Mathematical Biosciences and Engineering

MBE, 18(6): 8201–8222.
DOI: 10.3934/mbe.2021407
Received: 05 August 2021
Accepted: 12 September 2021
Published: 18 September 2021

http://www.aimspress.com/journal/MBE

Research article

Exploration of potential therapeutic and prognostic value of CXC chemokines in cervical squamous cell carcinoma and endocervical adenocarcinoma based on bioinformatics analysis

Caiyun Wu[1,†], Cong Ma[1,†], Jing Yuan[2] and Pei Zhou[2,*]

[1] Reproductive Medicine Center, Department of Obstetrics and Gynecology, First Affiliated Hospital of Anhui Medical University, Hefei 230022, China
[2] Prenatal Diagnosis Center, Department of Obstetrics and Gynecology, First Affiliated Hospital of Anhui Medical University, Hefei 230022, China

† The authors contributed equally to this work.

* **Correspondence:** Email: zhoupei7788@163.com; Tel: 055165908446.

Abstract: Cervical cancer, as the second most common female malignancy, brings a great health burden to women worldwide. Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) are the most common histological subtypes of cervical cancer. CXC chemokines (CXCLs) within the tumor microenvironment can modulate carcinogenesis and progression. The present study aimed to explore the therapeutic and prognostic value of different CXCLs in CESC. ONCOMINE, GEPIA, cBioPortal, TRRUST, GeneMANIA, STRING and TIMER were utilized to explore the expression, mutation and function of CXCLs in CESC, as well as their correlation with pathological and survival features of CESC patients. We found that the mRNA expression levels of CXCL1/8/9/10/11/13/16/17 in CESC were upregulated compared with normal cervical tissues, whereas CXCL12 was downregulated. No significant correlation was found between the expression levels and pathological stage of CESC patients. CESC patients with high expression of CXCL1/2/3/4/5/8 were significantly associated with poor overall survival, additionally, low mRNA level of CXCL3 was associated with better disease-free survival. Besides, a high mutation rate (43%) of CXCLs in CESC was observed. Depicted by co-expression analysis, the expression of CXCL1/2/3/6/8 showed a modest to strong correlation, while that of CXCL9/10/11/13 showed a very strong correlation. Differentially expressed CXCLs primarily functioned in chemokine signaling pathway and inflammation response, such as cell chemotaxis, chemokine activity and chemokine

receptor binding. We also found the association of CXCLs with the tumor-infiltration of six types of immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells) in CESC patients. The present study elucidated that CXCLs may have the potential to be novel therapeutic targets and prognosis predictors of CESC patients.

Keywords: bioinformatics; CXC chemokines; cervical squamous cell carcinoma and endocervical adenocarcinoma; therapy; prognosis

Abbreviations: CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma; CSCC: cervical squamous cell carcinoma; CXCL: CXC chemokine ligand; CXCR: CXC chemokine receptor; DFS: disease-free survival; ELR: glutamate-leucine-arginine; FIGO: International Federation of Gynecology and Obstetrics; GEPIA: Gene Expression Profiling Interactive Analysis; GO: gene ontology; GTEx: Genotype-Tissue Expression; HGCSIN: high-grade cervical squamous intraepithelial neoplasia; HPV: human papillomavirus; KEGG: Kyoto Encyclopedia of Genes and Genomes; OS: overall survival; PPI: protein-protein interaction; ROS: reactive oxygen species; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins; TCGA: The Cancer Genome Atlas; TF: transcription factor; Th: T helper; TIMER: Tumor Immune Estimation Resource; TRRUST: Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining

1. Introduction

Cervical cancer, a public health problem, ranked as the fourth leading cause of female cancer morbidity and mortality worldwide [1]. Due to the lack of primary prevention (human papillomavirus vaccination programs) and the secondary prevention (formalized screening procedures), the low-income and developing countries bear a high burden of cervical cancer [2]. Despite the progress in diagnosis and treatment, the prognosis of patients with advanced cervical cancer is still not optimistic. Two most common histological subtypes of cervical cancer are cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), making up about 75 and 25% of all cases respectively [3,4]. At present, the clinical management and prognosis prediction of CESC largely depend on the revised 2018 International Federation of Gynecology and Obstetrics (FIGO) staging guidelines. However, there is inconsistence between the clinical staging and prognosis; high FIGO staging doesn't always indicate worse prognosis [5]. Therefore, exploring new therapeutic targets and prognostic markers has long been an issue of concern.

CXC chemokine ligands 1-17 (CXCL1-17) in cancer are small proteins secreted by tumor cells and the cells within the tumor microenvironment, such as immune and stromal cells. Specially, CXCL15 was reported to express in murine organs with no homology found in human [6]. CXCLs are classified into two groups according to the presence of glutamate-leucine-arginine (ELR) motif as ELR^+ or ELR^-; ELR^+ CXCLs can attract neutrophils and are angiogenic, while most ELR^- CXCLs attract lymphocytes and are angiostatic [7,8]. They exert their effects by binding to specific CXC chemokine receptors (CXCRs), which in most cases are seven-transmembrane G-protein-coupled receptors, thereby activating various signaling pathways. CXC chemokines can recruit different types of immune cells to the tumor site, subsequently exerting cancer promotion or inhibition effects. These chemokines can also act directly on tumor cells to adjust cellular proliferation and metastasis, or act

on stromal cells to involve the process of angiogenesis and metastasis [7].

Previous studies have demonstrated the therapeutic and prognostic values of CXCLs in various cancer types, such as pancreas, prostate, colon cancer and glioblastoma [9–12]. It has been suggested that elevated CXCLs in glioblastoma could inhibit DNA damage response and promote tumor progression [12]. CXCLs were reported to correlate with colorectal cancer patients' survival [13]. Consistently, elevated CXCL1/2/8 in cervical cancer can promote tumor angiogenesis via CXCR2 on endothelial cells in a paracrine fashion, and promote cellular proliferation and survival in an autocrine manner [14]. On the contrary, inhibiting CXCL8 could suppress the cervical cancer cell proliferation and stimulate cellular apoptosis [15]. Thus, CXCLs may have the potential to be predictors and therapeutic targets of CESC. However, limited data was reported about the roles of different CXCLs in the development and prognosis of CESC. Our present study aimed to explore the expression, mutation and function of CXCLs in CESC, as well as their correlation with pathological and survival features by integrative bioinformatics analysis.

2. Materials and methods

2.1. ONCOMINE

The mRNA expression levels of CXCLs in cervical cancer versus corresponding normal cervical tissues were analyzed by the ONCOMINE database (https://www.oncomine.org), a free open platform which has collected 715 datasets including microarray data of 86733 samples [16]. Data was extracted with the significance thresholds set as a p value < 0.01, a fold change > 2 and a gene rank in the top 10%. Student's t-test was used to compare the differences in transcription levels.

2.2. GEPIA

GEPIA (Gene Expression Profiling Interactive Analysis) database (http://gepia.cancer-pku.cn/) provides integrated RNA sequencing data of 9736 tumors and 8587 normal samples based on The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) database [17]. It has three analysis modules, including single gene analysis, multiple gene analysis and cancer type analysis. In our present study, the mRNA expression comparison between the CESC and normal cervical tissues, the cancer pathological stage analysis and survival analysis of CXCLs were conducted by the single gene analysis module. We analyzed the association between CXCLs mRNA expression levels and overall survival (OS) or disease-free survival (DFS) in the CESC patients. Survival analysis was measured using Kaplan–Meier curves and log-rank test, also known as the Mantel-Cox test [18]. Multiple gene comparison was conducted via the multiple gene analysis module.

2.3. cBioPortal

The cBioPortal for Cancer Genomics (http://www.cbioportal.org) is a very practical tool for multidimensional cancer genome datasets analysis. It is a comprehensive open web platform based on TCGA database, which combines data mining, data integration and visualization [19]. We analyzed the genomic alterations of CXCL1-17 in CESC by the "Query" module of cBioPortal. We selected the genomic profiles as mutations, structural variant, putative copy-number alteration from GISTIC, and

mRNA Expression z-Score to all samples (log RNA Seq V2 RSEM), which ultimately included 275 complete samples. Using the "co-expression" module and Spearman's correlation, we evaluated the mRNA expression correlation of distinct CXCLs.

2.4. TRRUST

TRRUST (Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining) (https://www.grnpedia.org/trrust/) is a classical database of transcriptional regulatory networks. It provides information about transcription factors (TFs) regulation in human and mice, which not only contains the target genes corresponding to TFs, but also the regulatory relationships among these TFs [20]. We explored key TFs of CXCLs using the "Find key regulators for query genes" module in the TRRUST database.

2.5. GeneMANIA

GeneMANIA (http://www.genemania.org) is a simplified website that generates information about gene function prediction and gene lists analysis. It provides genetic interaction networks in co-expression, shared protein domains, predicted, co-localization, and pathways of a given gene list across nine organisms [21]. Via GeneMANIA, we constructed gene-gene interaction networks among CXCLs members as well as the top 50 neighbor genes.

2.6. STRING

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database (https://string-db.org/) strives to collect and integrate all types of protein-protein interaction (PPI), including primary and predicted interactions, based on all publicly available data. It stands out with high coverage, convenience of use and a consistent scoring system [22]. We constructed a PPI network of differentially expressed CXCLs to explore their interaction.

2.7. Function enrichment analysis

GO (gene ontology) term enrichment analysis includes three types: molecular function (GO-MF), which refers to the function of single gene product, such as binding activity or catalytic activity, cellular component (GO-CC), which is used to describe the location of gene products in cells, and biological process (GO-BP), which refers to orderly biological processes with multiple steps. KEGG (Kyoto Encyclopedia of Genes and Genomes) is utilized for systematic analysis of metabolic pathways of gene products in cells. In our study, we explored the potential functions of CXCLs and top 50 related genes using GO and KEGG pathway analysis. The results were visualized with R project using the "clusterProfiler" and "org.Hs.eg.db" package.

