Original Article

Functions of CXC chemokines as biomarkers and potential therapeutic targets in the hepatocellular carcinoma microenvironment

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Background: Several studies have indicated that CXC chemokines influence the prognosis and therapy in patients with hepatocellular carcinoma (HCC). However, there are limited studies on the roles of CXC chemokines in HCC based on data acquired from various databases. This study aimed to conduct an in-depth and comprehensive bioinformatic analysis of the expression and functions of CXC chemokines in HCC.

Methods: Data was obtained from various databases including ONCOMINE, UALCAN, STRING, GeneMinia, DAVID, Kaplan-Meier plotter, TIMER, GSCALite and NetworkAnalyst for the analysis of the expression and functions of the CXC chemokines in HCC.

Results: Analysis of the differential expression levels of CXC chemokines between HCC and adjacent normal tissues revealed that the mRNA expression levels of CXCL1/2/5/6/7/12/14 were significantly lower in HCC tissues than those in adjacent normal tissues, whereas the mRNA expression levels of CXCL9/16/17 were significantly higher in HCC tissues. Analysis of the relationship between CXC chemokines and overall survival revealed that high mRNA expression levels of CXCL1/3/5/6/8 were associated with poor overall survival, whereas high mRNA expression levels of CXCL2/4/7/9/10/12 were associated with better overall survival. The functions of CXC chemokines and related genes were associated with cytokine-cytokine receptor interactions and chemokine signaling pathway. Analysis of the association between CXC chemokines and activity of cancer pathways indicated that the DNA damage response and hormone androgen receptor (AR) signaling pathways were inhibited, whereas apoptosis, epithelial-mesenchymal transition (EMT) and Ras/mitogen-activated protein kinase (MAPK) signaling pathways were activated. The expression of CXC chemokines was positively correlated with the infiltration of six types of immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells).

Conclusions: This study has demonstrated that CXC chemokines can influence survival of patients with HCC by recruiting different types of immune cells into the tumor microenvironment.

Keywords: Hepatocellular carcinoma (HCC); chemokine; bioinformatics analysis; biomarker; prognosis

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Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, and is the fifth most common cancer globally and the third leading cause of cancer-related mortality (1). Numerous studies have indicated that hepatitis virus infection is a principal risk factor of the occurrence and progression of HCC (2). Several patients are diagnosed at the advanced stages of HCC due to the undefined symptoms of early-stage HCC, thereby missing the opportunity to receive hepatectomy and liver transplantation (3,4). In addition, the high rates of recurrence and metastasis in patients after resection contribute to poor prognosis (5,6). Therefore, it is necessary to investigate the underlying molecular mechanisms of HCC to enhance precise diagnosis and targeted treatment.

Studies conducted in the recent past have revealed that various chemokines within the tumor microenvironment affect the initiation and progression of HCC by binding to their receptors (7,8). Chemokines are small secreted proteins (8–12 kDa), which can significantly affect various aspects of the development of HCC including recruitment and infiltration of immune cells, modulating function of immune cells, promoting metastasis of tumor cells, influencing effects of immunotherapies, and regulating angiogenesis (9-12). Chemokines are subdivided into four classes based on the location of the first two N-terminal cysteine residues: CXC chemokines, CC chemokines, C chemokines and CX3C chemokines (13). CXC chemokine-chemokine receptor signaling pathway can affect the progression and therapy of HCC both directly and indirectly (14-18).

In this study, we conducted an in-depth bioinformatic analysis of 16 CXC chemokines (CXCL1-CXCL17, excluding CXCL15) based on data extracted from several public databases. An analysis of the differential expression levels of CXC chemokines in HCC tissues in comparison with adjacent normal tissues was performed, and the association between the expression levels of CXC chemokines and overall survival of patients with HCC explored. In addition, we investigated the roles of CXC chemokines in recruiting immune cells and activation of the cancer pathway. Moreover, external data were downloaded from the Gene Expression Omnibus (GEO) database to validate the results obtained from The Cancer Genome Atlas (TCGA) database. With the development of second-generation sequencing technology and the establishment of online databases, this study will help to improve the accuracy of evaluating prognosis and the efficiency of adopting therapies for patients with HCC.

