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Association Between EphA1 and Tumor Microenvironment in Gastric Carcinoma and its Clinical Significance

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: With the growing global burden of gastric carcinoma (GC) and the urgent need for biomolecular targeted therapies, this study aimed to elucidate the relationship between EphA1 and the tumor microenvironment (focusing primarily on the key inflammatory cytokines IL-6 and tumor angiogenic cytokine VEGF) to identify a new potential therapeutic target.

Material/Methods: IHC and qRT-PCR were performed to quantify the protein and gene expression levels of EphA1, IL-6, and VEGF in normal mucosal tissues, carcinoma tissues, and paracarcinomatous tissues from 57 GC patients. Spearman's rank correlation test was performed to determine the relationship between EphA1, IL-6, and VEGF expression levels. The relationships of EphA1 with clinicopathologic parameter and survival in GC patients were also evaluated.

Results: The protein and gene expression levels of EphA1 were all attenuated gradually from carcinoma tissues to paracarcinomatous tissues and then to normal mucosal tissues in GC patients. Additionally, significant correlations between the overexpression of EphA1 with aggressive clinicopathological features and shorter survival time of GC patients were verified. In particular, we found a significant positive correlation between the expression of EphA1 and tumor microenvironment hallmark proteins IL-6 and VEGF in carcinoma tissues and paracarcinomatous tissues.

Conclusions: EphA1 can promote the occurrence and development of GC by its selective high expression in cancer tissues and its relationship with malignant clinical features and prognosis of GC patients. The underlying potential mechanism appears to involve enhancement of the tumor microenvironment, which via drives the expression of tumor microenvironment hallmark proteins IL-6 and VEGF.

MeSH Keywords: Receptor, EphA1 • Receptors, Interleukin-6 • Stomach Neoplasms • Vascular Endothelial Growth Factor, Endocrine-Gland-Derived

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Background

According to the latest global cancer statistics, the incidence rate of gastric cancer (GC) ranks fourth and it is the third leading cause of cancer-related deaths worldwide [1]. The poor prognosis of gastric cancer is due to the high recurrence rate of postoperative and metastasis, as well as its multidrug resistance in chemotherapy-based treatments [2]. The available treatments for advanced-stage GC are still limited despite recent advances in molecular targeted therapies based on the molecular classification of gastric cancer, and identification of new biomarker of GC is urgently needed [3].

EphA1 belongs to the Ephs (erythropoietin-producing hepatocyte kinases) family, which was first discovered by Japanese scholar Hirai in the human liver cancer cell line etl-1, which produces erythropoietin, in 1987 [4]. Recently, accumulating evidence revealed that EphA1 is frequently overexpressed in various human cancers, including gastric carcinoma, and appears to influence many aspects of tumor biology and patient survival, and could be a novel potential therapeutic target [5–8]. However, research on EphA1 and gastric cancer has been limited and no investigations have reported the underlying mechanisms.

Gastric cancer, a typical inflammation-related solid cancer, is significantly correlated with chronic uncontrollable inflammatory stimulation and tumor angiogenesis, which are induced by the tumor microenvironment, in the occurrence and development of tumors [9–11]. VEGF can stimulate endothelial cell growth, migration, and survival of preexisting vasculature via a network of signaling processes triggered by activation of the VEGF-VEGFR signaling axis, which is the most important regulator of angiogenesis and specifically acts on vascular endothelial cells [11]. IL-6 is a chemokine that causes chronic active gastritis, mainly by inducing dense infiltration of neutrophils and macrophages in the gastric mucosa and inducing a proliferative response. Thus, a chronic inflammatory response appears to be an important condition for the occurrence of cancer and a vital step in malignant tumor cells to obtain malignant progression properties in inflammation-related cancers, including gastric carcinoma [12,13]. Ephs has been implicated in angiogenesis in the tumor microenvironment and in oxidative stress due to inflammation during tumorigenesis of solid tissues [14–16]. Thus, we hypothesized that EphA1 could be a key biomarker in the tumor microenvironment.

The association between EphA1 and the tumor microenvironment in gastric carcinoma is unclear. Considering the inflammatory-associated solid-tumor characteristics of gastric cancer, we examined the relationship between EphA1 and 2 important tumor microenvironmental markers (the key inflammatory cytokines IL-6 and tumor angiogenic cytokine VEGF) to understand whether EphA1 expression drives the increase of inflammatory factors and the promotion of angiogenesis in the tumor microenvironment. Here, we investigated the association of EphA1 with biological characteristics of tumors and the survival of patients to determine whether EphA1 is useful for predicting tumor malignant features and poor prognosis in GC patients.

