telephone call.

2.2 Immunohistochemical

Heat the tissue chips in an oven at 65 degrees Celsius for 45 minutes to soften the wax coating on the tissue chips. Tissues were fixed in 10% formalin, sectioned at 5 μ m, subsequently deparaffinized in xylene, and rehydrated in 100%, 95%, 80%, and 70% ethanol. All tissues were blocked with hydrogen peroxide for 10 min and heated in a microwave for antigen retrieval. After blocking with 1% goat serum, the sections were incubated with primary antibodies SOX17 (Bioss, bs-12205R, China, dilution, 1:300), MACC1 (Bioss, bs-4293R, China, 1:100), c-Met (Bioss, bs-0668R, China, 1:300) for 90 min at 37°C. After washing in PBS, the sections were incubated with secondary antibody (Goat anti-rabbit IgG) for 15 min at 37°C. After DAB staining, hematoxylin solution was incubated for 20 seconds, followed by gradient alcohol dehydration and xylene differentiation, and the tablets were sealed.

The cutoff values for positivity were as follows: Any five high-power fields ($\times 200$) were selected for measurement, and density of the staining was scored based on two pathological experts. For SOX17, the staining "intensity" measurements were translated into the two-tier system as positive "1" or negative "0" staining. The staining "percentage" measurements were graded using a four-tier system and was scored as "3" if $> 66\%$ tumor cells were immunostaining-positive; "2" for 33–66%; "1" for 1–33% and "0" if $< 1\%$ were positive. The IHC data were defined by "percentage $\times$ intensity." SOX17 protein expression level was graded as positive if nuclear staining "percentage $\times$ intensity" was "3"^[10]. For MACC1 and c-Met, the scores were as follows: "0" for no color, "1" for light yellow, "2" for yellow, and "3" for brown. Percentage of positive cells was calculated under the view, and scoring was performed according to the following standards: $\leq 5\%$, a score of "0"; 6–25%, a score of "1"; 26–50%, a score of "2"; and 51–100%, a score of "3"^[19]. The final score was obtained by multiplying the average staining intensity of each slice by the average percentage of positive cells, with 0–4 score for negative (–), and 5–9 for positive (+).

2.3 Statistical analysis

All statistical analyses were performed using SPSS 23.0 (SPSS Inc, Chicago, IL, USA). The characteristics of the ESCC patients were compared using the χ^2 test. Overall survival (OS) and progression-free survival (PFS) were assessed using the Kaplan–Meier method and the log-rank test. Multivariate analysis was carried out using the Cox proportional hazard regression model. $P < 0.05$ was considered statistically significant.

Results

3.1 Clinicopathological characteristics

The demographic data of the 232 patients with ESCC included in the study and the pathological characteristics are summarized in Table 1. The median age of patients at the time of diagnosis was 64 years (32–83 years). The patients were followed up for a mean of 35 months (range 0–108). 152 (65.5%) patients died during the follow-up period.

Table 1
General characteristics of ESCC patients

Clinicopathological characteristics	n(%)
Age (years)	
Range	32–83
Median	64
Tumor size (cm)	
Range	0.5–7.5
Median	3.2
Gender	
Male	165(71.1%)
Female	67(28.9%)
Ethnicity	
Han	118(50.9%)
Kazak	114(49.1%)
Differentiation	
Well	61(21.3%)
Moderate	123(53.0%)
Poor	48(20.7%)
Lymph metastasis	
Negative	159(68.5%)
Positive	73 (31.5%)
Invasive depth	
Mucosa	61(21.3%)
Muscularis	123(53.0%)
Full-thickness	48 (20.7%)
TNM stage	
IA + B	19(8.2%)
IIA + B	146(62.9%)
IIIA + B	45(20.7%)
IVA + B	61(19.4%)
Vascular invasion	
Negative	190(81.9%)
Positive	42(18.1%)
Nerve invasion	
Negative	182(78.4%)
Positive	50(21.6%)
Hematogenous metastasis	
Negative	201(86.6%)
Positive	31(13.4%)
Abbreviations: ESCC: Esophageal squamous cell carcinoma.	