We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/tcr-21-127).

Methods

ONCOMINE (www.oncomine.org) is a cancer microarray database that enables clinicians to study gene expression profiles of multiple cancer types (19). All the significant thresholds in the "Gene Summary" and "datasets" modules were set as follows: P value was set at 0.05, fold change was set at 2 and the gene rank was set in the top 10%. Student's t-test was used for differential expression analysis.

UALCAN (http://ualcan.path.uab.edu/) is an easy-to-use web-portal that allows cancer researchers to easily conduct analysis of TCGA gene expression data (20). In this study, we analyzed the relative expressions of CXC chemokines between primary tumor and normal tissue samples in liver hepatocellular carcinoma (LIHC) dataset. Differential expression analyses were performed using Student's t-test, and a P value <0.05 was considered statistically significant.

STRING (http://string-db.org/) is an online database with a collection of all available information on protein-protein interactions (PPIs), which aims to visualize a comprehensive PPI network (21). CXC chemokines were uploaded and the PPI network constructed based on an interaction score of 0.4.

GeneMinia (http://www.genemania.org) is a user-friendly website that characterizes the genes of interest based on functionally informative associations and displays the interaction network among genes (22). We identified CXC chemokine-related genes according to the networks using the following criteria: physical interactions, co-expression, predicted, pathway, co-localization, genetic interactions and shared protein domains.

DAVID (http://david.ncifcrf.gov/home.jsp) is a bioinformatic online tool that provides gene-annotation enrichment analysis for genes of interest, including the Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (23,24). The GO analysis consists of three categories: biological process (BP), cellular component (CC) and molecular function (MF). The annotation results were visualized using R software (https://www.r-project.org/) after completion of the functional enrichment analysis for CXC chemokines and related genes.

Kaplan-Meier plotter (http://kmplot.com/analysis) is an

online tool that enables researchers to assess the effect of 54k genes on survival in 21 cancer types (25). The effect of CXC chemokines on overall survival of patients with HCC was analyzed, and patients were split according to the most significant cut-off value.

TIMER (http://cistrome.shinyapps.io/timer/) is a user-friendly website that allows researchers to analyze infiltration of immune cells (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) in various cancer types based on a TIMER algorithm (26). The association between CXC chemokines and six immune cell subsets was assessed. Additionally, we built a multivariable Cox model to investigate the correlation between survival and six immune cell subsets, and CXC chemokines.

GSCALite (http://bioinfo.life.hust.edu.cn/web/GSCALite/) is a web-based platform that allows researchers to analyze cancer gene sets (27). In this study, the association between expression of CXC chemokines and activity of ten cancer related pathways was analyzed.

NetworkAnalyst (http://www.networkanalyst.ca) is a visual analytics platform that enables users to analyze gene expression profiles and obtain biological networks (28). We integrated and analyzed three GEO datasets, including GSE121248, GSE60502 and GSE57957, using the Multiple Gene Expression Tables module to further validate the differential expression of CXC chemokines in HCC and normal tissues. The significant thresholds were set as follows: false discovery rate (FDR) was 0.05 and fold change was 2. Differential expression analyses were performed using Fisher's method, and a combined adjusted P value <0.05 was considered statistically significant.

Ethical statement

TCGA and GEO belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

R software was used for all statistical analyses. The Student's *t*-test was used to perform the differential expression analysis between HCC tissues and adjacent normal tissues. The survival time in the high- and low-expression groups

was estimated using Kaplan-Meier curves. Besides, the log-rank test was used to analyze the differences. The Cox proportional hazard model was performed to investigate the correlation between clinical outcomes of HCC patients and six immune cell subsets, and CXC chemokines. P values less than 0.05 were considered statistically significant, and all P values were two-tailed.

