The effect of SOX17, MACC1 and c-Met expression in ESCC on prognosis

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Research

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Abstract

Aim: We mainly explored the expression of SOX17, MACC1 and c-Met in esophageal squamous cell carcinoma and their influence on the prognosis of patients.

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

The EC (esophageal cancer, EC) is the malignant tumor originating in the esophageal epithelium. In the global cancer Statistics of 2018, the EC was the 7th in the morbidity and the 6th in the mortality rate[1]. Esophageal squamous cell carcinoma is more common and has a worse prognosis. The incidence of esophageal cancer was significantly higher in males than in females[2]. The exact cause of esophageal cancer is unknown. But it happened and the living conditions of the residence, poor eating habits , there are strong carcinogen, lack of nutrients, virus infection, the lack of some anticancer factors and genetic susceptibility and so on[2, 3]. Although esophagectomy remains the primary treatment for esophageal cancer, it is highly invasive and is associated with a high incidence of morbidity and mortality. Furthermore, postoperative symptoms such as loss of appetite, early satiety, difficulty swallowing, aspiration and regurgitation can impair the patient's quality of life[4].The occurrence and development of esophageal cancer involve the changes of multiple genes and pathways, and the specific pathogenesis is still unclear. Therefore, it is of special importance and urgency to explore the molecular mechanisms of the occurrence, development and metastasis of esophageal cancer, establish the prevention and treatment targets, and seek new intervention strategies to improve the efficacy of esophageal cancer.

Recently, extensive studies have shown that some transcription factors related to stem cell self-renewal are abnormally regulated alter the signaling pathway during the carcinogenesis of esophageal squamous cell carcinoma[5].Our research group has been committed to the research on the progress of SOX family genes and ESCC.SOX17 plays an important role in the development of human[6], but its distribution in the esophagus has not been studied to date.

The SOX17 gene, A member of the SOXF 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].The research shows that SOX17 inhibits MACC1, MALAT1, NBN,

NFAT5, CSNK1A1, FN1 and SERBP1 genes through SRY binding mediated transcriptional regulation.FN1 and MACC1 may be novel inhibitory downstream genes of SOX17, and they may become potential therapeutic targets for ESCC[10]. SOX17 may be negative or poorly expressed in esophageal squamous cell carcinoma, and the effect of its expression in ESCC on prognosis remains to be further studied.

MACC1, Metastasis associated in colon cancer 1, is a newly identified prognostic biomarker for colorectal cancer metastasis and patient survival[11], when determined in the primary tumor or patient blood[12].The MACC1 gene is located on human chromosome 7p21.1 and has 7 exons, encoding a protein composed of 852 amino acid residues.MACC1 is a key regulator of the HGF/c-Met pathway. 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]. Dysregulation of HGF/c-Met signaling has been reported as a prognostic marker for tumorigenesis, early invasion, and metastasis[14]. MACC1 may be highly expressed in esophageal squamous cell carcinoma, and its high expression may be associated with poor prognosis of esophageal carcinoma, but its specific role and relationship remain to be 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]. 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[17]. c-Met may be overexpressed in esophageal cancer, but its specific mechanism remains to be further studied.

Our aim was to investigate the prognostic significance of expression of SOX17, MACC1 and c-Met in ESCC.

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: Uygur nationality, Mongolian, etc. 232 patients with ESCC and 160 adjacent nontumorous tissues were randomly selected in this study.

We collected 232 cases of ESCC paraffin-embedded tissues and 160 cases 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

On the day before the experiment, tissue chips were put into the 37◦C oven for the night, softening the wax layer that covered the tissue chip. Tissues were fixed in 10% formalin, sectioned at 5 μm, 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 for 15 min at 37°C. After using DAB for staining, the section was dehydrated, and tread with xylene.

The cutoff values for positivity were as follows: Any five high-power fields (×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 × intensity." SOX17 protein expression level was graded as positive if nuclear staining "percentage × 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: ≤5%, a score of "0"; 6–25%, a score of "1"; 26–50%, a score of "2"; and 51–100%, a score of "3"[18]. 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 $\chi 2$ test. 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 Clinicopathologic 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 31 months (range 1–72). 152 (65.5%) patients died during the follow-up period.

