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Original Article

Semaphorin4F is a potential biomarker for clinical progression and prognosis in gastric cancer

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Abstract: Background: Semaphorin4F (Sema4F) is a member of the semaphorin family and exhibits important regulatory functions in cancer biology. We aimed to explore the prognostic value and biologic function of Sema4F in gastric cancer (GC) through clinical data, laboratory studies, and bioinformatic methods. Methods: We investigated Sema4F-related data and the prognostic values of patients with GC based on several databases, including Tumor Immune Estimation Resource (TIMER), the Gene Expression Profiling Interactive Analysis 2 (GEPIA2), The University Of Alabama At Birmingham Cancer Data Analysis Portal (UALCAN) and Kaplan-Meier Plotter. We detected the expression of Sema4F in cell lines and tumor tissues by reverse transcription quantitative polymerase chain reaction (RT-qPCR), western blotting and immunohistochemistry. The prognostic value of Sema4F expression on patient overall survival was analyzed retrospectively using Kaplan-Meier survival and Cox regression analyses. Moreover, we used Kyoto encyclopedia of genes and genomes (KEGG), Gene Ontology (GO) and Gene-set enrichment analysis (GSEA) analyses to explore the relevant pathways of Sema4F in GC. Results: The expression of Sema4F was markedly increased in cancer tissues and cancer cell lines. Furthermore, high Sema4F expression was positively associated with various clinicopathologic data and independently predicted poor prognosis for overall survival in GC. Our functional enrichment analysis revealed that Sema4F was mainly involved in oxidative phosphorylation and tumor-related signaling pathways. Conclusions: Sema4F may be a valuable prognostic biomarker and a novel target for gastric cancer.

Keywords: Sema4F, gastric cancer, prognosis, biological function, biomarker

Introduction

Gastric cancer (GC) is a highly prevalent disease worldwide and has the fifth highest mortality rate among all cancers [1]. In China, gastric cancer is the third most frequently diagnosed cancer and the third leading cause of cancer-related death [2]. Because the symptoms of early gastric cancer are nonspecific, most patients are diagnosed with gastric cancer at an advanced stage and have a poor prognosis. Therefore, it is vital to search for novel biomarkers or effective therapeutic targets that will be helpful for improving the clinical outcomes of gastric cancer patients.

Semaphorins are a large family of developmental regulatory signals, and they are involved in the regulation of human cancers by controlling cell-cell communication, invasion metastasis, cell migration, inflammation, tumor angiogenesis and anticancer immune response [3]. Many studies have revealed that the role of semaphorins in cancer biology is complicated by the fact that semaphorins can be classified as either tumor-promoting or antitumorigenic depending on the cellular context [3]. Sema4F is a transmembrane family member whose function is poorly understood. Recently, Sema4F has been reported to serve as a tumor regulator in breast cancer [4], prostate cancer [5], and malignant peripheral nerve sheath tumors [6]. However, the role of Sema4F in gastric cancer is still unknown. To estimate the expression and clinical significance of Sema4F in gastric cancer, we analyzed a database. We found that Sema4F

was overexpressed in gastric cancer samples and was associated with clinicopathologic variables and poor prognosis in gastric cancer. Therefore, we speculated that Sema4F could be a biomarker for gastric cancer. To verify this hypothesis, we detected Sema4F expression in gastric cancer cells and tissues. Additionally, we analyzed the association of Sema4F expression with clinicopathologic characteristics and prognosis. Furthermore, we analyzed the biologic processes and signal transduction pathways that may mediate Sema4F activity in gastric cancer. Our findings provide new ideas for the role of Sema4F in gastric cancer.

Materials and methods

Database description

Transcription-related databases of Sema4F in patients with gastric cancer: Tumor Immune Estimation Resource (TIMER) is a comprehensive resource for systematic analysis of the associations between immune infiltrates and a wide spectrum of factors, including gene expression, clinical outcomes, somatic mutations, and somatic copy number alterations across diverse cancer types from TCGA (http://timer.comp-genomics.org). The mRNA levels of Sema4F in pan-cancer were examined by the online TIMER 2.0 database [7].

The University Of Alabama At Birmingham Cancer Data Analysis Portal (UALCAN) is a web resource that provides comprehensive cancer transcriptome data (http://ualcan.path.uab.edu/analysis.html) [8]. It includes 415 gastric cancer samples and 34 normal gastric tissue samples from The Cancer Genome Atlas (TCGA) and was used to analyze the difference in Sema4F expression between cancer and normal tissues.

The Gene Expression Profiling Interactive Analysis 2 (GEPIA2) database (http://gepia.cancer-pku.cn/) is an online database that facilitates the standardized analysis of RNA-seq data from 9,736 cancer samples and 8,587 normal control samples in the TCGA and Genotype-Tissue Expression Program (GTEx) datasets [9].

Kaplan-Meier plotter database: The Kaplan-Meier plotter (http://kmplot.com/analysis/) is a database that covers information on gene expression associated with the survival of patients with diverse cancer types [10]. We used the Kaplan-Meier plot database to analyze the prognostic significance of Sema4F mRNA expression. The optimal cutoff value was determined by selecting the "auto select best cutoff" option. Based on the cut-off, the patients were divided into high and low Sema4F expression cohorts, and overall survival (OS), first progression (FP), and post-progression survival (PPS) curves were plotted.

LinkedOmics database analysis: The LinkedOmics database (http://www.linkedomics.org/login.php) is a web-based platform for analyzing 32 TCGA cancer-associated multidimensional datasets [11]. Sema4F coexpression was examined using Pearson's correlation analysis and was presented in volcano plots, heatmaps, or scatter plots. The function module of LinkedOmics performs analysis of Gene Ontology biological process (GO_BP), KEGG pathways, kinase-target enrichment, miRNA-target enrichment and transcription factor-target enrichment by gene set enrichment analysis (GSEA). The rank criterion was FDR<0.1, and 1000 simulations were performed.

Functional enrichment analysis: SangerBox (http://SangerBox.com/Tool) is a helpful online portal for TCGA data analysis [12]. We explored the Gene Ontology Biological Process (GO_BP), Gene Ontology Molecular Function (GO_MF), Gene Ontology Cellular Component (GO_CC) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms of Sema4F. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed using Pearson's correlation coefficient. The rank criterion was FDR<0.1, and 1000 simulations were performed.

GSEA is a computational method used to determine whether a group of genes defined a priori shows a statistical difference between two biological states. GSEA was performed by the fgsea (v 1.12.0) package. Pathways with p.adj<0.05 and NES absolute ≥1 in the screening results were considered enriched pathways [13].

Patients

This study included 93 patients who underwent radical gastrectomy at Nantong Third People's

Hospital Affiliated with Nantong University (Nantong, China) between January 2016 and December 2021. The inclusion criteria were as follows: (I) gastric cancer was definitively diagnosed by postoperative pathology; (II) the patient's medical records were relatively complete; (III) no comprehensive antitumor treatments, such as radiotherapy, chemotherapy, targeted therapy, or immunotherapy, were performed before the operation; and (IV) OS follow-up data were complete. The exclusion criteria were as follows: (I) patients with any other types of malignant tumors; (II) patients with metastasis from other malignant tumors; and (III) death due to surgical complications. We retrospectively collected the medical record data of these patients, including demographic and clinicopathologic characteristics, and obtained the OS via telephone follow-up. The final follow-up was performed in December 2021. OS was defined as the duration from initial surgery to death or the last follow-up.

