Systematic analysis of CXC chemokine–vascular endothelial growth factor A network in colonic adenocarcinoma from the perspective of angiogenesis

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Abstract

Background

Tumor angiogenesis plays a vital role in tumorigenesis, proliferation, and metastasis. Recently, it has been gradually recognized that *VEGFA* and CXC chemokines are important players in angiogenesis. It is interesting to explore the expression level, gene regulatory network, prognostic value, and target prediction of CXC chemokine-VEGFA network in colon adenocarcinoma (COAD) from the perspective of tumor angiogenesis.

Methods

In this study, we analyzed gene expression and regulation, prognostic value, target prediction, and immune infiltrates related to CXC chemokine-VEGFA network in patients with COAD using multiple databases (cBioPortal, UALCAN, Human Protein Atlas, GeneMANIA, GEPIA, TIMER, TRRUST, LinkedOmics, and Metascape).

Results

Our results showed that *CXCL1/2/3/5/6/8/11/16/17* and *VEGFA* were significantly overexpressed, while *CXCL12/13/14* was underexpressed in patients with COAD. Moreover, genetic alterations in the CXC chemokine-VEGFA network found at varying rates in patients with COAD were as follows: *CXCL1/2/17* (2.1%), *CXCL3/16* (2.6%), *CXCL5/14* (2.4%), *CXCL6* (3%), *CXCL8* (0.8%), *CXCL11/13* (1.9%), *CXCL12* (0.6%), and *VEGFA* (1.3%). Promoter methylation of *CXCL1/2/3/11/13/17* was significantly lower in patients with COAD, while methylation of *CXCL5/6/12/14* and *VEGFA* was significantly higher. Furthermore, *CXCL9/10/11* and *VEGFA* expression was significantly correlated with the pathological stages of COAD. In addition, patients with COAD with high *CXCL8/11/14* or low *VEGFA* expression levels survived longer than patients without these characteristics. CXC chemokines and *VEGFA* form a complex regulatory network through co-expression, co-localization, and genetic interactions. Moreover, many transcription factor targets of the CXC chemokine-VEGFA network in patients with COAD were found: RELA, NFKB1, ZFP36, XBP1, HDAC2, SP1, ATF4, EP300, BRCA1, ESR1, HIF1A, EGR1, STAT3, and JUN. We further identified the top three miRNAs involved in regulating each CXC chemokine within the network: miR-518C, miR-369-3P, and miR-448 regulated *CXCL1*; miR-518C, miR-218, and miR-493 regulated *CXCL2*; miR-448, miR-369-3P, and miR-221 regulated *CXCL3*; miR-423 regulated *CXCL13*; miR-378, miR-381, and miR-210 regulated *CXCL14*; miR-369-3P, miR-382, and miR-208 regulated *CXCL17*; miR-486 and miR-199A regulated *VEGFA*. Furthermore, the CXC chemokine-VEGFA network in patients with COAD was significantly associated with immune infiltration.

Conclusions

This study revealed that the CXC chemokine-VEGFA network is a prognostic biomarker for patients with COAD. Moreover, our study provides new therapeutic targets for COAD which will act as a reference for further research in the future.

1. Background

Colon cancer is a common malignant tumor of the digestive tract. The incidence and mortality of colon adenocarcinoma (COAD) is the third highest of all cancer types [1]. Since early diagnosis of COAD remains difficult, its mortality is increasing significantly every year [2]. Approximately half of the patients relapsed or died within 5 years [3]. Therefore, finding new biomarkers and therapeutic targets for early diagnosis is the most important first step in the prevention and treatment of COAD.

Chemokines are a family of small heparin-binding proteins 8–10 kDa in size. Within the chemokine family, there are four subgroups (CXC, CC, CX3C, and C). The CXC subgroup has been shown to play a key role in angiogenesis in both physiological and pathological settings [4]. Recently, the role of CXCL in the regulation of tumor angiogenesis has attracted increasing interest [5]. Different members of the CXC chemokines subgroup can promote or inhibit angiogenesis, thus promoting or inhibiting tumor growth [6]. Multiple factors have been identified as regulators of angiogenesis. However, CXC chemokines are a unique family of cytokines that regulate angiogenesis in many different ways [7]. Vascular endothelial growth factor A (VEGFA) is a vital factor that plays an essential role in tumor angiogenesis and development [8]. Sunitinib has been used for treatment of advanced renal cell carcinoma as a VEGFA inhibitor. However, the side effects of sunitinib can be quite severe, such as kidney damage and cardiovascular damage [9]. CXC chemokines and *VEGFA* are heavily regulated during tumor angiogenesis. It has been shown that *CXCL12* can promote a malignant phenotype by promoting the clonal growth of colorectal cancer cells and regulating the expression of *VEGF* and *ICAM-1* [10].

Multiple online databases were used to explore the expression level, gene regulation network, prognostic value, and regulation targets of the CXC chemokine-VEGFA network in patients with COAD from an angiogenic perspective in this study. In addition, we want to identify the relationship between CXC chemokine and *VEGFA* expression and the development and prognosis of COAD, and to provide new insights into targeted therapies for patients with COAD.

2. Methods

2.1 UALCAN analysis

UALCAN (http://ualcan.path.uab.edu/analysis.html) is a free online database that provides analysis based on The Cancer Genome Atlas (TCGA) and MET500 cohort data [11]. The UALCAN database's "Expression Analysis" module was utilized to examine TCGA gene expression data, and the following screening criteria were applied: (1) gene: CXC chemokines and *VEGFA*, (2) dataset: COAD, and (3)

threshold setting conditions: *P*-value cutoff = 0.05. A Student's *t*-test was used for the comparative analysis [12].

2.2 Human Protein Atlas analysis

The Human Protein Atlas (https://www.proteinatlas.org/), an open access resource, provides analyses for specific human genes and proteins [13]. Screening condition: (1) gene: CXC chemokines and *VEGFA*, (2) choose section: tissue and pathology, (3) choose tissue: colon and COAD, and (4) choose a picture of tissue types: normal colon tissue and COAD.