Material and Methods

Patients and tissue specimens

All 57 tumor biopsies were obtained from patients with GC who had undergone gastrectomy at the Department of Gastrointestinal Oncology Surgery (Anhui Provincial Cancer Hospital, Hefei, China) from October 2015 to October 2016 and had not received adjuvant therapy such as chemotherapy or radiotherapy before the operation. There were 37 male patients and 20 female patients with age range 28 to 87 years and average age 62 years. All tissue specimens were diagnosed according to the standard of the National Gastric Cancer Cooperative Group, and were reexamined by pathologists. There were 9 cases of highly differentiated adenocarcinoma, 18 cases of moderately differentiated adenocarcinoma, 30 cases of poorly differentiated adenocarcinoma, 29 cases of positive lymph node metastasis, 28 cases of negative lymph node metastasis, 17 cases of TNM stage I, 20 cases of TNM stage II, and 20 cases of TNM stage IIII, among which 45 cases did not have penetrated serous membrane and 12 cases had penetrated serous membrane. All patients' clinical and pathological data, which procured from medical records, were used to analyze the correlation between EphA1, IL-6, and VEGF expression levels, and the last follow-up date was October 2019. This study was approved by the Institutional Review Committee of Anhui Provincial Cancer Hospital and we obtained written informed consent from all patients. Based on the literature and the practical working practice of consulting pathologists, the method of specimen selection was formulated: for each case, cancerous tissue, paracancerous tissue (1 to 2 cm from the margin of the tumor visible to the naked eye) and normal gastric mucosa tissue (>5 cm from the margin of the tumor visible to the naked eye) were taken for experimental use [17,18].

Immunochemical staining and analyses

Immunohistochemical staining of EphA1, IL-6, VEGF in cancer tissues, paracarcinomatous tissues, and adjacent normal tissues of GC was performed by the two-step method (Anhui Xin Le Biotechnology Co., Hefei, China). The negative

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control group and positive control group were treated with PBS and positive tissue sections, respectively.

After the staining by DAB, the location of positive expression of brown particles was observed under the microscope, and the yellow-brown particles located in the cytoplasm or cell membrane were identified as positive staining. The immunohistochemical results were evaluated by semi-quantitative integral method. Scores for the mean percentage of immunopositive cells were based on the number of positive staining cells per 100 cells in 10 high-magnification fields (magnification, ×400) from the hot spots in low magnification vision (magnification, ×100), and scored as follows: 0, 0–10% positive staining cells; 1, 10–25% positive staining cells; 2, 25–50% positive staining cells; 3, 50–75% positive staining cells; and 4, 75–100% positive staining cells. Scores of 0–1 were considered to be negative, a score of 2 was weak, a score of 3 was moderate, and a score of 4 was strong.

Molecular analyses

Gastric carcinoma tissues and their matching paracarcinomatous tissues, adjoining normal tissues were obtained fresh and snap-frozen for quantitative real-time reverse-transcriptase PCR (qRT-PCR) using Thermo Scientific PikoReal Real-Time PCR System (Thermo Scientific, USA). The total RNA was extracted using the RNA extraction reagent TRIzol (Life Technologies, USA) according to the manufacturer's protocol. Single-strand cDNA was synthesized using 1 μg total RNA with an oligo(dT) primer by RevertAid™ First-Strand cDNA Synthesis Kit (Thermo Scientific). The experiments were run in triplicate. The sense primer and antisense primer of EphA1, IL-6, and VEGF were designed according to the EphA1 mRNA, IL-6mRNA, VEGFmRNA sequence (GenBank accession number: NM_005232, NM_000600.5, NM_001025366.3) as summarized in Table 1. The relative gene expression levels were calculated using the comparative Ct (ΔΔCt) method, where the relative expression is calculated as $2^{-\Delta\Delta Ct}$, and Ct represents the threshold cycle.

Statistical analysis

SPSS 25.0 (SPSS, Inc., Chicago, IL, USA) software were used for statistical analysis. Correlations between EphA1 and tumor microenvironmental marker (IL-6 and VEGF) were assessed by Spearman's rank correlation test. Patient survival data were analysed using Kaplan-Meier analysis and log-rank test. Then, parameters which were significant in univariate analysis were selected for Cox multivariate analysis to identify their prognostic significance. $P<0.05$ indicates a statistically significant difference.

Results

Protein expression levels of EphA1, IL-6, and VEGF in the intratumoral tissues and their matching paracarcinomatous, and adjoining normal tissues of GC

EphA1 was found to be mainly expressed on the cell membranes and in cytoplasm, and was more abundant in carcinoma tissues compared with paracarcinomatous epithelium (75.4 *vs.* 35.1%; χ^2=18.769; $P<0.001$; Figure 1) and normal mucosal tissues (75.4 *vs.* 21.1%; χ^2=33.761; $P<0.001$; Figure 1). The expression level of EphA1 in paracancerous tissues was higher than that in normal tissues, but the difference was not statistically significant, possibly due to the small sample size. As with EphA1, expressions of IL-6 and VEGF also decreased gradually from carcinoma tissues to paracarcinomatous tissues and to normal mucosal tissues, and were mainly located in cytoplasm (Table 2).

Gene expression levels of EphA1, IL-6, and VEGF in intratumoral tissue and their matching paracarcinomatous and adjoining normal tissues of GC

Quantitative real-time RT-PCR was used to detect the expression of EphA1, IL-6, and VEGF transcript in 57 fresh specimens of gastric carcinoma and their matching paracarcinomatous epithelium and adjoining normal mucosa. We found that EphA1, IL-6, and VEGF were all significantly overexpressed in carcinoma compared to paracancerous tissues

Table 1. Primers Used in qRT-PCR.

