

Analysis of Therapeutic Targets and Prognostic Biomarkers of CXC Chemokines in Cervical Cancer Microenvironment

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Primary research

Keywords: cervical cancer, CXC chemokine, Q-PCR, immunohistochemistry, prognosis, tumor microenvironment

Posted Date: May 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-495925/v1>

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Version of Record: A version of this preprint was published at Cancer Cell International on July 28th, 2021. See the published version at <https://doi.org/10.1186/s12935-021-02101-9>.

Abstract

Background: It is well known that the tumor microenvironment (TME) has been received an increasing number of attention. CXC chemokines could regulate immune cell transport and tumor cell activity, thus exerting anti-tumor immunity. However, studies on the expression and prognosis of CXC chemokines in cervical cancer (CC) are more limited.

Methods: The study investigated the role of CXC chemokines in the TME and prognosis of CC using public databases. Moreover, quantitative real-time PCR (Q-PCR) and immunohistochemistry (IHC) of CXC chemokines were performed to further verify.

Results: The transcriptional levels of CXCL1/3/5/6/8/9/10/11/13/14/16/17 in CC tissues were significantly elevated while the transcriptional levels of CXCL2/4/7/12 were significantly reduced. We reached a consistent conclusion that the expression of CXCL9/10/11/13 was verified by Q-PCR and IHC. Moreover, CC patients with low transcriptional levels of CXCL1/2/3/4/5/8 were significantly associated with longer overall survival (OS). Our data suggest that RELA, NFKB1, and SP1 are key transcription factors for CXC chemokines, and the LCK, LYN and PAK are CXC chemokine kinases targets. We found significant correlations among the expression of CXC chemokines and the infiltration of six types of immune cells.

Conclusions: We performed a comprehensive analysis of CXC chemokines via clinical data and some online tumor database. Our results may provide new idea for the selection of immunotherapeutic targets and prognostic biomarkers for cervical cancer.

Background

Deaths due to cancers are a growing threat to human survival [1]. Cervical cancer is one of the leading causes of cancer death in women. Globally, CC accounts for almost 12% of all female cancers, making it the fourth most common female cancer in the world [2, 3]. According to the 2018 Global Cancer Statistics report, the incidence and mortality of CC account for 6.6% and 7.5% of all female cancer patients, respectively [4]. There have been improvements in the early detection and treatment of CC, which has improved patient survival. Yet, CC still causes many deaths, and the median overall survival rate of advanced cervical cancer was only 16.8 months [5]. To improve patient survival in CC, it is crucial to identify novel therapeutic targets to guide individualized treatment and predict the survival outcomes of patients with CC. Recently, many researchers have explored the therapeutic targets of CC, especially kinase and immune checkpoint inhibitors, and some progress has been made [6]; however, this is far from sufficient, and more therapeutic targets and prognostic biomarkers must be identified.

Chemokines are a family of soluble proteins with low molecular weight (8–15 kDa) that not only play important roles in development, homeostasis and angiogenesis, but also are involved in autoimmune

diseases, in tumor-related inflammation and immunity, as well as tumor growth and metastasis [7, 8]. Chemokines are secreted by tumor cells, immune cells and stromal cells in the tumor microenvironment, which can regulate the transport of immune cells and the growth of lymphatic tissue, and thus regulating anti-tumor immune response [9]. As a subfamily of a large family of chemokines, CXC chemokines have also been found to play a role in tumor genesis, development and prognosis [10]. CXC chemokines are potential therapeutic targets and prognostic biomarkers for many types of tumors, including colorectal cancer, renal cell carcinoma and breast tumor, et al [11–13].

CXC chemokine is an important component in the TME. Although previous studies have confirmed the expression and role of some members of the CXC chemokine family in cervical cancer, there is a lack of comprehensive and systematic research. Therefore, it is still worth exploring CXC chemokines as a therapeutic target and prognostic marker of cervical cancer. In this study, public databases were used to explore the mRNA expression, prognosis, and related target or kinase pathways of CXC chemokine family in cervical cancer. Q-PCR and immunohistochemistry further verified the expression. In brief, this study complements the function of CXC chemokines in cervical cancer, suggesting that some CXC chemokines can be used as potential therapeutic targets and prognostic biomarkers for CC.

Materials And Methods

Study population

In this study, clinical data and pathological specimens were collected retrospectively, and the patient's informed consent was obtained before the pathological specimens were collected. And cervical cancer and normal tissues specimens for Q-PCR and IHC analysis were obtained from patients undergoing surgery between January 1, 2017 and December 31, 2018 (N = 60). The current research work received approval from the Academic Committee of The Third Clinical Medical College of Xinjiang Medical University (affiliated Tumor Hospital) and was carried out in accordance with the rules put forward in the Declaration of Helsinki. This study had the relevant exemption certificate of informed consent issued by the Academic Committee. As the public dataset, neither ethics committee approval nor patient informed consent was needed for analyzing data.

Quantitative real-time PCR

Total RNA was isolated from normal tissues and tumor tissues using Trizol according to the manufacturer's instructions. Extracted RNA was converted into cDNA using 5×primescript buffer, primescript RT enzyme mix I, oligo dT primer and random 6 mers. The quantitative real-time PCR was performed in a BioRad CFX96 Real-Time PCR Detection System machine in the presence of GAPDH, CXCL9, CXCL10, CXCL11 and CXCL13. Target gene transcription levels were measured and normalized to GAPDH expression. Statistical analysis was performed using Student's *t*-test and Mann-Whitney U using Graphpad Prism 8 software (GraphPad Software). Differences with *p* values < 0.05 were considered statistically significant. The following primer sequences were used: GAPDH, 5'-GAAGGTGAAGGTCGGAGTC-3' and 5'-GAAGATGGTGATGGGATTC-3'; CXCL9, 5'-

TGAGAAAGGGTCGCTGTTCC-3' and 5'-GGGCTTGGGGCAAATTGTTT-3'; CXCL10, 5'-TGCCATTCTGATTTGCTGCC-3' and 5'-TGCAGGTACAGCGTACAGTT-3'; CXCL11, 5'-CCCTGGGGTAAAAGCAGTGA-3' and 5'-TAAGCCTTGCTTGCTTCGAT-3'; CXCL13, 5'-CGACATCTCTGCTTCTCATGC-3' and 5'-ACTGAGCTCTCTTGGACACAT-3'.

Immunohistochemistry

Formalin-fixed paraffin-embedded surgical specimens were used for immunohistochemistry study. The cervical cancer sections were dried at 60°C for 2 hour, subsequently were dewaxed in xylene and graded alcohols, hydrated and washed in phosphate-buffered saline. After antigen repair was treated in a microwave oven (15 min in citrate buffer, pH 6), the endogenous peroxidase was inhibited by 3% H₂O₂ for 30 min, then the sections incubated with 10% normal goat serum for 40 min. Primary antibodies composed of rabbit anti-CXCL9 antibody (diluted 1:100, bs-2551R, Beijing Boiyntesis Biotechnology Co, Beijing, China), rabbit Anti-CXCL10 antibody (diluted 1:150, bs-1502R, Beijing Boiyntesis Biotechnology Co, Beijing, China), rabbit anti-CXCL11 antibody (diluted 1:150, bs-2552R, Beijing Boiyntesis Biotechnology Co, Beijing, China) and rabbit anti-CXCL13 antibody (diluted 1:100, bs-2553R, Beijing Boiyntesis Biotechnology Co, Beijing, China) were applied overnight in a moist room at 4°C. Then the tissues were incubated with secondary antibodies, stained with diaminobenzidine and counterstained with hematoxylin. Positive staining was evaluated using computer aided image analysis and Image J software. The average CXC chemokines infiltration were determined by two independent pathologists who did not know the patient's pathological and clinical status from three randomized fields.