The study protocol was drafted following the ethical guidelines of the 1975 Declaration of Helsinki. The current study was reviewed and approved by the Ethics Committee of the Affiliated Nantong Hospital 3 of Nantong University. Informed consent was obtained from each patient.

Tissue samples

Gastric cancer tissues and adjacent normal gastric mucosa tissues were collected from 93 patients attending the Nantong Third People's Hospital Affiliated with Nantong University (Nantong, China) between January 2016 and December 2021. Histopathologic diagnosis of all patients was completed by the Department of Pathology. All tissues used in the study were frozen immediately after dissection and stored at -80°C until use.

Cell culture

Human GC cell lines (MKN-45, SGC-7901, AGS and MGC-803) and a normal human gastric epithelial cell line (GES-1) were kindly provided by the Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China). All cells were cultured in RPMI-1640 (Gibco, Thermo Fisher Scientific, Waltham, MA) with 10% fetal bovine serum (FBS; Cell Sciences, Canton, MA). All cells were cultured at 37°C in a humidified incubator under 5% CO_2 conditions.

Immunohistochemistry (IHC)

All tissues were fixed in 4% paraformaldehyde for 24 hours, embedded in paraffin and sectioned into 6-μm-thick slices. The slices were deparaffinized through a series of dimethyl benzene and graded alcohols. The slices were heated in citrate buffer (pH 6.0) for antigen retrieval, and endogenous peroxidase activity was blocked with H_2O_2. Then, the slices were incubated with an antibody against Sema4F (GeneTex, Texas, USA) at 4°C overnight and then incubated with a secondary antibody at room temperature for 1 hour (Novus Biologicals, Littleton, CO). After DAB staining, the sections were stained with hematoxylin. The tissue scores were assessed by two pathologists. The staining intensity observed in the gastric cancer tissues and normal gastric tissues was scored as 0 (negative), 1 (weak brown), 2 (medium brown) or 3 (strong brown); the degree of staining was scored as 0 (≤10%), 1 (>10%-25%), 2 (>25%-50%), 3 (>50%-75%) or 4 (>75%). Scoring was based on the product of the staining intensity score and degree score. A score ≥4 was considered high expression, and a score <4 was considered low expression.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from gastric cancer tissue samples or cell lines by TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA was reverse-transcribed to complementary DNA (cDNA) using PrimeScript RT Master Mix (Takara, Beijing, China). cDNA was used as a template to detect the expression of Sema4F with SYBR Green PCR Master Mix (Vazyme, NanJing, China). GAPDH was used as the internal control gene. Primer sequences are listed in **Table 1**.

Western blotting assay

Collected tissues were lysed in RIPA buffer supplemented with protease inhibitors (Beyotime, Shanghai, China) on ice for 15 minutes. The protein concentrations were measured with the BCA Protein Assay Kit (Beyotime) following the manufacturer's protocol. The protein extracts were separated by SDS-PAGE and then electrophoretically transferred to PVDF membranes (MilliporeSigma, St. Louis, USA). The

Table 1. Primer sequences

Name	Sequence (5'-3')
SEMA4F forward primer	ATGAAGATGGAGACGACGAAAT
SEMA4F reverse primer	GACTTTAATGCGCTCGTATGAG
GAPDH forward primer	CATGTTCCAATATGATTCCAC
GAPDH reverse primer	CCTGGAAGATGGTGATG

Table 2. Clinicopathologic features of 93 gastric cancer patients from Nantong Third People's Hospital Affiliated with Nantong University

General data of patients	
Clinical characteristic	Case (n = 93), n (%)
Gender	
Male	71 (76.3)
Female	22 (23.6)
Age (years)	
≥71	50 (53.8)
<71	43 (46.2)
Tumor size (cm)	
≥4	53 (57.0)
<4	40 (43.0)
TNM stage	
I+II	31 (33.3)
III+IV	62 (66.7)
Lymphatic metastasis	
Positive	58 (62.4)
Negative	35 (37.6)
Differentiation	
Poor	33 (35.5)
Moderate	55 (59.1)
Well	5 (5.4)
Lauren's classification	
Intestinal type	31 (33.3)
Diffuse type	25 (26.8)
Hybrid	37 (39.7)

membranes were incubated overnight at 4°C with primary antibodies against Sema4F (1:2000; GeneTex) and β-actin (1:5000; Proteintech), followed by detection with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000; MR Biotech). Protein bands were visualized using the ECL system (Thermo Fisher Scientific).

Statistical analysis

GraphPad Prism 8.0 software was used for data analysis and survival curve generation. Student's t test was used to determine the differences between the two groups, followed by the Cox proportional hazards regression model. SPSS 22.0 software (SPSS, Chicago, IL, USA) was used for all analyses. All the data are presented as the mean ± standard deviation. Statistical significance was defined as $P<0.05$. Kaplan-Meier Plotter analysis (http://kmplot.com/analysis) was used to compare overall survival (OS) in GC patients.

Results

Clinicopathologic features of 93 gastric cancer patients

The median age of the gastric cancer patients included in the study was 71, including 71 males (76.34%) and 22 females (23.66%). In terms of TNM stages, the majority of patients in the study were stage III and IV (66.7%), while stage I and II patients accounted for 33.3% of the sample. Among the 93 patients, pathologic analysis showed that the degree of differentiation was mainly low differentiation and moderate-low differentiation, accounting for 35.5% and 59.1%, respectively. All patients were followed up regularly. The last follow-up time was December 2021. During the follow-up period, 44 patients died. Specific clinicopathological features are shown in **Table 2**.

Sema4F expression is upregulated in public databases

Analysis of pan-cancer data in the TIMER 2.0 database showed that the mRNA levels of Sema4F were higher in 16 cancer types, including GC, than in normal tissues (**Figure 1A**). We further evaluated the Sema4F expression difference between the normal tissues and tumor tissues of various cancer types using GEPIA2 (**Figure 1B**). In addition, the protein expression profiles of 415 gastric cancer tissue samples and 34 normal gastric mucosa tissue samples from The Cancer Genome Atlas database were analyzed with UALCAN software. We found that the expression levels of Sema4F were increased in gastric cancer tissue samples compared to normal gastric mucosa tissue samples ($P<0.0001$; **Figure 1C**).

Sema4F expression was closely correlated with clinical variables and the prognosis of GC patients

To shed light on the role of Sema4F in GC, the association between Sema4F expression and