Gene	Amplicon size (bp)	Forward primer (5'→3')	Reverse primer (5'→3')
β-actin	96	CCCTGGAGAAGAGCTACGAG	GGAAGGAAGGCTGGAAGAGT
EPHA1	149	TGGCTGAAGCCTTATGTGGA	CTCAGGGTCCCTCGATACAC
VEGF	82	CTTCTGAGTTGCCCAGGAGA	CTGTCATGGGCTGCTTCTTC
IL-6	125	AGACAGCCACTCACCTCTTC	AGTGCCTCTTTGCTGCTTTC

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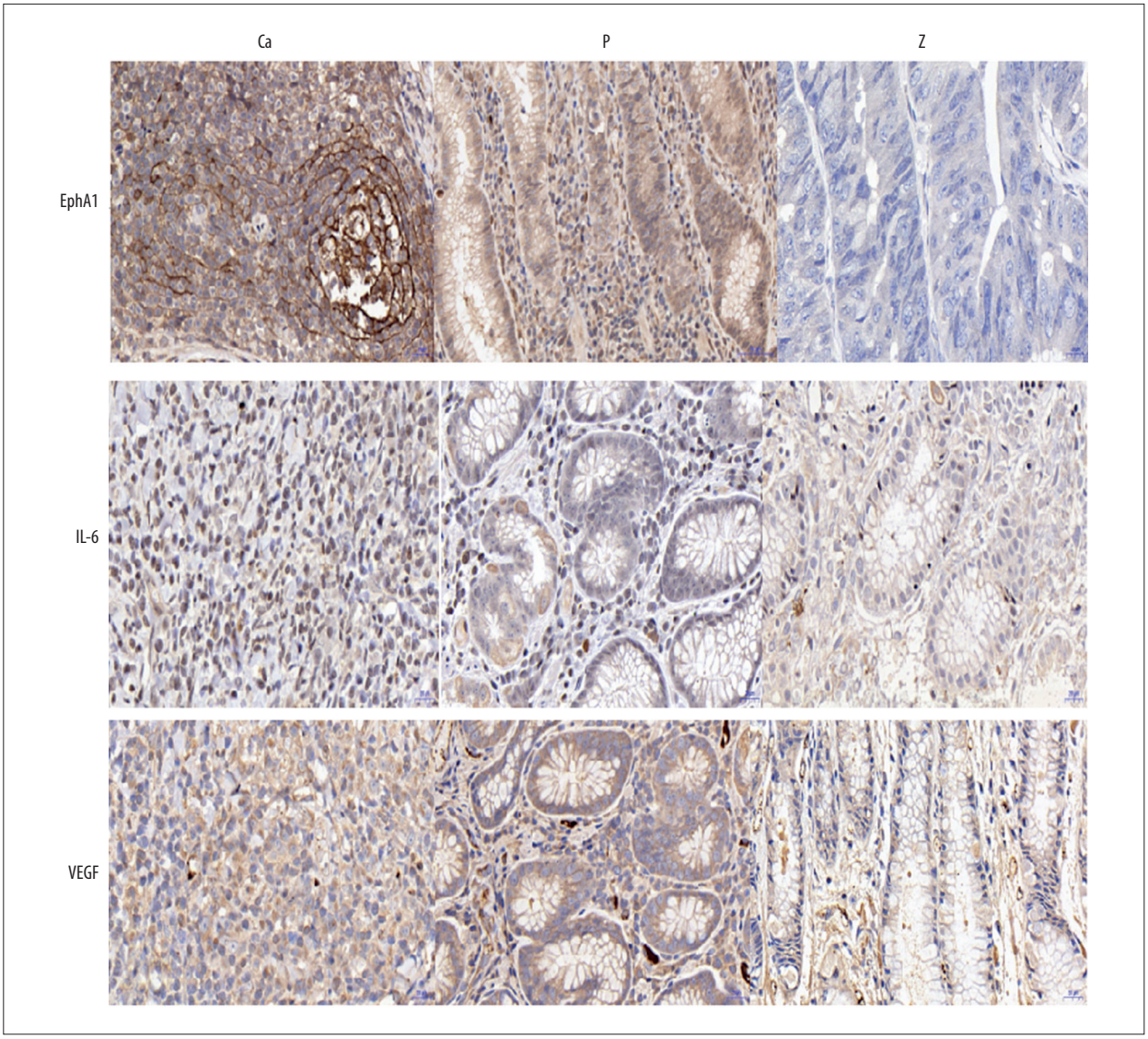

Figure 1. Immunohistochemical staining of EphA1, IL-6, and VEGF in adjacent normal tissues, carcinoma tissues and paracarcinomatous tissues of GC patients (Ca – cancer tissues, P – paracancer tissues, N – normal tissues, Bar=20 um).

Table 2. Positive rate of EphA1, IL-6, and VEGF expression in the normal mucosal tissues, carcinoma tissues and paracarcinomatous tissues of GC patients.