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

We detected *SOX17*, MACC1, and c-Met expression in both adjacent normal esophageal mucosa epithelium and ESCC tissues by IHC. We found that representative IHC images of *SOX17*, MACC1, and c-Met are presented in Figure 1. The blank controls for *SOX17*, MACC1, and c-Met are presented in Figure 1.

In normal esophageal mucosa, SOX17 was high expression, with positive expression located in the nucleus and cytoplasm, while MACC1 and c-Met were negatively expressed in normal tissues. We found that among 232 cases of normal esophageal mucosa,163 were SOX17 positive and 60 were SOX17 negative. Therefore, the positive rate of SOX17 in the normal esophageal mucosa was 73.1%.

Besides，we analyzed the relationship between positive expression of three proteins and clinicopathological parameters in 232 cases ESCC patients. *SOX17* expression was detected in ESCC tissues. *SOX17* was localized in cell nuclei and cytoplasm. Of the cancer specimens, *SOX17 low expression* was observed in 81.8% (190/232) of samples, *SOX17* status of the samples is shown in Figure 1. To evaluate the role of *SOX17* protein in ESCC progression, we analyzed the association between those protein expression and clinicopathological characteristic in ESCC using Pearson's $\chi 2$ test (Table 2). We observed that *SOX17 low* expression was associated with Vascular invasion (p=0.017) and Nerve invasion (p=0.014).

MACC1 is localized in the cytoplasm and cell membrane in ESCC patients. Among the 232 ESCC samples, 133 had positive MACC1 expression, so the positive rate of MACC1 was 57.3%.It is worth noting that the positive expression of MACC1 was significantly different from tumor size (p=0.039) and TNM stage (p =0.020), suggesting that MACC1 may be correlated with tumor size and TNM stage (Table 2).

c-Met were both localized in cell cytoplasm and cell nucleus. We also analyzed positive c-Met expression and observed these factors to be positive in 54.3% (126/232) of samples. c-Met positive expression was associated with hematogenous metastasis(p=0.045) (Table 2).

These results suggest that low expression of SOX17, high expression of MACC1 and high expression of c-Met may promote the progression of esophageal squamous cell carcinoma.

3.3 The relationship between SOX17, MACC1 and c-Met

In 232 cases of ESCC, the expression of SOX17 was not correlated with MACC1 (p = 0.092) or c-Met(p=0.948). In addition, 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).Then, the GEPIA database (http://gepia.cancer-pku.cn) was consulted, and it was found that the differential expression of c-Met in esophageal cancer was statistically significant (*p<0.05，Figure 2), and SOX17 expression was negatively correlated with MACC1 (p<0.001,R= -0.16) and c-Met (p<0.001,R= -0.16) expression, while MACC1 expression was positively correlated with c-Met (p<0.001,R= 0.39) expression，Figure 2）.

In conclusion, we conclude that SOX17 is often negative or 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 (Figure 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).

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 (Figure 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 feathers (Figure 5).Positive c-Met expression was significantly correlated with poor prognosis in the group with Tumor size≥3cm (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 significant 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).

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[19]. 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[20], small cell lung cancer[21], head and neck squamous cell cancer[22], cholangiocarcinoma[23],gastric cancer[24]and brain malignant glioma[25], 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 OSCC cells in a cancer-specific manner and that the transcriptional expression of these genes is regulated by promoter hypermethylation in OSCC[20]. The researches show that an inverse correlation between CpG hypermethylation and the mRNA expression level of SOX17 gene in ESCC patients[26].On the other hand, Low SOX17 protein expression was found in 47.4% (73 of 154) of ESCC patients with predicted poor prognosis.SOX17 protein low expression, TNM staging and recurrence were associated with a significantly increased risk of cancer-related death[10]. There is still controversy regarding the significance of *SOX17* expression for prognosis.

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[27]. 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)[28].

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).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. The correlation of MACC1 and c-Met in ESCC is confirmed by us, and this result can indicate that MACC1 may promote the development of esophageal squamous cell carcinoma.