2.3 GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) is an analysis tool that delivers RNA sequencing expression data from 9,736 cancers and 8,587 non-cancerous samples [14]. Gene (CXC chemokines and *VEGFA*), dataset (COAD), and threshold conditions (*P*-value cutoff = 0.05) were set as screening criteria. The expression of CXC chemokines and *VEGFA*, as well as the pathological stage of COAD, were analyzed using a Student's *t*-test. The prognosis of patients with COAD was analyzed using the Kaplan–Meier curve [12].

2.4 cBioPortal analysis

cBioPortal (http://cbioportal.org) is a free online database for visualizing, studying, and analyzing cancer genomic data [15]. The analysis of genetic alterations in the CXC chemokine-VEGFA network was conducted using cBioPortal in this study. 636 samples of COAD were analyzed. A *z*-score threshold of ± 2.0 was used to calculate mRNA expression z-scores for all samples (log RNA Seq V2 RSEM). CXC chemokines and *VEGFA* were the chosen gene [12].

2.5 STRING analysis

STRING (https://string-db.org/cgi/input.pl) is a free online database that helps researchers to analyses all publicly available sources of protein–protein interaction (PPI) data [16]. We created the PPI network interaction using STRING in this study. The screening criteria were set as follows: (1) confidence: 0.400, and species: *Homo sapiens* [12].

2.6 GeneMANIA analysis

GeneMANIA (http://www.genemania.org) is a free online database that used to create PPI networks, and analyze gene function [17]. The interaction networks were built using this database to explore the roles of CXC chemokines and *VEGFA* [12].

2.7 Metascape analysis

Metascape (https://metascape.org) is a free online gene function analysis tool that assists users in using current common bioinformatics analysis approaches to batch gene and protein analysis in order to predict function [18]. We conducted Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and

Genomes (KEGG) pathway enrichment analysis of the CXC chemokine-VEGFA network in COAD using Metascape [12].

2.8 TRRUST analysis

TRRUST (https://www.grnpedia.org/trrust/) is a free online database for human transcriptional regulatory networks [19]. We sought to discover key factors regulating the expression of the CXC chemokine-VEGFA network in COAD patients using TRRUST. The “Find key regulators for query genesa” module of TRRUST, species (human), and gene (CXC chemokines and *VEGFA*) were chosen in this study [12].

2.9 LinkedOmics analysis

LinkedOmics (http://www.linkedomics.org/) is a free database that provides methods for analyzing and comparing cancer multiomics data [20]. The “LinkInterpreter” module of LinkedOmics was used to derive biological insights into miRNA target enrichment, and transcription factor target enrichment of the CXC chemokine-VEGFA network. A minimum number of three genes (size), cancer type (COAD), a simulation of 500, gene (CXC chemokines and *VEGFA*), and target dataset (RNA-seq) were chosen in this study [12].

2.10 Timer analysis

TIMER (https://cistrome.shinyapps.io/timer/) is a free online platform for systematic analysis of tumor-infiltrating immune cells [21]. The “Gene module” of TIMER was used to assess the correlation between the expression level of CXC chemokine-VEGFA network and tumor-infiltrating immune cells [12].

3. Results

3.1 Aberrant expression of CXC chemokine-VEGFA network

The expression levels of the CXC chemokine-VEGFA network in patients with COAD to those without COAD were analyzed. We found that the transcriptional levels of *CXCL1/2/3/5/6/8/11/16/17* and *VEGFA* were remarkably upregulated in sex, pathological stage, and sample type ($P < 0.05$) (Fig. 1A1–G3, K1–M3). *CXCL12/13/14* expression level in patients with COAD was downregulated in sex, pathological stage, and sample type ($P < 0.05$) (Fig. 1H1–J3). In addition, immunohistochemical results validated the differential expression of CXC chemokine-VEGFA network between patient with COAD and those without COAD (Supplementary Fig. 1). The pathological stage of COAD and the differential expression of the CXC chemokine-VEGFA network were assessed in this study. The pathological stage in patients with COAD and the expression of *CXCL9/10/11* and *VEGFA* was found a significant correlation ($P < 0.05$) (Fig. 2). Next, the prognostic ability of the CXC chemokine-VEGFA network expression in COAD patients was evaluated. The overall survival was longer in COAD patients when levels of *CXCL8/11/14* expression were higher ($P \leq 0.05$) (Fig. 3A, 3B, and 3C) or when levels of VEGFA expression were lower ($P < 0.05$) (Fig. 3D).

3.2 Promoter methylation and genetic alteration analyses of CXC chemokine-VEGFA network

TCGA were utilized to analyze the genetic alterations of the CXC chemokine-VEGFA network in patients with COAD. As a result, the expression of *VEGFA* was altered by 1.3% in COAD patients (Supplementary Fig. 2). COAD patients had higher promoter methylation level of *VEGFA* than that in individuals without COAD (Supplementary Fig. 3). However, differences in chemokine expression levels in patients with COAD: *CXC1/2/17* (2.1%), *CXCL3/16* (2.6%), *CXCL5/14* (2.4%), *CXCL6* (3%), *CXCL8* (0.8%), *CXCL11/13* (1.9%), and *CXCL12* (0.6%) were found (Supplementary Fig. 2). Similarly, the promoter methylation level of *VEGFA* and *CXCL5/6/12/14* was higher in COAD patients than that in healthy people (Supplementary Fig. 3). Conversely, healthy people had higher promoter methylation level of *CXCL1/2/3/11/13/17* expression than in with COAD patients (Supplementary Fig. 3).

3.3 CXC chemokines and *VEGFA* interaction network

The potential interactions between CXC chemokines and *VEGFA* in patients with COAD were explored. 13 nodes and 68 edges were obtained in PPI network using STRING software. (Supplementary Fig. 4A). Moreover, cell chemotaxis, chemokine and cytokine receptor binding, chemokine and cytokine activity, leukocyte chemotaxis, and migration were the major functions of the CXC chemokine-VEGFA network in COAD patients (Supplementary Fig. 4B). In brief, CXC chemokines are connected to and interact with *VEGFA* in a complex network.

