Technology in Cancer Research & Treatment
Volume 19: 1-9

DOI: 10.1177/1533033820928117
journals.sagepub.com/home/tct
SAGE

Semaphorin 3F Serves as a Tumor Suppressor in Esophageal Squamous Cell Carcinoma and is Associated With Lymph Node Metastasis in Disease Progression

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Abstract

Background: Esophageal squamous cell carcinoma is one of the leading aggressive malignancies with high mortality. Semaphorin 3F has been reported to be involved in lymphangiogenesis by interacting the vascular endothelial growth factor C/neuropilin 2 axis. This study aimed to assess the clinical and functional role of semaphorin 3F and preliminarily evaluate the relationship between semaphorin 3F and lymph node metastasis in esophageal squamous cell carcinoma. **Methods:** The messenger RNA expression of semaphorin 3F was analyzed using quantitative real-time polymerase chain reaction. The expression differences of semaphorin 3F between patients having esophageal squamous cell carcinoma with and without lymph node metastasis were assessed, and the correlation of semaphorin 3F with vascular endothelial growth factor C and neuropilin 2 was estimated. The prognostic value of semaphorin 3F was evaluated using Kaplan-Meier survival curves and Cox regression analysis. Gain- and loss-functional cell experiments were performed to explore the biological function of semaphorin 3F, vascular endothelial growth factor C, and neuropilin 2. **Results:** The messenger RNA expression of semaphorin 3F was reduced in esophageal squamous cell carcinoma tissues compared with normal tissues, and lower semaphorin 3F expression was observed in patients having esophageal squamous cell carcinoma with positive lymph node metastasis. Semaphorin 3F expression was associated with lymph node metastasis and negatively correlated with vascular endothelial growth factor C and neuropilin 2. Lower semaphorin 3F expression was related to a poor overall survival of esophageal squamous cell carcinoma and served as an independent prognostic indicator. In esophageal squamous cell carcinoma cells, semaphorin 3F messenger RNA expression was also decreased compared with normal cells, and the overexpression of semaphorin 3F could significantly inhibit cell proliferation, migration, and invasion. The downregulation of vascular endothelial growth factor C and neuropilin 2 could inhibit cell proliferation, migration, and invasion of esophageal squamous cell carcinoma cells. **Conclusion:** All data indicate that semaphorin 3F serves as a potential prognostic biomarker and tumor suppressor of esophageal squamous cell carcinoma and may be involved in the lymph node metastasis development through regulating neuropilin 2.

Keywords

esophageal squamous cell carcinoma, prognosis, semaphorin 3F, proliferation, migration, invasion

Abbreviations

cDNA, complementary DNA; CCK-8, Cell Counting Kit 8; ESCC, esophageal squamous cell carcinoma; FBS, fetal bovine serum; mRNA, messenger RNA; NRP2, neuropilin-2; qRT-PCR, quantitative real-time polymerase chain reaction; SEMA3F, semaphorin 3F; TNM, tumor, node, metastasis; VEGF-C, vascular endothelial growth factor C.

Received: January 10, 2020; Revised: March 20, 2020; Accepted: April 16, 2020.

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Introduction

Esophageal squamous cell carcinoma (ESCC) is a frequent histological type of esophageal cancer and one of the leading aggressive malignancies with high mortality worldwide.[1] Some risk factors, such as smoking, alcohol, and lack of vegetables and fruits, contribute to the onset of ESCC.[2] Most of patients with ESCC are firstly diagnosed with advanced tumors with lymph node metastasis mainly due to no significant clinical manifestations.[3] Although great processes have been made in ESCC treatment, including surgical techniques, chemotherapy, and radiotherapy, the clinical outcomes of patients remain dismal, with a 5-year survival only of less than 10%.[4] Thus, it is important to identify novel molecular markers that may be involved in the pathogenesis of ESCC for improving the prognosis and treatment of ESCC.

The status of lymph node metastasis is closely associated with ESCC prognosis, and lymph node metastasis has been identified as an independent prognostic factor of ESCC.[5,6] Tumor lymphangiogenesis is a key process during the lymph node metastasis, involving the interactions between endothelial cells and tumor cells.[7] Vascular endothelial growth factor C (VEGF-C) is one of the most important molecules that promotes lymphangiogenesis and thus participates in the pathogenesis of various human cancers including ESCC.[8] Neuropilin 2 (NRP2) was identified as a coreceptor of VEGF-C and could mediate the promoting effect of VEGF-C on lymph node metastasis in some human malignancies.[9,10] In ESCC, NRP2 has also been reported to facilitate the tumorigenesis and metastasis.[11] Semaphorin 3F (SEMA3F) is a semaphorin ligand of NRP2 and can compete with VEGF-C to competitively bind NRP2.[12] It was first identified based on its role in neuronal development and axonal guidance.[13] Semaphorin 3F is located in chromosome 3p21.3, which is lowly expressed in lung cancer, indicating its potential tumor suppressor role.[14] Recently studies have demonstrated the functional role of SEMA3F in tumor progression of some malignancies, such as colorectal carcinoma[15] and oral squamous cell carcinoma.[16] More importantly, Zhang *et al* have reported that SEMA3F might be involved in the regulation of lymph node metastasis via interacting the VEGF-C/NRP2 axis.[10] However, there is litter evidence for the role of SEMA3F in ESCC.

