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Original Article

Evaluation of the correlativity of gender determining region Y-box 4, N-cadherin, CD44 and E-cadherin expression in the prognosis of esophageal squamous cell carcinoma

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Abstract: Background: SOX4 is highly expressed in many different tumor types, and SOX4 has been reported in the literature to participate in tumor proliferation, damaging and movement by leading Epithelial-Mesenchymal Transition. Cancer vital cells and Epithelial-Mesenchymal Transition have been repeatedly confirmed to participate during the proliferation, damaging and movement of cancer. This research examined the association of the Epithelial-Mesenchymal Transition-related molecules E-cadherin, N-cadherin, CD44, and SOX4 in the ESCC and aimed for providing inspiration for clinical treatment as well as to indicate a new direction for detecting invasion and forecasting the prospect of affected role using ESCC. Methods: Immunohistochemistry was utilized to observe the expression of the SOX4, N-cadherin, CD44 and E-cadherin proteins. Survival analysis of the positive and negative SOX4, E-cadherin, N-cadherin and CD44 protein expression groups was performed by the Kaplan-Meier approach. Outcomes: A confirming relationship was observed among the expression of SOX4, N-cadherin or CD44 and tumor diameter, distant metastasis, deepness of damaging, lymph node metastasis, pTNM stage and histological grade ($P<0.05$). Spearman correlativity calculation displayed that the expression of the SOX4 protein was obviously responded with the expression of the N-cadherin and CD44 proteins. Moreover, the expression of the N-cadherin and CD44 proteins was also positively correlated. The E-cadherin protein was negatively correlated with SOX4, N-cadherin and CD44 protein expression in ESCC. SOX4, N-cadherin, CD44, E-cadherin, age and distant metastasis were determined to be separate elements that influenced the prognosis of patients with ESCC. Conclusions: We found that suppression of ESCC providers can suppress the growth of bad tumors and change therapeutic results for ESCC patient since CD44 supports the induction of Epithelial-Mesenchymal Transition in ESCC.

Keywords: SOX4, N-cadherin, CD44, E-cadherin, ESCC

Introduction

Esophageal cancer (EC) is one of the worst neoplasms in the elements related to digestives pathway [1] with its occurrence, development, and outcomes involve multiple genes and processes. However, the pathogenesis of EC remains unclear. Squamous cell carcinoma is one of the most common histological classifications in EC. Main resource of treatment failure and death in patients with EC is infiltration and metastasis. Therefore, it is especially important to discover molecular providers for early diagnosis and forecast of ESCC.

Epithelial-Mesenchymal Transition, during which cells belonging to the epithelium develop a mesenchymal characteristic and motility through a cascade of biological events, is a complex program [2]. Epithelial-Mesenchymal Transition is a cellular procedure in which epithelial cells drop off polarize organ and cell-cell adjunction, experience varieties in cell form and in cytoskeletal organs and produce mesenchymal features, such as fibroblast-like cell morphology and growing cell movement and damage [3]. In this process, loss of polarity and adhesion of epithelial cells, cytoskeletal reorganization, and remodeling of the extracellular

matrix are observed, and the cells acquire a spindle shape with increased motility; this allows tumor cells to easily infiltrate and metastasize to other tissues. M Ricciardi, et al. indicated that Epithelial-Mesenchymal Transition is a well-displayed pathogenetic consequence that occurs in cancer advancement and movement, as cancer cells pass through Epithelial-Mesenchymal Transition get obviously damaging characteristics that support cancer spread [4]. Many studies have implicated Epithelial-Mesenchymal Transition in the damaging and movement of cancer. Epithelial-Mesenchymal Transition is described as a necessary procedure relying on which epithelial tumors can invade surrounding tissues [5]. Epithelial-Mesenchymal Transition requires the deprivation of epithelial providers, such as the nasty adjunction proteins claudin and occludin and the related adjunction proteins E-cadherin, α and β-catenin and cytokeratins. Simultaneously, the expression of many mesenchymal providers is increased, including matrix metalloproteinase, integrins α_v and β_1, vimentin, N-cadherin, and fibronectin [6, 7].

Gender deciding area Y-box 4 (SOX4) is one part of the SOX copy cause group and is featured by an obviously preserved race in its high-mobility DNA-binding area [8]. It has been reported that SOX4 is obviously displayed in ESCC [9]. The deregulated expression of SOX4 has been displayed to induce Epithelial-Mesenchymal Transition and movement in cancer organs [10].