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[29]. The findings found that there was a significant difference in OS between c-Met high expression patients and c-Met low expression or negative patients (median: 41.9 months vs. 56.7 months; p= 0.001). And multivariate analysis also showed that, of the covariates analyzed, c-Met high expression was the only prognostic factor for OS (HR: 0.459 [95 % confidence interval: 0.287-0.733]; p = 0.001)[30]. Another study uncovered that c-Met status was significantly correlated with tumor depth (p= 0.013) and pathological stage (p = 0.010).Survival analysis displayed that the 5-year overall survival rate of patients with c-Met high expression was significantly lower than that of those in the c-Met low expression group (p= 0.022).Univariate analysis revealed that patient survival was significantly associated with c-Met expression (p = 0.017) [31]. 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) [32].

In our study, we found c-Met positive patients accounted for 54.3% (126/232) of the total population. As well as, c-Met 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 negative c-Met expression had significantly better survival time than those with positive 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≥3cm (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 may serve as a biomarker for the prognosis of esophageal squamous cell carcinoma.

This is the first study to introduce the correlation between SOX17, MACC1 and c-Met expression in ESCC 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.

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.

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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Tables

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.

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
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.

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

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

Clinicopathological characteristics	OS		PFS	
	χ2	p-value	χ2	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.

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.