Considering the potential role of SEMA3F in development of lymph node metastasis, this study aimed to assess the expression of SEMA3F in patients with ESCC, analyze its relationship with ESCC lymph node metastasis, and explore the biological function in tumor progression. The results of this study may provide a novel insight in the role of SEMA3F in ESCC pathogenesis, especially in lymph node metastasis, and provide new prognostic biomarker and therapeutic target for ESCC treatment.

Materials and Methods

Patients and Tissue Collection

The protocols of this study were approved by the Ethics Committee of Binzhou Medical University Hospital, and each participant in this study provided informed consent for tissue sample collection and use. A total of randomly 118 patients with ESCC with complete clinicopathological data were recruited in this study, who were histologically diagnosed with ESCC in Binzhou Medical University Hospital between 2011 and 2013. Tumor tissues and nontumorous tissues were collected from the patients during a resection operation. None of the patients had received antitumor therapy prior to the surgery. The clinical characteristics of the patients were recorded and summarized in Table 1. All the patients were followed up for 5 years, and their survival information was analyzed for the subsequent survival analysis.

Table 1. Association of SEMA3F With Clinicopathological Characteristics of Patients With ESCC.

Features	No., n = 118	SEMA3F expression		*P* values
		Low (n = 64)	High (n = 54)	
Age, years				.664
≤60	44	25	19	
>60	74	39	35	
Gender				.617
Female	51	29	22	
Male	67	35	32	
Differentiation				.638
Well-moderate	76	40	36	
Poor	42	24	18	
Lymph node metastasis				.003
Negative	59	24	35	
Positive	59	40	19	
TNM stage				.027
I-II	59	26	33	
III-IV	59	38	21	

Abbreviations: ESCC, esophageal squamous cell carcinoma; SEMA3F, semaphorin 3F; TNM, tumor, node, metastasis.

Cell Culture and Transfection

Esophageal squamous cell carcinoma cell lines Eca109, EC9706, KYSE70, and TE1 and a normal human esophageal epithelial cell line HET1A were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Science (Shanghai, China). These cell lines were cultured in Dulbecco modified Eagle medium containing 10% fetal bovine serum (FBS; all from Gibco, Thermo Fisher Scientific) at 37 °C in a humidified atmosphere with 5% CO_2. To upregulate the expression of SEMA3F in Eca109 and TE1 cells, SEMA3F was cloned into pcDNA3.1 to construct an overexpression vector. The combined vectors or pcDNA3.1 were transfected into ESCC cells by Lipofectamine 2000 (Invitrogen) following the manufacturer instruction. In addition, NRP2 small interfering RNA (siRNA) and VEGF-C siRNA were transfected into ESCC cells using Lipofectamine 2000 following the manufacturer instruction, respectively. After 48 hours of transfection, cells were used for subsequent experiments.

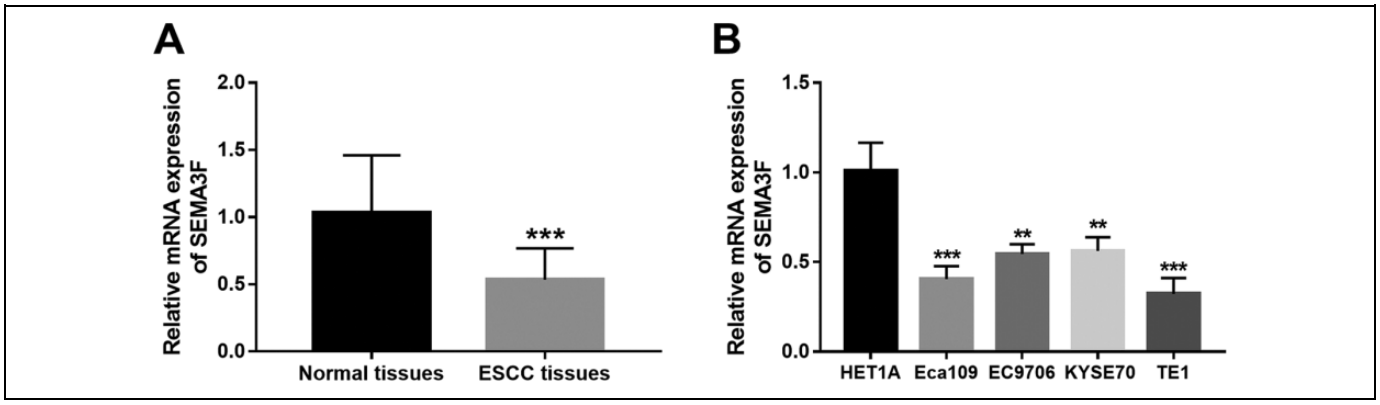

Figure 1. Expression of SEMA3F in patients with ESCC and ESCC cell lines. A, Expression of SEMA3F measured by qRT-PCR in ESCC tumor tissues and normal tissues. B, Expression of SEMA3F in ESCC cell lines (Eca109, EC9706, KYSE70, and TE1) and a normal human esophageal epithelial cell HET1A. $^{**}P < .01$, $^{***}P < .001$. ESCC indicates esophageal squamous cell carcinoma; SEMA3F, semaphorin 3F; qRT-PCR, quantitative real-time polymerase chain reaction.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from tissues and cells by TRIzol reagent (Invitrogen). The first-strand complementary DNA (cDNA) was synthesized from RNA using a PrimeScript RT reagent kit (TaKaRa) as per the manufacturer instruction. Quantitative real-time polymerase chain reaction (qRT-PCR) was then performed to evaluate the messenger RNA (mRNA) expression of SEMA3F, VEGF-C, and NRP2 using a 7500 Real-Time PCR System (Applied Biosystems) with the SYBR green I Master Mix Kit (Invitrogen). GAPDH was used as an internal control gene, and the relative expression data were normalized to GAPDH. All expression data were quantified using the $2^{-\Delta\Delta Ct}$ method.