Tumors are cellularly and molecularly heterogeneous, as they contain groups of undistinguished cancer organs that exhibit root cell-like characteristics [11]. Singh et al. highlighted in recent studies that implicated the use of TGF-β-regulated noncoding RNAs in riding Epithelial-Mesenchymal Transition and encouraging cancer cells that exhibit stem cell-like self-renewal [11]. Tumor stem cells mainly refer to a small number of cells that can induce tumors to a similar extent as tumour cells, and thus, they not only contribute to the quick increase of tumors but are also resistant to drugs used for treatment, which means that only differentiated cells are killed when patients receive chemotherapy. Additionally, tumor cells are retained, eventually leading to tumor recurrence [12]. CD44, a one-chain transmembrane glycoprotein that is encoded by a single gene, is highly heterogeneous and is an important sense organ for hyaluronic acid. CD44 is closely associated with tumor progression and primarily functions in the proliferation and infiltration of cancer cells. CD44 is also related to metastasis and promotes the generation of blood vessels around the tumor [13, 14]. CD44 expression is a phenotype of cancer stem cells, which have tumor-inducing functions, are highly invasive, and exhibit a certain resistance to chemoradiotherapy [15]. CD44 is considered one of the direct factors of drug resistance in cancer patients, and it is inextricably linked to disease recurrence [16].

Although the roles of CD44 and SOX4 have been reported in esophageal cancer, our experiments focused on the interaction of SOX4, CD44, N-cadherin and E-cadherin in ESCC and the mechanism by which they may be involved in the growth and invasiveness of ESCC.

Methods

Patients and specimens

We collected 271 cases of paraffin-embedded ESCC tissue specimens (patients received no anti-tumor or related treatment before surgery) and 80 cases of normal esophagus tissue samples from January 2010 to December 2010 from the Department of Pathology of the First Affiliated Hospital of Bengbu Medical College. The records of all patients contained complete clinical and pathological data and follow-up data. The patients were followed-up until the end or till October 2018. The follow-up stage lasted from 3 to 102 months. The data are shown in **Table 1**. The 80 normal controls were taken >5.0 cm from the ESCC mass and were confirmed by pathology after hematoxylin and eosin (HE) staining to be normal squamous tissue. This experiment was performed after approval by the Ethics Committee of Bengbu Medical College. Two experienced pathologists reviewed the pathology sections of the above patients, and then, we selected the archived patient specimens for paraffin block sectioning.

Immunohistochemistry (IHC)

All ESCC specimens were repaired in 4% neutral formalin solution, planted in paraffin, then cut continuously into 4 μm thick sections, deparaffinized in xylene and dehydrated in graded ethanol solutions. The endogenous peroxidase

Table 1. Clinicopathological factors

Patient characteristics	Numbers (n)	Percentage (%)
Gender		
Male	189	69.4
Female	82	30.6
Age (years)		
≤62	120	44.2
>62	151	55.8
Gross		
Medullary	107	39.5
Ulceration	125	46.1
Narrow	20	7.4
Fungating	19	7.0
Location		
Upper	28	10.3
Middle	185	68.3
Lower	58	21.4
Diameter (cm)		
≤3.5	156	57.6
>3.5	115	42.4
Distant metastasis		
No	253	93.4
Yes	18	6.6
Depth of invasion		
Over serous membrane	82	30.3
Above serous membrane	189	69.7
Lymph node metastasis		
No	193	71.2
Yes	78	28.8
TNM stage		
I and II	205	75.6
III and IV	66	24.4
Histological grade		
Well	131	48.3
Moderate	108	39.9
Poor	32	11.8

action was blocked by incubation of the sections in 4% H_2O_2 under room temperature for 10 min. The slides were then warmed up to 96°C during 31 min for antigen retrieval. It was then washed in PBS three times, and the slides were incubated with antibodies against SOX4, N-cadherin, CD44, and E-cadherin according to the kit instructions. Next, the slides were washed with water after diaminobenzidine (DAB) staining, followed by hematoxylin staining of the nuclei, differentiation in a hydrochloric acid-alcohol solution, bluing, dehydration, clearing and mounting. Known positive films were used as positive controls, whereas phosphate-buffered saline (PBS, pH 7.2) without the primary antibody served as the negative control.

Quantification of immunostaining

All immunohistochemical outcomes were determined by two pathologists blinded to the treatments. Ten areas of view were arbitrarily picked from each slide. The expression of the SOX4, N-cadherin, E-cadherin and CD44 proteins was primarily evaluated by the signal strength and area of spotting of the immunohistochemical markers. The staining intensity (that is, 0 indicates no staining, 1 indicates a light yellow color, 2 indicates a yellow color, 3 indicates a brownish yellow color) was examined; 10 high-power areas were arbitrarily picked, and the percentage of tumor cells was counted (<10% is 0, 11% to 25% is 1 point, 25% to 50% is 2 points, 51% to 75% is 3 points, and >76% is 4 points). The two values were multiplied to obtain the integral score. Finally, we decided the grade by multiplying the volume and the area of labeling to make an area of immunostaining graded from 0 to 13. Scores <3 were negative, and scores ≥3 were positive.