2.8. TIMER

TIMER (Tumor Immune Estimation Resource) database (https://cistrome.shinyapps.io/timer/) can analyze and visualize tumor infiltrating immune cells comprehensively and flexibly. It infers the

abundance of tumor infiltrating immune cells from gene expression profiles of different cancer types in TCGA [23]. We evaluated the correlation between CXCLs expression and the abundances of six immune infiltrates (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells) in CESC patients via the "Gene" module of TIMER database. We further explored the clinical relevance of tumor immune infiltrates by correcting for CXCLs gene expression in a multivariable Cox proportional hazard model via the "Survival" module. 302 patients with 73 dying were included in our analysis.

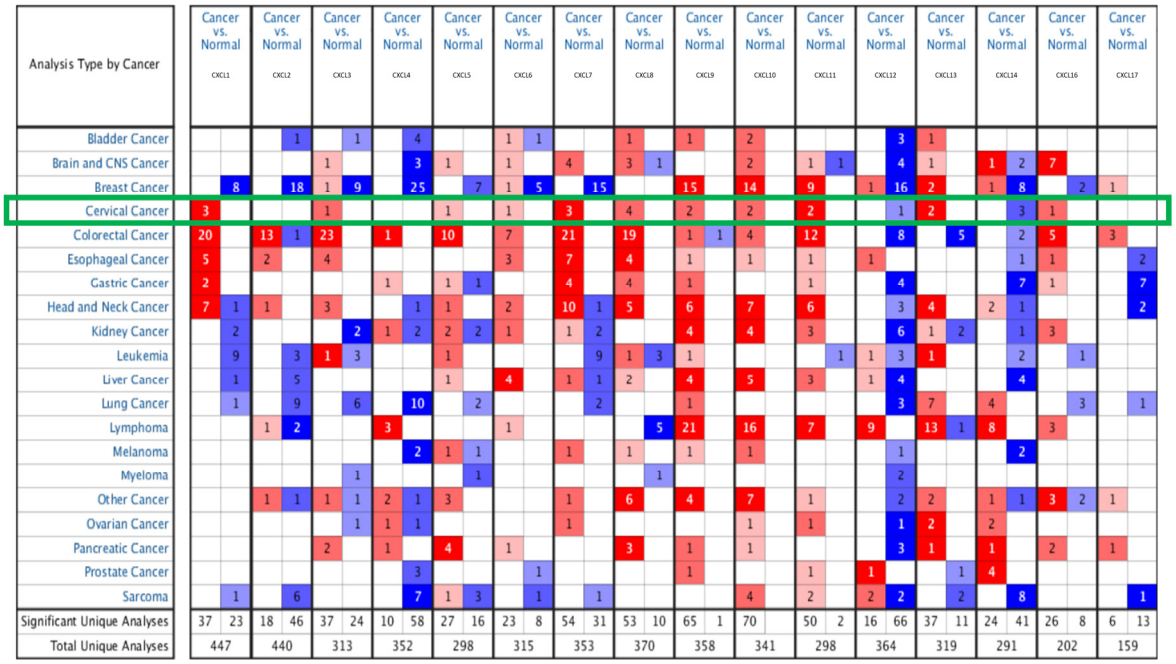


Figure 1. The transcription levels of CXCLs in different cancer types and respective normal tissues based on ONCOMINE database. The numbers in the graphic refer to the number of datasets that meet the criteria: p-value < 0.01, fold change > 2, gene rank: 10%, data type: mRNA. Red represents significantly upregulated expression and blue represents downregulated expression.

2.9. Statistical analysis

In ONCOMINE database, Student's t-test was used to perform differential analysis of CXCLs mRNA transcription levels between cervical cancer and normal cervical tissues. One-way ANOVA using disease state (Tumor/Normal) and pathological stage as variable respectively was used for differential expression analysis in GEPIA database. Log-rank test was used to perform univariate survival analysis in cohorts with different CXCLs expression levels. Spearman's correlation was used to describe the mRNA expression correlation of distinct CXCLs in cBioPortal database. The partial Spearman's correlation was used to evaluate the association between CXCLs expression and immune infiltration levels in TIMER database. The clinical relevance of tumor immune infiltrates was explored with a multivariable Cox proportional hazard model. The p-value < 0.05 was considered to be statistically significant.

3. Results

3.1. Dysregulated expression of CXCLs in CESC patients

Table 1. Effective variations in CXCLs expression at the transcription level between CESC and normal cervical tissues based on ONCOMINE database.

CXCLs	Types of CESC	Fold Change	*p*-value	Ref (PMID:)
CXCL1	HGCSIN	7.048	0.0000283	17974957 [24]
	CSCC	4.462	0.00000133	17974957 [24]
	CSCC	3.895	0.0000562	18506748 [25]
CXCL3	HGCSIN	2.797	0.00062	17974957 [24]
CXCL5	HGCSIN	5.994	0.002	17974957 [24]
CXCL6	HGCSIN	2.976	0.003	17974957 [24]
CXCL7	HGCSIN	7.048	0.0000283	17974957 [24]
	CSCC	4.462	0.00000133	17974957 [24]
	CSCC	3.895	0.0000562	18506748 [25]
CXCL8	CSCC	13.807	5.04E-09	18506748 [25]
	Cervical Cancer	7.47	3.14E-09	17510386 [27]
	CSCC	3.974	0.000000081	18191186 [26]
	CSCC	3.789	0.0000881	17974957 [24]
CXCL9	CSCC	8.529	8.45E-09	18191186 [26]
	CSCC	3.523	0.00000147	18506748 [25]
CXCL10	CSCC	3.982	4.89E-11	18191186 [26]
	CSCC	3.05	0.000115	18506748 [25]
CXCL11	CSCC	3.7	4.13E-16	18191186 [26]
	CSCC	3.555	0.0000209	18506748 [25]
CXCL13	CSCC	19.655	9.32E-11	18506748 [25]
	CSCC	5.835	0.0000349	17974957 [24]
CXCL16	Cervical Cancer	2.578	2.7E-10	17510386 [27]

CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; HGCSIN: High-Grade Cervical Squamous Intraepithelial Neoplasia; CSCC: Cervical Squamous Cell Carcinoma; CXCL: CXC chemokine; Ref: Reference.

We extracted the differential mRNA expression data of 16 CXCLs in cervical cancer and normal cervical tissues via the ONCOMINE database. As shown in Figure 1, the transcription levels of CXCL1/3/5/6/7/8/9/10/11/13/16 in cervical cancer were significantly higher than those in normal cervical tissues, while the transcription levels of CXCL12/14 were lower. We next compared their transcription levels in different types of cervical cancer versus normal cervical tissues. As Table 1 suggested, Zhai et al. found CXCL1/3/5/6/7 were overexpressed in high-grade cervical squamous intraepithelial neoplasia (HGCSIN) compared with normal tissues with fold changes of 7.048, 2.797, 5.994, 2.976 and 7.048 respectively. Besides, they reported the upregulation of CXCL1/7/8/13 in cervical squamous cell carcinoma (CSCC) with fold changes of 4.462, 4.462, 3.789 and 5.835 respectively [24]. Consistently, Scotto et al. also revealed the upregulated CXCL 1/7/8/9/10/11/13

mRNA expression in CSCC [25]. Biewenga also found significantly higher transcription levels of CXCL8/9/10/11 in CSCC in contrast with normal tissues [26]. Pyeon et al. found a 7.47-fold increase in CXCL8 (p = 3.14E-09) and a 2.578-fold increase in CXCL16 mRNA expression (p = 2.7E-10) in cervical cancer [27].

Furthermore, we compared the mRNA expression levels of CXCLs in CESC with normal tissues using the GEPIA database. As shown in Figure 2, the transcription levels of CXCL1/8/9/10/11/13/16/17 in CESC were significantly higher than those in normal samples, while the CXCL12 mRNA expression was significantly reduced in CSEC, which almost coincided with the results in ONCOMINE. We also analyzed the relative expression of CXCLs in CESC. We obtained that the relative expression of CXCL17 was the highest, while that of CXCL4 was the lowest (Figure 3).