Figures

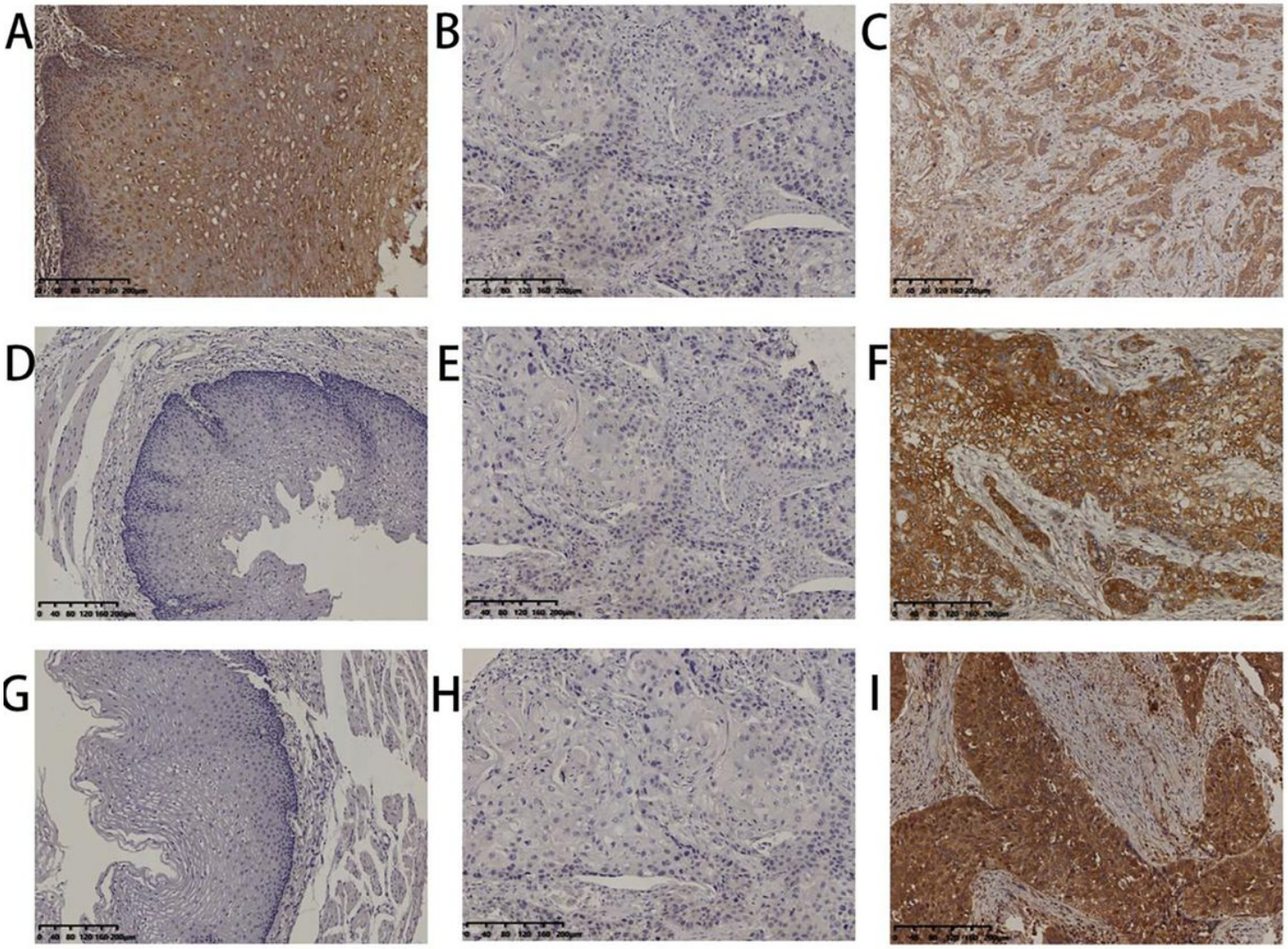

Figure 1

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.