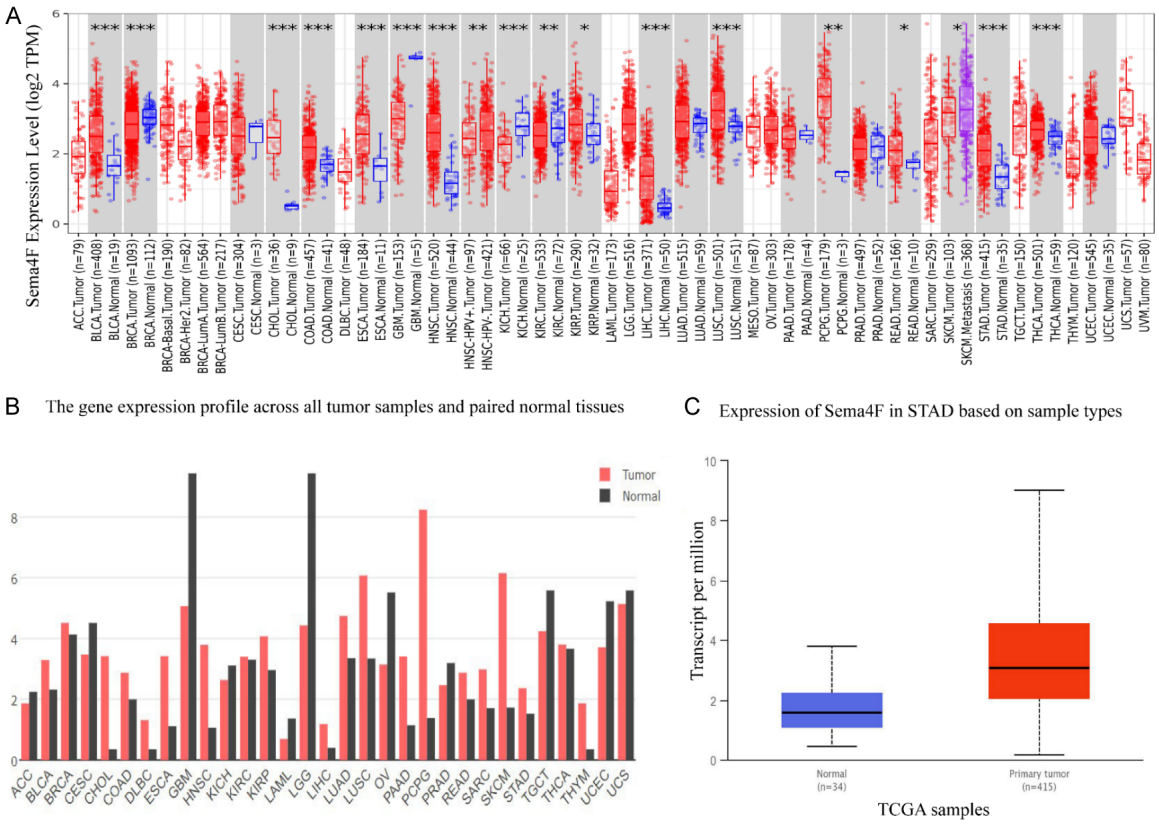

Figure 1. Database analysis of Sema4F expression in gastric cancer. A. Sema4F expression at the mRNA level in pan-cancer analysis using the TIMER 2.0 database. B. Higher expression of Sema4F in GC compared to normal tissues in the GEPIA2 database. C. The mRNA level of Sema4F was higher in 415 samples of primary tumor tissues than in 34 samples of normal gastric tissues in the UALCAN database.

clinical data was explored by the UALCAN database. GC patients were divided into several subgroups based on age, grade, H. pylori infection, nodal metastasis status, TP53 mutations, and tumor stage. The expression of Sema4F was significantly higher in GC patients than in normal controls in the subgroup analysis (**Figure 2**). Thus, these results showed that higher expression of Sema4F may be related to poor clinical features and clinical outcomes.

Then, Kaplan-Meier survival curves were used to assess the association between Sema4F expression and the survival outcomes of TCGA_STAD cohorts with available survival information. The OS, FP, and PPS survival curves indicated shorter survival in GC patients with high Sema4F expression than in patients with low Sema4F expression ($P<0.05$; **Figure 3A-C**). Collectively, these findings suggest that Sema4F levels may be a useful prognostic biomarker in GC.

Validation of Sema4F expression by RT-qPCR, western blot and IHC

To further confirm the expression of Sema4F in gastric cancer, we performed RT-qPCR to detect Sema4F expression in the normal human gastric epithelial cell line GES-1 and human gastric cancer cell lines MKN-45, SGC-7901, AGS and MGC-803. We found that the relative Sema4F mRNA expression in human gastric cancer cell lines was significantly higher than that in normal human gastric epithelial cell lines (**Figure 4A**).

Furthermore, we measured Sema4F expression in gastric cancer tissues and adjacent normal gastric mucosal tissues by RT-qPCR and western blotting and found that both the mRNA and protein levels of Sema4F were significantly increased in gastric cancer tissues (**Figure 4B**, **4C**). To further confirm this observation, we performed IHC and obtained comparable results

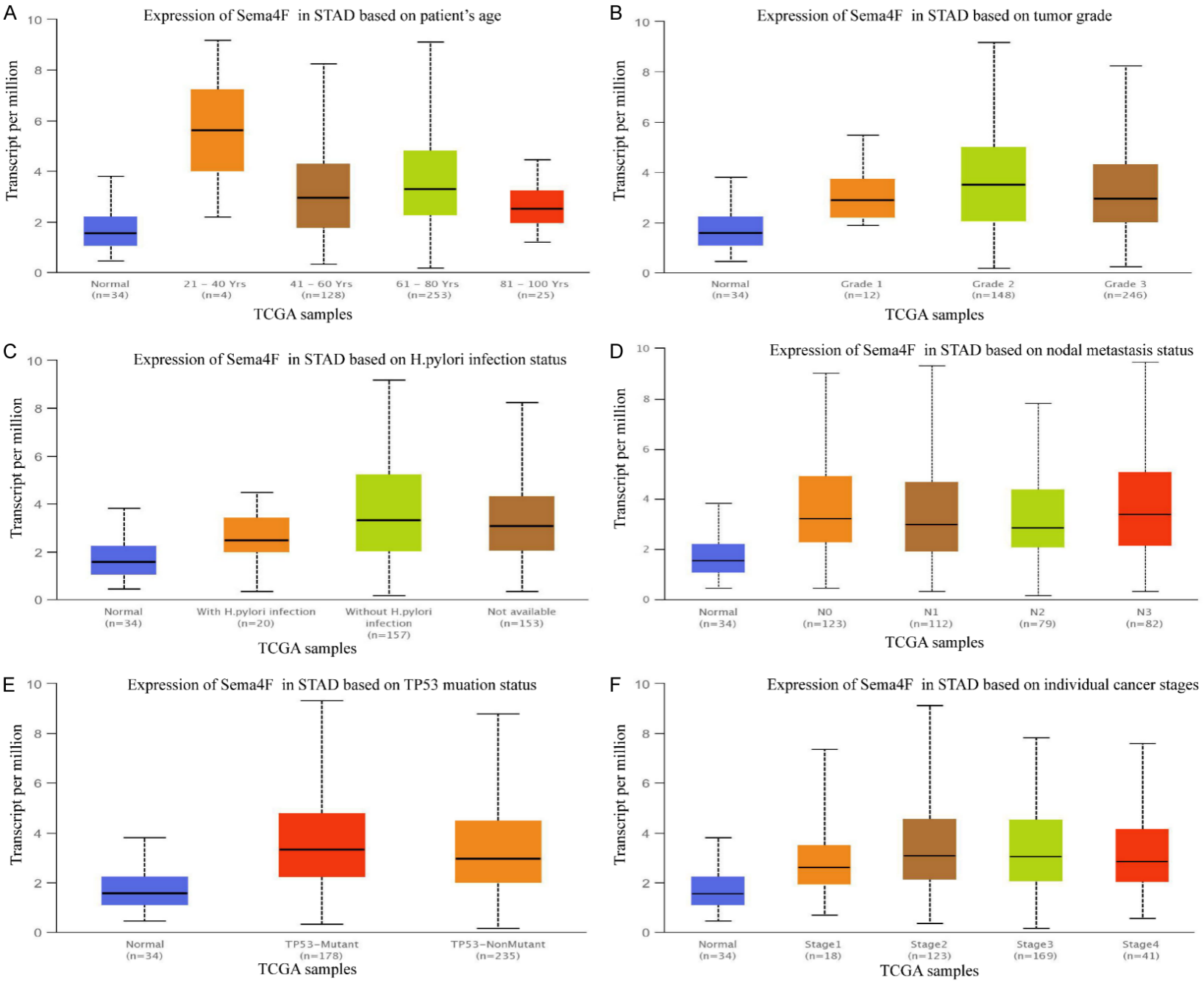
A
Expression of Sema4F in STAD based on patient's age
Transcript per million
Normal (n=34)
21 – 40 Yrs (n=4)
41 – 60 Yrs (n=128)
61 – 80 Yrs (n=253)
81 – 100 Yrs (n=25)
TCGA samples
B
Expression of Sema4F in STAD based on tumor grade
Transcript per million
Normal (n=34)
Grade 1 (n=12)
Grade 2 (n=148)
Grade 3 (n=246)
TCGA samples
C
Expression of Sema4F in STAD based on H.pylori infection status
Transcript per million
Normal (n=34)
With H.pylori infection (n=20)
Without H.pylori infection (n=157)
Not available (n=153)
TCGA samples
D
Expression of Sema4F in STAD based on nodal metastasis status
Transcript per million
Normal (n=34)
N0 (n=123)
N1 (n=112)
N2 (n=79)
N3 (n=82)
TCGA samples
E
Expression of Sema4F in STAD based on TP53 muation status
Transcript per million
Normal (n=34)
TP53–Mutant (n=178)
TP53–NonMutant (n=235)
TCGA samples
F
Expression of Sema4F in STAD based on individual cancer stages
Transcript per million
Normal (n=34)
Stage1 (n=18)
Stage2 (n=123)
Stage3 (n=169)
Stage4 (n=41)
TCGA samples