Transcription-related databases of CXC chemokines in patients of cervical cancer

We used the public databases ONCOMINE (www.oncomine.org) [14], GEPIA (<http://gepia.cancer-pku.cn/index.html>) [15] and UALCAN (<http://ualcan.path.uab.edu>) [16] that could provide cancer RNA sequence data and clinical data to analyze the differential expression of CXC chemokines in cervical cancer and adjacent cancer or normal tissues by using Student's *t*-test. The cut-off of *p* value was 0.05. In ONCOMINE, the fold change was 2.0, and the gene rank was in the top 10%. In addition, we conducted a prognostic study of CXC chemokines in GEPIA through the Kaplan-Meier curve.

cBioPortal

cBioPortal (www.cbioportal.org) is an online open-access website that involves in exploring, visualizing, and analyzing multidimensional cancer genomics data [17]. Genetic alterations of CXC chemokines was obtained from cBioPortal based on TCGA database. 293 cervical squamous cell carcinoma samples (TCGA, PanCancer Atlas) were analyzed. mRNA expression z-scores (log RNA Seq V2 RSEM) were obtained using a z-score threshold of ± 2.0 .

CXC chemokines-related networks

We studied the gene-association networks and protein-protein interaction (PPI) of CXC chemokines respectively by using GeneMANIA (<http://www.genemania.org>) [18], a website which could provide information on the protein and genetic interactions, pathways and co-expression of submitted genes, and the STRING database (<https://string-db.org/>) [19].

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

In this study, CXC chemokines were analyzed by GO, including biological processes (BP), cellular components (CC), molecular function (MF), and KEGG pathway enrichment on the website of DAVID 6.8 (<https://david.ncifcrf.gov/home.jsp>), which could clarify the biological functions of genes [20]. Then we visualized it with the "ggplot2" package of R software. P -value < 0.05 was considered to be statistically different.

Target analysis of kinases and transcription factors

The TRRUST (<https://www.grnpedia.org/trrust/>) database could provide transcription factor (TF)-target regulatory relationships [21]. Use the "LinkInterpreter" module in the LinkedOmics (<http://www.linkedomics.org/>) [22] database to obtain biological insights into kinase targets enrichment of CXC chemokines. We used TRRUST and LinkedOmics to perform TF and kinase targets analysis of CXC chemokines. Gene Set Enrichment Analysis (GSEA) was used to perform analyses with a minimum number of genes (size) of 3 and a simulation of 500. The results were analyzed by Spearman correlation test. The p -value cutoff was 0.05.

TIMER database

TIMER (<https://cistrome.shinyapps.io/timer/>) could provide a systematic assessment of the infiltration of different immune cells and their clinical effects [23]. This study used "Gene module" to evaluate the correlation between CXC chemokines level and immune cell infiltration in the tumor microenvironment.

Results

Abnormal expression of CXC chemokine in CC patients

In order to study the transcription levels of CXC chemokines between tumor and normal tissues in CC, we performed an analysis using the ONCOMINE, GEPIA and UALCAN database. Sixteen CXC chemokines were retrieved using the ONCOMINE database. The results are presented in Fig. 1 and Table 1. The transcriptional levels of CXCL1/3/8/9/10/11/13/16 in cervical cancer tissues were significantly elevated while the transcriptional levels of CXCL12/14 were significantly lower than that of normal cervical tissue. In GEPIA dataset, the results indicated that the expression levels of CXCL1/8/9/10/11/13/16/17 were increased while CXCL12 was reduced between tumor tissues and normal tissues in cervical (Fig. 2a). In UALCAN database, as expected, the transcriptional levels of CXCL6 ($p = 1.18e-4$), CXCL9 ($p = 1.63e-12$), CXCL10 ($p = 7.77e-16$), CXCL11 ($p = 8.36e-13$), CXCL13 ($p = 2.81e-7$) and CXCL17 ($p = 1.74e-3$) in cervical

tissues were significantly elevated (Fig. 2b-g). Taken together, these data suggest that these CXC chemokines play a significant role in the tumorigenesis and progression of cervical cancer.

Table 1

The significant changes of CXC chemokines expression in transcription level between different types of CC.

TLR	Type	Fold change	P-value	t-test	Reference
CXCL1	High Grade Cervical Squamous Intraepithelial Neoplasia Epithelia vs. Normal	7.048	2.83e-5	6.446	Zhai Cervix Statistics [24]
	Cervical Squamous Cell Carcinoma Epithelia vs. Normal	4.462	1.33e-6	5.866	Zhai Cervix Statistics [24]
	Cervical Squamous Cell Carcinoma vs. Normal	3.895	5.62e-5	4.166	Scotto Cervix 2 Statistics [25]
CXCL3	High Grade Cervical Squamous Intraepithelial Neoplasia Epithelia vs. Normal	2.797	6.20e-4	4.111	Zhai Cervix Statistics [24]
CXCL5	High Grade Cervical Squamous Intraepithelial Neoplasia Epithelia vs. Normal	5.994	0.002	4.393	Zhai Cervix Statistics [24]
CXCL6	High Grade Cervical Squamous Intraepithelial Neoplasia Epithelia vs. Normal	2.976	0.003	3.969	Zhai Cervix Statistics [24]
CXCL8	Cervical Squamous Cell Carcinoma vs. Normal	13.807	5.04e-9	6.765	Scotto Cervix 2 Statistics [25]
	Cervical Squamous Cell Carcinoma vs. Normal	3.974	8.10e-8	7.474	Biewenga Cervix Statistics [26]
	Cervical Squamous Cell Carcinoma Epithelia vs. Normal	3.789	8.81e-5	4.318	Zhai Cervix Statistics [24]
CXCL9	Cervical Squamous Cell Carcinoma vs. Normal	8.529	8.45e-9	11.614	Biewenga Cervix Statistics [26]

TLR	Type	Fold change	P-value	t-test	Reference
	Cervical Squamous Cell Carcinoma vs. Normal	3.523	1.47e-6	5.246	Scotto Cervix 2 Statistics [25]
CXCL10	Cervical Squamous Cell Carcinoma vs. Normal	3.982	4.89e-11	11.782	Biewenga Cervix Statistics [26]
	Cervical Squamous Cell Carcinoma vs. Normal	3.050	1.15e-4	3.947	Scotto Cervix 2 Statistics [25]
CXCL11	Cervical Squamous Cell Carcinoma vs. Normal	3.700	4.13e-16	12.455	Biewenga Cervix Statistics [26]
	Cervical Squamous Cell Carcinoma vs. Normal	3.555	2.09e-5	4.476	Scotto Cervix 2 Statistics [25]
CXCL13	Cervical Squamous Cell Carcinoma vs. Normal	19.655	9.32e-11	7.981	Scotto Cervix 2 Statistics [25]
	Cervical Squamous Cell Carcinoma Epithelia vs. Normal	5.835	3.49e-5	4.636	Zhai Cervix Statistics [24]

The expression of CXCL9/10/11/13 in cervical cancer tissues and normal tissues was also examined by Q-PCR (Fig. 3). We only verified CXCL9/10/11/13 because these factors were expressed in the intersection of the three common expression databases including ONCOMINE, GEPIA and UALCAN. CXCL9 ($p = 0.027$), CXCL10 ($p < 0.001$), CXCL11 ($p = 0.002$) and CXCL13 ($p < 0.001$) were significantly upregulated in cervical cancer tissues compared with normal tissues. We also carried out IHC for the purpose of testing the CXCL9/10/11/13 proteins expression in cervical cancer tissues as well as their counterparts. We found that CXCL9/10/11/13 proteins were more highly expressed in the cancer tissues than in the normal (Fig. 4). These were consistent with the findings in the database and further verified the transcriptional expression of CXCL9/10/11/13 in cervical cancer.