3.2 Association of SOX17, MACC1, and c-Met expression with clinicopathological parameters in ESCC

The expressions of SOX17, MACC1 and c-Met in normal esophageal epithelial cells and ESCC tissues were detected by immunohistochemistry. We found that the representative IHC images of SOX17, MACC1 and c-Met were shown in Fig. 1. And Fig. 1 shows blank controls for SOX17, MACC1, and c-Met. As is shown, SOX17 was diffusely and strongly expressed in normal esophageal mucosal tissue, and its positive expression was located in the nucleus and cytoplasm,

while both MACC1 and c-Met were negatively expressed in normal tissues. In esophageal squamous cell carcinoma, the expression of SOX17 was low (negative/weak positive) expression, and the expression of MACC1 and c-Met was positive. MACC1 is localized in the cytoplasm and cell membrane in ESCC patients. Otherwise, c-Met were both localized in cell cytoplasm and cell nucleus.

We analyzed the relationship between positive expression of three proteins and clinicopathological parameters in 232 ESCC patients. We found that the positive expression rate of SOX17 in 232 normal esophageal mucosal was 73.1% (163/232). Among all squamous cell carcinoma specimens, SOX17 positive specimens accounted for 81.8% (190/232). To assess the role of SOX17 proteins in the progression of ESCC, Pearson's χ^2 test was used to analyze the relationship between the expression of these proteins and the clinicopathological characteristics of ESCC (Table 2). SOX17 low expression was observed to be correlated with Vascular invasion ($p = 0.017$) and Nerve invasion ($p = 0.014$). Positive MACC1 expression was seen in 57.3% (133/232) of samples. Notably, positive MACC1 was correlated with tumor size ($p = 0.039$), TNM stage ($p = 0.020$) (Table 2). We also analyzed positive c-met expression and observed that these factors were positive in 54.3% (126/232) of samples. c-Met positive expression was significantly associated with hematogenous metastasis ($p = 0.045$) (Table 2).

Table 2
Correlation of SOX17, MACC1, and c-Met expression with clinicopathological features in 232 ESCC patients

	Total		p-value	Total		p-value	Total		p-value
	SOX17			MACC1			c-Met		
	Positive	Negative		Positive	Negative		Positive	Negative	
Gender									
Male	34(14.7%)	131(56.5%)		93(40.1%)	72(31.0%)		86(37.1%)	79(34.1%)	
Female	8(3.4%)	59(25.4%)	0.120	40(17.2%)	27(11.6%)	0.641	40(17.2%)	27(11.6%)	0.294
Age									
< 60	16(6.9%)	67(28.9%)		52(22.4%)	31(13.4%)		50(21.6%)	33(14.2%)	
≥ 60	26(11.2%)	123(53.0%)	0.729	81(34.9%)	68(29.3%)	0.221	76(32.8%)	73(31.5%)	0.176
Ethnicity									
Han	25(10.8%)	93(40.1%)		74(31.9%)	44(19.0%)		69(29.7%)	49(21.1%)	
Kazakh	17(7.3%)	97(41.8%)	0.215	59(25.4%)	55(23.7%)	0.092	57(24.6%)	57(24.6%)	0.195
Tumor location									
Upper	5(2.2%)	7(3.0%)		8(3.4%)	4(1.7%)		8(3.4%)	4(1.7%)	
Middle	23(9.9%)	118(50.9%)		79(34.1%)	62(26.7%)		69(29.7%)	72(31.0%)	
Lower	14(6.0%)	65(28.0%)	0.090	46(19.8%)	33(14.2%)	0.759	49(21.1%)	30(12.9%)	0.118
Tumor size									
< 3cm	11(4.7%)	59(25.4%)		33(14.2%)	37(15.9%)		35(15.1%)	35(15.1%)	
≥ 3cm	31(13.4%)	131(56.5%)	0.534	100(43.1%)	62(26.7%)	0.039	91(39.2%)	71(30.6%)	0.386
Degree of differentiation									
Poor	6(2.6%)	42(18.1%)		28(12.1%)	20(8.6%)		29(12.5%)	48(20.7%)	
Moderate	24(10.3%)	99(42.7%)		65(28.0%)	58(25.0%)		65(28.0%)	58(25.0%)	
Well	12(5.2%)	49(21.1%)	0.527	40(17.2%)	21(9.1%)	0.256	32(13.8%)	29(12.5%)	0.634
Lymph node metastasis									
Negative	28(12.1%)	131(56.5%)		93(40.1%)	66(28.4%)		83(35.8%)	76(32.8%)	
Positive	14(6.0%)	59(25.4%)	0.773	40(17.2%)	33(14.2%)	0.597	43(18.5%)	30(12.9%)	0.341
Invasive depth									
Mucosa	0(0.0%)	7(3.0%)		5(2.2%)	2(0.9%)		4(1.7%)	3(3.0%)	
Muscularis	18(7.8%)	80(34.5%)		52(22.4%)	46(19.8%)		53(22.8%)	45(19.4%)	
Full-thickness	24(10.3%)	103(44.4%)	0.448	76(32.8%)	51(22.0%)	0.443	69(29.7%)	58(25.0%)	0.988
TNM stage									
IA + B	3(1.3%)	16(6.9%)		17(7.3%)	2(0.9%)		13(5.6%)	6(2.6%)	
IIA + B	27(11.6%)	119(51.3%)		76(32.8%)	70(30.2%)		71(30.6%)	75(32.3%)	
IIIA + B	6(2.6%)	39(16.8%)		27(11.6%)	18(7.8%)		28(12.1%)	17(7.3%)	
IVA + B	6(2.6%)	16(6.9%)	0.568	13(5.6%)	9(3.9%)	0.020	14(6.0%)	8(3.4%)	0.149
Vascular invasion									
Negative	29(12.5%)	161(69.4%)		107(46.1%)	83(35.8%)		99(42.7%)	91(39.2%)	
Positive	13(5.6%)	29(12.5%)	0.017	26(11.2%)	16(6.9%)	0.508	27(11.6%)	15(6.5%)	0.152
Nerve invasion									
Negative	27(11.6%)	155(66.8%)		109(47.0%)	73(31.5%)		99(42.7%)	83(35.8%)	
Positive	15(6.5%)	35(15.1%)	0.014	24(10.3%)	26(11.2%)	0.132	27(11.6%)	23(9.9%)	0.960