Positive staining was presented as brown granular material. Positive SOX4 protein staining appeared as yellow or brownish orange particles in the nucleus or cytoplasm of tumor cells; positive E-cadherin, N-cadherin and CD44 staining appeared as yellow or brownish yellow particles located mainly in the tumor cell membrane (**Figure 1**).

Statistical analyses

Statistical analyses were performed utilizing the SPSS 22.0 statistical program system. Survival analysis of the positive and negative SOX4, E-cadherin, N-cadherin and CD44 protein expression groups was performed by the Kaplan-Meier approach. The log-rank trail was used for comparisons during sets, and a multivariate calculation was conducted using a Cox multivariate reversion model. The relation between SOX4, E-cadherin, N-cadherin and CD44 protein expression in ESCC and convention esophageal mucosa and clinical pathological parameters was calculated by χ^2 and the Spearman rank relation test. $P<0.05$ was considered statistically significant.

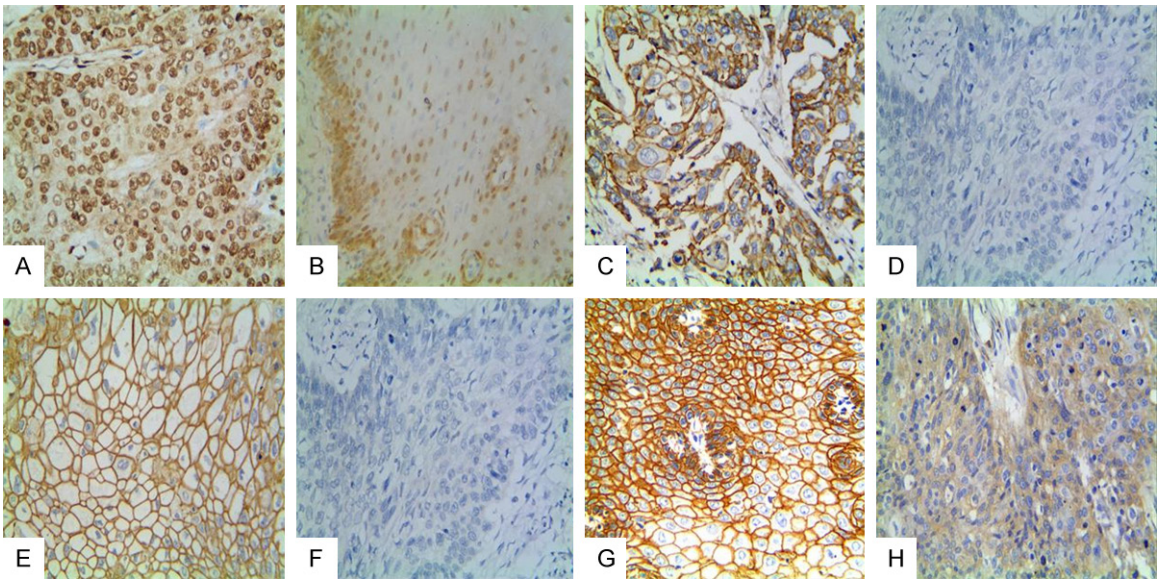

Figure 1. Expression of the SOX4, N-cadherin, CD44, and E-cadherin proteins in ESCC and control tissue (400× magnification). A. Positive SOX4 expression in the nuclei and cytoplasm of cancer cells. B. Positive SOX4 expression mainly in the nuclei of normal esophageal squamous epithelial cells. C. Positive N-cadherin expression in the membrane of cancer cells. D. Negative N-cadherin expression in normal esophageal squamous epithelial cells. E. Positive CD44 expression in the membrance of cancer cells. F. Negative CD44 expression in normal esophageal squamous epithelial cells. G. Positive E-cadherin expression in the membranes of normal esophageal squamous epithelial cells. H. Loss of E-cadherin expression in the membrane of cancer cells.

Outcomes

Expression of SOX4, E-cadherin, N-cadherin or CD44 in ESCC and normal samples

The positive expression rate of the SOX4 protein was 70.1% (190/271) and 18.8% (15/80) in ESCC and esophageal mucosa, separately, and the difference in expression was statistically important (P<0.001). The positive expression rate of the N-cadherin protein was 65.3% (177/271) and 30.0% (24/80) in ESCC and esophageal mucosa, respectively (P<0.001). The positive expression rate of the CD44 protein was 56.8% and 32.5% (26/80) in ESCC and esophageal mucosa, respectively (P< 0.001). The positive expression of E-cadherin in normal esophageal mucosa was 85.0% (68/80), which was significantly higher than that in esophageal squamous cell carcinoma 40.2% (109/271) (P<0.001).