3.4 GO and KEGG pathway enrichment analysis

Metascape were utilized to analyze the functions of the CXC chemokine-VEGFA network in patients with COAD. We found that the biological processes connected with CXC chemokines and *VEGFA* were mainly related to leukocyte chemotaxis, myeloid leukocyte migration, positive regulation of leukocyte chemotaxis, lymphocyte migration, and regulation of multi-organism processes (Supplementary Fig. 5A). Moreover, chemokine activity, cytokine activity, heparin binding, and growth factor activity were the main molecular functions of CXC chemokine-VEGFA network expression (Supplementary Fig. 5B). The KEGG pathway of the CXC chemokine-VEGFA network in COAD was mainly involved in cytokine-cytokine receptor interaction, rheumatoid arthritis, interleukin (IL)-17 signaling pathway, and nuclear factor kappa B (NF-κB) signaling pathway (Supplementary Fig. 5C).

3.5 Transcription factor targets involved with the CXC chemokine-VEGFA network

Potential transcription factors involved with the CXC chemokine-VEGFA network in COAD patients were identified (Table 1). v-rel reticuloendotheliosis viral oncogene homolog A (RELA) and nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1) were the key transcription factors involved with *CXCL1/2/5/8/12* and *VEGFA* in COAD patients ($P < 0.001$). In addition, *CXCL8* and *VEGFA* were found to be regulated by ZFP36 ring finger protein (ZFP36), X-box binding protein 1 (XBP1), histone

deacetylase 2 (HDAC2), activating transcription factor 4 (ATF4), E1A binding protein p300 (EP300), early growth response 1 (EGR1), signal transducer and activator of transcription 3 (STAT3), and Jun proto-oncogene (JUN) ($P < 0.01$). Furthermore, *CXCL1/5/14* and *VEGFA* were found to be regulated by Sp1 transcription factor (SP1) ($P < 0.001$). Breast cancer 1 (BRCA1) is the key transcription factor involved with *CXCL1* and *VEGFA* in COAD patients ($P < 0.01$). Finally, estrogen receptor 1 (ESR1) and hypoxia inducible factor 1 alpha subunit (HIF1A) regulated the functions of *CXCL12* and *VEGFA* ($P < 0.01$).

Table 1
Key regulated factor of CXCL and *VEGFA* in COAD (TRRUST).

Key TF	Description	Regulated gene	*P*-value	FDR
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	CXCL1, CXCL2, CXCL5,CXCL8, CXCL12,VEGFA	2.44e-08	1.78e-07
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	CXCL1, CXCL2, CXCL5,CXCL8, CXCL12,VEGFA	2.54e-08	11.78e-07
ZFP36	ZFP36 ring finger protein	CXCL8,VEGFA	1.22e-05	5.71e-05
XBP1	X-box binding protein 1	CXCL8,VEGFA	7.44e-05	0.000261
HDAC2	histone deacetylase 2	CXCL8,VEGFA	0.000152	0.000426
SP1	Sp1 transcription factor	CXCL1,CXCL5, CXCL14,VEGFA	0.000231	0.000515
ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)	CXCL8,VEGFA	0.000257	0.000515
EP300	E1A binding protein p300	CXCL8,VEGFA	0.000661	0.00106
BRCA1	breast cancer 1, early onset	CXCL1,VEGFA	0.000685	0.00106
ESR1	estrogen receptor 1	CXCL12,VEGFA	0.00121	0.0017
HIF1A	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	CXCL12,VEGFA	0.00144	0.00184
EGR1	early growth response 1	CXCL8,VEGFA	0.00162	0.00189
STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	CXCL8,VEGFA	0.00415	0.00447
JUN	jun proto-oncogene	CXCL8,VEGFA	0.00456	0.00456

3.6 miRNA targets of CXC chemokine-VEGFA network

The top three miRNA targets of the CXC chemokine-VEGFA network were obtained (Table 2). The miRNA targets of *CXCL1* were miR-518C, miR-369-3P, and miR-44. In addition, miR-518C, miR-218, and miR-493 were identified as potential miRNA targets that regulate *CXCL2*. Furthermore, we found that *CXCL3* was regulated by miR-448, miR-369-3P, and miR-221. An miRNA target of *CXCL13* is miR-423. Moreover, miR-378, miR-381, and miR-210 were identified as potential miRNA targets that regulate *CXCL14*. *CXCL17* is regulated by miR-369-3P, miR-382, and miR-208. Furthermore, our results showed that miR-486 and miR-199A are potential miRNA targets that regulate *VEGFA*.

Table 2
The miRNA target of CXCL and *VEGFA* in COAD (LinkedOmics).

Cancer type	Gene	Gene set	Leading Edge Number	*P*-value	FDR
COAD	CXCL1	TCCAGAG,MIR-518C	47	0	0.013584
		GTATTAT,MIR-369-3P	86	0	0.018113
		ATATGCA,MIR-448	64	0	0.024150
	CXCL2	TCCAGAG.MIR-518C	51	0	0
		AAGCACA,MIR-218	143	0	0
		ATGTACA,MIR-493	130	0	0
	CXCL3	ATATGCA,MIR-448	92	0	0
		GTATTAT,MIR-369-3P	100	0	0
		ATGTAGC, MIR-221, MIR-222	49	0	0.00052445
	CXCL13	ACCGAGC, MIR-423	3	0.015625	0.028853
	CXCL14	GTCAGGA,MIR-378	18	0.0051151	0.02937
		CTTGTAT,MIR-381	55	0	0.031719
		ACGCACA,MIR-210	3	0.0031348	0.037446
	CXCL17	GTATTAT,MIR-369-3P	97	0	0.0039402
		ACAACTT,MIR-382	18	0	0.023641
		CGTCTTA,MIR-208	5	0	0.029552
	VEGFA	GTACAGG,MIR-486	16	0.0030120	0.053189
		CTACTGT,MIR-199A	52	0	0.054125