Abbreviations: ESCC: Esophageal squamous cell carcinoma.

	Total		p-value	Total		p-value	Total		p-value
Hematogenous metastasis									
Negative	35(15.1%)	166(71.6%)		115(49.5%)	86(37.1%)	0.929	104(44.8%)	97(41.8%)	
Positive	7(3.0%)	24(10.3%)	0.487	18(7.8%)	13(5.6%)		22(9.5%)	9(3.9%)	0.045
Abbreviations: ESCC: Esophageal squamous cell carcinoma.									

In conclusion, SOX17, MACC1 and c-Met may be specific biomarkers for esophageal precancerous lesions and cancer screening.

3.3 The relationship between SOX17, MACC1 and c-Met

In 232 cases of ESCC, the expression of SOX17 was correlated with c-Met by Spearman correlation analysis ($p = 0.002$), but the expression of SOX17 was not correlated with MACC1 ($p = 0.092$; Table 3). We analyzed the relationship between MACC1 and c-Met by Spearman correlation analysis and found that the correlation between MACC1 and c-Met was statistically significant, and they were observably correlated ($p < 0.001$; Table 3).

Table 3
Correlation between SOX17, MACC1, and c-Met expression in 232 ESCC patients

Correlation between SOX17, MACC1, and c-Met expression in ESC ESCC patients															
Total (n,%)			r	p-value	Total (n,%)			r	p-value	Total (n,%)			r	p-value	
SOX17				MACC1				c-Met							
Positive		Negative		Positive		Negative		Positive		Negative					
SOX17															
Positive	-	-			82(35.3%)	108(46.4%)			87(37.5%)	103(44.4%)					
Negative	-	-	-	-	17(7.3%)	25(10.8%)	-0.021	0.750	19(8.2%)	23(9.9%)	-0.004	0.948			
MACC1															
Positive	25(10.8%)	108(46.4%)			-	-			95(40.90%)	38(16.4%)					
Negative	17(7.3%)	82(35.3%)	-0.021	0.750	-	-	-	-	31(13.4%)	68(29.3%)	0.398	<0.001			
c-Met															
Positive	23(9.9%)	103(44.4%)			95(40.90%)	31(13.4%)			-	-					
Negative	19(8.2%)	87(37.5%)	-0.004	0.948	38(16.4%)	68(29.3%)	0.398	<0.001	-	-	-	-			
Abbreviations: ESCC: Esophageal squamous cell carcinoma.															