Variable	Tumor tissues	Paracarcinomatous tissues	Normal mucosal tissues	χ^2	*P*-value
	Positive rate (%)	Positive rate (%)	Positive rate (%)		
EphA1	75.44(43/57)	35.08(20/57)	21.05(12/57)	36.90	<0.001
IL-6	77.19(44/57)	42.11(24/57)	29.82(17/57)	27.56	<0.001
VEGF	70.17(40/57)	33.33(19/57)	22.81(13/57)	28.93	<0.001

EphA1 – erythropoietin-producing hepatocellular carcinoma A1; IL-6 – interleukin-6; VEGF – vascular endothelial growth factor.

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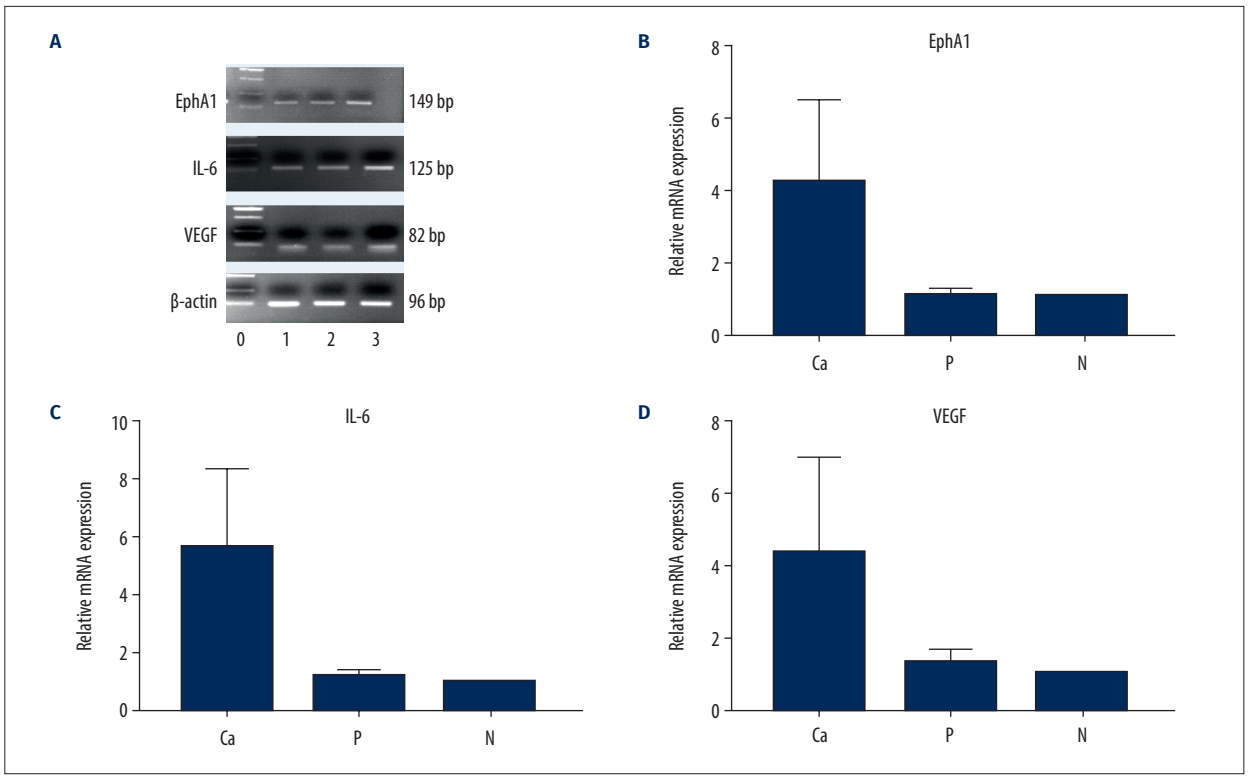

Figure 2. EphA1, IL-6, and VEGF mRNA levels in the intratumoral and their matching paracarcinomatous, adjoining normal tissues of GC patients. (**A**) The level of EphA1, IL-6, and VEGF mRNAs was detected by semi-quantitative RT-PCR. Lane 0, DNA molecular weight marker; lane 1, RNA sample from normal tissues; lane 2, RNA sample from paracancer tissues; lane 3, RNA sample from cancer tissues. (**B–D**): qRT-PCR to detect the relative mRNA expression levels of EphA1, IL-6 and VEGF in gastric cancer and their matching paracarcinomatous, adjoining normal tissues. All experiments were performed in triplicate. The results from 3 pairs of specimens analyzed by ANOVA are expressed as means±SD. * P<0.05 *vs.* cancer tissues. β-actin was used as the control (Ca – cancer tissues, P – paracancer tissues, N – normal tissues).

and adjoining normal tissues (EphA1: F=88.06; *P*<0.001; IL-6: F=152.20, *P*<0.001; VEGF: F=110.285, *P*<0.001; Figure 2). Although the 3 gene were all increasingly expressed in paracarcinomatous tissue compared to normal tissues, its expression was not significantly different in further multiple comparisons, suggesting that their upregulation takes place mainly after tumorigenesis (Figure 2).