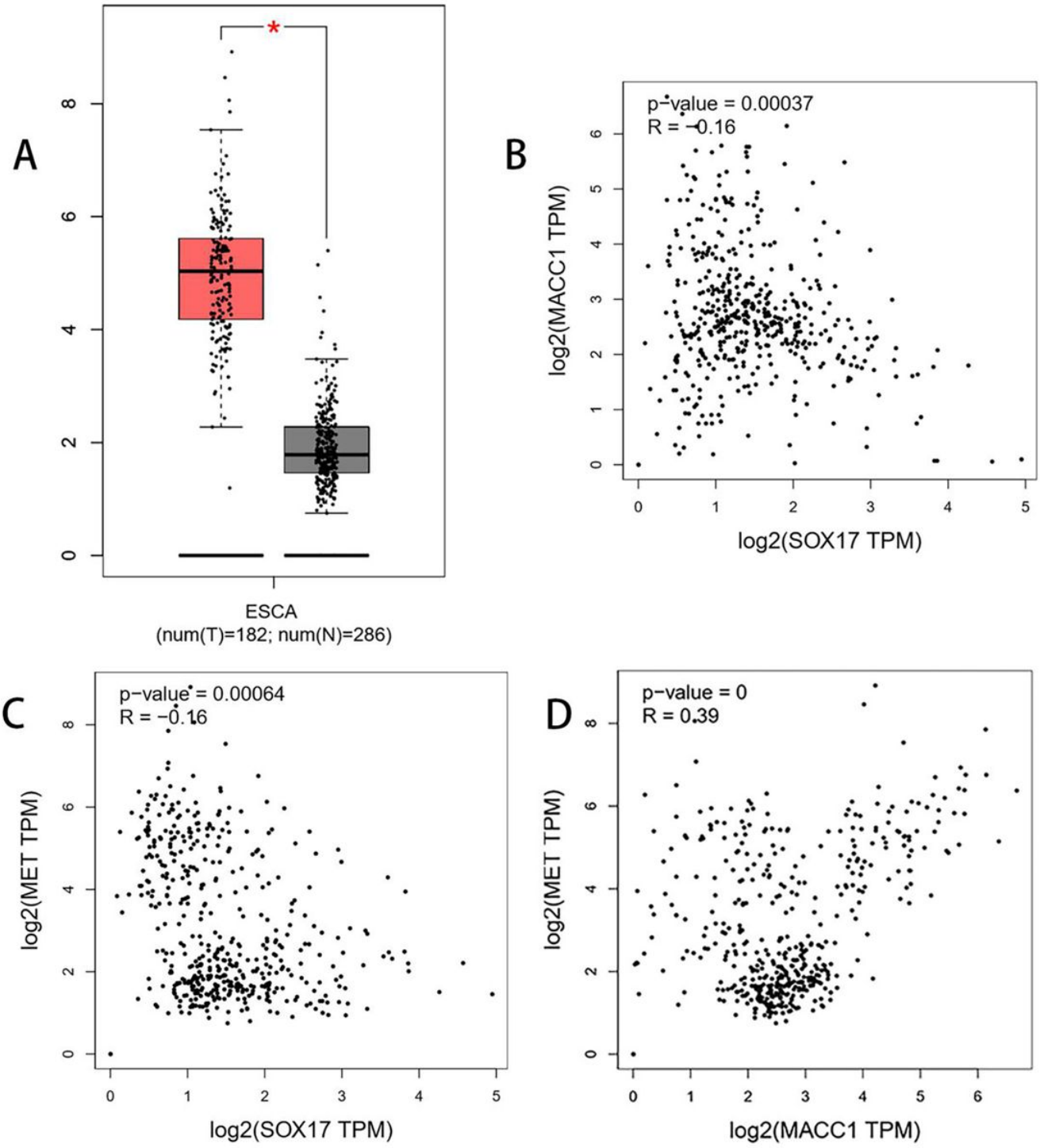

Figure 2

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).

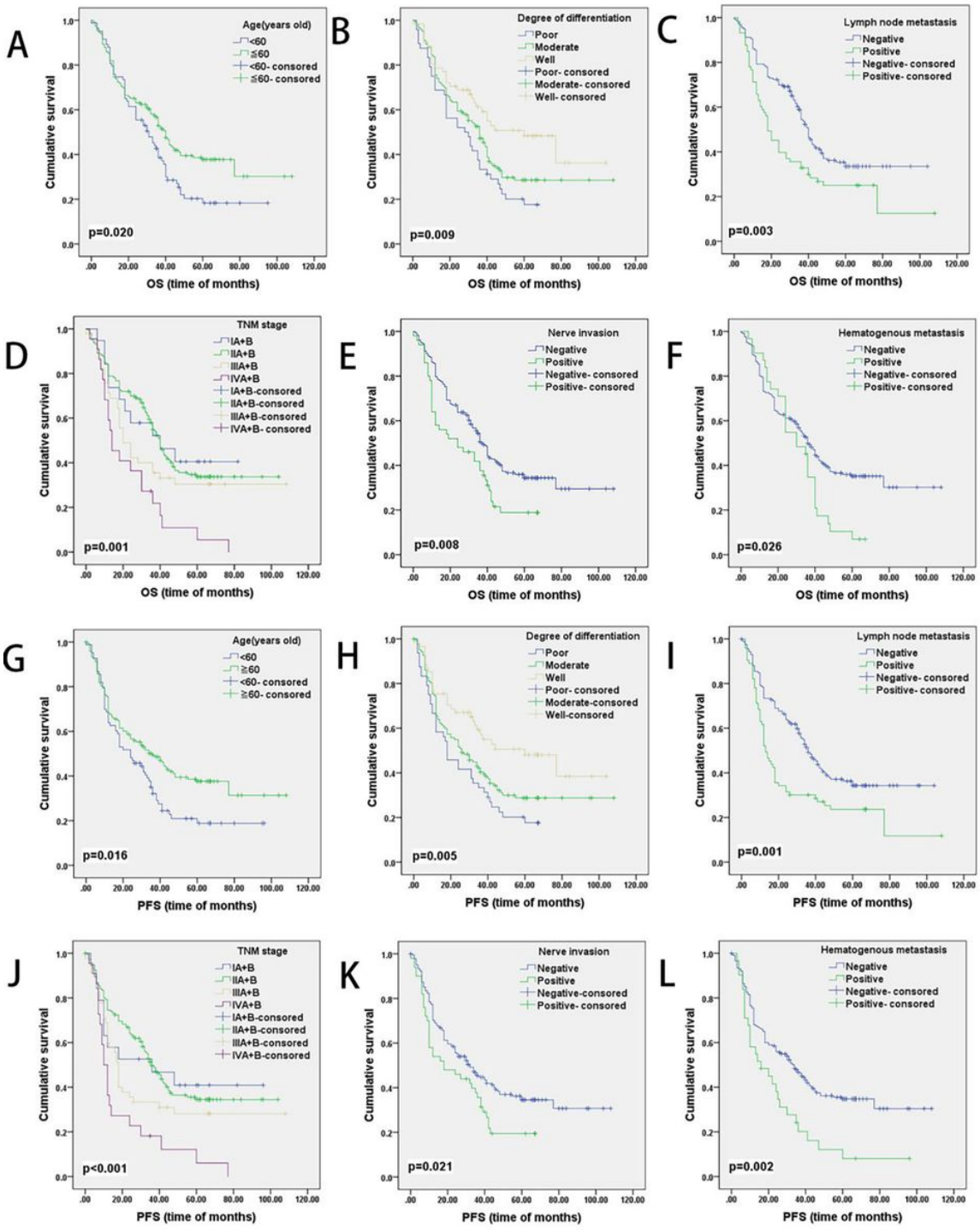

Figure 3

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.