CCK-8 Assay

This study used Cell Counting Kit 8 (CCK-8) Kit (Beyotime) to estimate ESCC cell proliferation. After 48 hours of cell transfection, cells were seeded into 96-well plates at a density of 3×10^3 cell/well and cultured for 3 days. At the time points of 0, 1, 2, and 3 days, CCK-8 solution was added into the cells with further 2-hour incubation at 37 °C. The optical density value at 450 nm of the cell culture was measured by a microplate reader (BioTek).

Transwell Assay

Esophageal squamous cell carcinoma cell migration and invasion were evaluated using the 24-well Transwell chambers (Corning). The chambers used for invasion assay were precoated with Matrigel (Corning), but those for migration assay had no Matrigel need. The transfected cells (cell density of 5×10^4 cells/well) with serum-free medium were seeded into the upper chambers. The lower chambers were filled up with culture medium containing 10% FBS. After 24 hours of incubation at 37 °C, the cells in lower chambers were stained with crystal violet and counted under a light microscope (Nikon).

Statistical Analysis

Data were presented as mean ± standard deviation and analyzed using SPSS 21.0 software (SPSS Inc) and GraphPad Prism 7.0 software (GraphPad Software, Inc). The significant differences between groups were assessed using Student *t* test, Chi-square test, or one-way analysis of variance followed by Tukey test. Correlation between indicators was analyzed using Pearson correlation coefficient. Multiple logistic regression analysis was used to assess independent association between SEMA3F expression and lymph node metastasis. Kaplan-Meier method was used to perform survival analysis with log-rank test to compare the differences between groups. Cox regression analysis was used to evaluate the prognostic value of SEMA3F in patients with ESCC. A *P* value of less than .05 was considered statistically significant.

Results

Expression of SEMA3F in ESCC Tissues and Cell Lines

According to qRT-PCR, the mRNA expression of SEMA3F was estimated in patients with ESCC and cell lines. As shown in Figure 1A, the mRNA expression of SEMA3F was significantly downregulated in ESCC tissues compared with the normal controls ($P < .001$). Similarly, the decreased expression of SEMA3F in 4 ESCC cell lines (Eca109, EC9706, KYSE70, and TE1) was demonstrated when compared to the normal cell line HET1A (all $P < .01$, Figure 1B).

Association of SEMA3F With Clinicopathological Characteristics of Patients With ESCC

The demographic and clinical characteristics of patients with ESCC were listed in Table 1, including age, gender, differentiation status, lymph node metastasis, and tumor, node, metastasis (TNM) stage. The patients were divided into low SEMA3F expression group (n = 64) and high SEMA3F expression group (n = 54) based on the mean SEMA3F mRNA

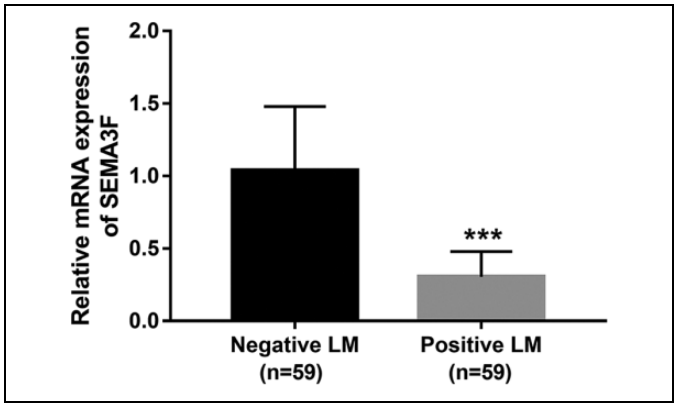

Figure 2. Expression of SEMA3F in patients having ESCC with or without lymph node metastasis. ***$P < .001$.

expression value in ESCC tissues, and clinical features in the 2 groups were compared by Chi-square test. The analysis results revealed that the mRNA expression of SEMA3F in ESCC tissues was associated with lymph node metastasis ($P = .003$) and TNM stage ($P = .027$). No significant association was found between SEMA3F and age, gender, or differentiation status (all $P > .05$).

Association of SEMA3F Expression With Patients Having ESCC With Different Lymph Node Metastasis Conditions

The published potential role of SEMA3F in lymphangiogenesis combined with the significant association we found between SEMA3F and lymph node metastasis in ESCC indicated that SEMA3F might be involved in the lymph node metastasis in ESCC pathogenesis. In the enrolled patients with ESCC, there were 59 cases with positive lymph node metastasis and 59 without lymph node metastasis. In the patients with lymph node metastasis, the mRNA expression of SEMA3F was lower than that in the patients without lymph node metastasis ($P < .001$, Figure 2), indicating that SEMA3F might be involved in the development of lymph node metastasis in ESCC.