Correlations between EphA1 and the tumor microenvironment hallmark proteins in GC

Our study evaluated the correlations between EphA1 and the tumor microenvironment-specific protein biomarkers IL-6 and VEGF in gastric cancer according to the immunohistochemical score. The associations between the expression of EphA1, IL-6, and VEGF are shown in Table 3. The positive expression levels of EphA1, IL-6, and VEGF were detected in 37 of 57 (64.9%) patients' intratumoral tissues, while negative expression was observed in 11 of 57 (19.3%) patients' intratumoral tissues. The positive expression levels of both EphA1, IL-6, and VEGF were detected in 19 of 57 (33.3%) patients' paracancerous tissues, while negative expression was observed in 34 of 57 (59.6%) patients' paracancerous tissues. The positive expression levels of both EphA1, IL-6 and VEGF were detected in 4 of 57 (7%) patients' normal tissues, while negative expression was observed in 31 of 57 (54.4%) patients' normal tissues. These results showed a positive relationship in the expression of EphA1 and IL-6 in carcinoma (r=0.826; *P*<0.001; Table 3) and paracancerous tissues (r=0.510; *P*<0.001; Table 3), and of EphA1 and VEGF (r=0.761, *P*<0.001 in carcinoma; r=0.307, *P*=0.020 in paracancerous tissues; Table 3). The trend of these preliminary findings indicated that EphA1 overexpression was associated with IL-6 overexpression and VEGF enrichment (Figure 3).

Associations between tumor biological characteristics and expression levels of EphA1, IL-6, and VEGF in GC

The associations between expression levels of EphA1, IL-6, and VEGF in GC cancer tissues and clinicopathological features were analyzed by the χ^2 test. The positive rate of EphAl expression was significantly higher in the tissues from

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Table 3. Associations between EphA1 and the tumour microenviroment hallmark proteins in GC patients.

	Tumor tissues				Paracarcinomatous tissues				Normal mucosal tissues			
	IL-6		VEGF		IL-6		VEGF		IL-6		VEGF	
	r-value	*P*-value	r-value	*P*-value	r-value	*P*-value	r-value	*P*-value	r-value	*P*-value	r-value	*P*-value
EphA1	0.826	<0.001	0.761	<0.001	0.510	<0.001	0.307	0.020	-0.008	0.954	0.241	0.071

EphA1 – erythropoietin-producing hepatocellular carcinoma A1; IL-6 – interleukin-6; VEGF – vascular endothelial growth factor.

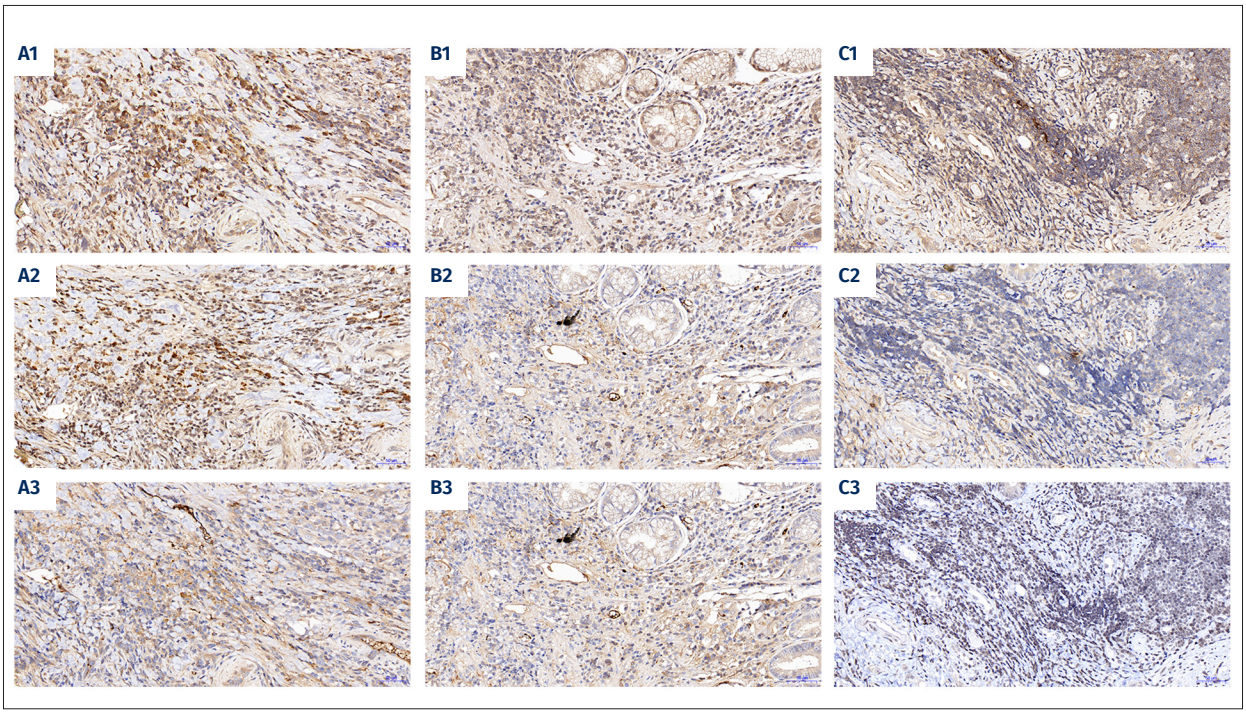

Figure 3. Representative immunohistochemical images of EphA1, IL-6, and VEGF coexpression in GC cancer tissues: (**A**) High expression of EphA1, IL-6, and VEGF in GC tissue; (**B**) Moderately expression of EphA1, IL-6, and VEGF in GC cancer tissue; (**C**) Low expression of EphA1, IL-6, and VEGF in GC tissue. Bar=50um.