Results

The differential expression of CXC chemokines in HCC and adjacent normal tissues

We identified 16 CXC chemokines (CXCL1-CXCL17, excluding CXCL15) using data from the ONCOMINE database. The mRNA expression levels of CXC chemokines in HCC and normal tissues were analyzed using the Gene Summary module of ONCOMINE (*Figure 1*). Results revealed that the mRNA expression levels of CXCL6, CXCL8, CXCL9, CXCL10 and CXCL11 in HCC tissues were significantly upregulated, whereas the mRNA expression levels of CXCL1, CXCL2, CXCL12 and CXCL14 were significantly downregulated. *Table 1* displays four studies on the expression levels of CXC chemokines between HCC and normal tissues with significant differences. Chen *et al.* demonstrated that mRNA expression levels of CXCL1, CXCL2, CXCL12 and CXCL14 were significantly lower in HCC tissues than those in normal tissues (29). Similarly, Roessler *et al.* reported that mRNA expression levels of CXCL2, CXCL12 and CXCL14 were significantly lower in HCC tissues compared to normal tissues (30). Wurmbach *et al.* established that mRNA expression levels of CXCL2, CXCL12 and CXCL14 were significantly downregulated in HCC tissues, whereas the mRNA expression level of CXCL10 was significantly upregulated in HCC tissues (31). Mas *et al.* revealed that mRNA expression level of CXCL2 was significantly downregulated in HCC tissues, whereas the mRNA expression levels of CXCL6, CXCL8, CXCL9, CXCL10 and CXCL11 were significantly upregulated in HCC tissues (32).

Additionally, the differential expression levels of CXC chemokines between HCC and normal tissues were analyzed using the UALCAN online tool, which was also used to analyze TCGA data. The analysis revealed that the mRNA expression levels of CXCL1, CXCL2, CXCL5, CXCL6, CXCL7 (PPBP), CXCL12 and CXCL14 were significantly lower in HCC tissues than those in adjacent
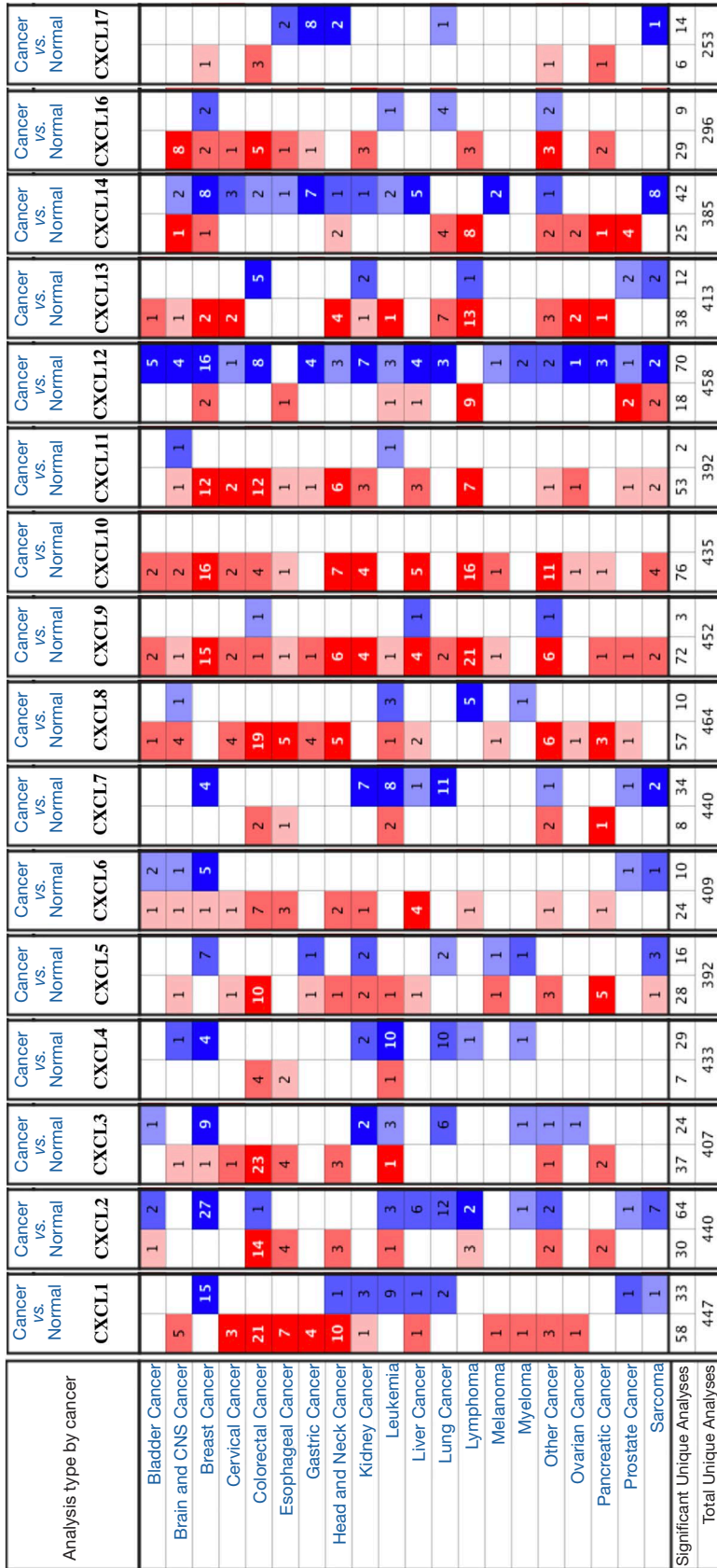
Figure 1 The transcription levels of CXC chemokines in different types of cancers (ONCOMINE). The figure shows the numbers of datasets with statistically significant mRNA high-expression (red) or low-expression (blue) of CXC chemokines.