Then, the GEPIA database (<http://gepia.cancer-pku.cn>) analysis showed that the expression of c-Met in esophageal cancer tissues was higher than that in normal esophageal mucosa tissues, which was statistically significant ($*p < 0.05$; Figure 2). Correlation analysis showed that the expression of SOX17 was negatively correlated with the expression of MACC1 ($p < 0.001$, $R = -0.16$) and c-Met ($p < 0.001$, $R = -0.16$), while the expression of MACC1 was positively correlated with the expression of c-Met ($p < 0.001$, $R = 0.39$; Figure 2).

In conclusion, we conclude that SOX17 is often low expression, MACC1 is positively expressed and c-Met is positively expressed in esophageal cancer, and MACC1 expression is positively correlated with c-Met expression. The relationship between the three proteins may play an important role in the development of esophageal carcinoma.

3.4 Association clinical features in ESCC with patient survival for OS and PFS

The survival rate of 232 ESCC patients in this study was 34.5%. OS and PFS were analyzed through Kaplan–Meier plots (Fig. 3). Univariate analysis showed that Age ($p = 0.020$), Degree of differentiation ($p = 0.009$), Lymph node metastasis ($p = 0.003$), TNM stage ($p = 0.001$), Nerve invasion ($p = 0.008$), Hematogenous metastasis ($p = 0.026$) were associated with OS (Table 4). Additionally, Age ($p = 0.016$), Degree of differentiation ($p = 0.005$), Lymph node metastasis ($p = 0.001$), TNM stage ($p < 0.001$), Nerve invasion ($p = 0.021$), and Hematogenous metastasis ($p = 0.002$) were significantly correlated with PFS (Table 4). Gender (OS : $p = 0.522$, PFS : $p = 0.511$), Ethnicity (OS : $p = 0.111$, PFS : $p = 0.282$), Tumor location (OS : $p = 0.369$, PFS : $p = 0.300$), Tumor size (OS : $p = 0.083$, PFS : $p = 0.050$), Invasive depth (OS : $p = 0.621$, PFS : $p = 0.258$), Vascular invasion (OS : $p = 0.965$, PFS : $p = 0.908$) was not associated with prognosis in both OS and PFS (Table 4).

Table 4
Univariate analysis of factors associated with OS and PFS in ESCC patients

Clinicopathological characteristics	OS		PFS	
	χ^2	p-value	χ^2	p-value
Gender				
Male/Female	0.411	0.522	0.432	0.511
Age(years)				
< 60/≥60	5.445	0.020	5.782	0.016
Ethnicity				
Han/Kazakh	2.546	0.111	1.159	0.282
Tumor location				
Upper/Middle/Lower	1.991	0.369	2.410	0.300
Tumor size(cm)				
< 3/≥3	3.011	0.083	3.857	0.050
Degree of differentiation				
Poor/Moderate/Well	9.340	0.009	10.440	0.005
Lymph node metastasis				
Negative/Positive	8.576	0.003	10.501	0.001
Invasive depth				
Mucosa/Muscularis/Full-thickness	0.954	0.621	2.713	0.258
TNM stage				
IA + B/IIA + B/IIIA + B/IVA + B	15.894	0.001	18.906	< 0.001
Vascular invasion				
Negative/Positive	0.002	0.965	0.013	0.908
Nerve invasion				
Negative/Positive	7.014	0.008	5.340	0.021
Hematogenous metastasis				
Negative/Positive	4.974	0.026	9.673	0.002
SOX17 expression				
Negative/Positive	0.237	0.626	0.506	0.477
MACC1 expression				
Negative/Positive	0.014	0.905	0.079	0.778
c-Met expression				
Negative/Positive	5.293	0.021	3.782	0.052

Abbreviations: ESCC, esophageal squamous cell carcinoma; OS, overall survival; PFS, progression-free survival.

To further confirm the role of SOX17, MACC1, and c-Met expression in ESCC, we analyzed OS and PFS using the Kaplan–Meier survival analysis with log-rank test (Fig. 4). We found that SOX17 expression was not associated with both OS ($p = 0.626$) and PFS ($p = 0.477$). Furthermore, MACC1 expression was not associated with both OS ($p = 0.906$) and PFS ($p = 0.778$). Notably, positive c-Met expression had a shorter OS ($p = 0.021$). However, c-Met expression was not associated with PFS ($p = 0.052$).