The prognostic value of CXC chemokines in cervical cancer

In order to evaluate the value of differentially expressed CXC chemokines in the progression of CC, we used GEPIA to evaluate the correlation between differentially expressed CXC chemokines and clinical outcomes. The low transcriptional levels of CXCL1 ($p = 0.033$), CXCL2 ($p = 0.046$), CXCL3 ($p = 0.017$), CXCL4 ($p = 0.027$), CXCL5 ($p = 0.011$) and CXCL8 ($p = 1.5e-5$) were significantly associated with longer OS of CC patients (Fig. 5a-f). In addition, the value of differentially expressed CXC chemokines in the disease free survival (DFS) of cervical patients was also evaluated. We found that cervical cancer patients with low transcriptional level of CXCL3 ($p = 0.018$) was significantly associated with longer DFS (Fig. 5g).

Analysis of genetic alteration, adjacent gene network and interaction of CXC chemokines in cervical cancer

We analyzed the genetic alterations of differentially expressed CXC chemokines by using the TCGA dataset in the cBioPortal. We found that high mutation rate of CXC chemokines (42%) was observed in CC patients. In the 293 sequenced cervical cancer patients, genetic alteration was found in 124 cervical cancer patients. As a result, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL6 and CXCL17 were altered in 6%, 3%, 4%, 4%, 6%, 5%, 5%, 5%, 6%, 6%, 5%, 5%, 6%, 5%, 7% and 6% of the cervical cancer samples, respectively (Fig. 6a). In addition, in order to explore the potential interactions of the differentially expressed CXC chemokines, we performed a protein-protein interaction (PPI) network analysis in STRING (Fig. 6b). The results revealed that the functions of these differentially expressed CXC chemokines were related to chemokine signaling pathways and inflammation response. In GeneMANIA website, the results also showed that the functions of differential expressed CXC chemokines were primarily related to cell chemotaxis, chemokine receptor binding, and chemokine activity (Fig. 6c).

Predicted functions and pathways of CXC chemokines in cervical cancer patients

The functions of differentially expressed CXC chemokines and their neighboring genes were analyzed using DAVID 6.8. The top 10 richest GO items are shown in Fig. 7a. GO term analysis showed that differentially expressed in correlation with CXC chemokines were located mainly in the extracellular space, extracellular region, cell, external side of plasma membrane and cell surface, where they participate chemotaxis, inflammatory response, monocyte chemotaxis, cellular response to interleukin-1 and immune response. They act as CCR chemokine receptor binding, CXCR chemokine receptor binding, heparin binding, chemokine receptor binding and chemoattractant activity. KEGG pathway analysis showed enrichment in the chemokine signaling pathway, cytokine-cytokine receptor interaction, rheumatoid arthritis, Toll-like receptor signaling pathway, TNF signaling pathway (Fig. 7b).

Kinase and transcription factor (TF) targets analysis in patients with cervical cancer

We used TRRUST and Linkedomics databases to explore the possible kinase and transcription factor targets of differential CXC chemokines in the cervical cancer microenvironment. We found that three transcription factors (RELA, NFKB1, and SP1) were associated with the regulation of CXC chemokines in the TRRUST (Table 2). RELA and NFKB1 were the key transcription factors for CXCL1/2/5/8/10/12. And

SP1 was the key transcription factor for CXCL1/5/14. We identified the top two kinase targets of CXC chemokines from the LinkedOmics database (Table 3). ATR and CHUK were the top two targets in the CXCL1 kinase-target network. Components of the CXCL2 kinase-target network were mainly associated with RPS6KB1 and ATM. GRK6 and ATM were suggested as the targets for the CXCL3 kinase-target network. PAK2 and PAK3 were primarily related to CXCL5. DAPK1 and EGFR were the top two targets in the CXCL6 kinase-target network. Components of the CXCL9 kinase-target network were mainly associated with ZAP70 and LCK. LYN and LCK were suggested as the targets for the CXCL10 and CXCL11 kinase-target network. RPS6KA4 and PLK3 were primarily related to CXCL12. LCK and SYK, and CDK1 and MAP3K8 were the top two targets in the CXCL13 and CXCL14 kinase-target networks, respectively. Components of the CXCL16 and CXCL17 kinase-target networks were mainly associated with LCK and LYN, as well as ADRBK1 and IGF1R.

Table 2
Key regulated factor of CXC chemokines in CC (TRRUST).

Key TF	Description	Regulated gene	P-value	FDR
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	CXCL1, CXCL2, CXCL5, CXCL8, CXCL10, CXCL12	1.09e-07	1.71e-07
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	CXCL1, CXCL2, CXCL5, CXCL8, CXCL10, CXCL12	1.14e-07	1.71e-07
SP1	Sp1 transcription factor	CXCL1, CXCL5, CXCL14	0.00683	0.00683

Table 3
The Kinase target networks of CXC chemokines in CC (LinkedOmics).

CXC chemokines	Enriched kinase target	Description	Leading EdgeNum	P-value
CXCL1	Kinase_ATR	ATR serine/threonine kinase	16	0
	Kinase_CHUK	conserved helix-loop-helix ubiquitous kinase	10	0
CXCL2	Kinase_RPS6KB1	ribosomal protein S6 kinase B1	6	0
	Kinase_ATM	ATM serine/threonine kinase	38	0
CXCL3	Kinase_GRK6	G protein-coupled receptor kinase 6	2	0
	Kinase_ATM	ATM serine/threonine kinase	37	0
CXCL5	Kinase_PAK2	p21 (RAC1) activated kinase 2	7	0
	Kinase_PAK3	p21 (RAC1) activated kinase 3	3	0
CXCL6	Kinase_DAPK1	death associated protein kinase 1	6	0
	Kinase_EGFR	epidermal growth factor receptor	20	0
CXCL9	Kinase_ZAP70	zeta chain of T-cell receptor associated protein kinase 70	4	0
	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	23	0
CXCL10	Kinase_LYN	LYN proto-oncogene, Src family tyrosine kinase	21	0
	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	21	0
CXCL11	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	22	0
	Kinase_LYN	LYN proto-oncogene, Src family tyrosine kinase	21	0
CXCL12	Kinase_RPS6KA4	ribosomal protein S6 kinase A4	11	0
	Kinase_PLK3	polo like kinase 3	6	0
CXCL13	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	22	0
	Kinase_SYK	spleen associated tyrosine kinase	15	0
CXCL14	Kinase_CDK1	cyclin dependent kinase 1	74	0
	Kinase_MAP3K8	mitogen-activated protein kinase kinase kinase 8	7	0

CXC chemokines	Enriched kinase target	Description	Leading EdgeNum	P-value
CXCL16	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	23	0
	Kinase_LYN	LYN proto-oncogene, Src family tyrosine kinase	22	0
CXCL17	Kinase_ADRBK1	G protein-coupled receptor kinase 2	8	0
	Kinase_IGF1R	insulin like growth factor 1 receptor	7	0