patients with a larger tumor size (χ^2=4.734; *P*=0.030), inferior differentiation grade (χ^2=8.126; *P*=0.004), terminal TNM stage (χ^2=5.549; *P*=0.018), and lymphatic metastasis (χ^2=6.440; *P*=0.011). IL-6 expression was higher in tumor tissues with advanced TNM stage (χ^2=9.103; *P*=0.003), poor tumor differentiation (χ^2=10.326; *P*=0.001), larger tumor size (χ^2=14.399; *P*<0.001), and lymphatic metastasis (χ^2=12.567; *P*<0.001). Notably, as summarized in Table 4, the overexpression of VEGF was also positively correlated with all of the aforementioned clinicopathological characteristics in present study, including tumor size (χ^2=7.083; *P*=0.008), TNM stage (χ^2=5.786; *P*=0.016), lymphatic metastasis (χ^2=7.249; *P*=0.007), and tumor differentiation degree(χ^2=4.985; *P*=0.026).

Survival analysis

As shown in Figure 4, we found that the OS of patients with positive EphA1 expression was obviously shorter than that of patients with EphA1 negative expressed (32.23±2.60 months *vs.* 47.14±0.83 months, χ^2=4.924, *P*=0.026) according to the Kaplan-Meier analysis. Similarly, patients with positive IL-6 and VEGF expression tended to have a worse OS (IL-6: 34.97±2.71 *vs.* 44.23±2.66 months; χ^2=3.894; *P*=0.048; VEGF: 34.23±2.92 *vs.* 43.52±2.36 months; χ^2=4.162; *P*=0.041). Compared with the patients with negative expression of EphA1, IL-6, and VEGF, the patients with positive expression of EphA1, IL-6, and VEGF had significantly worse prognosis, which might stratify patients more accurately (OS of all 3 are positive expression: 33.07±3.10 *vs.* OS of all 3 are negative expression: 44.91±1.99 months; χ^2=6.215; *P*=0.013; Figure 4).

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Table 4. Associations between clinicopathological characteristics and protein expression levels of EphA1, IL-6 and VEGF in GC.

Variable	n	EphA1			IL-6			VEGF		
		Positive rate, %	χ^2	*P*-value	Positive rate, %	χ^2	*P*-value	Positive rate, %	χ^2	*P*-value
Tumor length										
<3cm	19	57.8	4.734	0.030	47.3	14.399	<0.001	47.3	7.083	0.008
>3cm	38	84.2			92.1			81.5		
Differentiation degree										
Low	31	90.3	8.126	0.004	93.5	10.326	0.001	87.1	4.985	0.026
Moderate/high	26	57.7			57.6			61.5		
TNM stage										
I/II	37	67.5	5.549	0.018	64.8	9.103	0.003	59.4	5.786	0.016
III/IV	20	95			100			90		
Lymphatic metastasis										
Positive	29	89.6	6.440	0.011	96.5	12.567	<0.001	86.2	7.249	0.007
Negative	28	60.7			57.1			53.5		

EphA1 – erythropoietin-producing hepatocellular carcinoma A1; IL-6 – interleukin-6; VEGF – vascular endothelial growth factor.

Univariate analysis revealed that prognosis was also significantly associated with lymphatic metastasis (*P*=0.030) and TNM stage (*P*=0.034; Table 5). Additionally, as summarized in Table 6, EphA1 positive expression was an independent prognostic factors for the OS of GC patients by multivariate analysis using the Cox regression model. The risk of death for GC patients with high expression of EphA1 was 10.298 times (*P*=0.025) higher than that for patients with low expression of EphA1 (Table 6).

Discussion

In this study, the expression of EphA1 attenuated gradually from carcinoma tissues to paracarcinomatous tissues and then to adjacent normal tissues, regardless of the protein- or gene-level expressions in GC patients. Further analysis showed that higher expression level of EphA1 was associated with stronger tumor proliferation and invasion ability (i.e., the larger the tumor diameter, the lower the differentiation degree, and the worse the TNM stage and the lymph node metastasis). It revealed that the positive rate of EphA1 in gastric mucosa gradually enriched as the tumor grade increased from zero to one, from smallest to largest, and from weak to progressive. These results further confirm previous studies on the promoting effect of EphA1 on gastric cancer, but our research is more complete and systematic [19].

Because of the simultaneous detection and comparison of EphA1 expression change in cancer tissues, para-cancer tissues and normal gastric mucosal tissues in patients with gastric cancer, we basically simulated the development process of gastric cancer from clinical samples, so we can in a clearer and more detailed way understand how the EphA1 gene affects the occurrence and development of gastric cancer.