To explore whether SEMA3F downregulation was purely contributed to lymph node metastasis, we further carried out the multiple logistic regression analysis. The results in Table 2 showed that SEMA3F expression was closely associated with lymph node metastasis after the removal of confounding factors (odds ratio = 0.290, 95% CI: 0.128-0.655, $P = .003$).

Correlation of SEMA3F With NRP2 and VEGF-C in Patients With ESCC

Considering the promoting effect of VEGF-C/NRP2 axis on lymphangiogenesis in various tumors including ESCC, the mRNA expression of VEGF-C and NRP2 was estimated in this study. As shown in Figure 3A, the mRNA expression levels of VEGF-C and NRP2 were both elevated in ESCC tissues compared with the normal tissues (both $P < .001$). In addition, the expression of VEGF-C and NRP2 was as expected to be upregulated in positive lymph node metastasis patients with ESCC when compared to the negative lymph node metastasis cases with ESCC (all $P < .001$, Figure 3B), and a positive correlation between VEGF-C and NRP2 ($r = 0.801$, $P < .001$) in patients with ESCC is shown in Figure 3C. Of note, the expression of SEMA3F was found to be negatively correlated with both NRP2 ($r = -0.740$, $P < .001$) and VEGF-C ($r = -0.640$, $P < .001$) in patients with ESCC (Figure 3D and E).

Table 2. Multiple Logistic Regression Analysis of Lymph Node Metastasis Status.

	Multiple logistic regression analysis		
	Odds ratios	95% CI	*P* values
SEMA3F expression	0.290	0.128-0.655	.003
Age	1.205	0.607-2.742	.635
Gender	1.269	0.564-2.855	.564
Differentiation	1.055	0.462-2.411	.899
TNM stage	1.189	0.537-2.631	.670

Abbreviations: SEMA3F, semaphorin 3F; TNM, tumor, node, metastasis.

Clinical Significance of SEMA3F in the Prognosis of ESCC

This study used the survival information obtained from a 5-year follow-up survey to construct the Kaplan-Meier survival curves. The curves shown in Figure 4 revealed that patients having ESCC with low SEMA3F expression had shorter survival time compared with those with high SEMA3F expression (log-rank $P = .007$). The subsequent Cox regression analysis results (Table 3) demonstrated that SEMA3F was an independent prognostic indicator for the overall survival of patients with ESCC (hazard ratio = 2.324, 95% CI = 1.283-4.209, $P = .005$).

Inhibiting Effect of SEMA3F on ESCC Cell Proliferation, Migration, and Invasion

To understand the functional role of SEMA3F in ESCC progression, *in vitro* manipulation of SEMA3F was performed by cell transfection with pcDNA3.1-SEMA3F in Eca109 and TE1 cells. The results shown in Figure 5A and B indicated that SEMA3F expression was successfully upregulated in both ESCC cell lines Eca109 and TE1 by pcDNA3.1-SEMA3F ($P < .001$). The CCK-8 assay results revealed that the overexpression of SEMA3F could significantly inhibit ESCC cell proliferation ($P < .05$, Figure 5C and D). The migration and invasion results evaluated by Transwell assay showed that ESCC cell migration and invasion abilities also were suppressed by the upregulation of SEMA3F (all $P < .01$, Figure 5E-H).

The Effect of VEGF-C and NRP2 on ESCC Cell Proliferation, Migration, and Invasion

Considering the expression of SEMA3F was negatively correlated with both VEGF-C and NRP2 expression, we further