Analysis of CXC chemokine immune cell infiltration in patients with cervical cancer

As a component of the TME, CXC chemokines are involved in inflammation responses and immune cell infiltration, thereby affecting the clinical outcome of cervical cancer patients. Therefore, we comprehensively explore the correlation between differentially expressed CXC chemokines and immune cell infiltration using the TIMER database (Fig. 8). There was a negative correlation between CXCL1 expression and the infiltration of CD4 + T cells (Cor = -0.201, $p = 7.75e-4$), macrophages (Cor = -0.175, $p = 3.41e-3$) and dendritics (Cor = -0.163, $p = 6.61e-3$). The infiltration of CD4 + T cells was negatively associated with CXCL2 (Cor = -0.198, $p = 9.10e-4$) and CXCL3 (Cor = -0.224, $p = 1.76e-4$). CXCL4 expression was negatively associated with the infiltration of CD4 + T cells (Cor = -0.136, $p = 2.37e-2$), and positively associated with the infiltration of macrophages (Cor = 0.128, $p = 3.34e-2$). There was a positive correlation between the expression of CXCL9, CXCL10, CXCL11, CXCL13 and CXCL16, and the infiltration of CD8 + T cells, CD4 + T cells, neutrophils and dendritic cells (all $p < 0.05$). There was a positive correlation between the expression of CXCL12, and the infiltration of B cells, CD4 + T cells, macrophages and dendritic cells (all $p < 0.05$). CXCL14 expression was negatively associated with the infiltration of neutrophils (Cor = -0.198, $p = 9.01e-4$), and positively associated with the infiltration of B cells (Cor = 0.125, $p = 3.78e-2$) and macrophages (Cor = 0.123, $p = 4.07e-2$). CXCL17 expression was positively associated with the infiltration of CD4 + T cells (Cor = 0.19, $p = 1.48e-03$) and neutrophils (Cor = 0.174, $p = 3.58e-3$).

Discussion

CC remains one of the major causes of cancer-related death among woman world-wide [1]. For patients with early cervical cancers, surgery or radiotherapy or a combination of both is recommended for the best cure rate [27, 28]. However, in most cases, the majority of patients with cervical cancer present with an advanced stage of disease, with limited access to adequate treatment. As a result, the mortality rates are still high [27]. Nowadays, an increasing number of studies have demonstrated the importance of a new type of regulation by epigenetics in specific genes in the progression of cervical cancer [29]. This provides new ideas for the treatment of cervical cancer. The present findings demonstrate that the CXC chemokines was correlated with survival of CC patients, indicating that CXC chemokines could be a potential therapeutic targets and prognostic biomarkers of CC.

Intercellular communication between CC cells and stromal cells affects the expression patterns of chemokines in various cell types, thus facilitating specific microenvironments for tumor invasion and metastasis. CXC chemokines' altered expression in malignancies dictates leukocyte recruitment and activation, angiogenesis, tumorigenesis, cancer cell proliferation, and metastasis in all the stages of the disease [30–32]. Studies have reported correlations between CXC chemokine, tumor microenvironment and tumor immunotherapy, suggesting that CXC chemokines may regulate tumor progression and immunotherapy effect [33]. However, the prognostic value and biological function of CXC chemokines in CC have not been comprehensively characterized.

We comprehensively analyzed the expression and function of CXC chemokines in cervical cancer by using public databases. This study found that the expression of CXCL1/3/5/6/8/9/10/11/13/14/16/17 was up-regulated in cervical cancer tissues, while the expression of CXCL2/4/7/12 was down-regulated. Q-PCR and IHC also showed that CXCL9/10/11/13 was highly expressed in cervical cancer tissues. In addition, CC patients with low transcription levels of CXCL1/2/3/4/5/8 had longer OS, while CC patients with low levels of CXCL3 had longer DFS. We also found that RELA, NFKB1 and SP1 are the key TFs for CXC chemokines, and LCK, LYN and ATM are the kinases targets of CXC chemokines. In brief, these data indicated that differentially expressed CXC chemokines may play an important role in CC.

Tumorigenesis and the progression of cervical cancer is a complex and multifaceted. Due to the significant differential expression of multiple chemokines in CC, we explored the molecular characteristics of these chemokines in CC. CXC chemokines differentially expressed in CC had frequent genetic alterations with a mutation rate of 42%, of which increased mRNA expression was the most alteration. The CCL family was associated with CXC chemokines neighboring alteration, including CCL1, CCL2, CCL8 and CCL21, etc. These CCL factors play different but similar functions in tumors. It has been reported that CCL1, CCL2 and CCL21 affect tumor metastasis and progression through direct or indirect interactions with lymph nodes [34–36]. CCL8 was independent prognostic factors in cervical cancer patients, and increased CCL8 secretion promoting tumor progression [37, 38].

Then via GO enrichment analysis and KEGG pathway enrichment analysis, we found that the functions of these genes are primarily related to the chemokine signaling pathway, cytokine-cytokine receptor interaction, and the Toll-like receptor signaling pathway. Studies have shown that chemokine signaling pathways play a key role in the proliferation, angiogenesis, immune evasion and metastasis of various cancers [39–41]. TLRs are type I intermembrane proteins, containing a leucinerich domain (that recognizes the PAMPs) and an intracellular domain that activates signal transduction. It has been demonstrated that TLR signaling pathways in tumor cells can affect cancer progression. Stimulation of TLRs in tumor cells fosters chronic inflammation that drives cancer cell proliferation, migration, and angiogenesis, and establishes a tumor microenvironment that impairs the immune system, thereby allowing tumors to establish themselves and to thrive [42, 43]. These data indicated that CXC chemokines differentially expressed in CC were potential therapeutic targets.

By analyzing the differential expression of CXC chemokines in cervical cancer, we found three key transcription factors (RELA, NFKB1 and SP1). RELA phosphorylation contributes to disease progression, especially inflammatory diseases and cancer, by regulating NF-κB signaling [44]. Some studies have revealed an association between elevated RELA expression and poor survival [45]. NFKB1 display increased inflammation and susceptibility to certain forms of DNA damage, leading to cancer, and a rapid ageing phenotype [46]. NFKB1 was a tumour suppressor by inhibiting cell proliferation, colony formation and migration in cervical cancer, while the mutation could weaken the tumour suppressing functions of NFKB1 [47]. Sp1 may contribute to radioresistance through inhibiting G2/M phase arrest by targeting CDK1, affecting the prognosis of patients [48]. Moreover, our data indicated that the SRC family tyrosine kinases (LCK, LYN) and PAK family kinase (PAK2, PAK3) were the probable targets of the differentially expressed CXC chemokines. These kinases are involved in tumor development and progression by regulating tumor cell proliferation, migration, invasion, and apoptosis [49–51]. In CC, differentially expressed CXC chemokines may affect tumor development and progression by regulating these kinases.

In the cervical cancer microenvironment, CXC chemokines could control the migration and localization of immune cells, and immune cells could be a factor of immunotherapy and clinical outcome by affecting tumor development and progression [52, 53]. In this study, we found a significant correlation between the expression of CXC chemokines and the infiltration of the six immune cell types, B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells. Previous studies have shown that the interaction of CD4 + T cell and CD8 + T cells influence the immune response and prognosis of tumors [54, 55]. Macrophages influence tumor development via interacting with tumor cells to induce lymphangiogenesis in CC⁵⁶. These results indicated that CXC chemokines are not only as prognostic indicators, but also may reflect immune status.