Figure 2. Correlation of Sema4F expression with clinical parameters in GC. A. Sema4F correlated with GC patient age. B. Tumor grade. C. H. pylori infection status. D. Nodal metastasis status. E. TP53 mutation. F. Stages.

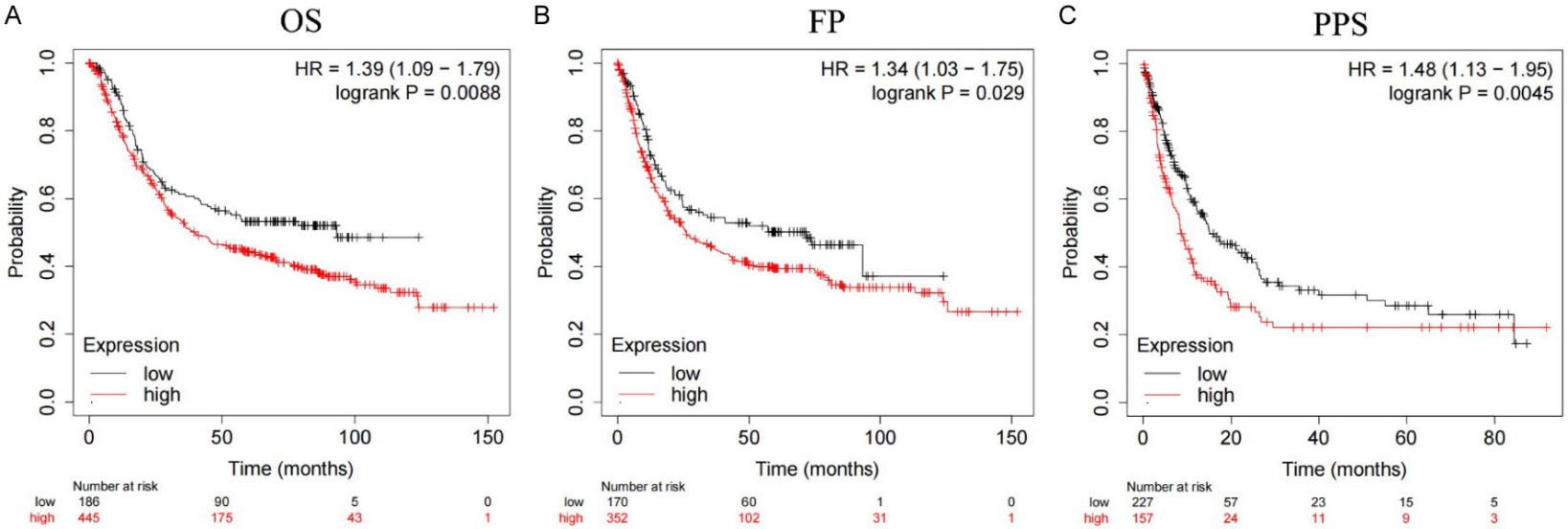

Figure 3. Kaplan-Meier curves of OS (A), FP survival (B), and PPS (C) show that mRNA levels of Sema4F are significantly related to survival in GC.

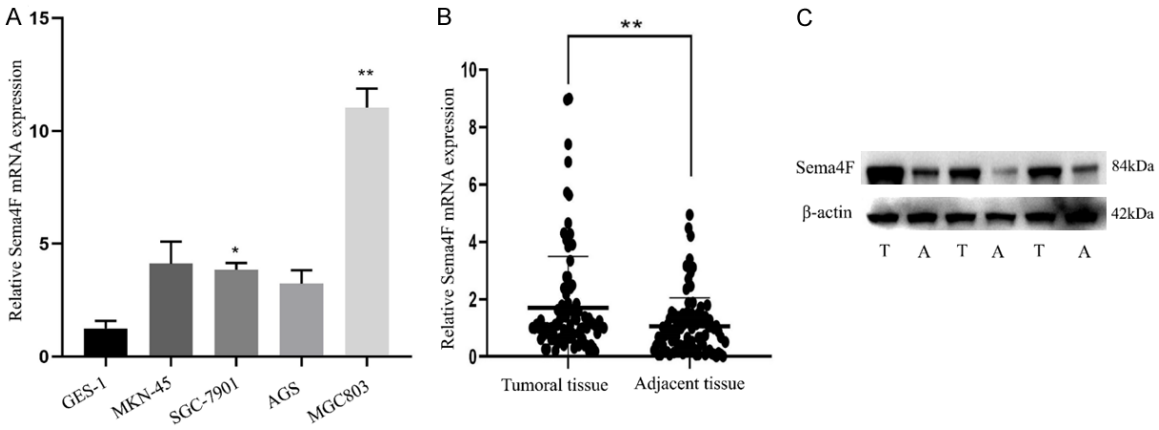

Figure 4. Increased Sema4F expression in gastric cancer cells and tissues. A. Expression of Sema4F mRNA in human gastric cancer lines compared to normal human gastric epithelial cell lines, as detected by RT-qPCR (*P<0.05, **P<0.01). B. Expression of Sema4F mRNA in human gastric cancer tissues compared to normal human gastric epithelial tissues, as detected by RT-qPCR (**P<0.01). C. The expression of Sema4F in gastric cancer tissues and adjacent normal gastric mucosal tissues was detected by western blotting. Representative images are shown.