Table 1 The significant changes of CXC chemokines expression in transcription level between hepatocellular carcinoma and normal tissues (ONCOMIINE)

Gene	Type	Fold change	P value	t-test	Ref
CXCL1	Hepatocellular carcinoma *vs.* Normal	−4.161	1.76E-20	−10.489	Chen
CXCL2	Hepatocellular carcinoma *vs.* Normal	−6.540	8.33E-60	−20.350	Roessler
CXCL2	Hepatocellular carcinoma *vs.* Normal	−3.601	4.65E-17	−9.237	Chen
CXCL2	Hepatocellular carcinoma *vs.* Normal	−5.737	1.45E-7	−6.632	Wurmbach
CXCL2	Hepatocellular carcinoma *vs.* Normal	−7.403	2.59E-7	−6.414	Roessler
CXCL2	Hepatocellular carcinoma *vs.* Normal	−2.273	9.36E-7	−5.344	Mas
CXCL6	Hepatocellular carcinoma *vs.* Normal	3.749	3.15E-7	5.637	Mas
CXCL8	Hepatocellular carcinoma *vs.* Normal	3.323	7.84E-6	4.972	Mas
CXCL9	Hepatocellular carcinoma *vs.* Normal	5.334	6.20E-17	12.031	Mas
CXCL10	Hepatocellular carcinoma *vs.* Normal	9.272	6.33E-17	11.988	Mas
CXCL10	Hepatocellular carcinoma *vs.* Normal	5.928	1.78E-4	4.269	Wurmbach
CXCL11	Hepatocellular carcinoma *vs.* Normal	2.188	2.26E-6	5.355	Mas
CXCL12	Hepatocellular carcinoma *vs.* Normal	−5.338	2.32E-93	−26.987	Roessler
CXCL12	Hepatocellular carcinoma *vs.* Normal	−2.387	4.32E-31	−14.115	Chen
CXCL12	Hepatocellular carcinoma *vs.* Normal	−4.036	4.43E-11	−9.560	Roessler
CXCL12	Hepatocellular carcinoma *vs.* Normal	−4.233	3.82E-9	−7.239	Wurmbach
CXCL14	Hepatocellular carcinoma *vs.* Normal	−10.940	6.98E-154	−41.196	Roessler
CXCL14	Hepatocellular carcinoma *vs.* Normal	−9.667	1.24E-21	−18.539	Roessler
CXCL14	Hepatocellular carcinoma *vs.* Normal	−12.903	1.96E-43	−18.813	Chen
CXCL14	Hepatocellular carcinoma *vs.* Normal	−13.977	6.81E-10	−8.892	Wurmbach