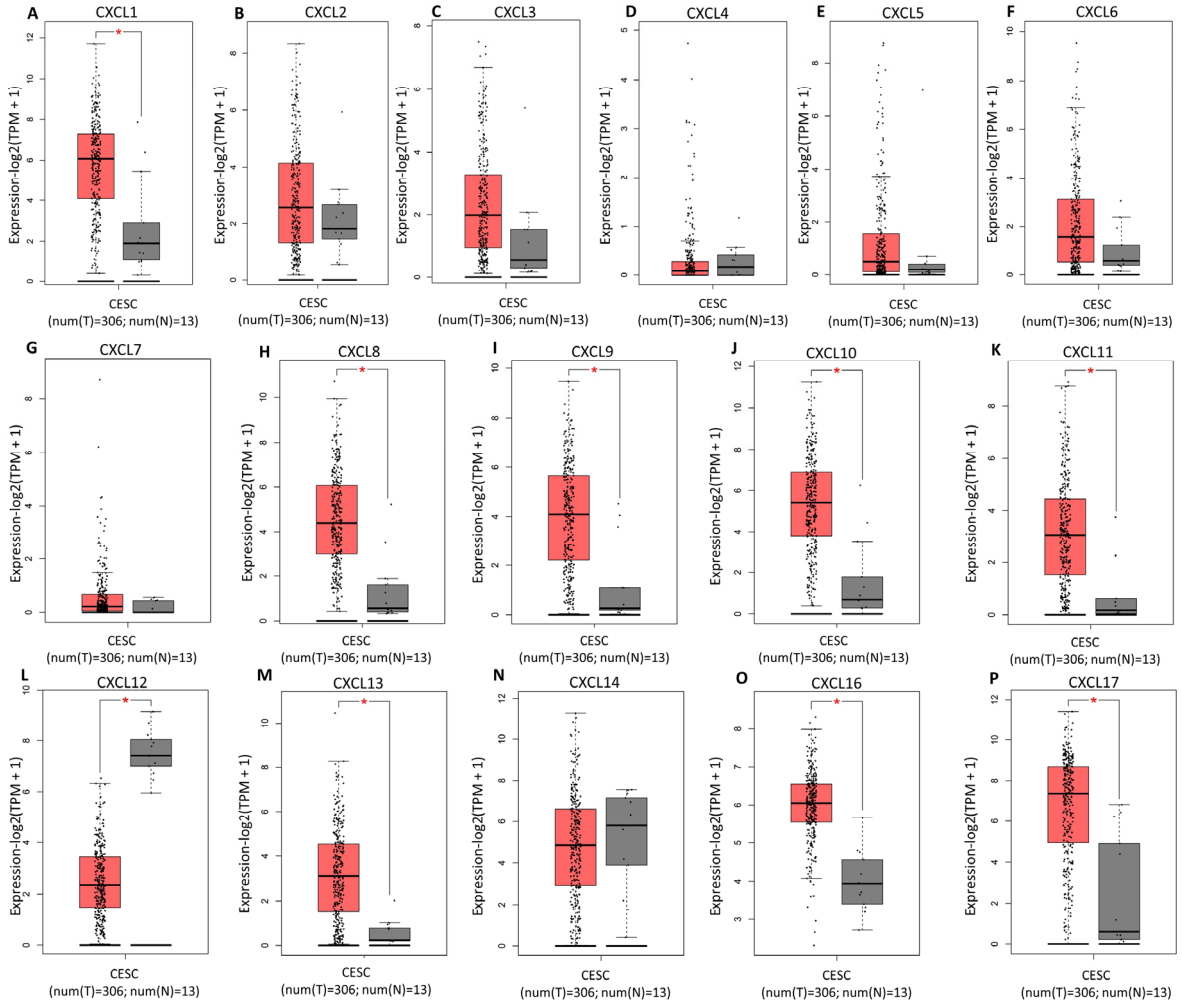


Figure 2. The comparison of CXCLs mRNA expression levels between CESC and normal cervical tissues based on GEPIA database. (A-P) depict mRNA expression box plots of CXCL1-17 respectively except CXCL15. 306 CESC samples were matched with 13 normal samples from TCGA and GTEx database. The expression data was converted to $\log_2$(TPM + 1) for differential analysis. Genes with higher $|\log_2 FC|$ values than 1 and lower p-values than 0.01 were considered differentially expressed genes. Log_2FC was defined as median (Tumor) - median (Normal). T: tumor; N: normal; TPM: transcripts per million reads; FC: fold change.

Asterisks indicate differential expression between CESC and normal control group.

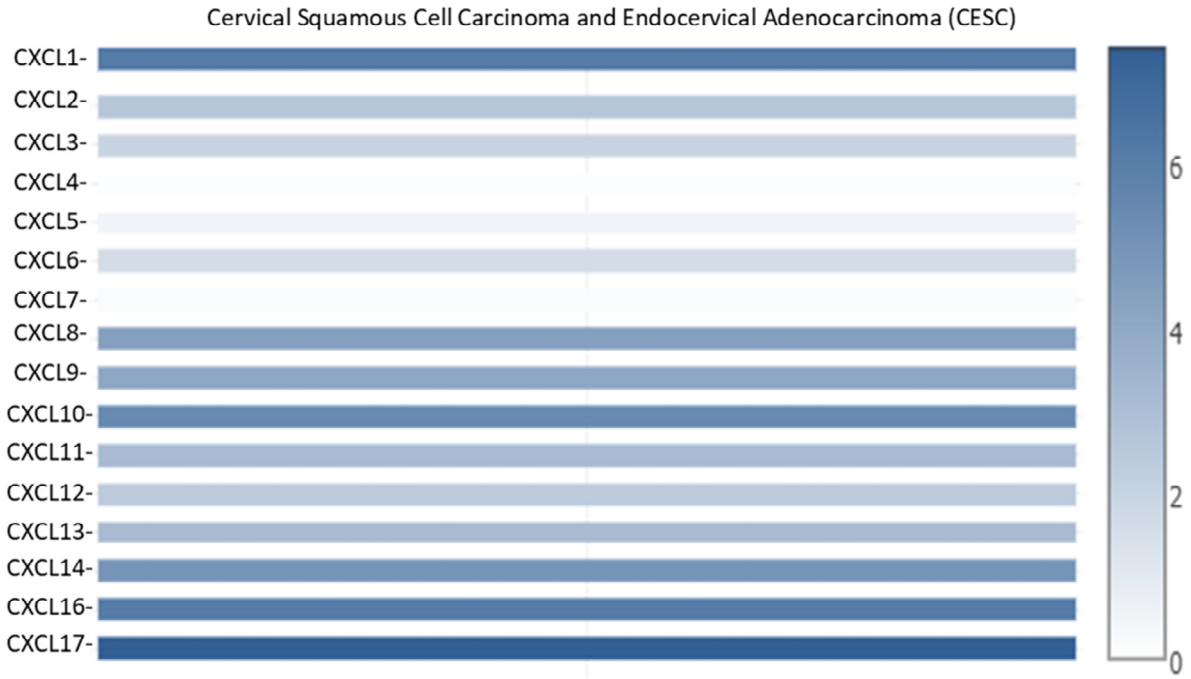


Figure 3. The relative expression levels of CXCLs in CESC based on multiple gene analysis module in GEPIA database. The right column indicates the median expression value of distinct CXCLs in CESC, which was transformed from $\log_2$(TPM + 1). The darker the color, the higher the corresponding expression level, which was lowest in CXCL4 (0.1) and highest in CXCL17 (7.4). The CESC sample size was 306.

3.2. Association of CXCLs mRNA expression with pathological stage and prognosis in CESC patients

We then explored the relationship between mRNA expression levels of CXCLs with the cancer pathological stage via GEPIA database. The results showed that no significant correlation was found in all CXCLs groups. The CXCLs expressed constantly along with the progression of CESC.

To study the prognostic values of distinct CXCLs in CESC, we analyzed the correlation between CXCLs mRNA expression levels with clinical outcome via GEPIA database. Figures 4 and 5 illustrate the OS and DFS curves, respectively. Patients with low expression 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-05) were significantly associated with favorable OS (Figure 4). Other CXCLs except these were found no significant effect on OS. Additionally, the DFS curves showed that low mRNA level of CXCL3 was associated with better DFS outcome (Figure 5).

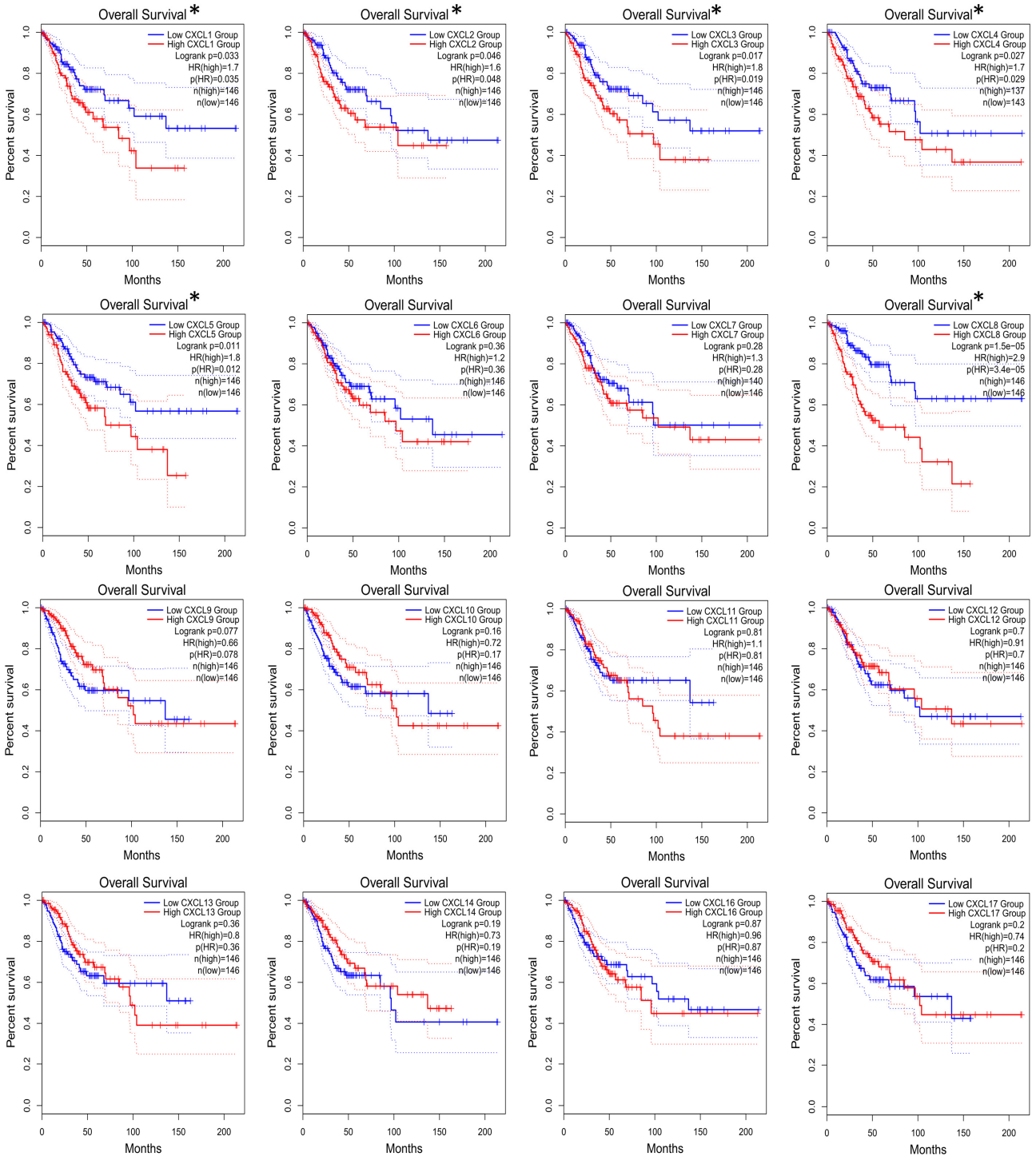


Figure 4. Prognostic value of distinct CXCLs in the overall survival (OS) outcome of CESC patients based on GEPIA database. The expression threshold for splitting high-expression and low-expression cohorts was set as the median. Samples with expression level higher than 50% were considered as the high-expression cohort, and the opposite were considered as the low-expression cohort. The sample size of high/low expression cohort was both 146. Log-rank test was used to perform the difference analysis. The hazards ratio (HR) was calculated based on Cox proportional hazard model. The dotted lines indicate the 95% confidence interval. *$p < 0.05$.