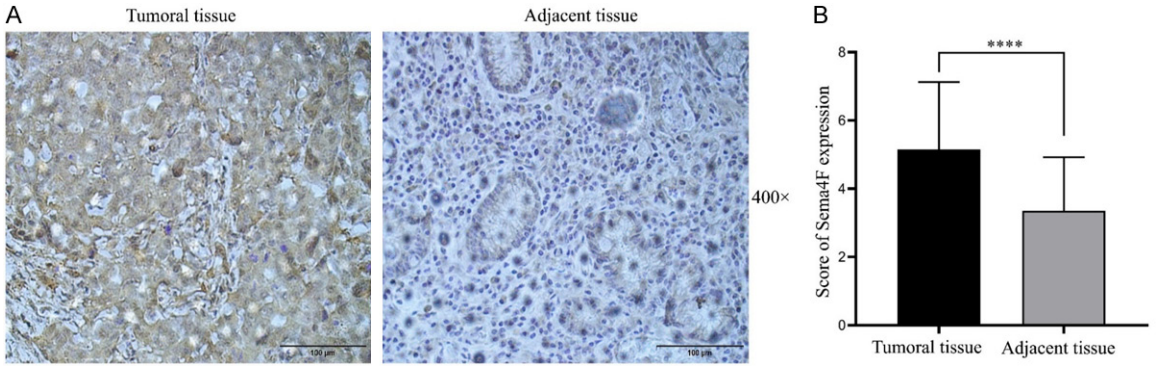

Figure 5. Immunohistochemistry was used to detect the intensity of Sema4F expression in gastric cancer tissues (****P<0.0001).

(**Figure 5**). We thus concluded that in gastric cancer, the expression of Sema4F is very upregulated.

Correlation between Sema4F expression and the clinicopathologic characteristics of gastric cancer

To evaluate the clinical value of Sema4F expression in gastric cancer patients, we divided all gastric cancer samples into two groups: a high Sema4F expression group (n = 41, more than the median value of Sema4F expression) and a low Sema4F expression group (n = 52, less than the median value of Sema4F expression). Then, we analyzed the correlation between Sema4F expression and the clinicopathologic characteristics of gastric cancer through the chi-square test. As shown in **Table 3**, we found that high Sema4F expression was significantly correlated with tumor size (P = 0.004), TNM stage (P = 0.001) and lymph node metastasis (P = 0.019) in gastric cancer patients. However, age (P = 0.894), sex (P = 0.105), differentiation degree (P = 0.192) and Lauren classification (P = 0.065) were not associated with Sema4F expression.

Upregulation of Sema4F protein expression was associated with poor prognosis in GC

We plotted the Kaplan-Meier survival curves of the two groups to investigate the prognostic value of Sema4F expression for the overall survival of gastric cancer patients. Similar to the results of the database analysis, we found that

Table 3. Correlations between Sema4F expression and the clinicopathologic characteristics of gastric cancer

Characteristic	N	SEMA4F expression		*P* value
		Low (N = 52)	High (N = 41)	
Gender				0.105
Male	71	43	28	
Female	22	9	13	
Age (years)				0.894
≥71	50	29	21	
<71	43	23	20	
Tumor size (cm)				0.004
<4	40	27	13	
≥4	53	25	28	
TNM stage				0.001
I+II	31	27	4	
III+IV	62	25	37	
Lymphatic metastasis				0.019
Positive	58	27	31	
Negative	35	25	10	
Differentiation				0.192
Well + moderate	59	36	23	
Poor	34	16	18	
Lauren's classification				0.065
Intestinal type	31	22	9	
Diffuse type	25	10	15	
Hybrid	37	20	17	

the overall survival of gastric cancer patients was shorter in the high Sema4F expression group than in the low Sema4F expression group (P = 0.003; **Figure 6A**). Moreover, worse differentiation and TNM stage indicated the worst outcomes (P<0.05; **Figure 6B**, **6C**). Then, univariate analysis of each clinicopathologic value was performed to investigate the prognostic value for patient survival time. The results showed that Sema4F expression (P = 0.004; **Table 4**) and TNM stage (P = 0.03; **Table 4**) were prognostic factors for overall survival in gastric cancer patients. Furthermore, we analyzed those two parameters by multivariate Cox proportional hazards model analysis. The results suggested that Sema4F expression (P = 0.041; **Table 4**) was an independent prognostic factor for overall survival, with a hazard ratio of 2.028 and a 95% confidence interval of 1.029-3.997.

Functional enrichment analysis of Sema4F

To gain insight into the biological meaning of Sema4F in GC, the functional module of LinkedOmics was used to examine Sema4F coexpression in the TCGA_STAD cohort. As shown in **Figure 7A**, 12,622 genes (dark red dots) showed significant positive correlations with Sema4F, whereas 7,603 genes (dark green dots) showed significant negative correlations (false discovery rate, FDR<0.1). The top 50 significant genes positively and negatively correlated with Sema4F are shown in the heatmap (**Figure 7B**, **7C**).

The results of the GO analysis showed significant enrichment of the biologic process (BP) terms cell-cell signaling by Wnt, aerobic respiration, positive regulation of JUN kinase activity, glycoprotein catabolic process, and actin polymerization-dependent cell motility (**Figure 8A**). The significantly enriched cellular component (CC) terms mainly included endomembrane system, plasma membrane part, RNA polymerase II transcription factor complex, and transcription factor TFIIH holo complex (**Figure 8B**). The significantly enriched molecular function (MF) terms mainly included histone binding, ATPase activity, metallopeptidase activity, structural constituent of cytoskeleton, and Rac GTPase binding (**Figure 8C**). Most importantly, KEGG analysis revealed that Sema4F tended to be enriched in the following terms: metabolic pathways, endocytosis, oxidative phosphorylation, Notch signaling pathway, Wnt signaling pathway, and MAPK signaling pathway (**Figure 8D**).

Additionally, we performed gene-set enrichment analysis (GSEA). The results showed that Sema4F was mainly enriched in oxidative phosphorylation, the Notch signaling pathway, the Wnt signaling pathway and the MAPK signaling pathway (**Table 5**; **Figure 9**). These results provide good insight into the mechanisms of Sema4F in GC.

Discussion

Gastric cancer is a common global health problem, as it is a lethal malignancy that is difficult to treat. In China, the incidence of gastric cancer ranks second only to that of liver cancer [2]. Despite improvements in new diagnostic and

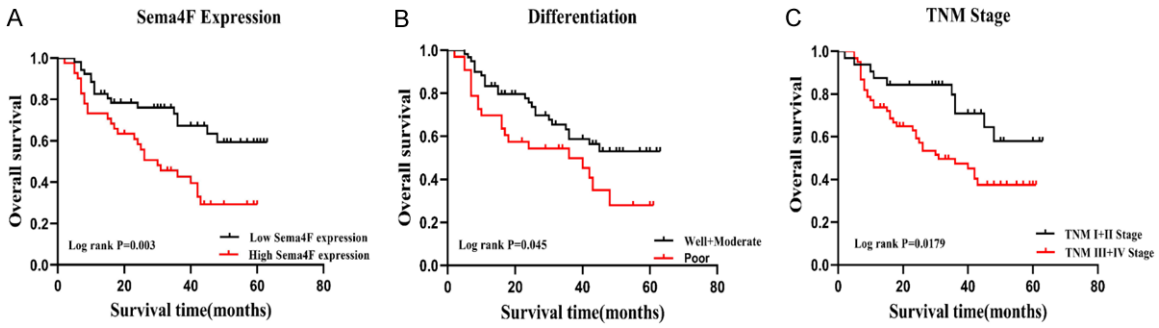

Figure 6. Association between the expression level of Sema4F and gastric cancer patient prognosis. Kaplan-Meier overall survival (OS) curves by clinicopathologic characteristics. A. Sema4F expression. B. Differentiation. C. TNM stage.