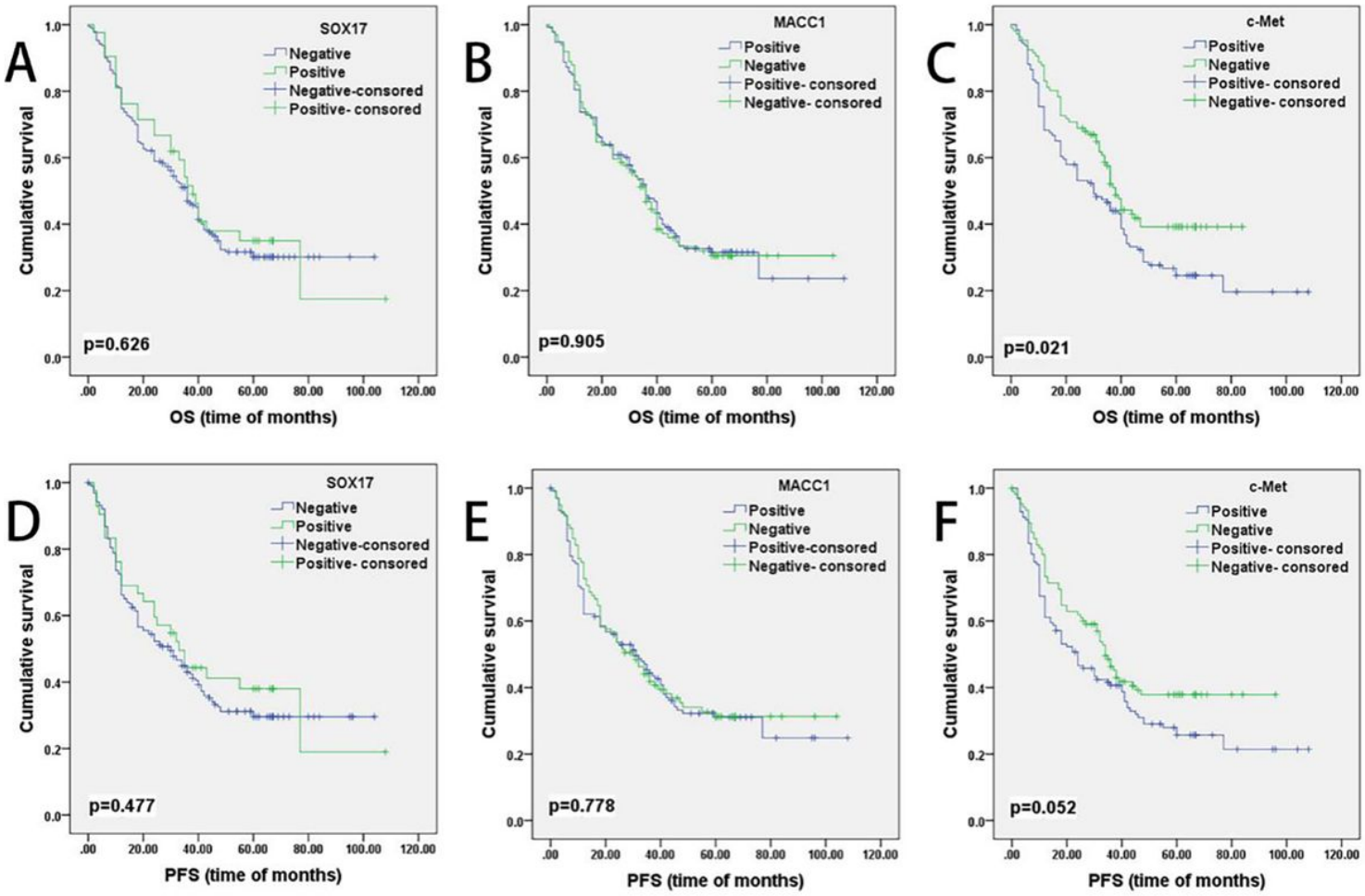

Figure 4

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.