The tumor microenvironment has long been suspected to play a major role in the pathogenesis of cancer by interactions between the various components of the tumor microenvironment [20]. More and more evidence shows that targeting the tumor microenvironment can be used as a supplement to traditional therapies to improve the therapeutic effect in malignant tumors [21]. IL-6 is regarded as a key player in tumor cell proliferation, survival, and metastatic dissemination through activation of numerous signaling pathways and downstream mediators like signal transducer and activator of transcription 3 (STAT3), and accumulating evidence suggests a link between the chemokine IL-6 and tumor microenvironment [22,23]. Vascular endothelial growth factor (VEGF) is the most notable proangiogenic factor and plays a key role in the generation of new blood vessel networks. Within the tumor microenvironment, activation of the VEGF-VEGFR signaling axis enhances the permeability of blood vessels and promotes the progression of diverse malignancies [24]. Many clinical trials have confirmed

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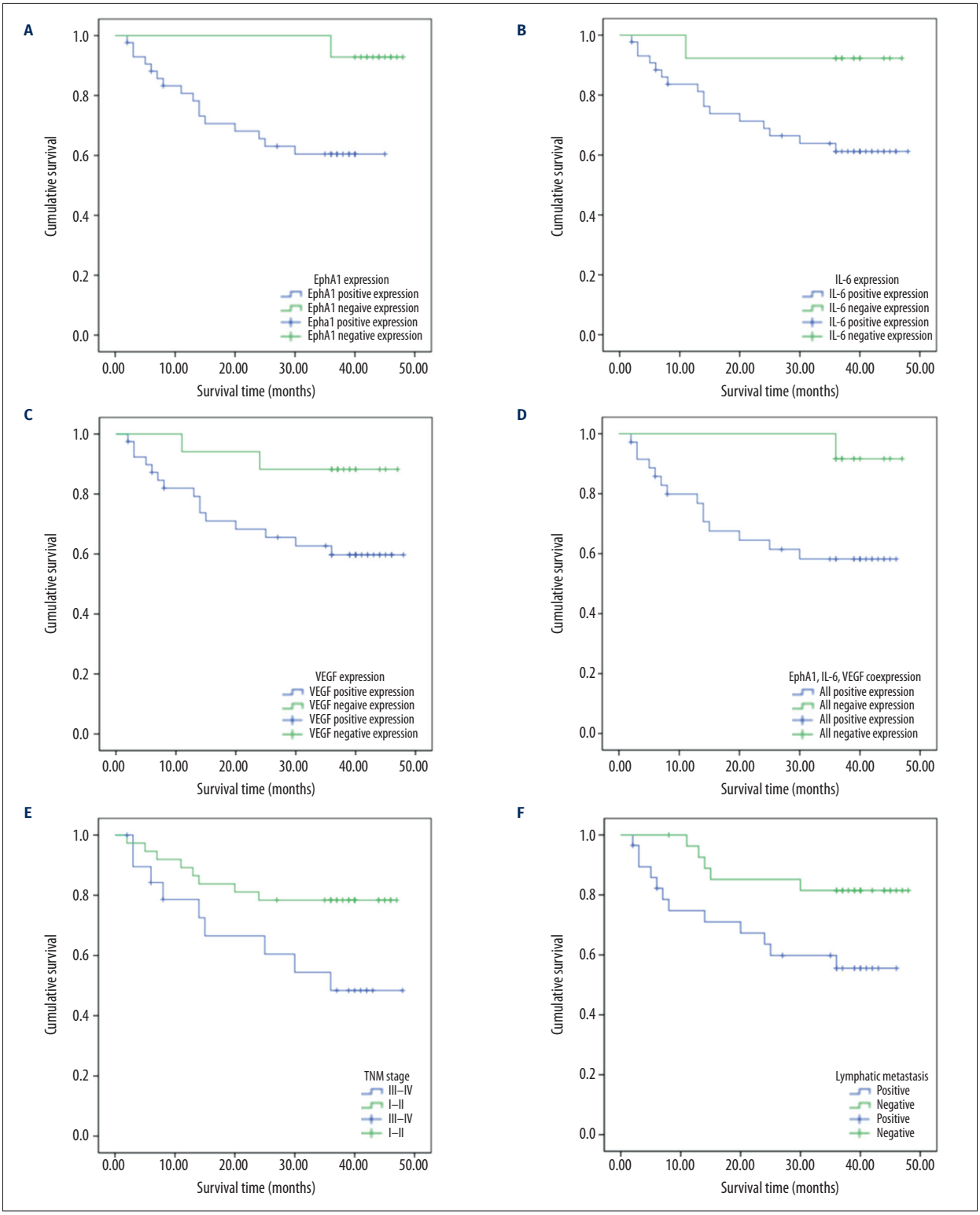

Figure 4. Kaplan-Meier analysis of overall survival (OS) curves of GC patients based on EphA1, IL-6 and VEGF expressions and other significantly meaningful clinicopathological parameters. (**A**) OS curve based on EphA1 expression (positive versus negative); (**B**) OS curve based on IL-6 expression (positive versus negative); (**C**) OS curve based on VEGF expression (positive versus negative); (**D**) OS curve based on EphA1, IL-6 and VEGF coexpressions (all positive versus all negative); (**E**) OS curve based on TNM stages (I–II versus III–IV); (**F**) OS curve based on Lymphatic metastasis (positive versus negative).