Next, we used Kaplan–Meier and a log-rank test to analyze OS rate in ESCC after stratification by c-Met expression and clinical features (Fig. 5). Positive c-Met expression was significantly correlated with poor prognosis in the group with Tumor size ≥ 3 cm ($p = 0.011$), the depth of invasion (Muscularis) ($p = 0.013$), negative lymph node metastasis ($p = 0.045$), TNM IIA + B pathological stage ($p = 0.015$), negative vascular invasion ($p = 0.030$), and negative hematogenous metastasis ($p = 0.045$). Moreover, c-Met expression correlated significantly with PFS among the depth of invasion (Muscularis) ($p = 0.019$), TNM IIA + B pathological stage ($p = 0.020$), and negative vascular invasion ($p = 0.046$).

Cox proportional multivariate analysis of relationships between all the significant variables and patient survival are shown in Table 5. We found that age (hazard ratio [HR]: 0.686, 95% confidence interval [CI]: 0.469–0.949, $p = 0.023$), degree of differentiation (HR: 0.7, 95% CI: 0.555–0.885, $p = 0.003$), lymph node

metastasis (HR:1.62, 95% CI: 1.163–2.258, p = 0.004),TNM stage (HR:1.438, 95% CI:1.168–1.77, p = 0.001), nerve invasion (HR:1.617, 95% CI: 2.331–1.122, p = 0.01),and hematogenous metastasis (HR:1.577, 95% CI: 1.044–2.381, p = 0.03),c-Met expression (HR:1.453, 95% CI:1.049–2.012, p = 0.025) were significant independent predictors of OS. In the multivariate analysis, age (HR:0.678, 95% CI:0.49–0.938, p = 0.019), degree of differentiation (HR:0.689, 95% CI:0.546–0.868, p = 0.002) lymph node metastasis (HR:1.703, 95% CI:1.223–2.372, p = 0.002)TNM stage (HR:1.485, 95% CI:1.204–1.832, p < 0.001), nerve invasion (HR:1.522, 95% CI:1.056–2.194, p = 0.024), and hematogenous metastasis (HR:1.899, 95% CI:1.25–2.885, p = 0.003) were also found to be independent factors affecting PFS. But c-Met expression (HR:1.37, 95% CI:0.991–1.894, p = 0.057) is not PFS related(Table 5).

Table 5
Multivariate analysis of factors associated with OS and PFS for ESCC

Clinicopathological characteristics	OS			PFS		
	95%CI	HR	p-value	95%CI	HR	p-value
Age (years)						
< 60/≥60	0.469–0.949	0.686	0.023	0.49–0.938	0.678	0.019
Degree of differentiation						
Poor/Moderate/Well	0.555–0.885	0.7	0.003	0.546–0.868	0.689	0.002
Lymph node metastasis						
Negative/Positive	1.163–2.258	1.62	0.004	1.223–2.372	1.703	0.002
TNM stage						
IA + B/IIA + B/IIIA + B/IVA + B	1.168–1.77	1.438	0.001	1.204–1.832	1.485	< 0.001
Nerve invasion						
Negative/Positive	2.331–1.122	1.617	0.01	1.056–2.194	1.522	0.024
Hematogenous metastasis						
Negative/Positive	1.044–2.381	1.577	0.03	1.25–2.885	1.899	0.003
c-Met expression						
Negative/Positive	1.049–2.012	1.453	0.025	0.991–1.894	1.37	0.057

Abbreviations: CI, confidence interval; ESCC, esophageal squamous cell carcinoma; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

In summary, c-Met may be a prognostic target for patients with esophageal squamous cell carcinoma.

Discussion

Esophageal squamous cell carcinoma (ESCC) is the most difficult subtype of esophageal cancer to treat due to the paucity of effective targeted therapy. Our research group has been committed to the study of the role of the SOX family in ESCC. It is found that SOX2 was related to lymph node metastasis (p = 0.004) and vascular invasion (p = 0.041). Patients with SOX2 overexpression had poor prognosis^[20]. SOX17, like SOX2, is a stem cell transcription factor of the SOX family gene.

SOX17,as a marker of embryonic stem cells, contains the DNA-binding domain of HMG-box, which can bind to specific DNA target sequences with high affinity, thus affecting the transcription of target genes^[9]. This makes it the most effective therapeutic target. SOX17 is expressed in a variety of tumors such as esophageal cancer^[10],oral cancer^[21], small cell lung cancer^[22], head and neck squamous cell cancer^[23], cholangiocarcinoma^[24],gastric cancer^[25]and brain malignant glioma^[26], and plays a key role in the occurrence, development, recurrence and metastasis of tumors.