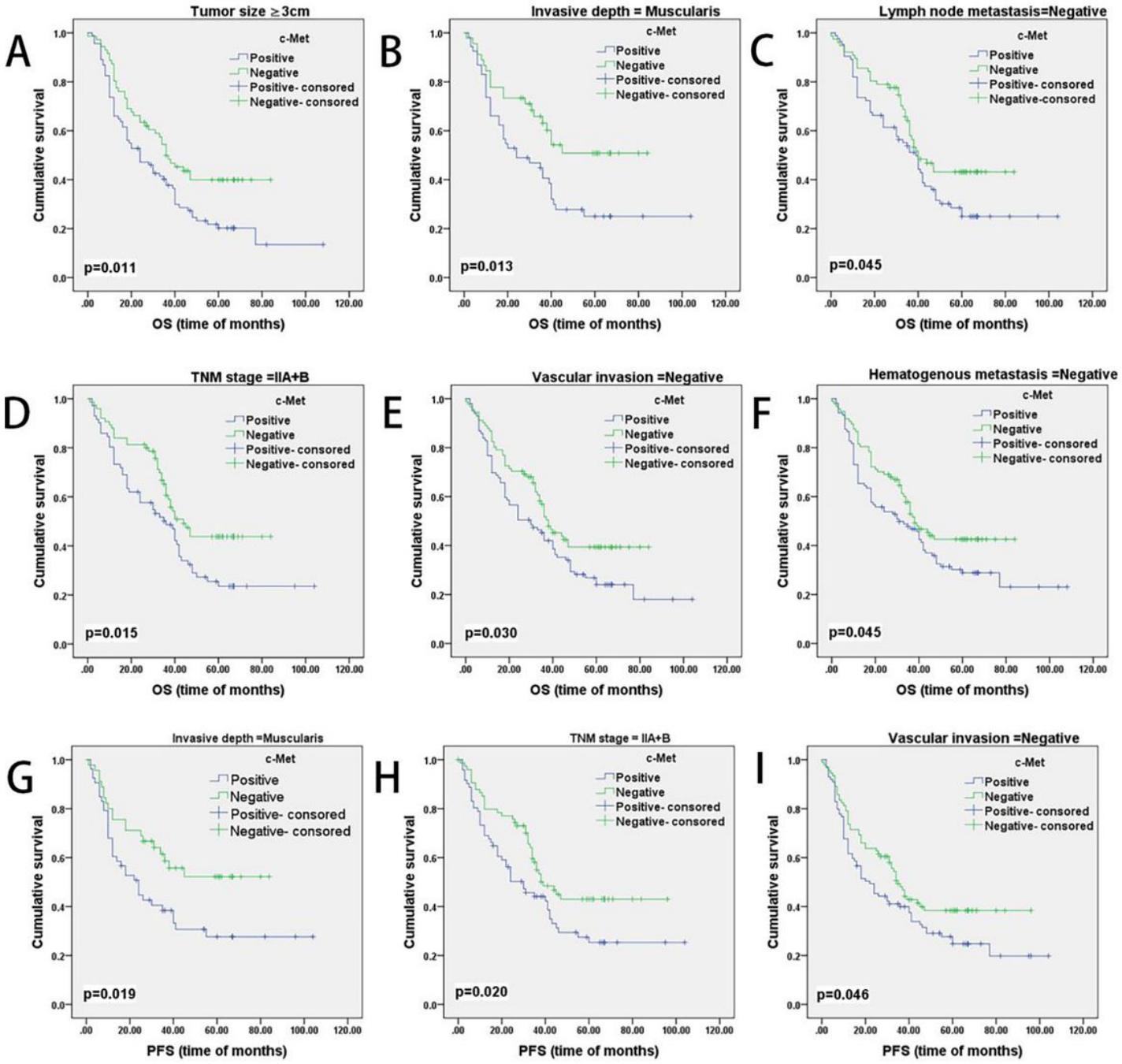

Figure 5

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≥3cm (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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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- Table3.xlsx
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