Correlativity between SOX4, E-cadherin, N-cadherin or CD44 and clinicopathological causes

A close relation was observed among the expression of SOX4, N-cadherin or CD44 with tumor diameter, distant metastasis, degree of damaging, lymph node movement, pTNM stage and tumor histological grade (P<0.05). Moreover, as patient's age increased, the expression of the N-cadherin protein was higher in ESCC; the change was statistically significant (P < 0.05). A negative relationship was observed between the expression of E-cadherin and tumor diameter, distant metastasis, degree of damage, lymph node movement, pTNM stage and histological grade (P<0.05). The expression of SOX4, E-cadherin, N-cadherin or CD44 was not correlated with gender, age, tumor location, or tumor gross (P>0.05). Moreover, the expression of SOX4 and CD44 was not correlated with age (P>0.05) (**Table 2**).

Correlativity of SOX4, E-cadherin, N-cadherin and CD44 protein expression in esophageal squamous cell tumor

Spearman correlativity calculation displayed that the expression of the SOX4 organ was positively correlated with the expression of the N-cadherin and CD44 proteins (r = 0.591, 0.456; separately, P<0.001). Also, the expression of the N-cadherin and CD44 proteins was also positively correlated (r = 0.695, P<0.001). However, E-cadherin protein expression was nega-

Table 2. Correlation of SOX4, N-cadherin, CD44, or E-cadherin expression with the clinicopathological characteristics of ESCC

Variable	SOX4		X^2	P	N-cadherin		X^2	P	CD44		X^2	P	E-cadherin		X^2	P
	+	-			+	-			+	-			+	-		
Gender			0.022	0.883			0.015	0.902			0.025	0.873			0.070	0.791
Male	132	57			123	66			108	81			77	112		
Female	58	24			54	28			46	36			32	50		
Age (years)			1.691	0.194			7.108	0.008			3.153	0.076			11.733	0.001
≤62	89	31			68	52			61	59			62	58		
>62	101	50			109	42			93	58			47	104		
Gross			6.769	0.080			4.769	0.190			1.556	0.669			1.186	0.756
Medullary	82	25			72	35			63	44			42	65		
Ulceration	78	47			76	49			69	56			53	72		
Narrow	14	5			12	7			9	10			8	11		
Fungating	16	4			17	3			13	7			6	14		
Location			0.045	0.978			1.154	0.562			0.197	0.906			3.080	0.214
Upper	20	8			20	8			15	13			9	19		
Middle	129	56			117	68			105	80			81	104		
Lower	41	17			40	18			34	24			19	39		
Diameter (cm)			6.333	0.012			16.834	<0.001			40.507	<0.001			23.292	<0.001
≤3.5	100	56			86	70			63	93			82	74		
>3.5	90	25			91	24			91	24			27	88		
Distant metastasis			5.448	0.020			4.730	0.030			43.310	<0.001			6.795	0.009
No	173	80			161	92			138	115			107	146		
Yes	17	1			16	2			16	2			2	16		
Depth of invasion			105.105	<0.001			181.986	<0.001			123.329	<0.001			147.391	<0.001
Over serous membrane	168	21			172	17			149	40			31	158		
Above serous membrane	22	60			5	77			5	77			78	4		
Lymph node metastasis			7.452	0.006			23.114	<0.001			37.727	<0.001			31.075	<0.001
No	126	67			109	84			87	106			98	95		
Yes	64	14			68	10			67	11			11	67		
pTNM stage			18.010	<0.001			34.987	<0.001			43.310	<0.001			38.673	<0.001
I and II	130	75			114	91			94	111			104	101		
III and IV	60	6			63	3			60	6			5	61		
Histological grade			20.024	<0.001			17.931	<0.001			12.928	0.002			9.949	0.007
Well	75	56			70	61			60	71			65	66		
Moderate	89	19			86	22			74	34			32	76		
Poor	26	6			21	11			20	12			12	20		

Table 3. Correlation among SOX4, N-cadherin, CD44, and E-cadherin expression in ESCC

Variable	SOX4		*rs*	*P*	N-cadherin		*rs*	*P*	CD44		*rs*	P
	+	-			+	-			+	-		
N-cadherin												
+	159	18	0.591	<0.001#								
-	31	63										
CD44												
+	136	18	0.456	<0.001#	145	9	0.695	<0.001#				
-	54	63			32	85						
E-cadherin												
+	46	63	-0.500	<0.001*	25	84	-0.730	<0.001*	13	96	0.744	<0.001*
-	144	18			152	10			142	21		

#positive association, *negative association.