Table 4. Univariate and multivariate Cox regression analyses for overall survival in gastric cancer

Characteristic	Univariate analysis				Multivariate analysis			
	P value	HR	95% CI for exp (b)		*P* value	HR	95% CI for exp (b)	
			Lower	Upper			Lower	Upper
Gender	0.811	1.090	0.538	2.208				
Age	0.117	1.625	0.885	2.985				
Tumor size	0.163	1.560	0.836	2.912				
TNM stage	0.030	2.88	1.078	4.442	0.271	1.556	0.708	3.421
I+II								
III+IV								
Lymphatic metastasis	0.193	1.528	0.807	2.891				
Positive								
Negative								
Differentiation	0.093	0.601	0.601	0.332				
SEMA4F expression	0.004	2.431	1.320	4.478	0.041	2.028	1.029	3.997
Low								
High								

treatment techniques, the 5-year survival rate is still below 35% [14]. Therefore, more efficient methods are needed to increase the survival rate. With the rapid development of molecular medicine, many biomarkers have been found to be related to the occurrence, progression, and prognosis of gastric cancer. For example, the overexpression of HER2 is associated with poor prognosis in gastric cancer patients [15], and the tumor markers CEA, CA199, and CA125 have been widely used in recent years to predict the prognosis of gastric cancer patients [16]. At present, researchers are devoted to finding new biomarkers with higher sensitivity and specificity than the existing biomarkers. Sema4F is a transmembrane semaphorin family member that has been found in breast-, central nervous system-, and prostate-related cells [5, 6, 17]. However, the expression pattern of Sema4F in gastric cancer patients is still unknown.

In our study, we provide several lines of evidence for a possible role for Sema4F in gastric cancer.

The results of multiple databases indicated that Sema4F is upregulated in a variety of cancers, including GC, and that patients with poor clinicopathologic features often have high expression levels of Sema4F. Kaplan-Meier survival curves for OS, FP, and PPS showed that GC patients with higher Sema4F levels had poorer and shorter overall survival times. These findings strongly suggest that Sema4F may play a tumor-promoting role and that high Sema4F

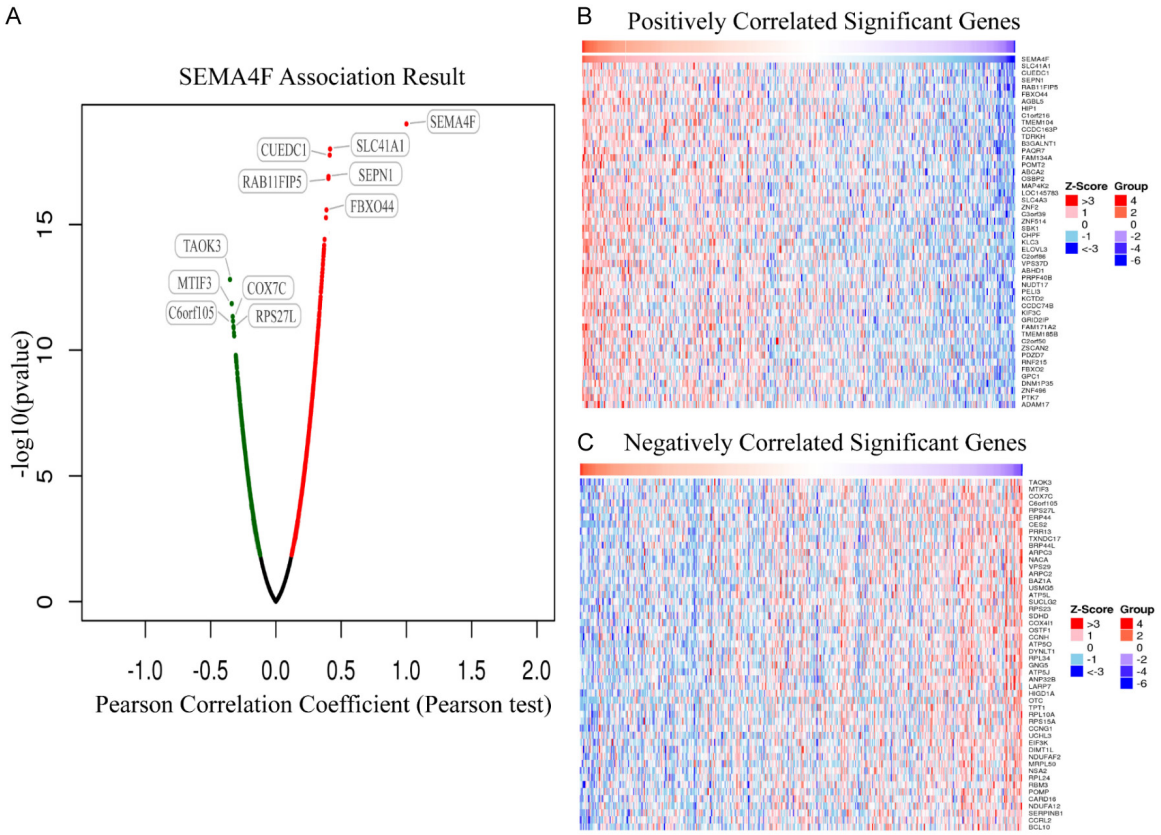

Figure 7. Sema4F coexpression genes in GC (LinkedOmics). (A) The global Sema4F highly correlated genes identified by Pearson's test in the TCGA_STAD cohort. Heatmaps showing the top 50 genes positively (B) and negatively (C) correlated with Sema4F in STAD. Red indicates positively correlated genes, and blue indicates negatively correlated genes.

expression is strongly associated with poorer outcomes in GC patients.

Then, we performed RT-qPCR to confirm Sema4F expression in gastric cancer cells. We detected Sema4F expression in gastric cancer tissues by RT-qPCR, western blotting, and immunohistochemistry. Our results indicated that in gastric cancer, Sema4F could be a tumor promoter and may contribute to disease progression.

Furthermore, we analyzed the association between Sema4F expression and clinicopathologic data and observed that high Sema4F expression was positively associated with tumor size, TNM stage and lymph node metastasis in gastric cancer patients. To confirm the usefulness of Sema4F as a prognostic factor of GC, Kaplan-Meier curves were analyzed to determine the correlation between survival and Sema4F expression. The results showed that GC patients with higher Sema4F expression had significantly shorter survival than those with lower Sema4F expression. Other clinicopathological parameters also associated with OS were analyzed using Kaplan-Meier survival curves. Then, we conducted univariate and multivariate Cox regression analyses to identify independent prognostic factors, and the results demonstrated that the Sema4F expression level could be used as an independent prognostic predictor in gastric cancer patients.

Sema4F has been shown to act as a tumor promoter in tumorigenesis. In breast cancers, Gabrovska et al. showed that Sema4F was involved in tumor progression [17]. Moreover, Simon Grelet et al. demonstrated that the upregulation of Sema4F was involved in metastatic progression through the TGFβ/Platr18/Sema4F axis [18]. In prostate cancer, Ding et al. reported that Sema4F overexpression accelerated cell proliferation and migration in vitro