Kim Soo Yeon identified that TFPI2, SOX17, and GATA4 are frequently hypermethylated in human oral squamous cell carcinoma cells in a cancer-specific manner and that the transcriptional expression of these genes is regulated by promoter hypermethylation in OSCC^[21]. Methylated SOX17 is mainly expressed in tumor tissues, while SOX17 is highly expressed in normal tissues, suggesting that SOX17 may play an inhibitory role in tumor progression. The researches show that an inverse correlation between CpG hypermethylation and the mRNA expression level of SOX17 gene in ESCC patients^[27]. Methylated SOX17 is mainly expressed in tumor tissues, while SOX17 is highly expressed in normal tissues, suggesting that SOX17 may play an inhibitory role in tumor progression. Other studies have reported that the expression of SOX17 is low in ESCC patients, and the low expression of SOX17 is significantly associated with TNM staging, tumor recurrence, and higher risk of death^[10]. The effect of SOX17 expression in esophageal cancer on prognosis and its specific mechanism are still controversial.

In the current study, we found that SOX17 was low expressed in 81.8% (190/232) of primary ESCC patients. More importantly, we observed that SOX17 low expression was associated with Vascular invasion (p = 0.017), and Nerve invasion (p = 0.014). SOX17 expression was not correlated with MACC1 (p = 0.750) and c-Met (p = 0.948). With regard to survival, we found that SOX17 expression was not significantly correlated (OS: p = 0.626, PFS: p = 0.477) with patient survival time in ESCC by Kaplan–Meier analysis. The difference between the above results may be related to the individual differences and sample size of the subjects. Our findings reminder that SOX17 may be a key tumor suppressor gene in ESCC.

The metastasis-associated in colon cancer-1 (MACC1) gene was identified in 2008 by Stein Ulrike^[11]. Recent findings have shown that MACC1 expression is upregulated, statistically significantly, in ESCC when compared with normal esophageal squamous epithelial. Recent study found that 47 of 85 cancer lesions (55.2 %) were stained positive, and high expression of MACC1 was correlated with the node metastasis and TNM stage ($p < 0.05$). The Kaplan-Meier survival curve showed that patients with high MACC1 expression had significantly reduced overall 5-year survival rates ($p = 0.004$). Cox regression analysis revealed that high expression of MACC1 was associated with increased risk of death (hazard ratio [HR] = 2.25) in patients with esophageal cancer^[28]. Song et al demonstrated that OS time of MACC1 positive expression (39.0 ± 15.1 months) for patients with ESCC by Kaplan-Meier analysis was significantly lower than that of MACC1 negative for patients (53.7 ± 11.3 months; log-rank = 36.601, $p < 0.001$)^[29].

In the present study, we found that MACC1 was highly expressed in 57.3% (133/232) of primary ESCC patients. In addition, we discovered that positive MACC1 was correlated with tumor size ($p = 0.039$), TNM stage ($p = 0.020$), and c-Met expression ($p < 0.001$). In addition, the results of GEPIA database analysis showed that MACC1 significantly positively correlated with c-Met ($p < 0.001$, $R = 0.39$), while MACC1 was negatively correlated with SOX17 ($p < 0.001$, $R = -0.16$). This is partly consistent with our research results. This finding can illustrate that MACC1 is also an important factor in c-Met signaling pathways. With regard to survival, the Kaplan-Meier analysis demonstrated that the expression of MACC1 was not significantly related to the survival and prognosis of ESCC patients (OS: $p = 0.905$, PFS: $p = 0.778$). Although MACC1 has been reported to be a prognostic factor, findings from our study were not consistent with those from previous studies. The reason may be the primary anti-MACC1 antibody used and the diagnostic criteria were different among the studies. We confirmed the correlation between MACC1 and c-Met in ESCC, which may suggest that MACC1 may promote the development of esophageal squamous cell carcinoma, and this process may be related to the c-Met signaling pathway.

c-Met is a receptor-type tyrosine kinase that is involved in a wide range of cellular functions, including proliferation, motility, migration, invasion and tumor angiogenesis^[30]. Liu et al. found that c-Met overexpression was significantly correlated with the depth of invasion, lymph node metastasis, and AJCC stage in colon cancer^[31]. Huang et al. found that the high expression of c-Met played an important role in the invasion and lymph node metastasis of gastric cancer^[32]. Ozawa found that c-Met overexpression was significantly associated with tumor depth ($p = 0.013$) and pathological stage ($p = 0.010$) of esophageal squamous cell carcinoma, and the 5-year survival rate was significantly reduced in patients with high c-Met expression ($p = 0.022$)^[33]. Another study showed that high c-Met expression was an independent predictor of poor prognosis in patients for OS with ESCC (HR: 0.459 [95 % confidence interval: 0.287–0.733]; $p = 0.001$)^[34]. In addition, a recent meta-analysis found that c-Met overexpression was significantly associated with shorter OS (HR: 2.17, 95% CI: 1.62–2.90, $p < 0.001$) in esophageal squamous cell carcinoma (ESCC) ^[35].