Table 4. Results of multivariate logistic regression analyses of overall survival (OS)

	B	SE	Wald	p	Exp (B)	95.0% CI for Exp (B)	
						Lower	Upper
Age (years)	1.057	0.200	27.906	<0.001	2.878	1.944	4.260
Gender	0.304	0.175	3.009	0.083	1.355	0.961	1.910
Location	0.186	0.134	1.936	0.164	1.204	0.927	1.565
Gross	0.103	0.080	1.646	0.199	1.108	0.947	1.297
Diameter (cm)	0.234	0.173	1.840	0.175	1.264	0.901	1.773
Depth of invasion	0.470	0.564	0.693	0.405	1.599	0.529	4.832
Lymph node metastasis	-0.910	0.505	3.253	0.071	0.403	0.150	1.082
Histological grade	-0.028	0.132	0.045	0.832	0.972	0.751	1.259
Distant metastasis	2.388	0.326	53.781	<0.001	10.890	5.753	20.616
pTNM stage	0.772	0.531	2.113	0.146	2.163	0.764	6.122
SOX4	0.706	0.321	4.856	0.028	2.026	1.081	3.798
N-cadherin	2.974	0.585	25.837	<0.001	19.573	6.217	61.618
CD44	1.624	0.305	28.436	<0.001	5.075	2.793	9.219
E-cadherin	0.713	0.281	6.431	0.011	2.040	1.176	3.540

tively correlated with SOX4, N-cadherin and CD44 protein expression in ESCC (r = -0.500, -0.730, -0.744; *P*<0.001) (**Table 3**).

Prognosis and multivariate analyses

Gender, year, tumor position, tumor gross, tumor histological grade, tumor diameter, distant metastasis, degree of damaging, lymph node movement, pTNM stage, SOX4 protein expression, N-cadherin protein expression, CD44 protein expression, and E-cadherin protein expression (plus group and bad group) were introduced into the Cox model for analysis. Follow-up data showed that SOX4, N-cadherin, CD44, E-cadherin, age and distant metastasis were absolute causes that influenced the prognosis of patients with ESCC (**Table 4**).

The whole survival (OS) rate of this set of patients was 31.4%. A Kaplan-Meier survival calculation displayed that the OS rate of the SOX4 protein positive set was lower than that of the negative set (7.0%, 21.8% for the SOX4 positive and negative groups, respectively; *P*<0.001); the OS rate of the N-cadherin protein positive set was lower than that of the negative set (0.0%, 31.4% for the N-cadherin positive set and negative groups, respectively; *P*<0.001); the OS rate of the CD44 protein positive set was more lower than that of the negative set (1.1%, 30.3% for the CD44 positive and negative groups, respectively; *P*<0.001); the OS rate of the E-cadherin protein negative set was more higher than that of the positive set (29.5%, 1.8% for the E-cadherin positive and negative groups, respectively; *P*<0.001). Overexpression of SOX4, N-cadherin or CD44 forecasted a bad

outcome because of the OS time (log rank = 83.561, 216.615, 180.951, respectively; P< 0.001; **Table 5**; **Figure 2A-C**), whereas low-expression of E-cadherin forecasted a poor prognosis in terms of overall survival (OS) time (log rank = 146.740, P<0.001, **Table 5**, **Figure 2D**). Moreover, the unit of negative E-cadherin expression and positive expression of SOX4, N-cadherin and CD44 was correlated with a poor prognosis compared with the opposite unit (log-rank = 52.882; P<0.001, **Table 5**, **Figure 2E**).

Discussion

It has always been difficult to find effective treatment methods to manage tumor recurrence and metastasis. Damaging and metastasis are the most basic biological features of malignant tumors and are also some of the most important factors that often lead to patient death. During the research, our team studied the expression of the four tumor invasion-related factors SOX4, N-cadherin, CD44, and E-cadherin in patients with ESCC to provide guidance for clinical treatment and to indicate a new direction for detecting invasion and predicting the prognosis of patients with ESCC.