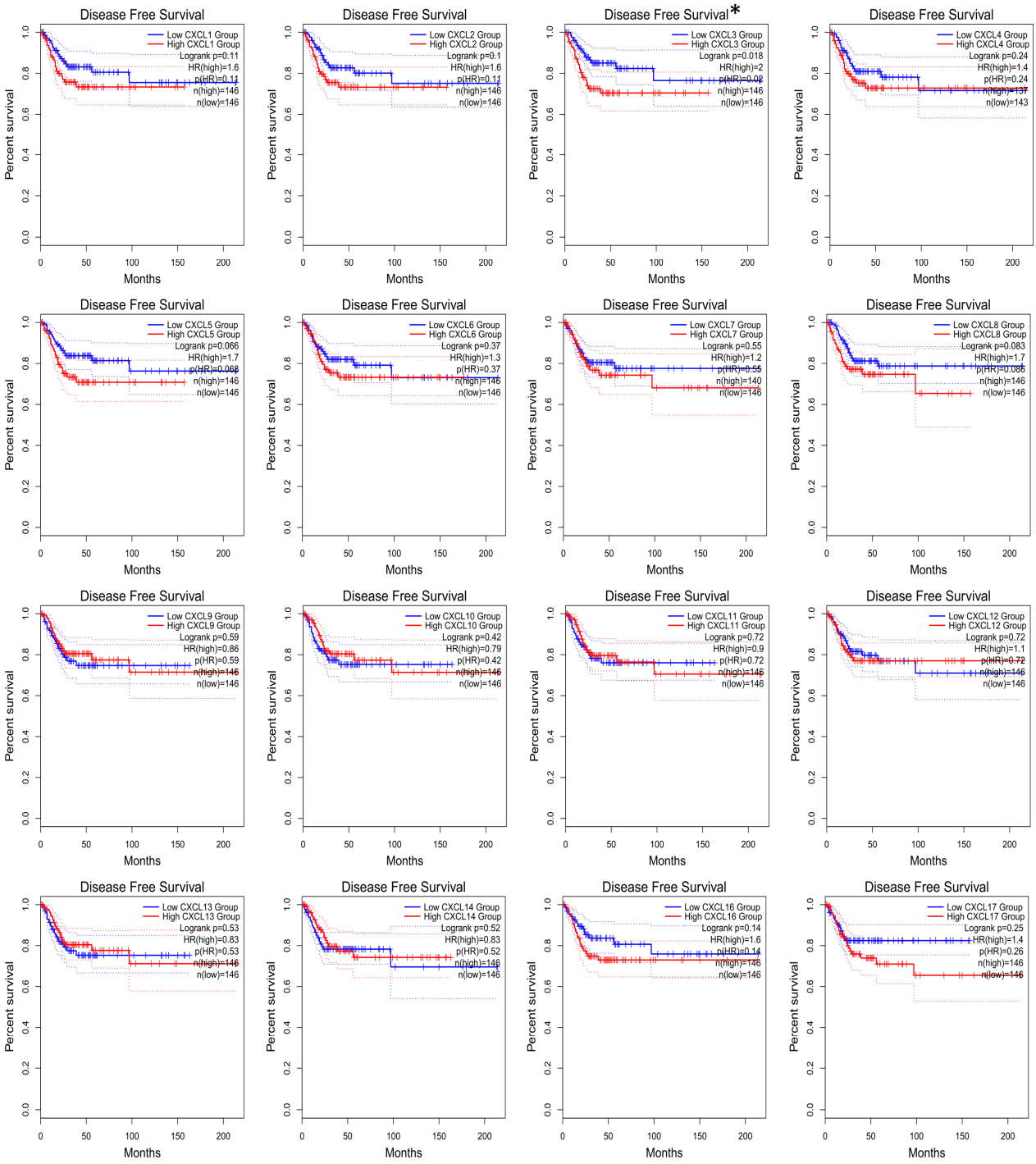


Figure 5. Prognostic value of distinct CXCLs in the disease-free survival (DFS) outcome of CESC patients based on GEPIA database. The threshold for splitting high/low expression cohort and statistical analysis was same as that in the OS analysis. *$p < 0.05$.

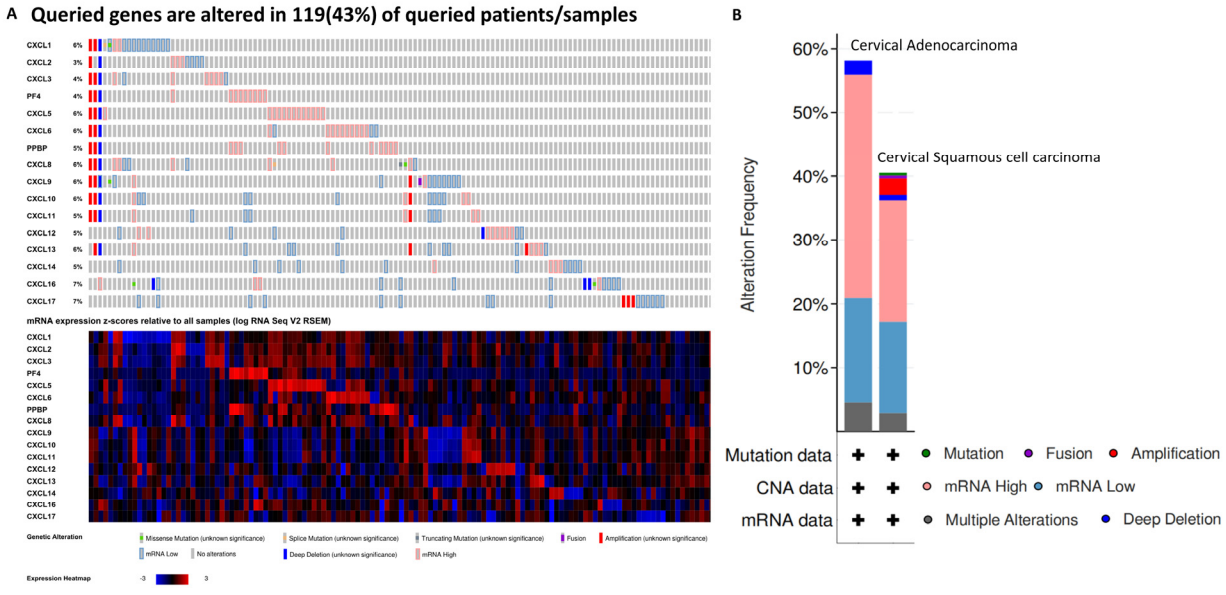
3.3. Genetic alteration, co-expression and key transcription regulators of CXCLs in CESC patients

We next explored the genetic alteration of the differentially expressed CXCLs in CESC via the cBioPortal database. As Figure 6A suggested, queried genes were altered in 119 (43%) of queried

patients/samples. The altered/profiled ratio of CXCL1-17 (except CXCL15) ranged from 3% to 7%. Several kinds of alterations were detected in cervical squamous cell carcinoma and cervical adenocarcinoma respectively, including mutation, fusion, deep deletion and so on (Figure 6B). Upregulated expression of CXCLs mRNA was the dominant alteration among the profiled samples.

Owing to the different expression levels of CXCLs in CESC patients, we tried to investigate if there were correlations among their expression. A Spearman's correlation heatmap was constructed (Figure 6C). The expression of CXCL1 was positively correlated to that of CXCL2/3/4/5/6/7/8/16 with different correlation coefficient. There was a modest to strong relation among the expression of CXCL1/2/3/6/8. CXCL9 was positively related to CXCL10/11/13, and a very strong correlation among the CXCL9/10/11/13 was showed. Besides, a weak relation among the CXCL12/13/14 was found.

Based on the differential expression of CXCLs in CESC and normal samples, we further analyzed the key regulators of CXCLs at transcription level in CESC via TRRUST. As Table 2 demonstrated, RELA, NFKB1, and SP1 were three key TFs targeting CXCLs. RELA and NFKB1 regulated the expression of CXCL1/10/12/2/5/8 (p-value = 4.22E-08 and 4.39E-08, respectively), while SP1 targeted CXCL1/14/5 (p-value = 0.00461).



C

	CXCL1	CXCL2	CXCL3	CXCL4	CXCL5	CXCL6	CXCL7	CXCL8	CXCL9	CXCL10	CXCL11	CXCL12	CXCL13	CXCL14	CXCL16	CXCL17
CXCL1	1.000	0.631	0.651	0.235	0.361	0.547	0.319	0.732	-0.108	-0.033	0.018	-0.156	-0.063	-0.242	0.237	0.133
CXCL2	0.631	1.000	0.862	0.281	0.487	0.254	0.351	0.632	-0.094	-0.002	0.076	-0.088	-0.078	-0.267	0.359	-0.069
CXCL3	0.651	0.862	1.000	0.358	0.510	0.295	0.436	0.604	-0.211	-0.118	-0.055	-0.125	-0.149	-0.182	0.298	-0.024
CXCL4	0.235	0.281	0.358	1.000	-0.024	0.116	0.458	0.262	-0.086	-0.141	-0.067	0.084	-0.044	-0.112	0.056	-0.138
CXCL5	0.361	0.487	0.510	-0.024	1.000	0.358	0.328	0.476	-0.073	-0.043	0.033	0.078	-0.064	-0.045	0.093	-0.227
CXCL6	0.547	0.254	0.295	0.116	0.358	1.000	0.185	0.448	0.043	0.064	0.083	0.103	0.019	-0.069	0.037	0.138
CXCL7	0.319	0.351	0.436	0.458	0.328	0.185	1.000	0.358	-0.091	-0.113	-0.055	-0.010	-0.061	-0.062	0.127	-0.086
CXCL8	0.732	0.632	0.604	0.262	0.476	0.448	0.358	1.000	-0.152	-0.040	0.001	-0.233	-0.132	-0.236	0.106	-0.069
CXCL9	-0.108	-0.094	-0.211	-0.086	-0.073	0.043	-0.091	-0.152	1.000	0.842	0.816	0.316	0.773	-0.031	0.124	0.062
CXCL10	-0.033	-0.002	-0.118	-0.141	-0.043	0.064	-0.113	-0.040	0.842	1.000	0.915	0.153	0.709	-0.0726	0.13	0.0481
CXCL11	0.018	0.076	-0.055	-0.067	0.033	0.083	-0.055	0.001	0.816	0.915	1.000	0.151	0.657	-0.124	0.177	-0.00458
CXCL12	-0.156	-0.088	-0.125	0.084	0.078	0.103	-0.010	-0.233	0.316	0.153	0.151	1.000	0.243	0.257	-0.0729	0.0336
CXCL13	-0.063	-0.078	-0.149	-0.044	-0.064	0.019	-0.061	-0.132	0.773	0.709	0.657	0.243	1.000	0.0379	0.135	0.0798
CXCL14	-0.242	-0.267	-0.182	-0.112	-0.045	-0.069	-0.062	-0.236	-0.031	-0.0726	-0.124	0.257	0.0379	1.000	-0.226	0.0589
CXCL16	0.237	0.359	0.298	0.056	0.093	0.037	0.127	0.106	0.124	0.13	0.177	-0.0729	0.135	-0.226	1.000	0.261
CXCL17	0.133	-0.069	-0.024	-0.138	-0.227	0.138	-0.086	-0.069	0.062	0.0481	-0.00458	0.0336	0.0798	0.0589	0.261	1.000