In our study, experiment and several online bioinformatic platforms were systematically analyzed the expression, mutations, correlated genes, and prognostic value of CXC chemokines in CC. At the same time, there were some limitations in our study. First, a multicenter, prospective research is needed to validate the conclusions because our study is the retrospective design. Second, analysis on the transcriptional and translational level can reflect some aspects of immune status, but not global changes. Third, we should collect more clinical specimen data for experimental verification. Moreover, only the mRNA and protein expressions of CXCL9/10/11/13 were verified in the experiment, and more indicators should be further verified in the experiment.

Conclusions

In conclusion, we performed a comprehensive analysis of CXC chemokines via clinical data and some online tumor database. Patients with low transcriptional levels of CXCL1/2/3/4/5/8 were significantly associated with better prognosis. We hope our results could provide novel insights for the selection of immunotherapeutic targets and prognostic biomarkers for cervical cancer.

Abbreviations

TME: Tumor microenvironment; CC: Cervical cancer; Q-PCR: Quantitative real-time PCR; IHC: Immunohistochemistry; OS: Overall survival; PPI: Protein-protein interaction; GSEA: Gene Set Enrichment Analysis; DFS: Disease free survival; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: Biological processes; CC: Cellular components; MF: Molecular function; TF: Transcription factor.

Declarations

Acknowledgments

Thanks to Xinjiang Medical University for helping authors in manuscript. And we sincerely thank all the authors and study participants for their support in this study.

Author Contributions

WNK and FG conceived and designed the research. WNK, GZ, FG performed the experiments, interpreted the data and wrote the manuscript. HXC, WNW, XQS and QNS discussed the data and provided constructive suggestions. FG and XMM supervised the study. All authors read and approved the manuscript.

Funding

This work was supported by grants from Xinjiang Medical University's clinical medicine peak discipline (33-0104006020801#) and the special scientific research project of young medical scientific and technological talents of Xinjiang Uygur Autonomous region Health and Family Planning Commission (WJWY-202154).

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

The current research work received approval from the Academic Committee of The Third Clinical Medical College of Xinjiang Medical University (affiliated Tumor Hospital) and was carried out in accordance with the rules put forward in the Declaration of Helsinki. This study had the relevant exemption certificate of informed consent issued by the Academic Committee.

Consent for publication

All listed authors have actively participated in the study and have read and approved the submitted manuscript.

Competing Interests

The authors declare that they have no competing interests.

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Figures

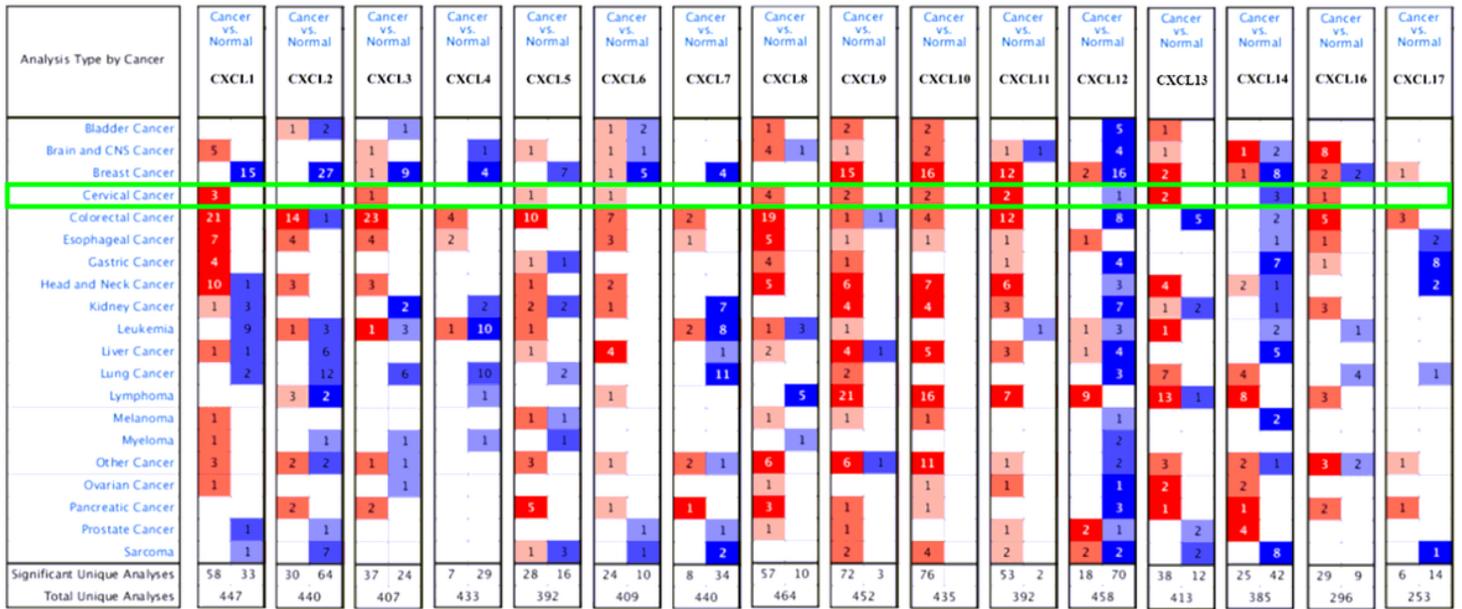


Figure 1

mRNA levels of CXC chemokines in cervical cancer (ONCOMINE). It shows the numbers of datasets with statistically significant mRNA over-expression (red) or down-regulated expression (blue) of CXC chemokines.