normal tissues, whereas the mRNA expression levels of CXCL9, CXCL16 and CXCL17 were significantly higher in HCC tissues (*Figure 2*). A heatmap was constructed to illustrate the expression patterns of CXC chemokines in the LICH dataset (*Figure 3*).

Construction of protein-protein interaction networks and prediction of related genes of CXC chemokines

A protein-protein interaction network containing 16 nodes and 111 edges was constructed using data from the STRING database based on a minimum interaction score of 0.4 (*Figure 4A*). Furthermore, the top 100 related genes of CXC chemokines were predicted to further explore functional enrichment of genes using GeneMinia online tool (*Figure 4B*).

Functional enrichment analysis of CXC chemokines and related genes

Functional enrichment analyses of the 116 genes including 16 CXC chemokines and 100 related genes were performed using DAVID database. *Figure 5A* presents the top 10 GO terms for three functional categories comprising BP, CC and MF, respectively. In the BP category, cell chemotaxis, leukocyte chemotaxis, chemokine-mediated signaling pathway, response to chemokines, cellular responses to chemokine, myeloid leukocyte migration, granulocyte chemotaxis, granulocyte migration, neutrophil chemotaxis and neutrophil migration were the top 10 terms. In the CC category, external side of plasma membrane, secretory granule lumen, cytoplasmic vesicle lumen, vesicle lumen, tertiary granule lumen, platelet alpha granule lumen, platelet alpha granule, invadopodium, immunological synapse and