Figure 6. Genetic alteration and co-expression of CXCLs in CESC patients based on cBioPortal database. (A) OncoPrint summary of alteration and expression heatmap of CXCLs. (B) Cancer types summary of alteration in two subtypes of CESC. (C) A Spearman's correlation heat map of differentially expressed CXCLs in CESC.

Table 2. Key regulated factors of CXCLs in CESC based on TRRUST database.

Key TF	Description	Regulated gene	*P*-value	FDR
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	CXCL1, CXCL10, CXCL12, CXCL2, CXCL5, CXCL8	4.22E-08	6.58E-08
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	CXCL1, CXCL10, CXCL12, CXCL2, CXCL5, CXCL8	4.39E-08	6.58E-08
SP1	Sp1 transcription factor	CXCL1, CXCL14, CXCL5	0.00461	0.00461

3.4. Interaction and function enrichment analysis of CXCLs in CESC patients

To explore the neighbor genes and interaction among differentially expressed CXCLs in CESC, we extracted protein-protein interaction (PPI) network by GeneMANIA and STRING database. As reported by GeneMANIA, there were 20 nodes surrounding the 16 CXCLs members and displaying interactions in shared protein domains, co-expression, predicted, co-localization and pathways (Figure 7A). PF4V1 (platelet factor 4 variant 1), CX3CL1 (C-C motif chemokine ligand 3 like 1), and XCL2 (X-C motif chemokine ligand 2) were the top three CXCLs-related genes. GeneMANIA report also showed that these CXCLs and their associated genes functioned in terms of cell chemotaxis, chemokine or cytokine activity, and chemokine receptor or G-protein coupled receptor binding, with the most remarkable correlation with cytokine activity (FDR = 8.57e-83) (Figure 7A). We also isolated the top 50 neighbor genes of CXCLs as well as their interaction network using the GeneMANIA. As the results in Figure 8 suggested, CCL20, RBP4, TTLL2, CDC42EP2, SYT16, CCDC105, SAA1, TNF, NNMT, SAA2, GUCA2B, SLC16A3, IER3, GOLM1, AGR3, SPAG1, IL17C, GMDS, BCL2L15, TMC5, DRC1, C2orf50, RND1, VNN2, C9orf152, DNAH7, ARHGEF38, AP4B1, CFAP43, TUBB1, LY6G6F, RP11-302M6.4, AMELY, LIM2, FAM9C, HBG1, METTL21C, BEND2, VMO1, CMTM2, TAAR9, OR8B8, HGF, DIRAS3, PTGFR, PSPN, ITGA5, MMP9, SLAMF8, TFEC exhibited structural, expression or functional association with CXCLs in CESC. Moreover, as depicted in Figure 7B, a PPI network among CXCLs was conducted via the STRING database. 16 nodes and 111 edges were obtained with average local clustering coefficient = 0.958 and PPI enrichment p-value < 1.0e-16.

As the results above suggested, CXCLs played an important role in chemokine signaling pathway and inflammation response. To further validate the results, we performed function enrichment analysis. Under the condition of p.adj < 0.1 and $q$value < 0.2, the enrichment results included 14 MFs, 124 BPs, 11 CCs and 19 KEGG pathways. According to GO enrichment analysis, the molecular functions of CXCLs were mainly in chemokine activity, chemokine receptor binding and receptor ligand activity, involving the biological process about cell chemotaxis and granulocyte migration (Figure 7C), which were largely consistent with the results in GeneMANIA report. The cellular components mainly consisted of plasma lipoprotein particle, high-density lipoprotein particle and axonemal dynein complex (Figure 7C). KEGG pathway analysis showed that CXCLs and their neighbor genes involved the pathway closely related to cervical carcinogenesis and progression, including viral protein interaction with cytokine and cytokine receptor, cytokine-cytokine receptor interaction as well as chemokine signaling pathway (Figure 7D).

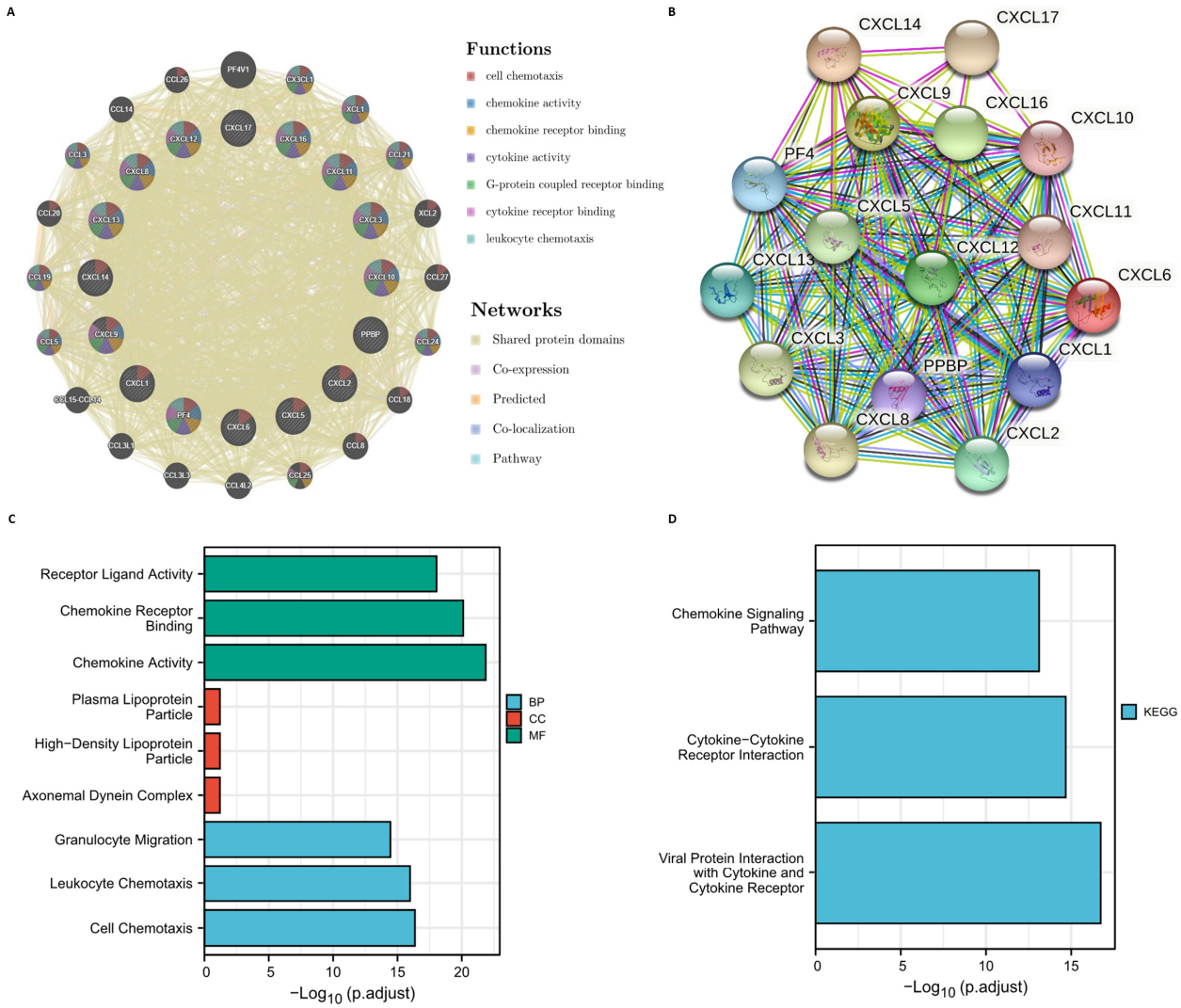


Figure 7. Neighbor gene network, interaction analysis and function enrichment analysis of differentially expressed CXCLs in CESC patients. (A) Gene-gene interaction network among CXCLs members based on GeneMANIA. (B) Protein-protein interaction network among CXCLs members based on STRING. (C) GO term enrichment analysis of CXCLs and top 50 neighbor genes in molecular function (GO-MF), cellular component (GO-CC) and biological process (GO-BP). (D) KEGG pathway analysis of CXCLs and top 50 neighbor genes.