In our study, we found c-Met positive patients accounted for 54.3% (126/232) of the total population. As well as, c-Met high expression was significantly associated with hematogenous metastasis ($p = 0.045$) and MACC1 expression ($p < 0.001$). With regard to survival, we found that ESCC patients with low c-Met expression had significantly better survival time than those with high c-Met expression ($p = 0.021$) by Kaplan–Meier analysis. Subgroup analysis found that in the positive c-Met expression group, age, gender, ethnicity, tumor location, degree of differentiation, and nerve invasion did not lead to any significant survival benefit. Moreover, subgroup analysis showed that positive c-Met expression was significantly correlated with favorable prognosis in the group with Tumor size ≥ 3 cm ($p = 0.011$), negative lymph node metastasis ($p = 0.045$), the depth of invasion (Muscularis) ($p = 0.013$), TNM IIA + B pathological stage ($p = 0.015$), negative vascular invasion ($p = 0.030$), and negative hematogenous metastasis ($p = 0.045$). However, we found that c-Met expression was not associated with PFS ($p = 0.052$). The results of the multi-factor analysis were consistent with the single-factor K-M analysis, and patients with positive c-Met expression had worse OS ($p = 0.025$). Our results suggest that c-Met is highly expressed in esophageal cancer, and its expression may be closely related to the expression of MACC1. Overexpression of c-Met promotes the progression of esophageal cancer, and it is an independent influencing factor for poor prognosis in ESCC patients. c-Met can be used as a biomarker to diagnose and predict the prognosis of esophageal cancer.

Previous studies only analyzed the expression of SOX17, MACC1 and c-Met in esophageal cancer individually. This is the first study to introduce the correlation between the expression of SOX17, MACC1 and c-Met in ESCC and clinicopathological parameters and prognosis of patients. And what our study found that c-Met expression was significantly associated with MACC1 expression ($p < 0.001$). However, SOX17 expression was independent of either MACC1 ($p = 0.750$) or c-Met ($p = 0.948$). And the survival analysis and COX regression analysis showed that the expression of c-Met was associated with OS in ESCC ($p = 0.021$, $p = 0.025$). MACC1 may be an important factor in c-Met pathways, and their expression may be related to the occurrence of ESCC. It was suggested that c-Met could be used as a molecular target for ESCC therapy.

Our study had several limitations. First, the diagnostic criteria for SOX17, MACC1 and c-Met status were tentative and not standardized. Second, we analyzed SOX17, MACC1 and c-Met only for protein overexpression using IHC, and not for gene amplification. In terms of clinical utility, standardized methods and diagnostic criteria should be established on the basis of the ability to evaluate pharmacological response to therapeutic intervention. Since recent clinical trials often use the diagnostic criteria with IHC for ESCC patient enrichment, the correlation between IHC overexpression and the affinity of each drug, and tumor heterogeneity may define the success of clinical development of each agent. Our existing studies have laid a foundation for further exploring the mechanisms of these three factors in the occurrence, development, invasion and metastasis of esophageal cancer, and provided a reference for clinical diagnosis and searching for potential therapeutic targets.

Conclusions

In conclusion, low expression of SOX17 was related to vascular invasion and nerve invasion. The high expression of MACC1 was closely associated with the tumor size and TNM stage. Besides, high expression of c-Met is associated with blood metastasis. There was a significant correlation between c-Met and MACC1. SOX17, MACC1 and c-Met participate in the occurrence and development of ESCC. We also found that positive c-Met expression was significantly associated with worse OS. Taken together, c-Met could be used as prognostic factors in patients with ESCC. These data could be used as the basis for future clinical trials for targeting agents in the treatment of ESCC patients.