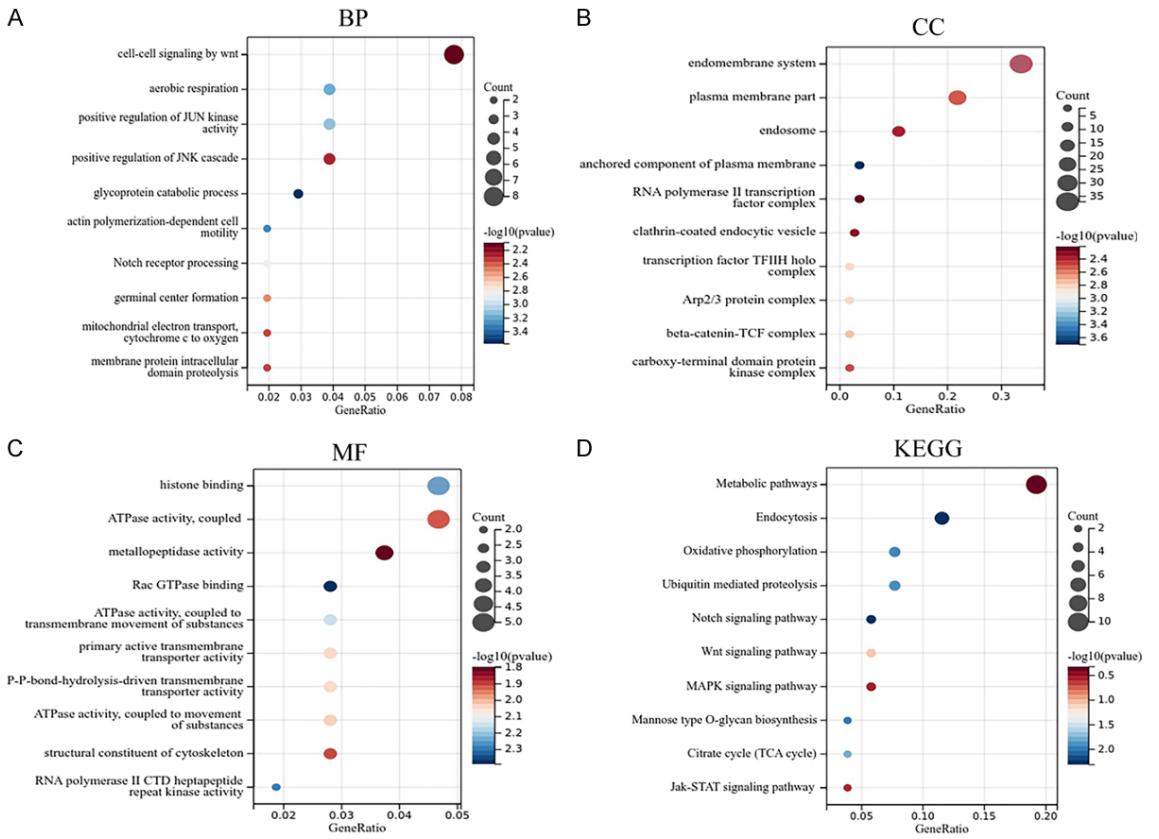

Figure 8. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis results for Sema4F in GC from TCGA database. A. Top 10 enrichment terms in biologic process (BP) categories in GC. B. Top 10 enrichment terms in cellular component (CC) categories in GC. C. Top 10 enrichment terms in molecular function (MF) categories in GC. D. Top 10 KEGG enrichment pathways in GC.

Table 5. Enriched pathways associated with Sema4F expression

Gene set	ES	NES	NOM *p*-val	FDR q-val
Oxidative phosphorylation	0.53	2.59	≤0.001	≤0.001
Notch signaling pathway	-0.58	-1.74	≤0.001	0.005
Wnt signaling pathway	-0.51	-1.71	≤0.001	0.006
MAPK signaling pathway	-0.48	-1.66	≤0.001	0.009

ES, enrichment score; NES, normalized enrichment score; NOM, nominal; FDR, false discovery.

[5]. In our study, we found that Sema4F is overexpressed in gastric cancer and is associated with clinical progression and poor prognosis. However, the effect of Sema4F on the biological function of gastric cancer needs to be verified by experiments.

With the development of high-throughput sequencing and new computational methods, evidence suggests that multiple genes interact with each other and influence the occurrence and progression of tumors [19]. In the present study, we successfully identified 7,603 genes that were negatively correlated with Sema4F and 12,622 genes that were positively correlated with Sema4F in the GC group by using LinkedOmics. To investigate the function of Sema4F, we carried out GO and KEGG analyses of genes coexpressed with Sema4F. The biologic processes enriched by genes coexpressed with Sema4F include signal transduction, positive regulation of JUN kinase activity, RNA polymerase II transcription factor complex, ATPase activity and Rac GTPase binding. KEGG pathway analysis showed that genes coexpressed with Sema4F were significantly enriched in oxidative phosphorylation and pathways in cancer. GSEA can be used to elucidate biological pathways in which genes are involved [13]. GSEA using TCGA data further showed that oxidative phosphorylation, the Notch signaling

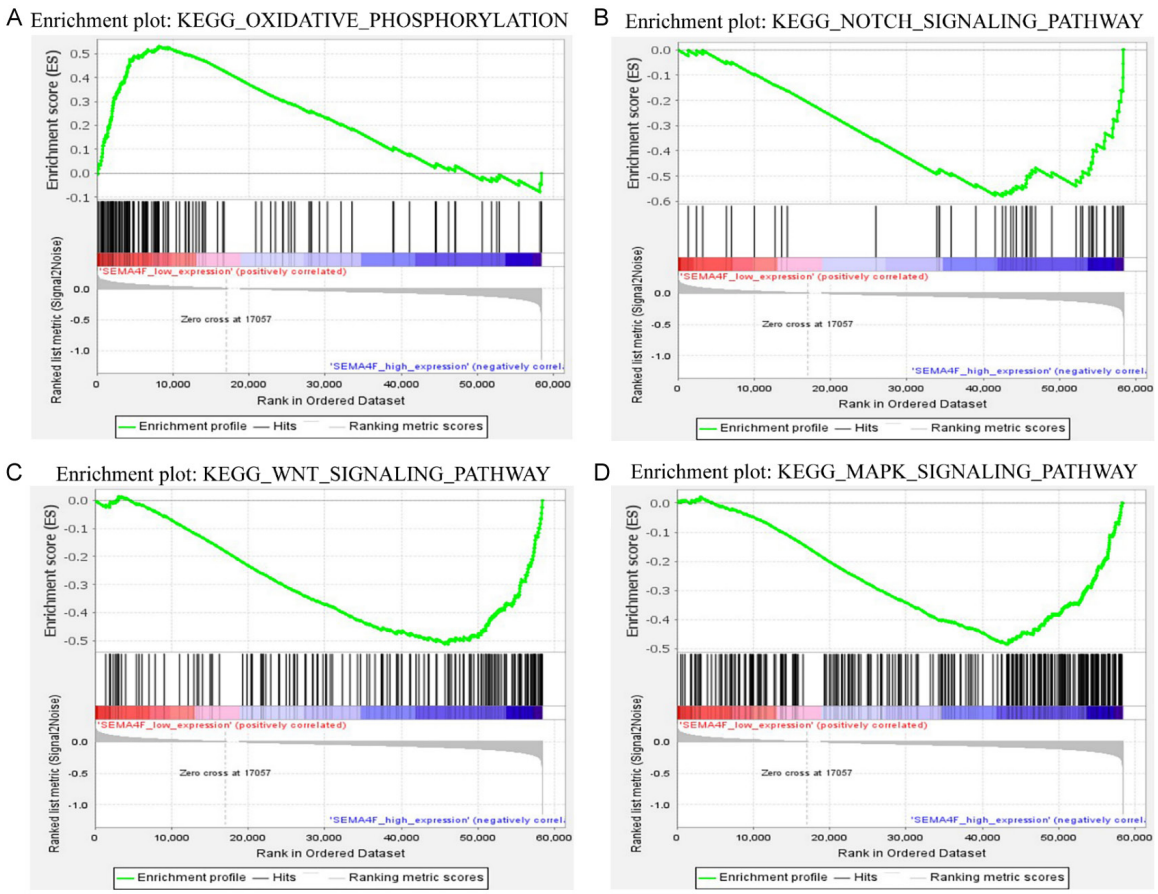

Figure 9. Gene set enrichment analysis of Sema4F in GC. (A) Oxidative phosphorylation, (B) the Notch signaling pathway, (C) the Wnt signaling pathway, and (D) the MAPK signaling pathway were differentially enriched with Sema4F expression.

pathway, the Wnt signaling pathway, and the MAPK signaling pathway were differentially enriched with the expression of Sema4F.