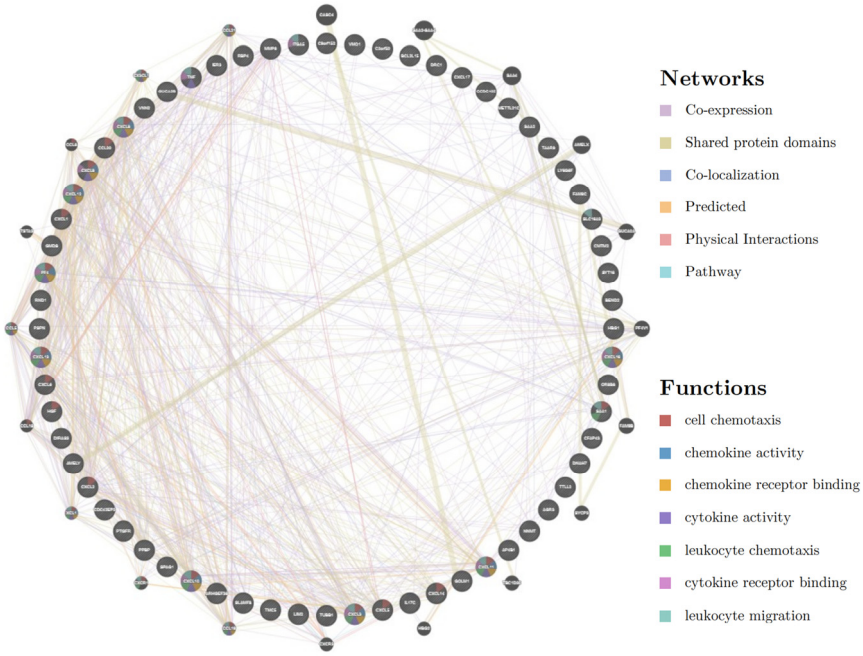


Figure 8. Interaction network among CXCLs and top 50 neighbor genes based on GeneMANIA database.

3.5. Tumor-infiltrating immune cells associated with CXCLs in CESC patients

The composition and abundance of immune cells in tumor microenvironment strongly affect the tumor progression and the effect of immunotherapy. Thus, as tumor microenvironment-related prognostic genes, we explored the correlation of CXCLs expression with immune infiltrating levels in CESC via TIMER database (Figure 9). The expression levels of CXCL1/2/6/9/10/11/12/13/14 were negatively correlated with tumor purity ($p < 0.05$), indicating their high expression in the tumor microenvironment. CXCL1 expression level showed an inverse correlation with the infiltration of CD4+ T cell (partial-cor = -0.201, p = 7.75e-04), macrophage (partial-cor = -0.175, p = 3.41e-03), and dendritic cell (partial-cor = -0.163, p = 6.61e-03). CXCL2 was negatively related with the infiltration of only CD4+ T cell (partial-cor = -0.198, p = 9.10e-04). CXCL3 expression level was inversely proportional to the infiltration level of CD4+ T cell (partial-cor = -0.224, p = 1.76e-04) and dendritic cell (partial-cor = -0.199, p = 9.00e-04). A positive relation was observed between CXCL4 expression level and macrophage infiltration level (partial-cor = 0.128, p = 3.34e-02), while there was a negative correlation with that of CD4+ T cell (partial-cor = -0.136, p = 2.37e-02). No significant relation was found between the expression of CXCL5/6/7 and the six types of immune cells infiltration ($p > 0.05$). CXCL9/13 expression levels were positively related to almost all immune cells except the macrophage. Additionally, except for B cell, CXCL10/11 expression levels were significantly associated with all immune cell types (all $p < 0.05$). CXCL12 expression correlated positively with the infiltration of B cell (partial-cor = 0.385, p = 3.41e-11), CD4+ T cell (partial-cor = 0.342, p = 5.04e-09), macrophage (partial-cor = 0.439, p = 1.74e-14) and dendritic cell (partial-cor = 0.19, p = 1.52e-03). As for CXCL14, the infiltration levels of B cell, macrophage and neutrophil had significant relation in CESC patients. CXCL16 expression had a positive relation to almost all immune cells except B cell and macrophage.

CXCL17 expression was closely tied with only the infiltration of CD4+ T cell (partial-cor = 0.19, p = 1.48e-03) and neutrophil (partial-cor = 0.174, p = 3.58e-03).

We further evaluated the influence of CXCLs expression and six tumor-infiltrating immune cells on survival of CESC patients using Cox proportional hazard model via TIMER database (Table 3). The results showed tumor- infiltrating CD8+ T cell was significantly related to the clinical outcome of CESC patients (p = 0.040), which was a protective factor for the survival of CESC patients (coefficient = -5.291, Hazard Ratio = 0.005). Other immune cells and CXCL members showed no significant correlation with the survival of CESC (p > 0.05).

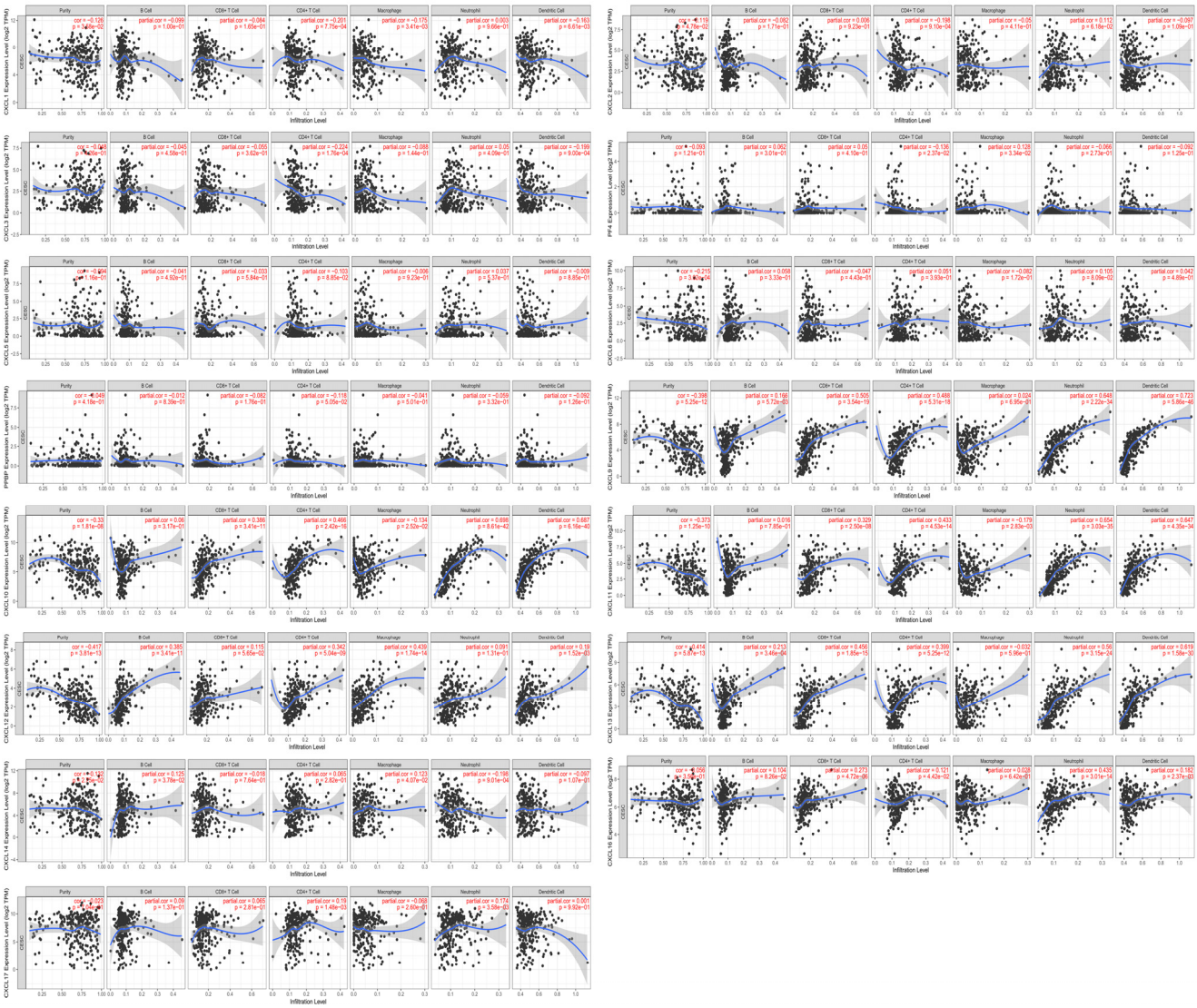


Figure 9. Correlation of CXCLs expression with tumor purity and immune cell infiltration levels in CESC patients based on TIMER database. The relationships between CXCLs expression levels and tumor purity, as well as tumor-infiltration levels of B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil and dendritic cell were displayed. The plots showed the purity-corrected partial Spearman's correlation (partial-cor) and p-value. The number of CESC patients was 306.

Table 3. The Cox proportional hazard model of CXCLs and six tumor-infiltrating immune cells in CESC based on TIMER database.

	coef	HR	95%CI_l	95%CI_u	*p*-value
B cell	-3.103	0.045	0.000	554.044	0.519
CD8 + T cell*	-5.291	0.005	0.000	0.791	0.040
CD4 + T cell	-6.207	0.002	0.000	8.421	0.145
Macrophage	1.536	4.645	0.001	24566.920	0.726
Neutrophil	-7.691	0.000	0.000	43.505	0.189
Dendritic	4.950	141.192	0.739	26979.025	0.065
CXCL1	0.104	1.109	0.908	1.355	0.310
CXCL2	0.003	1.003	0.760	1.325	0.982
CXCL3	0.116	1.123	0.843	1.495	0.428
PF4 (CXCL4)	0.222	1.248	0.868	1.794	0.231
CXCL5	0.030	1.030	0.890	1.192	0.689
CXCL6	-0.066	0.936	0.799	1.097	0.414
PPBP (CXCL7)	-0.247	0.781	0.590	1.034	0.084
CXCL9	-0.123	0.884	0.660	1.184	0.408
CXCL10	-0.145	0.865	0.622	1.201	0.386
CXCL11	0.323	1.381	0.991	1.923	0.056
CXCL12	0.177	1.193	0.950	1.499	0.128
CXCL13	0.055	1.056	0.850	1.313	0.621
CXCL14	-0.100	0.905	0.810	1.010	0.075
CXCL16	-0.200	0.818	0.572	1.171	0.273
CXCL17	0.015	1.015	0.896	1.150	0.815

Coef: coefficient; HR: hazard ratio; 95%CI_l: lower 95% confidential interval; 95%CI_u: upper 95% confidential interval; PF-4: platelet factor-4, also known as CXCL4; PPBP: pro-platelet basic protein, also known as CXCL7. $^{*}p < 0.05$.