Abbreviations

EC Esophageal carcinoma

ESCC: Esophageal squamous cell carcinomas

PBS: phosphatebuffered saline

DAB: 3,3'-Diaminobenzidine tetrahydrochloride

IHC: immunohistochemistry

OS Overall survival

PFS Progression free survival

HR: Hazard ratio

CI: Confidence intervals

Declarations

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (approval no. 20180223-08). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YS and CL designed and performed the experiments, analyzed the data and were the main contributors to the manuscript. ML and HW performed the experiments and interpreted data. GA and LS were used for supplementary experiments. Literature review and database analysis of SX and WZ. WL were involved in the experiments and data collection. YM designed the experimental program. All authors read and approved the final manuscript.

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Figures

Figure 1

IHC staining of SOX17, MACC1, and c-Met in ESCC tissues. Notes: (A) SOX17-positive localized in cell nuclei and cytoplasm expression in normal esophageal tissues (10×); (B) SOX17-negative expression in ESCC (10×);(C) SOX17-low expression in ESCC (10×); (D) MACC1-negative expression in normal esophageal tissues (10×); (E) MACC1-negative expression in ESCC (10×); (F) MACC1-positive expression localized both in cell cytoplasm and cytomembrane in ESCC (10×); (G) c-Met-negative expression in normal esophageal tissues (10×); (H) c-Met-negative expression in ESCC (10×);(I) c-Met-positive expression localized in cell cytoplasm and cell nucleus in ESCC (10×); Abbreviations: ESCC, esophageal squamous cell carcinoma; IHC, immunohistochemistry.

Figure 2

SOX17, MACC1, c-Met query results in GEPIA database. Notes: (A) The expression difference of c-Met in esophageal cancer (red represents tumor tissue, black represents normal esophageal tissue), c-Met expression in esophageal cancer is higher than that in normal esophageal tissue ($*p < 0.05$); (B) SOX17 and MACC1 were negatively correlated in esophageal cancer ($p < 0.001$, $R = -0.16$); (C) The expressions of SOX17 and c-Met in esophageal cancer were negatively correlated ($p < 0.001$, $R = -0.16$); (D) The expressions of MACC1 and c-Met were positively correlated in esophageal cancer ($p < 0.001$, $R = 0.39$).

Figure 3

OS and PFS analysis of patients clinicopathological parameter with ESCC using the Kaplan–Meier method. Notes: OS according to (A)Age($p=0.020$); (B)Degree of differentiation($p=0.009$); (C)Lymph node metastasis ($p=0.003$); (D) TNM stage($p=0.001$); (E) Nerve invasion($p=0.008$); (F)Hematogenous metastasis($p=0.026$). PFS according to (G)Age($p=0.016$); (H)Degree of differentiation($p=0.005$); (I)Lymph node metastasis ($p=0.001$); (J)TNM stage($p<0.001$); (K) Nerve invasion ($p=0.021$); (L)Hematogenous metastasis($p=0.002$). Abbreviations: OS, overall survival; PFS, progression-free survival; ESCC, esophageal squamous cell carcinoma.

Figure 4

OS and PFS analysis of patients SOX17, MACC1, and c-Met expression with ESCC using the Kaplan–Meier method. Notes: OS according to (A) SOX17 expression($p=0.626$); (B)MACC1 expression($p=0.906$); (C)c-Met expression($p=0.021$).PFS according to(D) SOX17 expression($p=0.477$); (E)MACC1 expression($p=0.778$); (F)c-Met expression($p=0.052$). Abbreviations: OS, overall survival; PFS, progression-free survival; ESCC, esophageal squamous cell carcinoma.

Figure 5

OS and PFS based on c-Met expression. Notes: The OS and PFS of ESCC patients was analyzed using Kaplan–Meier and stratified by c-Met expression level. OS according to (A) Tumor size $\geq 3\text{cm}$ ($p=0.011$); (B) Invasive depth (Muscularis) ($p=0.013$); (C) Negative lymph node metastasis ($p=0.045$); (D) TNM IIA+B pathological stage ($p=0.015$); (E) Negative vascular invasion ($p=0.030$); (F) Negative hematogenous metastasis ($p=0.045$). PFS according to (G) Invasive depth (Muscularis) ($p=0.019$); (H) TNM IIA+B pathological stage ($p=0.020$); (I) Negative vascular invasion ($p=0.046$). Abbreviations: OS, overall survival; PFS, progression-free survival; ESCC, esophageal squamous cell carcinoma.