Relative cellular features including decreased cell adhesion as well as increased motility and invasiveness could result from Epithelial-Mesenchymal Transition [17]. Cadherins have been displayed to take a significant role in the cancer metastasis procedure [18]. N-cadherin is primarily discovered in neural organ and fibroblasts, where it is seen to take a less stubborn and more various part of cell-cell illness [19]. N-cadherin has been displayed to take place in breast cancer and melanoma [20, 21]. In this study, by IHC, our team discovered that the expression of N-cadherin within ESCC was significantly higher than in adjacent normal tissues. Moreover, high expression of the N-cadherin tissue was positively correlated with tumor histological score, pTNM stage, degree of damaging and lymph node metastasis. The outcomes suggest that high expression of N-cadherin is implicated in the invasion and migration of ESCC. Several researches have shown that non-tissue-special expression of N-cadherin in cancers takes an important part in cell damaging, and migration [22] that is related with the outcomes of our experimental research. E-cadherin is discovered almost in the epithelia, at which place it suggests tight cell-cell relations known as adherent adjunctions [23]. Little E-cadherin expressions have been displayed among much carcinomas originating from epithelial organs including gastric, breast, pancreatic, and hepatic tumors, and its downregulation is often linked to the degree of movement and degree of damaging [24, 25]. In this study, the expression of E-cadherin in ESCC was obviously lower than in adjacent common organs according to immunohistochemistry. The absence of or low N-cadherin expression has been correlated with invasion and dissemination in ESCC. It has also been reported that loss of E-cadherin save cancer organs from apoptosis [26].

It has been displayed that SOX4 acts either as a cancer-promoting gene or as a tumor gene in different kinds of cancers. Wang B, et al. found growing expression of SOX4 during colorectal cancer organs and reported that the gene of SOX4 contained colorectal cancer cell proliferation and damaging [27]. SOX4 acts as a negative role of the growing of glioblastoma too, separately by acting through p53-p21 marking to take down G0/G1 cell cycle arrest [28]. SOX4 is also overexpressed in different malignant tumor organs, included carcinomas of the lung, beast, colorectum, liver, uterus, prostate and urinary bladder, as well as in melanoma, glioma and leukemia [8]. In our research, we discovered that SOX4 is obviously expressed in ESCC and that its expression is much higher in ESCC organs than in common esophageal mucosa. This result is consistent with the outcomes of study done before [9, 29]. We confirmed that SOX4 expression is positively correlated with degree of damaging, lymph node movement, pTNM stage, cancer histological grade, and distant migration in ESCC. These outcomes suggest that SOX4 is included in the invasion and metastasis of ESCC as a tumor-promoting gene. Han R, et al. proved that SOX4 was upregulated in ESCC and that SOX4 expression was inversely correlated with a cohort of race pointers. In another study, SOX4 knockdown decreased cell proliferation and strengthened doxorubicin-induced cellular senescence in vitro [9].

CD44 was copied in 1989 and was pointed as a part of the cartilage link egg family [30, 31].

Table 5. Results of univariate logistic regression analyses of overall survival (OS)

Variable	N	Mean OS time (months)	*Log-rank*	*P*
Gender			1.059	0.304
Male	189	55.375±2.743		
Female	82	49.976±4.343		
Age (years)			11.079	0.001
≤62	120	55.375±2.743		
>62	151	49.976±4.343		
Gross			1.998	0.573
Medullary	107	54.149±3.605		
Ulceration	125	54.336±3.603		
Narrow	20	45.300±6.894		
Fungating	19	55.842±8.155		
Location			3.029	0.220
Upper	28	57.857±6.460		
Middle	185	55.384±2.857		
Lower	58	46.549±4.917		
Diameter (cm)			28.885	<0.001
≤3.5	156	65.136±3.024		
>3.5	115	39.643±3.195		
Distant metastasis			137.431	<0.001
No	253	57.009±2.360		
Yes	18	7.833±0.883		
Depth of invasion			155.713	<0.001
Over serous membrane	82	35.523±2.081		
Above serous membrane	189	95.768±2.291		
Lymph node metastasis			45.853	<0.001
No	193	62.611±2.795		
Yes	78	31.683±2.933		
pTNM stage			87.267	<0.001
I and II	205	64.034±2.672		
III and IV	66	21.773±1.379		
Differentiation			21.299	<0.001
Well	131	64.989±3.293		
Moderate	108	40.611±3.223		
Poor	32	50.750±7.038		
SOX4			83.561	<0.001
Negative	81	86.366±3.288		
Positive	190	39.568±2.337		
N-cadherin			216.615	<0.001
Negative	94	95.450±2.329		
Positive	177	31.593±1.783		
CD44			180.951	<0.001
Negative	117	86.350±2.518		
Positive	154	28.946±1.959		
E-cadherin			146.740	<0.001
Negative	162	33.159±2.106		
Positive	109	84.358±3.047		

Ample evidence now exists for the significance of CD44 expression in the improvement of many tumor kinds [32] and for its expression on cancer stem cells [33]. Cancer root organs includes mutations which cause the achievement of additional attributes in addition to the properties of 'tumor-initiating cells' said before, that are important attributes of common organ root cells [34]. CD44 is a significant pointer of CSCs and is a membrane glycoprotein included in cell-cell and cell-extracellular matrix adhesion and thus taking a significant part in cell migration, differentiation, and survival [35, 36]. During the current research, our team found high expression of CD44 in ESCC. What's more, our outcomes indicated that CD44 expression in ESCC was obviously correlated with degree of damaging, lymph node movement, pTNM stage, tumor histological grade, and distant migration. We hypothesized that high expression of the CD44 protein allows tumor cells to easily break through the esophageal serosa and participate in lymph node metastasis and distant metastasis of ESCC. Overexpression of CD44 has been discovered in different kinds of cancers and can take its part as a prognostic pointer in different solid cancers, including ESCC [37], ovarian cancer [38], and gastric cancer [39].