4. Discussion

Cervical cancer, as the second most prevalent female malignancy, brings a great health and economic burden to women worldwide [28]. CESC is the leading pathological type of cervical cancer. According to the statistics, the 5-year survival rate for advanced cervical cancer is less than 50% in some areas [29]. Therefore, oncologists have been trying to search for therapeutic targets and prognostic biomarkers to improve the survival of CESC patients, such as novel genes [30], mRNA [31] and protein [32]. Tumor microenvironment has been a hotspot recently. As important components of tumor microenvironment, CXC chemokines have been widely reported to regulate tumor growth, metastasis and tumor-related immunity directly or indirectly [33]. However, the expression and roles of CXCLs in CESC are rarely investigated to date. In this study, we explored the potential value of CXCLs as therapeutic targets and prognostic biomarkers in CESC patients using integrative bioinformatic methods with the benefit of various public online databases.

Our results showed the upregulated mRNA expression levels of CXCL1/8/9/10/11/13/16/17 in CESC via GEPIA database, although there is no significant correlation between the expression levels

and pathological stage. A number of studies have elucidated the disorder of CXCL expression and their critical role in tumor occurrence and progression [34–36]. For example, Paczek et al. revealed that serum CXCL8 levels of colorectal cancer patients were higher than that of healthy controls, and they were significantly correlated with tumor stages and metastases [37]. CXCLs exert their effects on the tumor via direct as well as indirect ways. Tokunaga et al. reported that cancer cells-derived CXCL9/10/11 functioned as autocrine signals and bound to their own CXCR3A, thereby directly promoting cell proliferation and metastasis [38]. Besides, CXCL3-transfected cervical cancer Hela cells exhibited enhanced proliferation and migration as well as downregulated apoptosis via an autocrine way [39]. On the other hand, CXCLs are primarily responsible for various immune cells chemotaxis. CXCL9/10/11 can recruit CD8+ T cells, T helper 1 (Th1) cells and natural killer (NK) cells to tumor site by binding to CXCR3 on their surface, and further restrain colorectal cancer development [40]. On the contrary, CXCL9/10/11 can also promote Th2, Treg and myeloid derived suppressor cells migration to tumor site and establish a microenvironment conducive to tumor growth [38]. Thus, the abnormal expression of CXCLs would affect the attracted immune cells exerting right function in the right site. Although more mechanism researches are needed, dysregulated CXCLs-CXCRs axis may be promising interference targets to inhibit the cancer cells directly or indirectly by activating anti-tumor immune response.

In our study, high expression of CXCL1/2/3/4/5/8 were significantly associated with poor OS, similarly, low mRNA level of CXCL3 was associated with better DFS, indicating their potential as prognostic biomarkers of CESC. In various human cancers, CXCLs were reported to be linked with prognosis. For example, advanced high-grade serous ovarian cancer patients with high CXCL9/10 expression exhibited improved survival [41]. High serum CXCL13 level in penile cancer patients was associated with unfavorable DFS [42]. Hepatocellular carcinoma patients with high CXCL10/12/14 expression implied favorable survivals [43]. However, above CXCLs showed no significant correlation with the survival of CESC patients in our study. Renal cell carcinoma patients with high CXCL1/2/3/4/5/6/8 levels had shorter DFS and OS [44], which was largely consistent with our results. Notably, CXCL1/2/3/4/5/6/8 except CXCL4 all belong to ELR^+ CXC chemokines and exhibit pro-angiogenesis and pro-metastasis ability [7,45,46], which may partly explain the poor clinical outcome of patients with overexpressed CXCL1/2/3/5/6/8. CXCL4, also known as platelet factor-4 (PF-4), is an ELR^- CXC chemokine mainly derived from platelets [47]. It exerts effects by binding to CXCR3 on target cells. Depending on the presence of splice variants CXCR3A or CXCR3B, it promotes endothelial cells survival (CXCR3A) or apoptosis (CXCR3B). It was reported that CXCL4 showed high affinity binding to CXCR3B but not CXCR3A isoform, which may account for its angiostatic effects [48]. Consistently, high CXCL1/3/5/8 levels were reported to be linked with poor overall survival in hepatocellular carcinoma patients, while differently, high CXCL2 level was showed to be correlated with better survival [49]. Therefore, more studies are still needed to validate the exact prognostic value of CXCLs in CESC patients.

In addition, we observed high mutation rate (43%) of CXCLs in CESC patients. The most mutation we detected was abnormal CXCLs mRNA expression. Next, we found RELA, NFKB1, and SP1 were three key TFs regulating the expression of CXCLs at transcription level. RELA and NFKB1 are two subunits of NF-κB TFs with and without a transcription activation domain respectively. NF-κB signaling pathway regulates immune response and apoptosis, thereby involving in carcinogenesis [50]. SP 1 was reported to activate various long noncoding RNAs (lncRNAs) transcription thereby promoting cervical cancer proliferation and invasion [51–53]. Our

study proposed a new perspective that SP1 may participate in the occurrence and development of cervical cancer by regulating the expression of CXCLs.

Numerous studies have illustrated the importance of immune response in carcinogenesis. It is widely accepted that the pathogenesis of cervical cancer is aetiologically associated with the persistent infection of high-risk human papillomavirus (HPV) [54], which is highly immunogenic and stimulates chronic inflammation. The immune cells attracted to the local site secrete proinflammatory cytokines, triggering various signaling pathways then facilitating the reactive oxygen species (ROS) formation, which may cause DNA damage. HPV infection-derived genetic instability and the inflammation-mediated DNA damage provide conditions for viral DNA integrating to host genomes and subsequent immune evasion [55,56]. In our study, interaction and function analysis elucidated the close involvement of distinct CXCLs and their neighbor genes in chemokine signaling pathway and inflammation response, such as cell chemotaxis, chemokine or cytokine activity, and viral protein interaction with cytokine and cytokine receptor. Besides, CXCLs expression levels were found highly relevant to immune cell infiltration levels. Therefore, our study provided the information that CXCLs may be potential immunotherapeutic targets from the perspective of inflammation response.

5. Conclusions

Our study elucidated the dysregulated expression of CXCLs in CESC patients via bioinformatic methods, and provided a potential insight to target CXCLs in CESC treatment. Besides, we found that overexpressed CXCL1/2/3/4/5/8 could be candidates to predict shorter survival of CESC patients. However, we analyzed the overall survival rather than CESC specific survival which may be more representative with less confounding bias. In addition, we can't avoid a quite common failing of bioinformatic analysis; we measured the mRNA expression level of CXCLs which may not represent global expression status of CXCLs. Also, more fundamental experiments are necessary to explore the exact role of CXCLs in CESC and underlying molecular mechanism. Further clinical studies to confirm their accuracy in prognosis prediction as well as effectiveness as therapeutic targets are required before application in clinical practice.

Acknowledgments

There was no funding support for this study.

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. M. Arbyn, E. Weiderpass, L. Bruni, S. de Sanjosé, M. Saraiya, J. Ferlay, et al., Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis, *The Lancet Global Health*, **8** (2020), e191–e203.
2. M. Vu, J. Yu, O. A. Awolude, L. Chuang, Cervical cancer worldwide, *Curr. Probl. Cancer*, **42** (2018), 457–465.

3. W. J. Small, M. A. Bacon, A. Bajaj, L. T. Chuang, B. J. Fisher, M. M. Harkenrider, et al., Cervical cancer: A global health crisis, *Cancer*, **123** (2017), 2404–2412.
4. K. J. Park, Cervical adenocarcinoma: integration of HPV status, pattern of invasion, morphology and molecular markers into classification, *Histopathology*, **76** (2020), 112–127.
5. J. D. Wright, K. Matsuo, Y. Huang, A. I. Tergas, J. Y. Hou, F. Khoury-Collado, et al., Prognostic Performance of the 2018 International Federation of Gynecology and Obstetrics Cervical Cancer Staging Guidelines, *Obstet Gynecol.*, **134** (2019), 49–57.
6. J. M. Schmitz, V. J. McCracken, R. A. Dimmitt, R. G. Lorenz, Expression of CXCL15 (Lungkine) in murine gastrointestinal, urogenital, and endocrine organs, *J. Histochem. Cytochem.*, **55** (2007), 515–524.
7. S. Cabrero-de L. Heras, E. Martinez-Balibrea, CXC family of chemokines as prognostic or predictive biomarkers and possible drug targets in colorectal cancer, *World J. Gastroenterol.*, **24** (2018), 4738–4749.
8. J. Vandercappellen, J. Van Damme, S. Struyf, The role of CXC chemokines and their receptors in cancer, *Cancer Lett.*, **267** (2008), 226–244.
9. N. H. Lee, M. Nikfarjam, H. He, Functions of the CXC ligand family in the pancreatic tumor microenvironment, *Pancreatology*, **18** (2018), 705–716.
10. N. Nagaya, G. T. Lee, S. Horie, I. Y. Kim, CXC chemokine/receptor axis profile and metastasis in prostate cancer, *Front. Mol. Biosci.*, **7** (2020), 579874.
11. K. Liu, M. Lai, S. Wang, K. Zheng, S. Xie, X. Wang, Construction of a CXC chemokine-based prediction model for the prognosis of colon cancer, *Biomed. Res. Int.*, **2020** (2020), 6107865.
12. C. Li, H. Deng, Y. Zhou, Y. Ye, S. Zhao, S. Liang, et al., Expression and clinical significance of CXC chemokines in the glioblastoma microenvironment, *Life Sci*., **261** (2020), 118486.
13. X. Li, Q. Zhong, D. Luo, Q. Du, W. Liu, The prognostic value of CXC subfamily ligands in stage I-III patients with colorectal cancer, *PLoS One*, **14** (2019), e0214611.
14. W. Zhang, Q. Wu, C. Wang, L. Yang, P. Liu, C. Ma, AKIP1 promotes angiogenesis and tumor growth by upregulating CXC-chemokines in cervical cancer cells, *Mol. Cell Biochem.*, **448** (2018), 311–320.
15. H. M. Ding, H. Zhang, J. Wang, J. H. Zhou, F. R. Shen, R. N. Ji, et al., miR302c3p and miR520a3p suppress the proliferation of cervical carcinoma cells by targeting CXCL8, *Mol. Med. Rep.*, **23** (2021).
16. D. R. Rhodes, S. Kalyana-Sundaram, V. Mahavisno, R. Varambally, J. Yu, B. B. Briggs, et al., Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles, *Neoplasia*, **9** (2007), 166–180.
17. Z. Tang, C. Li, B. Kang, G. Gao, C. Li, Z. Zhang, GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, *Nucleic Acids Res.*, **45** (2017), W98–W102.
18. A. Nagy, A. Lanczky, O. Menyhart, B. Gyorffy, Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets, *Sci. Rep.*, **8** (2018), 9227.
19. J. Gao, B. A. Aksoy, U. Dogrusoz, G. Dresdner, B. Gross, S. O. Sumer, et al., Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, *Sci. Signal*, **6** (2013), pl1.
20. H. Han, J. W. Cho, S. Lee, A. Yun, H. Kim, D. Bae, et al., TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions, *Nucleic Acids Res.*, **46** (2018), D380–D386.
21. M. Franz, H. Rodriguez, C. Lopes, K. Zuberi, J. Montojo, G. D. Bader, et al., GeneMANIA update 2018, *Nucleic Acids Res.*, **46** (2018), W60–W64.