3.7 Correlation of CXC chemokine-VEGFA network expression and differentially expressed genes

mRNA sequencing data of 379 patients with COAD were obtained from the TCGA database of LinkedOmics. 19,828 genes were closely related to *CXCL1/2/3/5/6/8/11/12/13/14/16/17* and *VEGFA* (Supplementary Fig. 6). Among them, we found that 11,701 and 8,127 genes were negatively and positively correlated with *CXCL1* expression, respectively (Supplementary Fig. 6A1). Moreover, 50 genes had a notable positive or negative correlation with *CXCL1* expression in COAD patients ($P < 0.05$) (Supplementary Fig. 6A2 and 6A3). *CXCL1* expression was strongly positively associated with expression of *CXCL3* (Pearson correlation coefficient (PCO) = 0.8921, $P = 4.226e{-}132$) (Supplementary Fig. 7A1), *CXCL2* (PCO = 0.8121, $P = 3.304e{-}90$) (Supplementary Fig. 7A2), and *ZC3H12A* (Pearson correlation = 0.6531, $P = 1.882e{-}47$) (Supplementary Fig. 7A3).

Furthermore, we found that 11,137 and 8,691 genes were negatively and positively correlated with *CXCL2* expression, respectively (Supplementary Fig. 6B1). 50 genes had a marked positive or negative correlation with *CXCL2* expression in COAD patients (Supplementary Fig. 6B2 and 6B3). Moreover, the expression of *CXCL2* was positively associated with the expression of *CXCL3* (PCO = 0.8728, $P = 1.601e{-}119$) (Supplementary Fig. 7B1), *CXCL1* (Pearson correlation = 0.8121, $P = 3.304e{-}90$) (Supplementary Fig. 7B2), and *ZC3H12A* (PCO = 0.6447, $P = 6.735e{-}46$) (Supplementary Fig. 7B3). Furthermore, 12,096 and 7,732 genes were negatively and positively correlated with *CXCL3* expression, respectively (Supplementary Fig. 6C1). 50 genes had a notable positive or negative correlation with *CXCL3* expression in COAD patients (Supplementary Fig. 6C2 and 6C3). Expression of *CXCL3* was positively associated with the expression of *CXCL1* (PCO = 0.8921, $P = 4.226e{-}132$) (Supplementary Fig. 7C1), *CXCL2* (PCO = 0.8728, $P = 1.601e{-}119$) (Supplementary Fig. 7C2), and *ZC3H12A* (PCO = 0.6707, $P = 7.446e{-}51$) (Supplementary Fig. 7C3). Our results showed that 8,680 and 11,148 genes were negatively and positively correlated with *CXCL5* expression, respectively (Supplementary Fig. 6D1). 50 genes had a notable positive or negative correlation with *CXCL5* expression in COAD patients (Supplementary Fig. 6D2 and 6D3). *CXCL5* expression was positively associated with the expression of *IL24* (PCO = 0.7438, $P = 5.884e{-}68$) (Supplementary Fig. 7D1), *IL8* (PCO = 0.7269, $P = 1.632e{-}63$) (Supplementary Fig. 7D2), and *MMP3* (PCO = 0.7213, $P = 4.269e{-}62$) (Supplementary Fig. 7D3). Our results showed that 8,605 and 11,223 genes were negatively and positively correlated with *CXCL6* expression, respectively (Supplementary Fig. 6E1). 50 genes had a marked positive or negative correlation with *CXCL6* expression in COAD patients (Supplementary Fig. 6E2 and 6E3). *CXCL6* expression was positively associated with the expression of *CXCL5* (PCO = 0.7105, $P = 1.689e{-}59$) (Supplementary Fig. 7E1), *MMP3* (PCO = 0.6904, $P = 5.921e{-}55$) (Supplementary Fig. 7E2), and *IL8* (PCO = 0.6833, $P = 1.935e{-}53$) (Supplementary Fig. 7E3). In addition, 9,079 and 10,749 genes were negatively and positively correlated with *CXCL8* expression, respectively (Supplementary Fig. 6F1). 50 genes had a significant positive or negative correlation with *CXCL8* expression in COAD patients (Supplementary Fig. 6F2 and 6F3). *CXCL8* expression was positively associated with *GPR109B* (PCO = 0.7712, $P = 5.939e{-}76$) (Supplementary Fig. 7F1), *IL1B* (PCO = 0.7623, $P = 3.25e{-}73$) (Supplementary Fig. 7F2), and *OSM* (PCO = 0.7593, $P = 2.368e{-}72$) (Supplementary Fig. 7F3). Furthermore, 9,517 and 10,311 genes were negatively and positively correlated with *CXCL11* expression, respectively (Supplementary Fig. 6G1). 50 genes had a significant positive or negative correlation with *CXCL11* expression in COAD patients (Supplementary