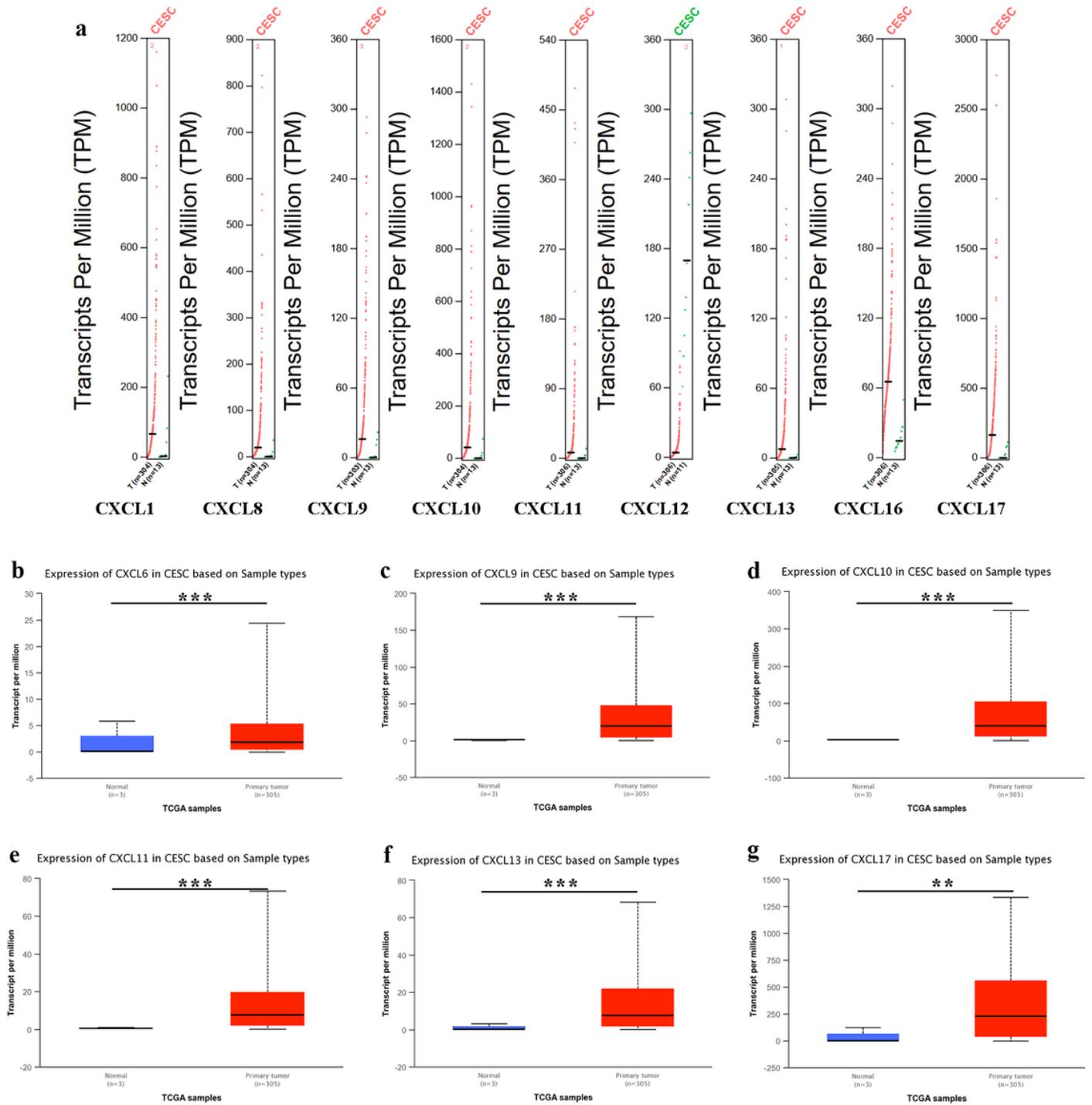


Figure 2

The expression of distinct CXC chemokines in CC tissues and adjacent normal cervical tissues. a The color of red represents high expression, while green color shows low expression between cervical tumor and normal tissues in the GEPIA database. b-g The color of blue represents normal cervical tissues, red color represents CC tissue in the UALCAN database. ** p < 0.01, *** p < 0.001.

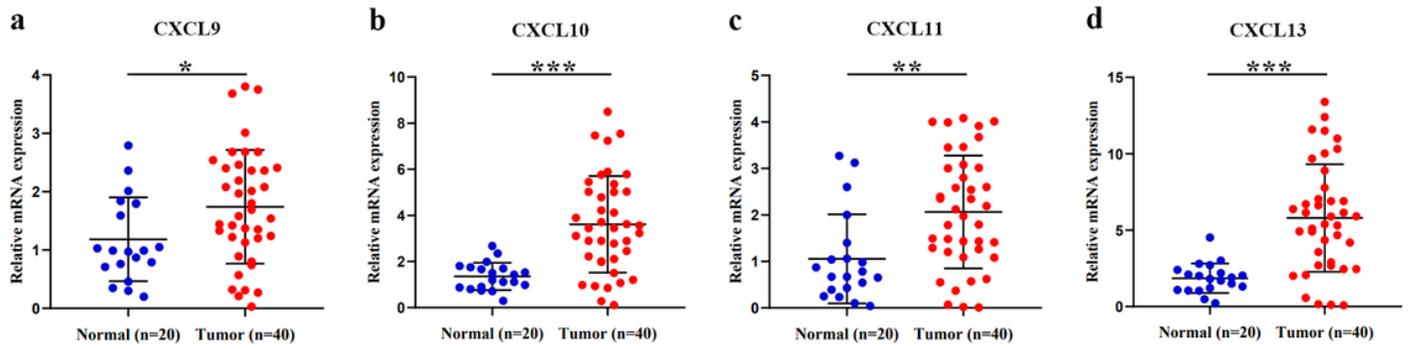


Figure 3

Expression of CXC chemokines between CC tissues and normal tissues were analyzed by Q-PCR. a CXCL9. b CXCL10. c CXCL11. d CXCL13. * p < 0.05, ** p < 0.01, *** p < 0.001.

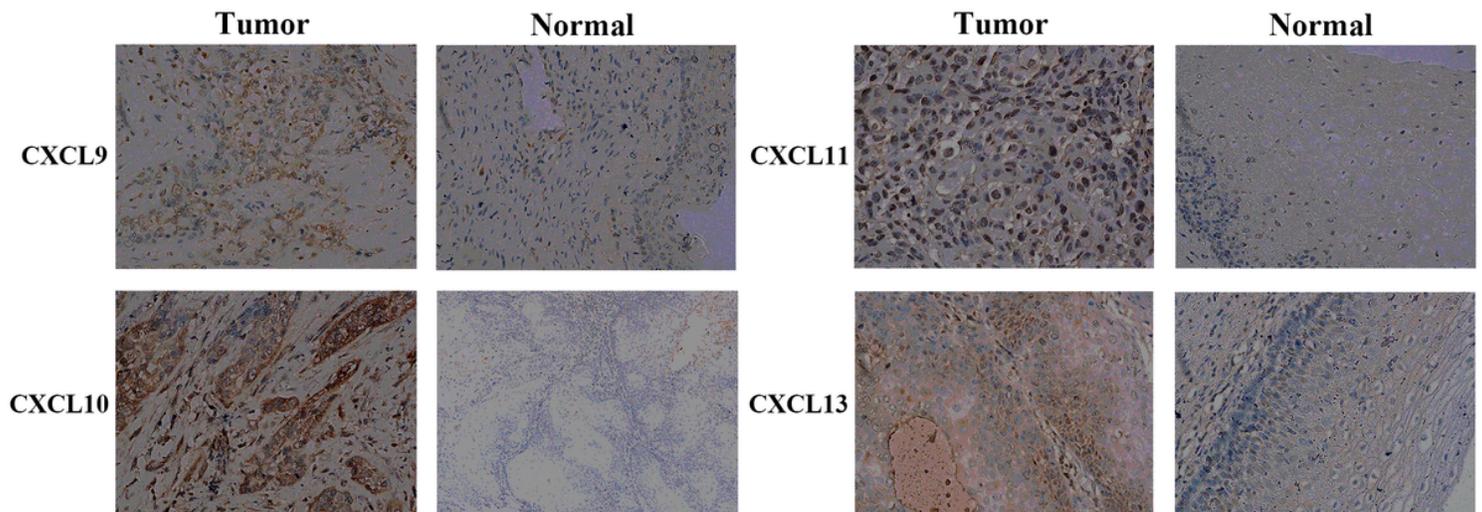


Figure 4

Representative immunohistochemistry images of distinct CXC chemokines in cervical cancer tissues and normal cervical tissues.