Oxidative phosphorylation (OXPHOS) is reportedly involved in the development of cancer [20]. Additionally, it has been reported that OXPHOS and glycolysis promote tumor cell growth by producing enough ATP [21]. Zhou et al. [22] found that Notch signaling can both promote and inhibit tumor development in various types of cancer. Yann Duchartre et al. [23] reported that Wnt signaling is related to the initiation and/or maintenance and development of many cancers. Sun et al. [24] showed that the MAPK signaling pathway is involved in the regulation of glucose uptake in malignant cells to regulate the occurrence of tumors. Therefore, we speculated that Sema4F is involved in regulating cell metabolism, cell proliferation, apoptosis, metastasis, angiogenesis and drug resistance through OXPHOS and various signaling pathways to promote the occurrence and development of tumors. The study also has some limitations. We were unable to provide information regarding the cellular biological functions and mechanisms of Sema4F in GC. Thus, more experiments are needed to validate these findings in the future.

Conclusion

In this research, Sema4F was overexpressed in gastric cancer tissues and cells. High Sema4F expression is associated with clinical progression and poor prognosis in gastric cancer patients. We found a possible mechanism of Sema4F in GC by using bioinformatic methods. Overall, Sema4F can be used as a hypothetical target for the diagnosis and treatment of gastric cancer.

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Disclosure of conflict of interest

None.

Abbreviations

BP, Biological Process; CC, Cell Composition; MF, Molecular Function; GESA, Gene-set enrichment analysis; KEGG, Kyoto encyclopedia of genes and genomes; GO, Gene Ontology; GC, Gastric cancer; OS, Overall survival; FP, First progression; PPS, Post-progression survival; GTEx, Genotype-Tissue Expression Program; TIMER, Tumor Immune Estimation Resource; GEPIA2, The Gene Expression Profiling Interactive Analysis 2; UALCAN, The University Of Alabama At Birmingham Cancer Data Analysis Portal.

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References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-249.

[2] Cao W, Chen HD, Yu YW, Li N and Chen WQ. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. Chin Med J (Engl) 2021; 134: 783-791.

[3] Mastrantonio R, You H and Tamagnone L. Semaphorins as emerging clinical biomarkers and therapeutic targets in cancer. Theranostics 2021; 11: 3262-3277.

[4] Grelet S, Fréreux C, Obellianne C, Noguchi K, Howley BV, Dalton AC and Howe PH. TGFβ-induced expression of long noncoding lincRNA Platr18 controls breast cancer axonogenesis. Life Sci Alliance 2021; 5: e202101261.

[5] Ding Y, He D, Florentin D, Frolov A, Hilsenbeck S, Ittmann M, Kadmon D, Miles B, Rowley D and Ayala G. Semaphorin 4F as a critical regulator of neuroepithelial interactions and a biomarker of aggressive prostate cancer. Clin Cancer Res 2013; 19: 6101-6111.

[6] Ma KH, Duong P, Moran JJ, Junaidi N and Svaren J. Polycomb repression regulates Schwann cell proliferation and axon regeneration after nerve injury. Glia 2018; 66: 2487-2502.

[7] Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B and Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020; 48: W509-W514.

[8] Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon AR, Athar M, Netto GJ, Qin ZS, Kumar S, Manne U, Creighton CJ and Varambally S. UALCAN: an update to the integrated cancer data analysis platform. Neoplasia 2022; 25: 18-27.

[9] Tang Z, Kang B, Li C, Chen T and Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019; 47: W556-W560.

[10] Nagy Á, Munkácsy G and Győrffy B. Pancancer survival analysis of cancer hallmark genes. Sci Rep 2021; 11: 6047.

[11] Vasaikar SV, Straub P, Wang J and Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res 2018; 46: D956-D963.

[12] Wang D, Wang Y, Zou X, Shi Y, Liu Q, Huyan T, Su J, Wang Q, Zhang F, Li X and Tie L. FOXO1 inhibition prevents renal ischemia-reperfusion injury via cAMP-response element binding protein/PPAR-γ coactivator-1α-mediated mitochondrial biogenesis. Br J Pharmacol 2020; 177: 432-448.

[13] Hu J, Qiu D, Yu A, Hu J, Deng H, Li H, Yi Z, Chen J and Zu X. YTHDF1 is a potential pan-cancer biomarker for prognosis and immunotherapy. Front Oncol 2021; 11: 607224.

[14] Zeng H, Chen W, Zheng R, Zhang S, Ji JS, Zou X, Xia C, Sun K, Yang Z, Li H, Wang N, Han R, Liu S, Li H, Mu H, He Y, Xu Y, Fu Z, Zhou Y, Jiang J, Yang Y, Chen J, Wei K, Fan D, Wang J, Fu F, Zhao D, Song G, Chen J, Jiang C, Zhou X, Gu X, Jin F, Li Q, Li Y, Wu T, Yan C, Dong J, Hua Z, Baade P, Bray F, Jemal A, Yu XQ and He J. Changing cancer survival in China during 2003-15: a pooled analysis of 17 population-based cancer registries. Lancet Glob Health 2018; 6: e555-e567.

[15] Lei YY, Huang JY, Zhao QR, Jiang N, Xu HM, Wang ZN, Li HQ, Zhang SB and Sun Z. The clinicopathological parameters and prognostic significance of HER2 expression in gastric cancer patients: a meta-analysis of literature. World J Surg Oncol 2017; 15: 68.

[16] Huang C, Liu Z, Xiao L, Xia Y, Huang J, Luo H, Zong Z and Zhu Z. Clinical significance of serum CA125, CA19-9, CA72-4, and fibrinogen-

to-lymphocyte ratio in gastric cancer with peritoneal dissemination. Front Oncol 2019; 9: 1159.
[17] Gabrovska PN, Smith RA, Tiang T, Weinstein SR, Haupt LM and Griffiths LR. Semaphorin-plexin signalling genes associated with human breast tumourigenesis. Gene 2011; 489: 63-69.
[18] Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstråle M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D and Groop LC. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003; 34: 267-273.
[19] Lin Y, Zhang L, Zhang X, Wei X, Liu X, Xie Y and Han G. Identification of potential diagnostic and prognostic biomarkers for gastric cancer based on bioinformatic analysis. J Environ Pathol Toxicol Oncol 2023; 42: 61-68.
[20] Greene J, Segaran A and Lord S. Targeting OXPHOS and the electron transport chain in cancer; Molecular and therapeutic implications. Semin Cancer Biol 2022; 86: 851-859.
[21] Vander Heiden MG and DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. Cell 2017; 168: 657-669.
[22] Zhou B, Lin W, Long Y, Yang Y, Zhang H, Wu K and Chu Q. Notch signaling pathway: architecture, disease, and therapeutics. Signal Transduct Target Ther 2022; 7: 95.
[23] Duchartre Y, Kim YM and Kahn M. The Wnt signaling pathway in cancer. Crit Rev Oncol Hematol 2016; 99: 141-149.
[24] Sun Y, Liu WZ, Liu T, Feng X, Yang N and Zhou HF. Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. J Recept Signal Transduct Res 2015; 35: 600-604.