Fig. 6G2 and 6G3). *CXCL11* expression was positively associated with *CXCL10* (PCO = 0.8389, *P* = 1.299e−101) (Supplementary Fig. 7G1), *UBD* (PCO = 0.7214, *P* = 3.935e−62) (Supplementary Fig. 7G2), and *IDO1* (PCO = 0.7116, *P* = 9.137e−60) (Supplementary Fig. 7G3). Moreover, 8,017 and 11,811 genes were negatively and positively correlated with *CXCL12* expression, respectively (Supplementary Fig. 6H1). 50 genes had a significant positive or negative correlation with *CXCL12* expression in COAD patients (Supplementary Fig. 6H2 and 6H3). *CXCL12* expression was positively associated with *NPR1* (PCO = 0.804, *P* = 3.835e−87) (Supplementary Fig. 7H1), *SLIT3* (PCO = 0.8013, *P* = 3.915e−86) (Supplementary Fig. 7H2), and *SHE* (PCO = 0.7966, *P* = 1.928e−84) (Supplementary Fig. 7H3). Our results showed that 8,779 and 11,049 genes were negatively and positively correlated with *CXCL13* expression, respectively (Supplementary Fig. 6I1). 50 genes had a significant positive or negative correlation with *CXCL13* expression in COAD patients (Supplementary Fig. 6I2 and 6I3). *CXCL13* expression was positively associated with expression of *TIGIT* (PCO = 0.8089, *P* = 5.598e−89) (Supplementary Fig. 7I1), *SH2D1A* (PCO = 0.7857, *P* = 1.229e−80) (Supplementary Fig. 7I2), and *SIRPG* (PCO = 0.7854, *P* = 1.508e−80) (Supplementary Fig. 7I3). In addition, 8,724 and 11,104 genes were negatively and positively correlated with *CXCL14* expression, respectively (Supplementary Fig. 6J1). 50 genes had a significant positive or negative correlation with *CXCL14* expression in COAD patients (Supplementary Fig. 6J2 and 6J3). *CXCL14* expression was positively associated with the expression of *D4S234E* (PCO = 0.7057, *P* = 2.24e−58) (Supplementary Fig. 7J1), *TNFSF11* (PCO = 0.6172, *P* = 3.643e−41) (Supplementary Fig. 7J2), and *COL9A1* (PCO = 0.6154, *P* = 7.338e−41) (Supplementary Fig. 7J3). Furthermore, 9,737 and 10,091 genes were negatively and positively correlated with *CXCL16* expression, respectively (Supplementary Fig. 6K1). 50 genes had a significant positive or negative correlation with *CXCL16* expression in COAD patients (Supplementary Fig. 6K2 and 6K3). *CXCL16* expression was positively associated with the expression of *ZMYND15* (PCO = 0.7944, *P* = 1.175e−83) (Supplementary Fig. 7K1), *FLII* (PCO = 0.6123, *P* = 2.248e−40) (Supplementary Fig. 7K2), and *NDEL1* (PCO = 0.6072, *P* = 1.486e−39) (Supplementary Fig. 7K3). We also found that 10,483 and 9,345 genes were negatively and positively correlated with *CXCL17* expression, respectively (Supplementary Fig. 6L1). 50 genes had a significant positive or negative correlation with *CXCL17* expression in COAD patients (Supplementary Fig. 6L2 and 6L3). *CXCL17* expression was positively associated with the expression of *FAM83A* (PCO = 0.4586, *P* = 4.148e−21) (Supplementary Fig. 7L1), *GPR110* (PCO = 0.435, *P* = 6.333e−19) (Supplementary Fig. 7L2), and *SEMG1* (PCO = 0.402, *P* = 3.753e−16) (Supplementary Fig. 7L3). Finally, we found that 10,446 and 9,382 genes were negatively and positively correlated with *VEGFA* expression, respectively (Supplementary Fig. 6M1). 50 genes had a significant positive or negative correlation with *VEGFA* expression in COAD patients (Supplementary Fig. 6M2 and 6M3). *VEGFA* expression was positively associated with the expression of *GTPBP2* (PCO = 0.5773, *P* = 4.639e−35) (Supplementary Fig. 7M1), *CCNL1* (PCO = 0.5422, *P* = 2.411e−30) (Supplementary Fig. 7M2), and *CREBZF* (PCO = 0.516, *P* = 3.606e−27) (Supplementary Fig. 7M3).

3.8 Immune cell infiltration and CXC chemokine-VEGFA network expression

CXCL1 expression in COAD patients was positively associated with CD8 + T cells infiltration, neutrophils, and dendritic cells ($P < 0.05$) (Supplementary Fig. 8A). However, macrophages were negatively associated

with *CXCL1* expression ($P < 0.01$) (Supplementary Fig. 8A). In addition, neutrophil infiltration was positively associated with the expression of *CXCL2* and *CXCL3* ($P < 0.001$) (Supplementary Fig. 8B and 8C). However, macrophages were negatively associated with *CXCL2* and *CXCL3* expression ($P < 0.001$) (Supplementary Fig. 8B and 8C). Furthermore, expression levels of *CXCL5/6/8* in patients with COAD were positively associated with the infiltration of CD8 + T cells, macrophages, neutrophils, and dendritic cells ($P < 0.01$) (Supplementary Fig. 8D, 8E, and 8F). B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells were positively associated with *CXCL11/12/13/16* expression ($P < 0.01$) (Supplementary Fig. 8G, 8H, 8I, and 8K). The expression level of *CXCL14* in patients with COAD was positively associated with the infiltration of CD8 + T cells, CD4 + T cells, neutrophils, and dendritic cells ($P < 0.05$) (Supplementary Fig. 8J). B cells were positively associated with *CXCL17* expression ($P < 0.001$) (Supplementary Fig. 8L). CD4 + T cells were positively associated with *VEGFA* expression ($P < 0.01$) (Supplementary Fig. 8M).

4. Discussion

Tumor angiogenesis plays a vital role in tumorigenesis, proliferation, and metastasis. In recent years, studies have identified *VEGFA* and CXC chemokines as important participants in angiogenesis, particularly tumor angiogenesis [12, 22, 23]. The expression levels of CXC chemokines and *VEGFA* have been studied in a range of tumor types; however, findings are contradictory with regard to colonic adenocarcinomas [24, 25]. This study investigated expression level, gene regulatory network, prognostic value, and target prediction of the CXC chemokine-VEGFA network for COAD from the perspective of tumor angiogenesis.

In this study, we also examined whether there was correlation between pathological stage and differential expression of COAD. The expression of *CXCL1/2/3/5/6/8/11/16/17* and *VEGFA* was upregulated in patients with COAD compared to individuals without COAD. Patients with COAD also showed downregulated *CXCL12/13/14* expression. The results were same with those in a previous study in patients with COAD [25], and contradict previous findings in patients with colorectal cancer [24]. This may be due to the small sample size and the many different types of colorectal cancer. We further attempted to explain the different expression levels by investigating promoter methylation and gene alteration in patients with COAD, as these are the factors that affect tumor cell proliferation, angiogenesis, and metastasis. We found that patients with COAD had different rates of genetic alteration in their genes. Moreover, the promoter methylation levels of *CXCL5/6/12/14* and *VEGFA* were higher in patients with COAD than those in normal people. Conversely, the promoter methylation levels of *CXCL1/2/3/11/13/17* were lower in patients with COAD. So, we hypothesized that genetic methylation and alteration within the CXC chemokine-VEGFA network may be the leading cause of abnormal gene expression levels in patients with COAD.