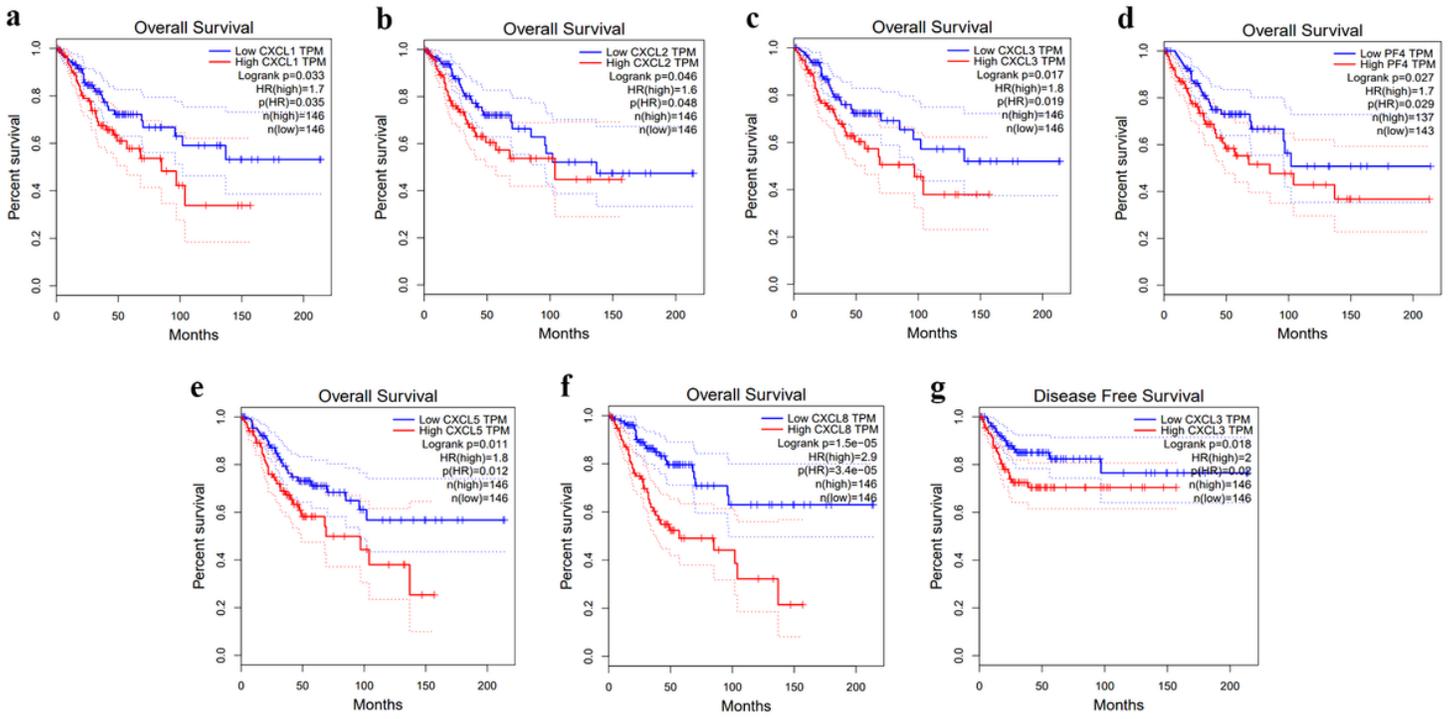


Figure 5

The prognostic value of different expressed CXCL chemokines in survival curve. a CXCL1 (OS). b CXCL2 (OS). c CXCL3 (OS). d CXCL4 (OS). e CXCL5 (OS). f CXCL8 (OS). g CXCL3 (DFS).

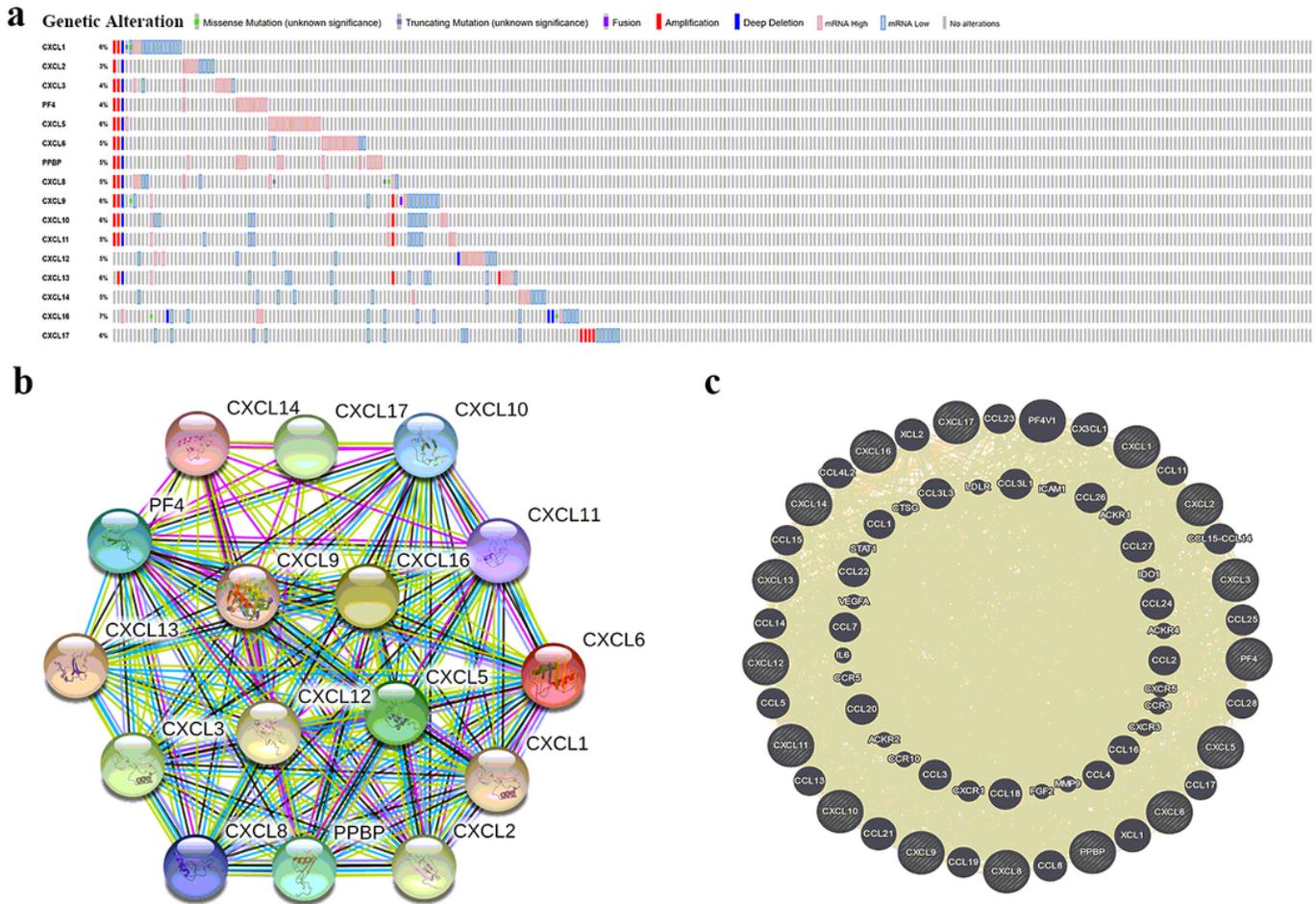


Figure 6

Genetic alteration, neighbor gene network and interaction analyses of different expressed CXC chemokines. a CXC chemokines' mutation analysis in CC (cBioPortal). b-c Protein-protein interaction network of different expressed CXC chemokines (STRING and GeneMANIA).