The controversy that whether Epithelial-Mesenchymal Transition marker expression and cancer stem cell marker expression are associated with ESCC prognosis still exists. SOX4 was reported to be essential to Epithelial-Mesenchymal Transition because it regulates the epigenetic modifier Ezh2 [40]. Aya Sasaki, et al.

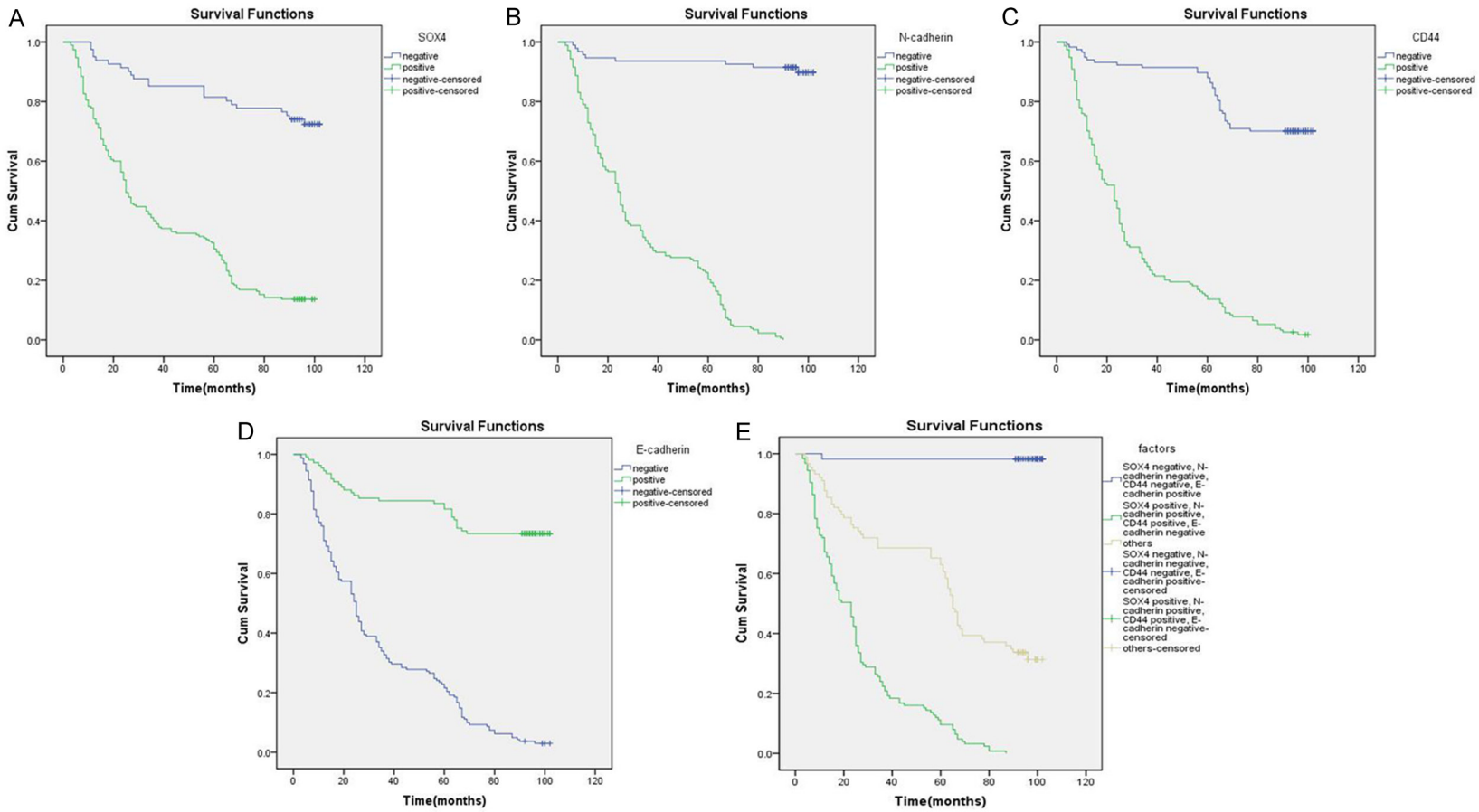

Figure 2. Kaplan-Meier analysis of the overall survival (OS) rate of patients with ESCC. The y-axis indicates the percentage of patients; the x-axis indicates their survival in months. A. Overall survival of all patients in relation to SOX4 expression (log-rank = 83.561, $P<0.001$). B. Overall survival of all patients in relation to N-cadherin expression (log-rank = 216.615, $P<0.001$). C. Overall survival of all patients in relation to CD44 expression (log-rank = 180.951, $P<0.001$). D. Overall survival of all patients in relation to the combination of E-cadherin expression (log-rank = 146.740, $P<0.001$). E. Overall survival of all patients in relation to the combination of SOX4, N-cadherin, CD44, and E-cadherin expression (log-rank = 196.086, $P<0.001$). The green line represents negative E-cadherin expression and positive expression of SOX4, N-cadherin and CD44, while the blue line represents positive E-cadherin expression and negative expression of SOX4, N-cadherin and CD44. The yellow line represents positive or negative expression of the other proteins. In all analyses, ⊥ in blue, green or yellow represents censored observations.

have demonstrated that ADAM28 is a transcriptional target of SOX4 and that both CD44 and ADAM28 are coexpressed by carcinoma cells at the invasive front of breast and lung carcinoma tissues. Furthermore, they speculated that SOX4-induced ADAM28 coordinately functions to maintain and/or enhance SOX4-mediated Epithelial-Mesenchymal Transition at the invasive sites in breast and lung carcinomas [41]. N-cadherin, also known as cadherin-2, is expressed in place of E-cadherin during Epithelial-Mesenchymal Transition where it promotes tumor progression in many types of carcinomas [42]. It had previously been proposed that cancer stem cells in carcinomas undergo Epithelial-Mesenchymal Transition to gain a migratory, mesenchymal phenotype that enables them to migrate away from the primary tumor to colonize distant sites, where they then undergo mesenchymal-to-epithelial transition (MET) to establish a metastatic tumor of the same epithelial character as the parent tumor [43]. Spearman correlativity analysis confirmed the synergistic positive correlativity between high expression of SOX4 and high expression of both N-cadherin and CD44, but low expression of E-cadherin was associated with high expression of SOX4, N-cadherin and CD44. By reviewing the literature and the results of this study, we speculated that the abnormal expression of SOX4 