We also found a notable correlation between the *CXCL9/10/11* and *VEGFA* expression, and the pathological stage of COAD. Furthermore, the survival time of patients with COAD was longer with low *VEGFA* expression levels or high *CXCL8/11/14* expression levels. Therefore, the expression levels of

CXCL8/11/14 and *VEGFA* may be potential prognostic indicators for COAD patients. *CXCL8/11/14* and VEGFA promote tumor angiogenesis in different ways [26–28]. Thus, they may affect the prognosis of patients with COAD through multiple biological functions.

The potential functions and interactions of the CXC chemokine-VEGFA network were explored in this study. They were found to be complex and tightly connected. Genes in the network were mainly involved in cytokine receptor binding, chemokine and cytokine activity, leukocyte chemotaxis, and migration. All were closely related to angiogenesis. For example, IL-8 (*CXCL8*) promotes tumor angiogenesis by binding to CXCR1 and CXCR2 receptors [29]. In addition, increasing the anti-tumor activity of cytokine-induced killer cells could reduce tumor proliferation and angiogenesis [30]. Taken together, these results suggest that the CXC chemokine-VEGFA network may influence the development of COAD by increasing tumor angiogenesis.

Furthermore, the functions of the CXC chemokine-VEGFA network in patients with COAD were mainly related to chemokine activity, cytokine activity, and growth factor activity with GO enrichment analysis, all functions closely related to tumor angiogenesis. More studies are needed to confirm the mechanism by which this happens. In this study, we further found through KEGG pathway analysis that the cytokine–cytokine receptor interaction signaling pathway, IL-17 signaling pathway, and NF-κB signaling pathway were highly involved in the CXC chemokine-VEGFA network in COAD patients. All of them are highly related to tumor angiogenesis [31, 32]. Therefore, regulation of them may be a potential treatment strategy for patients with COAD.

Mutated or altered transcription factors represent a unique class of drug targets that mediate aberrant gene expression, and the development of these drugs may impact future cancer treatments. Thus, the targets and regulators of the CXC chemokine-VEGFA network in COAD patients were further analyzed. The transcription factor targets of the CXC chemokine-VEGFA network in patients with COAD were identify. RELA, NFKB1, ZFP36, XBP1, HDAC2, SP1, ATF4, EP300, BRCA1, ESR1, HIF1A, EGR1, STAT3, and JUN are crucial regulatory factors. Our results showed that these factors have potential functions in regulating tumor angiogenesis by targeting *VEGFA*. Studies have shown that RELA, NFKB1, HDAC2, SP1, ATF4, EP300, BRCA1, ESR1, HIF1A, EGR1, STAT3, and JUN regulate tumor angiogenesis, thus affecting tumor growth and prognosis [33–44]. However, the role of ZFP36 and XBP1 in tumor angiogenesis has not yet been reported. miRNAs also play a crucial role in regulating gene expression. miRNAs suppress target genes expression by targeting at their 3'-untranslated regions. miRNA target discovery may ultimately help elucidate the underlying mechanisms of tumorigenesis. Thus, CXC chemokine-VEGFA network-associated miRNA targets in patients with COAD were further explored. Most of them (miR-218, miR-493, miR-221, miR-222, miR-423, miR-378, miR-381, miR-210, miR-382, and miR-199A) have been shown to regulate tumor angiogenesis [45–48]. In short, our study provides potential therapeutic strategies for the treatment of COAD through the prediction of regulated factors and miRNA targets.

The correlation between CXC chemokine-VEGFA network expression and differentially expressed genes in COAD patients was explored in this study. We found that in patients with COAD, close to 20,000 genes

were negatively or positively correlated with CXC chemokine-VEGFA network expression. From these, we screened for genes with the highest correlation with CXC chemokines and *VEGFA*. Some of the genes with the highest correlation (*ZC3H12A*, IL24, *MMP3*, *IL1B*, *OSM*, *IDO1*, *NPR1*, and *TIGIT*) were positively associated with tumor angiogenesis [49, 50]. Regulation of these cancer-related genes may offer an alternative therapeutic strategy for the treatment of patients with COAD. Immune infiltration is highly related to the clinical prognosis of tumors. Immune cells reach the tumor site through vascular transport, and vascularization of tumors is a process mediated by angiogenesis. We found that CXC chemokine-VEGFA network expression, which regulates angiogenesis, is correlated with the infiltration of immune cell. This infiltration involved CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells. Improving immune cell infiltration in COAD by developing drugs that act on the CXC chemokine-VEGFA network or CXC chemokines and *VEGFA*-related regulatory targets is a viable therapeutic oncology strategy.

5. Conclusion

In this study, we determined the expression level and gene regulatory network of the CXC chemokine-VEGFA network, which plays an important role in angiogenesis in COAD. We also identified new prognostic biomarkers and therapeutic targets. These findings will provide insight for the study and treatment of COAD.

Abbreviations

COAD
Colon adenocarcinoma
VEGFA
Vascular endothelial growth factor A
GEPIA
Gene expression profiling analysis
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
TCGA
The Cancer Genome Atlas
RELA
v-rel reticuloendotheliosis viral oncogene homolog A
NFKB1
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
ZFP36
ZFP36 ring finger protein

XBP1
X-box binding protein 1
HDAC2
Histone deacetylase 2
ATF4
Activating transcription factor 4
EP300
E1A binding protein p300
EGR1
Early growth response 1
STAT3
Signal transducer and activator of transcription 3
JUN
Jun proto-oncogene
SP1
Sp1 transcription factor
BRCA1
Breast cancer 1
ESR1
Estrogen receptor 1
HIF1A
Hypoxia inducible factor 1 alpha subunit

Declarations

Ethics approval and consent to participate

Not applicable. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and analyzed during the current study are available in the UALCAN (http://ualcan.path.uab.edu/analysis.html), The Human Protein Atlas (https://www.proteinatlas.org/), GEPIA (http://gepia.cancer-pku.cn/index.html), cBioPortal (http://cbioportal.org), STRING (https://string-db.org/cgi/input.pl), GeneMANIA (http://www.genemania.org), and Metascape (https://metascape.org).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZS and JC performed data analysis work and aided in writing the manuscript. YLST designed the study and assisted in writing the manuscript. QYX, LD, XYL, YSC, and HL edited the manuscript. All authors read and approved the final manuscript.