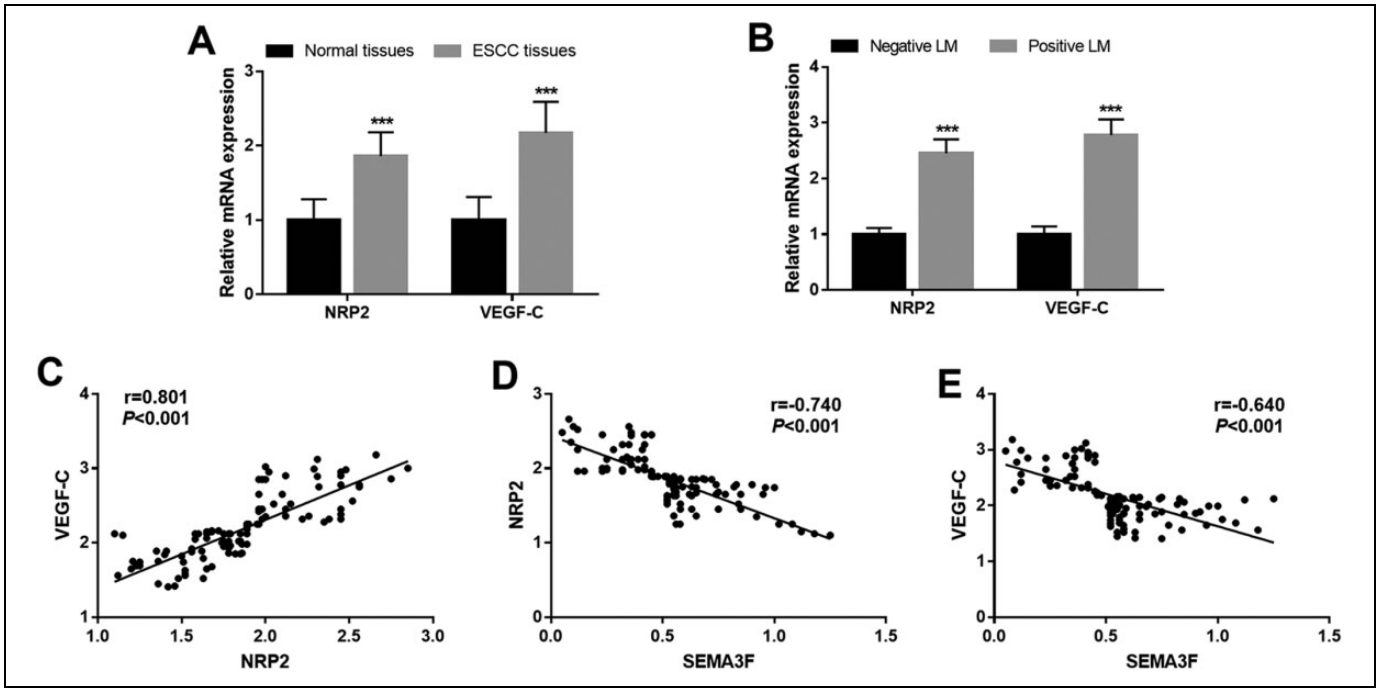

Figure 3. Expression of NRP2 and VEGF-C and their correlation with SEMA3F. A, Expression of NRP2 and VEGF-C in ESCC tissues. B, Expression of NRP2 and VEGF-C in patients having ESCC with different status of lymph node metastasis. C, Correlation between NRP2 and VEGF-C. D and E, Correlation of SEMA3F with NRP2 (D) and VEGF-C (E). ***$P < .001$. ESCC indicates esophageal squamous cell carcinoma; NRP2, neuropilin 2; SEMA3F, semaphorin 3F; VEGF-C, vascular endothelial growth factor C.

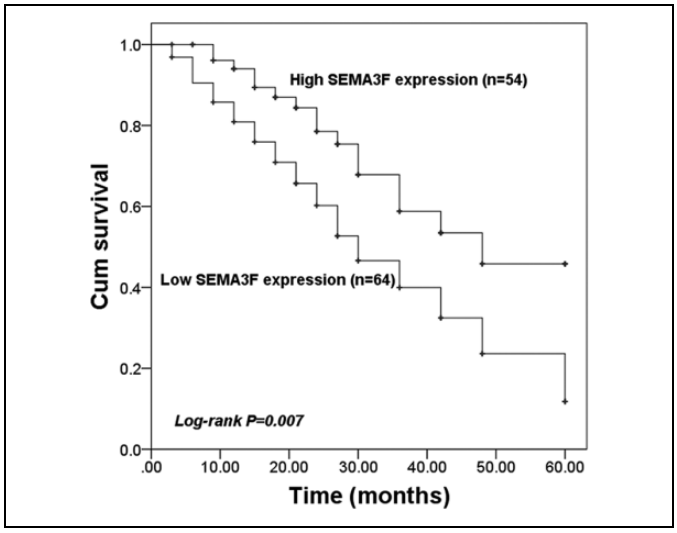

Figure 4. Kaplan-Meier survival curves based on different expression of SEMA3F in patients with ESCC. Log-rank $P = .007$. ESCC indicates esophageal squamous cell carcinoma; SEMA3F, semaphorin 3F.

explored the effect of VEGF-C and NRP2 in ESCC cells, respectively. As shown in Figure 6A and B, VEGF-C siRNA downregulated the expression of VEGF-C in both Eca109 and TE1 cells ($P < .001$). The functional experiment results indicated that knockdown of VEGF-C expression inhibited cell proliferation, migration, and invasion of Eca109 and TE1 cells (All $P < .05$, Figure 6C-H).

Table 3. Cox Regression Analysis for Patients With ESCC.

	Multivariate analysis		
Variables	HR	95% CI	*P* value
SEMA3F	2.324	1.283-4.209	.005
Age	1.049	0.585-1.880	.873
Gender	1.011	0.586-1.744	.969
Differentiation	1.090	0.626-1.898	.760
Lymph node metastasis	1.587	1.011-2.829	.049
TNM stage	1.758	1.023-3.020	.041

Abbreviations: ESCC, esophageal squamous cell carcinoma; SEMA3F, semaphorin 3F; TNM, tumor, node, metastasis.

Furthermore, we explored the potential effect of NRP2 in ESCC using Eca109 and TE1 cells. The qRT-PCR results showed that NRP2 expression was downregulated by NRP2 siRNA in both Eca109 and TE1 cells ($P < .001$, Figure 7A and B). The CCK-8 assay and Transwell assay results demonstrated that downregulation of NRP2 suppressed cell proliferation, migration, and invasion of Eca109 and TE1 cells, respectively, compared with mock (Figure 7C-H).