and N-cadherin and the deletion of E-cadherin promote the occurrence and maintenance of Epithelial-Mesenchymal Transition, which further promotes the occurrence of ESCC. The occurrence of Epithelial-Mesenchymal Transition may be an early event in ESCC infiltration and metastasis, which can promote cancer stem cell expression of CD44. Enhanced expression gives tumor cells a special phenotype, while high expression of CD44 further promotes the invasion and metastasis of ESCC. The initiation of Epithelial-Mesenchymal Transition and the increase in CD44 expression indicate a poor prognosis of ESCC patients. Through the above process, tumor cells acquire greater invasive and metastatic potential. Adrian Biddle, et al. have also indicated that cancer stem cells undergo Epithelial-Mesenchymal Transition in the primary tumor and that the resulting motile cancer stem cells invade through the stroma to reach the circulatory and lymphatic systems through which they travel to distant sites. At these secondary sites, they undergo mesenchymal-to-epithelial transition (MET) to resume their proliferative phenotype and produce a metastatic growth [44].

This research discovered that loss of E-cadherin expression was positively correlated with overexpression of SOX4, N-cadherin and CD44 in ESCC. The OS time of ESCC patients with concurrent loss of E-cadherin expression and overexpression of SOX4, N-cadherin and CD44 was significantly worse than that of ESCC patients with heterogeneous expression of these markers or co-expression of SOX4, N-cadherin, CD44 and E-cadherin expression. Therefore, concurrent loss of E-cadherin expression and overexpression of SOX4, N-cadherin and CD44 may play an important role in predicting prognosis, which is helpful for understanding the progression and exploring treatments for ESCC. Multivariate analysis showed that positive expression of the SOX4, N-cadherin, CD44 and E-cadherin proteins and distant metastasis were independent prognostic factors for postoperative survival in ESCC patients.

Conclusion

Our findings suggest that suppression of ESCC markers may inhibit the development of malignant tumors and improve the therapeutic outcomes of ESCC patients since CD44 supports the induction of Epithelial-Mesenchymal Transition in ESCC. Therefore, early combined detection of SOX4, N-cadherin, CD44, E-cadherin protein expression can be used as an indicator by which the infiltration, metastasis and prognosis of ESCC patients can be evaluated. Furthermore, the specific mechanisms by which SOX4 interacts with N-cadherin and the high expression of CD44 during Epithelial-Mesenchymal Transition are not well understood and will be the focus of our future work.

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Disclosure of conflict of interest

None.

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