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Figures

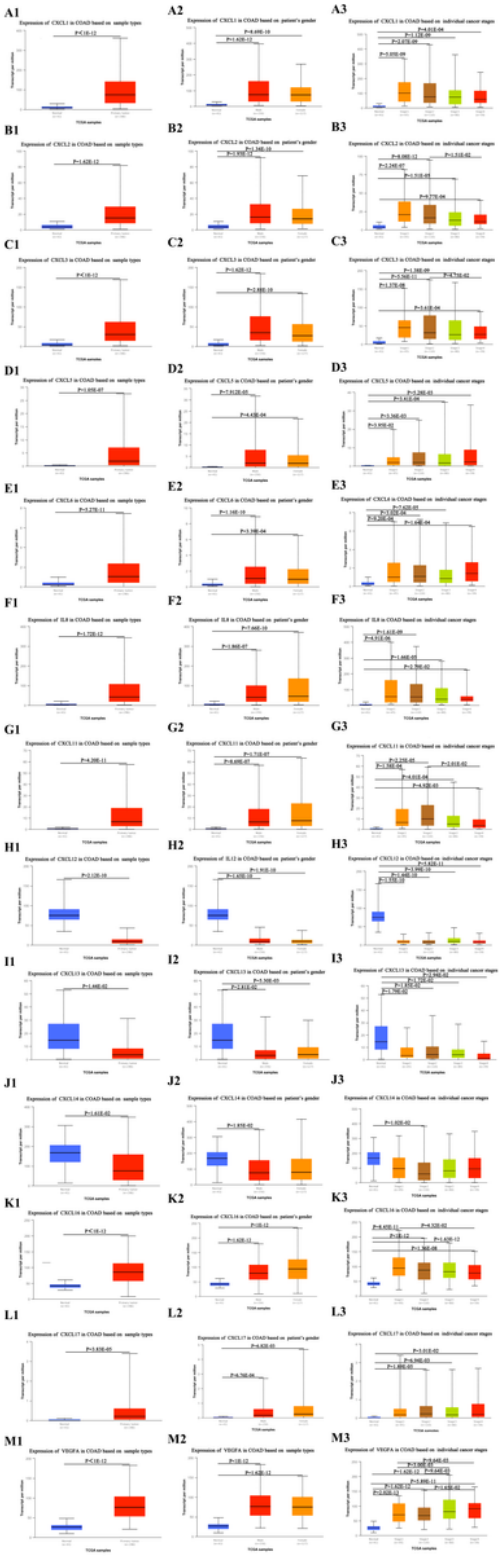

Figure 1

The transcription of CXC chemokines-*VEGFA* network in COAD (UALCAN). (A1-M1) the transcription expression of *CXCL1/2/3/5/6/8/11/12/13/14/16/17* and *VEGFA* in COAD based on sample types. (A2-M2) the transcription expression of *CXCL1/2/3/5/6/8/11/12/13/14/16/17* and *VEGFA* in COAD based on patient's gender. (A3-M3) the transcription expression of *CXCL1/2/3/5/6/8/11/12/13/14/16/17* and *VEGFA* in COAD based on individual cancer stages.