Discussion

Esophageal squamous cell carcinoma remains one of the leading aggressive malignancies with poor clinical outcomes. The incidence rate of lymph node metastasis in ESCC is reported to be approximately 38.2% to 43%.[17] The reported evidence

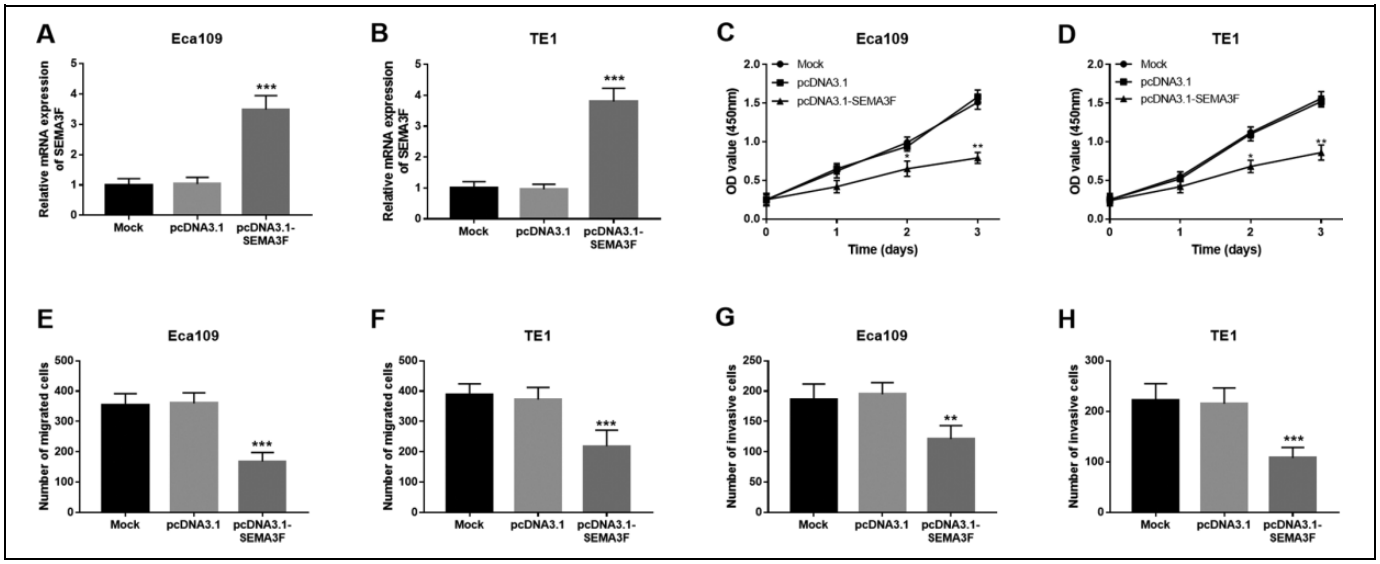

Figure 5. Effect of SEMA3F on cell proliferation, migration, and invasion of Eca109 and TE1 cells. A and B, Cell transfection efficient examination via evaluating the expression of SEMA3F by qRT-PCR. C and D, The overexpression of SEMA3F inhibited ESCC cell proliferation. E and F, Cell migration of ESCC was inhibited by the overexpression of SEMA3F. G and H, The upregulation of SEMA3F in ESCC cells led to suppressed cell invasion. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. ESCC indicates esophageal squamous cell carcinoma; SEMA3F, semaphorin 3F; qRT-PCR, quantitative real-time polymerase chain reaction.

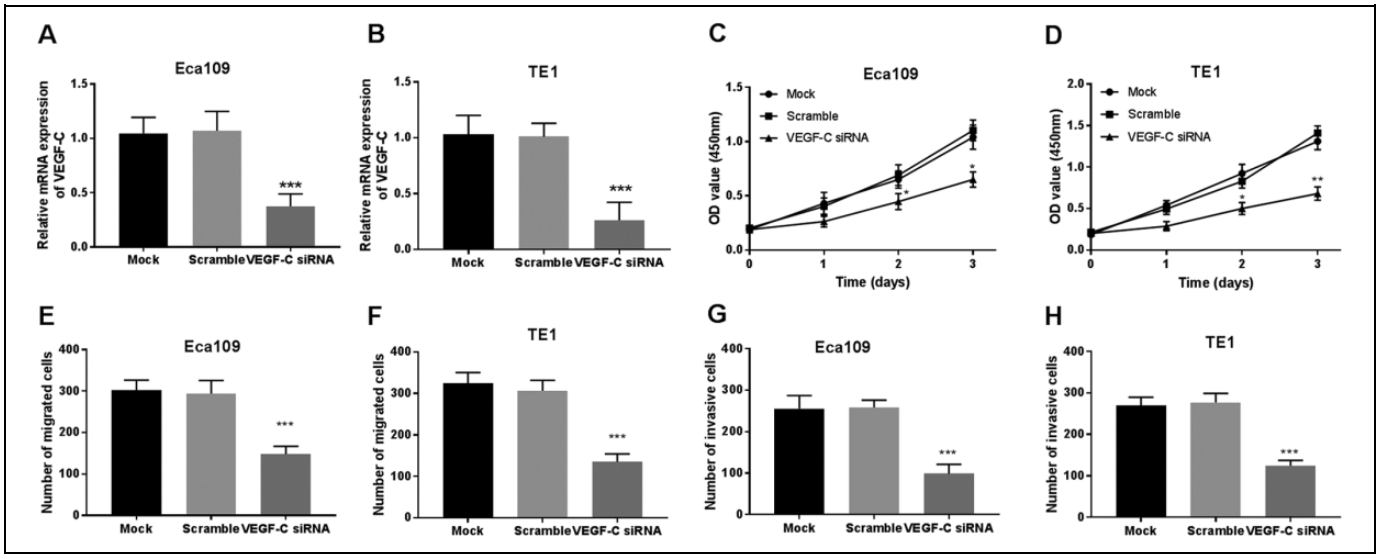

Figure 6. Effect of VEGF-C on cell proliferation, migration, and invasion of Eca109 and TE1 cells. A and B, Cell transfection efficient via evaluating the expression of VEGF-C by qRT-PCR. C and D, Knockdown of VEGF-C inhibited ESCC cell proliferation. E and F, ESCC cell migration was suppressed by the downregulation of VEGF-C. G and H, Downregulation of VEGF-C inhibited cell invasion. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. ESCC indicates esophageal squamous cell carcinoma; VEGF-C, vascular endothelial growth factor C; qRT-PCR, quantitative real-time polymerase chain reaction.