22. D. Szklarczyk, A. L. Gable, D. Lyon, A. Junge, S. Wyder, J. Huerta-Cepas, et al., STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets, *Nucleic Acids Res.*, **47** (2019), D607–D613.
23. T. Li, J. Fu, Z. Zeng, D. Cohen, J. Li, Q. Chen, et al., TIMER2.0 for analysis of tumor-infiltrating immune cells, *Nucleic Acids Res.*, **48** (2020), W509–W514.
24. Y. Zhai, R. Kuick, B. Nan, I. Ota, S. J. Weiss, C. L. Trimble, et al., Gene expression analysis of preinvasive and invasive cervical squamous cell carcinomas identifies HOXC10 as a key mediator of invasion, *Cancer Res.*, **67** (2007), 10163–10172.
25. L. Scotto, G. Narayan, S. V. Nandula, H. Arias-Pulido, S. Subramaniyam, A. Schneider, et al., Identification of copy number gain and overexpressed genes on chromosome arm 20q by an integrative genomic approach in cervical cancer: potential role in progression, *Genes Chromosomes Cancer*, **47** (2008), 755–765.
26. P. Biewenga, M. R. Buist, P. D. Moerland, E. Ver Loren van Themaat, A. H. van Kampen, F. J. ten Kate, et al., Gene expression in early stage cervical cancer, *Gynecol. Oncol.*, **108** (2008), 520–526.
27. D. Pyeon, M. A. Newton, P. F. Lambert, J. A. den Boon, S. Sengupta, C. J. Marsit, et al., Fundamental differences in cell cycle deregulation in human papillomavirus-positive and human papillomavirus-negative head/neck and cervical cancers, *Cancer Res.*, **67** (2007), 4605–4619.
28. G. Menderes, J. Black, C. L. Schwab, A. D. Santin, Immunotherapy and targeted therapy for cervical cancer: an update, *Exp. Rev. Anticancer Ther.*, **16** (2016), 83–98.
29. M. Wassie, Z. Argaw, Y. Tsige, M. Abebe, S. Kisa, Survival status and associated factors of death among cervical cancer patients attending at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia: a retrospective cohort study, *BMC Cancer*, **19** (2019), 1221.
30. J. Liu, S. Nie, M. Gao, Y. Jiang, Y. Wan, X. Ma, et al., Identification of EPHX2 and RMI2 as two novel key genes in cervical squamous cell carcinoma by an integrated bioinformatic analysis, *J. Cell Physiol.*, **234** (2019), 21260–21273.
31. H. Ding, X. X. Xiong, G. L. Fan, Y. X. Yi, Y. R. Chen, J. T. Wang, et al., The new biomarker for cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) based on public database mining, *Biomed. Res. Int.*, **2020** (2020), 5478574.
32. Q. Xie, W. Ou-Yang, M. Zhang, H. Wang, Q. Yue, Decreased expression of NUSAP1 predicts poor overall survival in cervical cancer, *J. Cancer.*, **11** (2020), 2852–2863.
33. A. Bikfalvi, C. Billottet, The CC and CXC chemokines: major regulators of tumor progression and the tumor microenvironment, *Am. J. Phys. Cell Phys..*, **318** (2020), C542–C554.
34. Q. Zeng, S. Sun, Y. Li, X. Li, Z. Li, H. Liang, Identification of therapeutic targets and prognostic biomarkers among CXC chemokines in the renal cell carcinoma microenvironment, *Front. Oncol.*, **9** (2019), 1555.
35. Y. Li, T. Wu, S. Gong, H. Zhou, L. Yu, M. Liang, et al., Analysis of the prognosis and therapeutic value of the CXC chemokine family in head and neck squamous cell carcinoma, *Front. Oncol.*, **10** (2020), 570736.
36. X. Chen, R. Chen, R. Jin, Z. Huang, The role of CXCL chemokine family in the development and progression of gastric cancer, *Int. J. Clin. Exp. Pathol.*, **13** (2020), 484–492.
37. S. Paczek, M. Lukaszewicz-Zajac, M. Gryko, P. Mroczko, A. Kulczynska-Przybik, B. Mroczko, CXCL-8 in preoperative colorectal cancer patients: significance for diagnosis and cancer progression, *Int. J. Mol. Sci.*, **21** (2020).

38. R. Tokunaga, W. Zhang, M. Naseem, A. Puccini, M. D. Berger, S. Soni, et al., CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation–A target for novel cancer therapy, *Cancer Treat. Rev.*, **63** (2018), 40–47.
39. Y. L. Qi, Y. Li, X. X. Man, H. Y. Sui, X. L. Zhao, P. X. Zhang, et al., CXCL3 overexpression promotes the tumorigenic potential of uterine cervical cancer cells via the MAPK/ERK pathway, *J. Cell Physiol.*, **235** (2020), 4756–4765.
40. T. J. Zumwalt, M. Arnold, A. Goel, C. R. Boland, Active secretion of CXCL10 and CCL5 from colorectal cancer microenvironments associates with GranzymeB+ CD8+ T-cell infiltration, *Oncotarget*, **6** (2015), 2981–2991.
41. H. Bronger, J. Singer, C. Windmüller, U Reuning, D Zech, C Delbridge, et al., CXCL9 and CXCL10 predict survival and are regulated by cyclooxygenase inhibition in advanced serous ovarian cancer, *Br. J. Cancer*, **115**(2016), 553–563.
42. M. Mo, S. Tong, T. Li, X. Zu, X. Hu, Serum CXCL13 level is associated with tumor progression and unfavorable prognosis in penile cancer, *Oncol. Targets Ther.*, **13** (2020), 8757–8769.
43. T. Lin, E. Zhang, P. P. Mai, Y. Z. Zhang, X. Chen, L. S. Peng, CXCL2/10/12/14 are prognostic biomarkers and correlated with immune infiltration in hepatocellular carcinoma, *Biosci. Rep.*, **41** (2021).
44. M. Dufies, O. Grytsai, C. Ronco, O. Camara, D. Ambrosetti, A. Hagege, et al., New CXCR1/CXCR2 inhibitors represent an effective treatment for kidney or head and neck cancers sensitive or refractory to reference treatments, *Theranostics*, **9** (2019), 5332–5346.
45. J. H. Distler, M. H. A. Fau-Kurowska-Stolarska, R. E. K. M. Fau-Gay, S. G. R. Fau-Gay, O. G. S. Fau-Distler, O. Distler, Angiogenic and angiostatic factors in the molecular control of angiogenesis, *Q J. Nucl. Med.*, **47** (2003), 149–161.
46. G. Opdenakker, J. Van Damme, The countercurrent principle in invasion and metastasis of cancer cells. Recent insights on the roles of chemokines, *Int. J. Dev. Biol.*, **48** (2004), 519–527.
47. P. Ruytinx, P. Proost, S. Struyf, CXCL4 and CXCL4L1 in cancer, *Cytokine*, **109** (2018), 65–71.
48. L. Lasagni, M. Francalanci, F. Annunziato, E. Lazzeri, S. Giannini, L. Cosmi, et al., An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4, *J. Exp. Med.*, **197** (2003), 1537–1549.
49. Y. H. Wang, J. H. Huang, Z. F. Tian, Y. F. Zhou, J. Yang, The role of CXC cytokines as biomarkers and potential targets in hepatocellular carcinoma, *Math. Biosci. Eng.*, **17** (2019), 1381–1395.
50. J. Concetti, C. L. Wilson, NFKB1 and Cancer: Friend or Foe?, *Cells*, **7** (2018).
51. L. Zhang, S. K. Liu, L. Song, H. R. Yao, SP1-induced up-regulation of lncRNA LUCAT1 promotes proliferation, migration and invasion of cervical cancer by sponging miR-181a, *Artif. Cells Nanomed. Biotechnol.*, **47** (2019), 556–564.
52. S. Chang, L. Sun, G. Feng, SP1-mediated long noncoding RNA POU3F3 accelerates the cervical cancer through miR-127-5p/FOXD1, *Biomed. Pharmacother.*, **117** (2019), 109133.
53. W. G. Ma, S. M. Shi, L. Chen, G. Lou, X. L. Feng, SP1-induced lncRNA FOXD3-AS1 contributes to tumorigenesis of cervical cancer by modulating the miR-296-5p/HMGA1 pathway, *J. Cell Biochem.*, **122** (2021), 235–248.
54. E. J. Crosbie, M. H. Einstein, S. Franceschi, H. C. Kitchener, Human papillomavirus and cervical cancer, *Lancet*, **382** (2013), 889–899.

55. S. R. Georgescu, C. I. Mitran, M. I. Mitran, C. Caruntu, M. I. Sarbu, C. Matei, et al., New insights in the pathogenesis of HPV infection and the associated carcinogenic processes: the role of chronic inflammation and oxidative stress, *J. Immunol. Res.*, **2018** (2018), 5315816.
56. V. M. Williams, U. F. M. Fau-Soto, P. J. S. U Fau-Duerksen-Hughes, P. J. Duerksen-Hughes, HPV-DNA integration and carcinogenesis: putative roles for inflammation and oxidative stress, *Future Virol.*, **6** (2011), 45–57.

AIMS Press