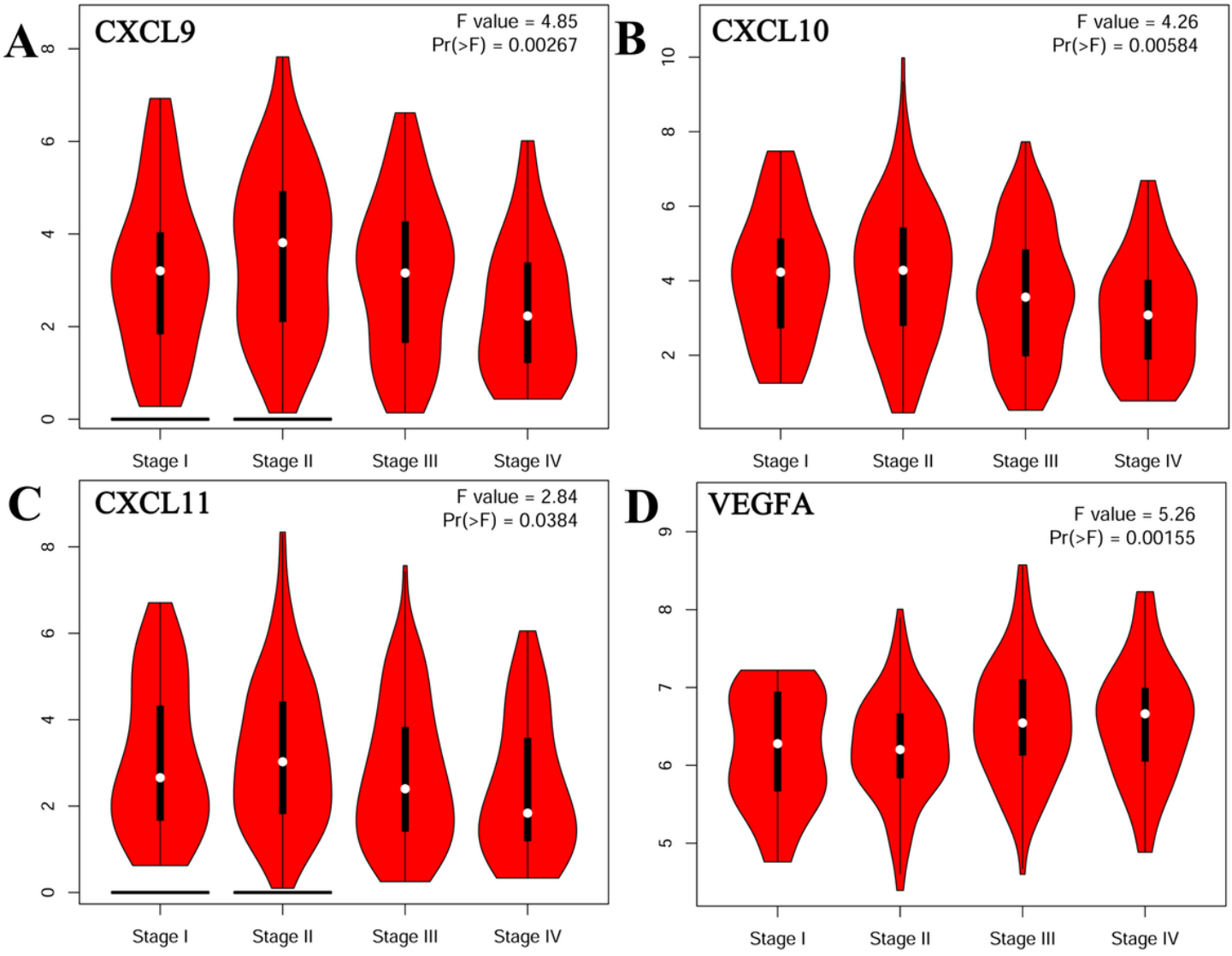

Figure 2

Correlation between the pathological stage and different expressed CXC chemokines-*VEGFA* network of COAD patients (GEPIA). (A) *CXCL9*; (B) *CXCL10*; (C) *CXCL11*; (D) *VEGFA*.

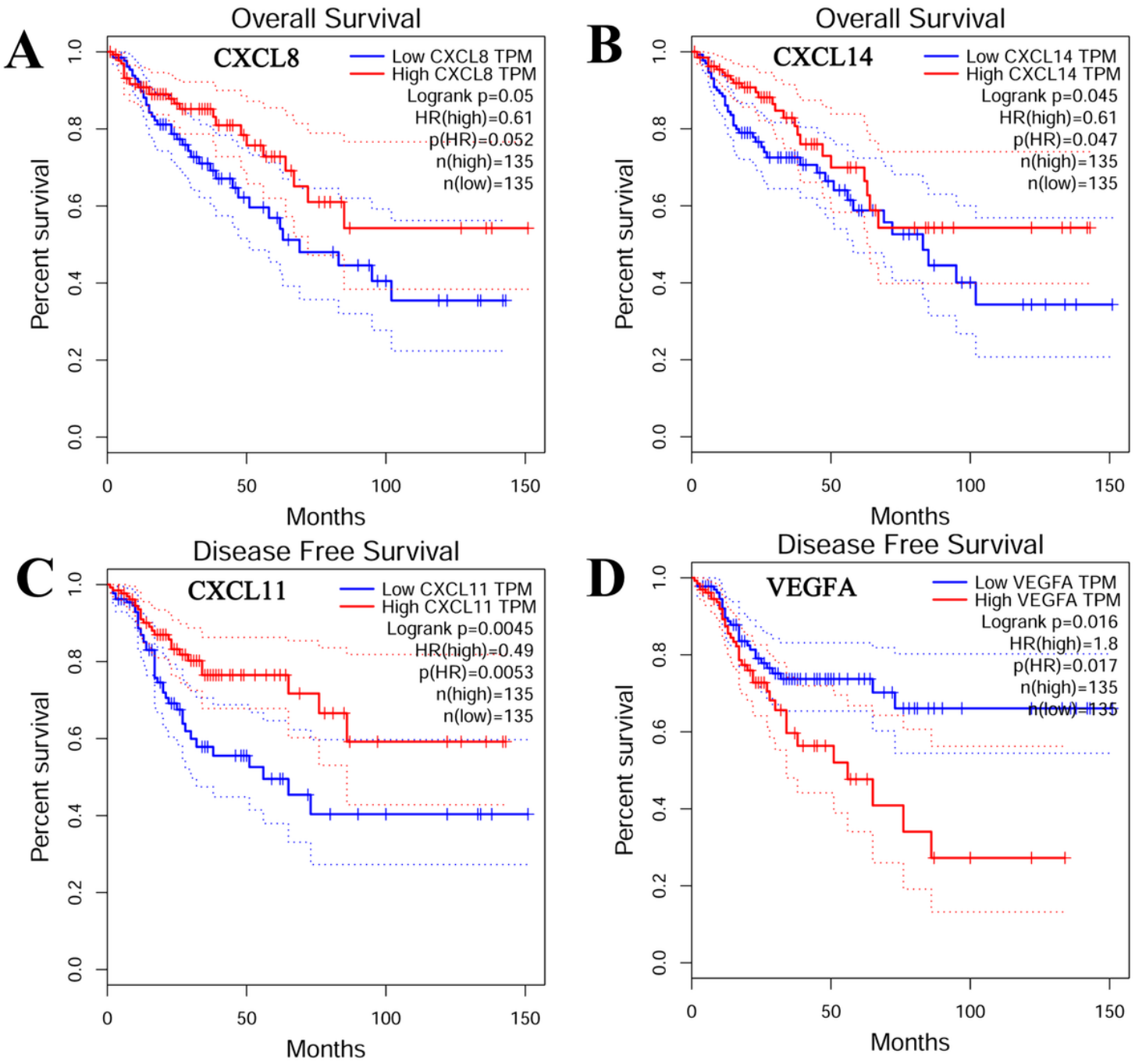

Figure 3

The prognostic value of CXC chemokines-*VEGFA* network in COAD (GEPIA). The overall survival curve of (A) *CXCL8*; (B) *CXCL14*; (C) *CXCL11*; (D) *VEGFA*.

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