indicated that patients having ESCC with positive lymph node metastasis had a poor prognosis than those without nodal metastasis.[5] Thus, lymph node metastasis has been considered to be a prognostic factor of ESCC and an important issue that has been focused on in the management of ESCC. Lymph vessels constitute the metastatic pathway into lymph nodes in the pathogenesis of various cancers, including ESCC.[18] The formation of new lymph vessels can be promoted in malignancies through the lymphangiogenesis, and the development of lymph node metastasis involves lymphangiogenesis.[19] Thus, the methods to regulate lymphatics and lymphangiogenesis may be efficient therapeutic approaches to restrict tumor progression.[20]

Lymphangiogenesis is a complex process, and some key molecules have been reported to be involved in the regulation of this process.[21] Vascular endothelial growth factor C is one of

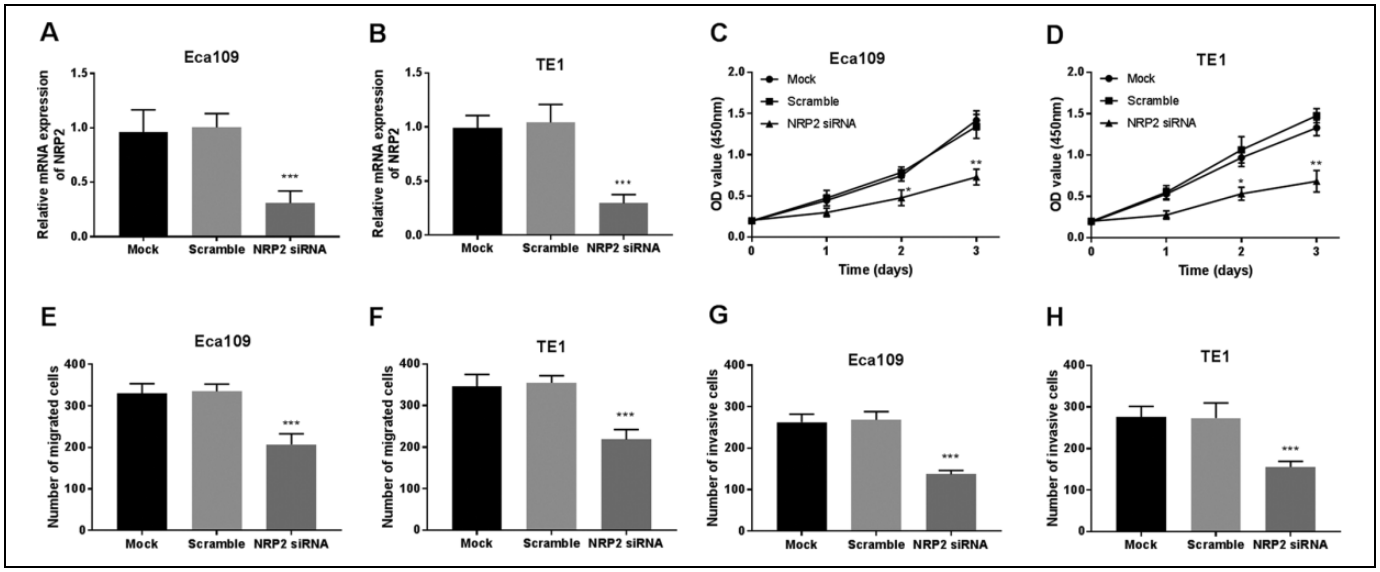

Figure 7. Effect of NRP2 on cell proliferation, migration, and invasion of Eca109 and TE1 cells. A and B, Cell transfection efficient via detecting the expression of NRP2 using qRT-PCR. C and D, The downregulation of NRP2 suppressed ESCC cell proliferation. E and F, Cell migration of ESCC was suppressed by the knockdown of NRP2. G and H, The downregulation of NRP2 in ESCC cells led to suppressed cell invasion. $*P < .05$, $**P < .01$, $***P < .001$. ESCC indicates esophageal squamous cell carcinoma; NRP2, neuropilin 2; qRT-PCR, quantitative real-time polymerase chain reaction.

the most important factors that can promote lymphangiogenesis. In addition, VEGF-C has been investigated in a number of human cancers with high expression, which was closely correlated with tumor lymph node metastasis.[22] Neuropilin 2, as a nontyrosine kinase transmembrane receptor, has been found to bind to VEGF-C and thereby lead to the developmental lymphangiogenesis.[23] Overexpression of NRP2 has been detected in various human cancers, and its promoting effect on cancer pathogenesis has also been reported.[24] In the present study, the expression levels of VEGF-C and NRP2 were also upregulated in ESCC tissues compared with the normal controls, which were consistent with the previous publications. In addition, the higher expression levels of VEGF-C and NRP2 were further demonstrated in patients having ESCC with positive lymph node metastasis, which further confirm the important regulatory roles of VEGF-C and NRP2 in the development of lymph node metastasis.