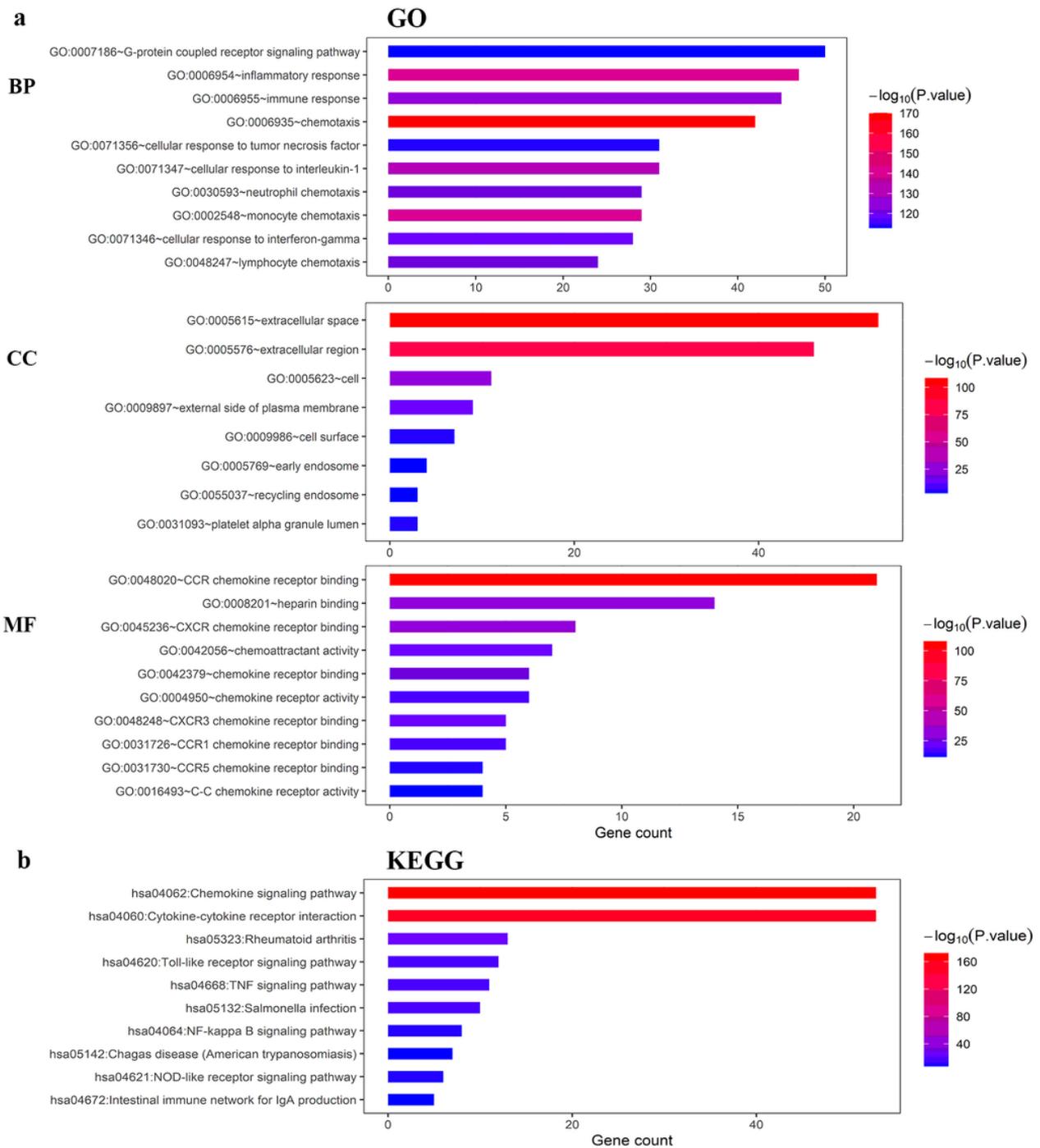


Figure 7

Analysis of GO and KEGG (DAVID). a Bar-plot of GO enrichment in biological process terms, cellular component terms and molecular function terms. b Bar-plot of KEGG enriched terms.

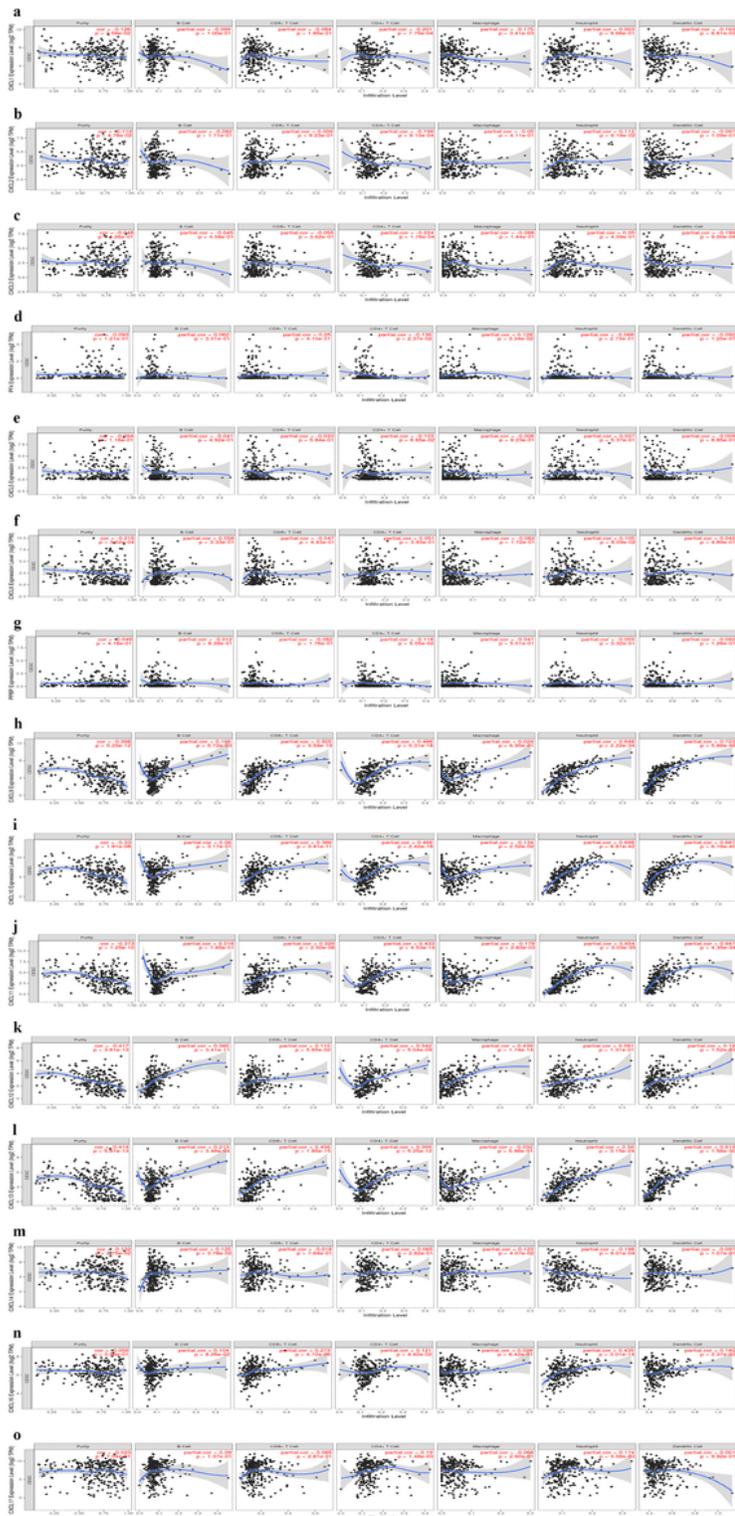


Figure 8

The correlation between different expressed CXC chemokines and immune cell infiltration (TIMER).