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Table 5. Kaplan-Meier survival analysis of EphA1, IL-6 and VEGF expressions and other clinicopathological parameters in GC patients.

Variables	Mean survival time (months)	95% CI	*P* value
EphA1 expression			
Negative	47.143	45.524–48.762	0.026
Pisitive	32.236	27.131–37.340	
IL-6 expression			
Negative	44.231	39.016–49.446	0.048
Pisitive	34.977	29.659–40.295	
VEGF expression			
Negative	43.529	38.889–48.170	0.041
Pisitive	34.239	28.510–39.968	
Coexpression			
All posituve	33.072	25.906–37.757	0.013
All negative	44.913	44.363–47.804	
TNM stage			
I–II	39.432	34.674–44.191	0.034
III–IV	31.552	23.215–39.889	
Differentiation degree			
Low	34.137	27.804–40.470	0.108
Moderate/high	40.381	34.817–45.946	
Lymphatic metastasis			
Positive	31.429	24.761–38.097	0.030
Negative	42.185	37.453–46.918	

Table 6. Cox multivariate analysis of EphA1 and other clinicopathological parameters in GC patients.

Covariates	HR	95% CI for HR	*P* value
EphA1 (negative *vs.* postive)	10.298	1.335–79.467	0.025
VEGF (negative *vs.* positive)	3.437	0.747–15.824	0.113
TNM stage (I–II *vs.* III–IV)	2.671	0.984–7.248	0.054

VEGF inhibitors as important therapeutic agents in multiple solid tumors, including gastric cancer [25,26]. The relationship between Ephs and the tumor microenvironment has been studied in several other types of tumors [27,28]. Increasing evidence indicates that Ephs and ephrins mediate cell-cell interactions in tumor cells and in the tumor microenvironment, especially the tumor stroma and tumor vasculature, to promote tumor development, progression, metastasis, and prognosis by increasing angiogenesis and movement of inflammatory cells in cancer [15,29–31]. Few GC studies have assessed the relationships between EphA1 and the tumor microenvironment, or their combined effect on prognosis. Our study id the first to find a significant positive correlation between EphA1 protein and tumor microenvironmental hallmark proteins VEGF and IL-6 in gastric cancer. We found that their combined effect promoted the occurrence and development of gastric cancer. Further research is needed on which of these 3 indicators influences the others and which is the initiator.

In terms of the mechanism of EphA1 with tumor microenvironment, the available cumulative findings demonstrate that EphA1 promotes tumorigenicity and tumor progression in tumor cells and in the tumor microenvironment by binding to the ligand Ephrin and then generating cell contact-dependent bidirectional signals. The Eph-Ephrin signaling pathway can regulate cancer cell shape, movement, survival, and proliferation [32], and it may also interact with other signaling systems or trigger a network of signaling processes or downstream mediators to affect malignancy [33,34]. This suggests that the mechanisms of the Eph/Ephrin signaling pathways are complex and need to be further investigated. Regardless of the mechanism by which EphA1 is activated, it cannot be ruled out that EphA1 acts on other signaling systems on the cell surface after activation, such as important inflammation-related signaling pathways and angiogenic signaling pathways. Therefore, it is reasonable to speculate that abnormal EphA1 overexpression triggers the Eph-Ephrin signaling pathway and then activates the downstream inflammatory signaling pathway and angiogenesis signaling pathway, forming a malevolent tumor microenvironment to accelerate tumor progression and then affect the prognosis of GC patients.

Further investigations are needed to more precisely define the molecular mechanisms involved in EphA1 and the tumor microenvironment. In future GC research, we plan to further investigate the relationships between EphA1 and the tumor microenvironment hallmark proteins IL-6 and VEGF *in vivo* and *in vitro*.

There are some limitations to our study: it was a retrospective study, and the small sample size may have biased our

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results. These limitations make the present conclusions preliminary and they need further study.

We found that EphA1 can be regarded as an upregulated factor in GC cells, enhancing the tumor microenvironment with increasing IL-6 and VEGF expression, and facilitating the occurrence and progression of tumors, thus leading to a poor prognosis. EphA1 could be a new potential therapeutic target for GC in future, and suppressing the interactions between EphA1 and the tumor microenvironment may provide an effective measure to regulate tumor progression and postoperative recurrence in GC.

Conclusions

This study simulated the process of gastric cancer (from normal mucosal tissues to atypical hyperplasia paracarcinomatous tissues and then to carcinoma tissues) from clinical samples and confirmed that EphA1 can promote the development of gastric cancer. We found for the first time that the mechanism by which EphA1 promotes the occurrence and development of gastric cancer is related to the tumor microenvironment, which suggests that EphA1 could be a new target for gene therapy of gastric cancer, providing new ideas and a theoretical basis for the diagnosis and treatment of gastric cancer.

Conflict of interest

None.

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