Recently studies have paid attention to the regulatory effect of SEMA3F on tumor progression.[25] Zhou *et al* have provided evidence for SEMA3F as a tumor suppressor of colorectal carcinoma by inhibiting tumor cell metastasis through the phosphatidylinositol 3-kinase/protein kinase B signaling.[15] Liu *et al* have reported that SEMA3F decreased expressed in oral squamous cell carcinoma tissues and cells and could suppress tumor cell migration, invasion, and proliferation.[16] In head and neck squamous carcinoma, downregulated SEMA3F has been described to be an antilymphangiogenic metastasis suppressor gene and thus was proposed as a therapeutic target.[26] What is worth noting is that Bagri and his colleagues found that NRP-2, as a receptor, is common to both VEGF-C and SEMA3F, and these 2 ligands might be a competitive relationship in binding NRP-2 and subsequent lymphangiogenesis.[27] A study by Zhang *et al* have investigated the effect of VEGF-C on lymphangiogenesis in oral squamous cell carcinoma and found that SEMA3F might be involved in the regulation of lymph node metastasis via interacting the VEGF-C/NRP2 axis.[10] This study firstly assessed the expression and functional role of SEMA3F in ESCC. The expression results revealed that the expression of SEMA3F was significantly elevated in ESCC tissues and cell lines and closely associated with lymph node metastasis in patients with ESCC. Furthermore, a higher expression of SEMA3F was observed in patients having ESCC with positive lymph node metastasis compared with those patients without lymph node metastasis, and SEMA3F was negatively correlated with NRP2 and VEGF-C. In addition, the multiple logistic regression analysis of lymph node metastasis status showed the independent association between SEMA3F expression and lymph node metastasis. These data indicated that SEMA3F might be involved in the development of lymph node metastasis in ESCC by interacting the VEGF-C/NRP2 axis, but this deduction needs to be confirmed in further studies by investigating the regulatory role of SEMA3F/VEGF-C/NRP2 in lymphangiogenesis in ESCC.

The decreased expression of SEMA3F has been found to be related to shorter survival time in some human cancers, such as osteosarcoma[28] and oral squamous cell carcinoma[10] and thus been proposed as candidate prognostic biomarker. Given the deregulated expression of SEMA3F in patients with ESCC, this study analyzed the overall survival in patients with different SEMA3F expression levels. The Kaplan-Meier survival analysis and Cox analysis results implied that patients having ESCC with low SEMA3F expression had a poor overall survival and

that the reduced SEMA3F expression served as an independent prognostic indicator for patients with ESCC. It is concluded that the decreased expression of SEMA3F may predict a poor prognosis in patients with ESCC.

In addition to the potential effect of SEMA3F on lymph node metastasis in cancer pathogenesis, its regulatory role in tumor cell processes, such as cell proliferation, migration, and invasion, has also been reported in some malignancies, including colorectal carcinoma, oral squamous cell carcinoma, and breast cancer.[15,16,29] Therefore, this study conducted cell functional gain and loss experiments to explore the functional role of SEMA3F in tumor progression. By analyzing ESCC cell biological processes, this study found that the overexpression of SEMA3F in ESCC cells led to the inhibiting of cell proliferation, migration, and invasion, indicating the tumor suppressor role of SEMA3F. Considering SEMA3F might be involved in the development of lymph node metastasis in ESCC by interacting the VEGF-C/NRP2 axis, furthermore, we also investigated the potential effect of VEGF-C and NRP2 in ESCC cell processes. The results showed that downregulation of VEGF-C and NRP2 inhibited cell proliferation, migration, and invasion of Eca109 and TE1 cells. These results demonstrated that ESCC cells may be the points of action of SEMA3F, NRP2, and VEGF-C. However, how does SEMA3F regulate ESCC cell proliferation, migration, and invasion remain unclear, which warrant further studies to understand the underlying mechanisms.

In conclusion, the results of this study suggested that the decreased expression of SEMA3F is found in ESCC tissues, and this downregulation is more significant in patients having ESCC with positive lymph node metastasis, and that the expression of SEMA3F is negatively correlated with NRP2 and VEGF-C in ESCC, indicating that SEMA3F may play an important role in lymph node metastasis involving the VEGF-C/NRP2 axis. In addition, SEMA3F can be used as a potential prognostic biomarker of ESCC and may serve as a tumor suppressor by inhibiting tumor cell proliferation, migration, and invasion. This study provides a novel insight into the relationship between SEMA3F and lymph node metastasis of ESCC, and SEMA3F may be a new therapeutic target for the treatment of ESCC.

Authors' Note

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Zhen Xie, Tianyue Li, and Bingtao Huang. The first draft of the manuscript was written by Shuai Liu and Lianguo Zhang. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The protocols of this study were approved by the Ethics Committee of Binzhou Medical University Hospital, and each participant in this study provided informed consent for tissue sample collection and use.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by A Project of Shandong Province Higher Educational Science and Technology Program [J18KB114].

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