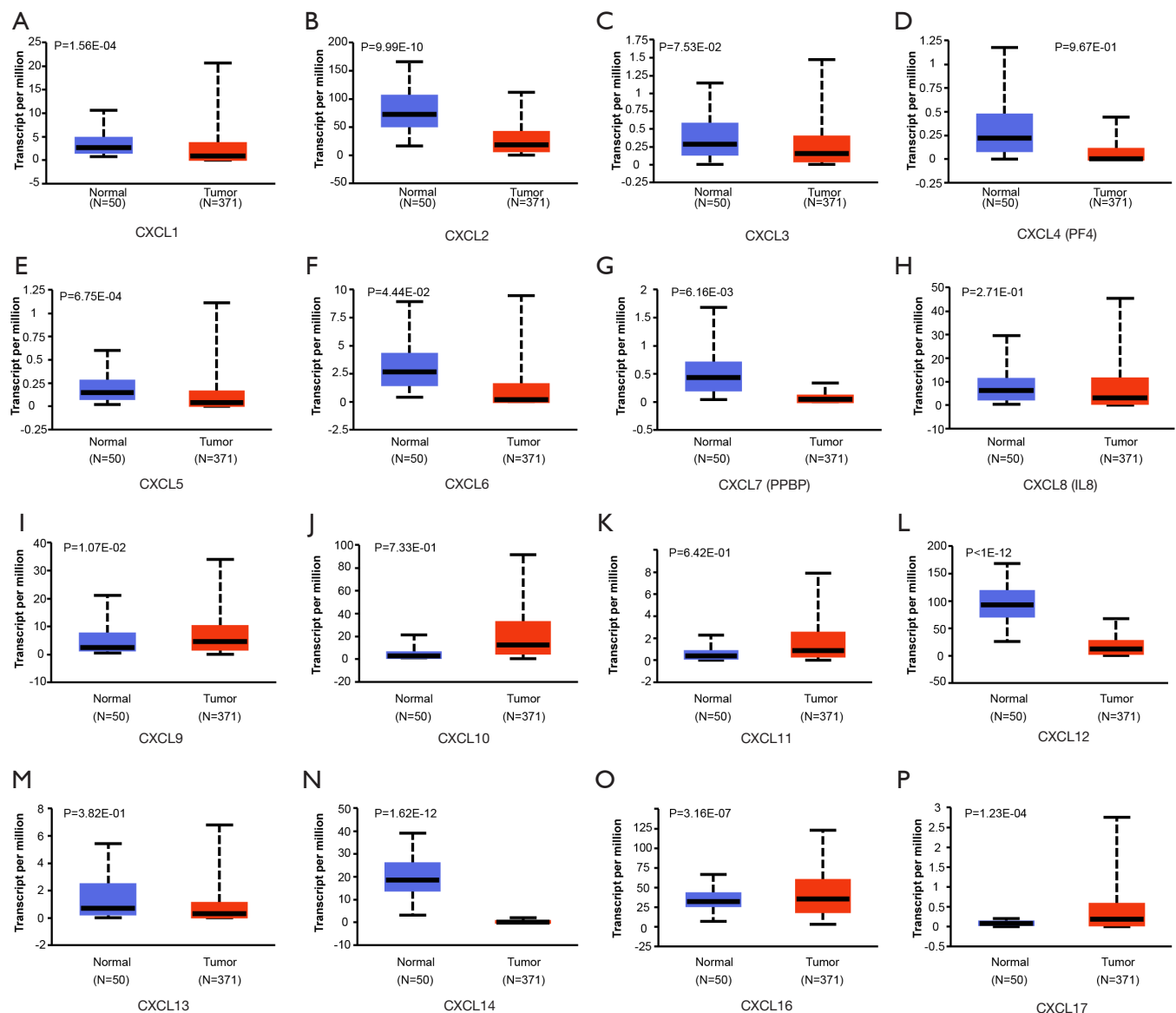
Figure 2 The expression levels of CXC chemokines between HCC and normal tissues (UALCAN). The mRNA expression levels of (A) CXCL1, (B) CXCL2, (E) CXCL5, (F) CXCL6, (G) CXCL7, (L) CXCL12, and (N) CXCL14 were significantly lower in HCC tissues than those in adjacent normal tissues, whereas the mRNA expression levels of (I) CXCL9, (O) CXCL16, and (P) CXCL17 were significantly higher in HCC tissues. Besides, six genes, including (C) CXCL3, (D) CXCL4, (H) CXCL8, (J) CXCL10, (K) CXCL11, and (M) CXCL13, do not show significantly differential expression between HCC tissues and adjacent normal tissues. HCC, hepatocellular carcinoma.

invadopodium membrane were the top 10 terms. Cytokine activity, cytokine receptor binding, G protein-coupled receptor binding, chemokine receptor binding, chemokine activity, CCR chemokine receptor binding, chemokine binding, C-C chemokine binding, C-C chemokine receptor activity and G protein-coupled chemoattractant receptor activity were the top 10 terms in the MF category. The

top 10 KEGG pathways identified included the following: cytokine-cytokine receptor interaction, chemokine signaling pathway, viral protein interaction with cytokines and cytokine receptors, human cytomegalovirus infection, IL-17 signaling pathway, rheumatoid arthritis, TNF signaling pathway, Kaposi sarcoma-associated herpesvirus infection, NF-kappa B signaling pathway and toll-like receptor
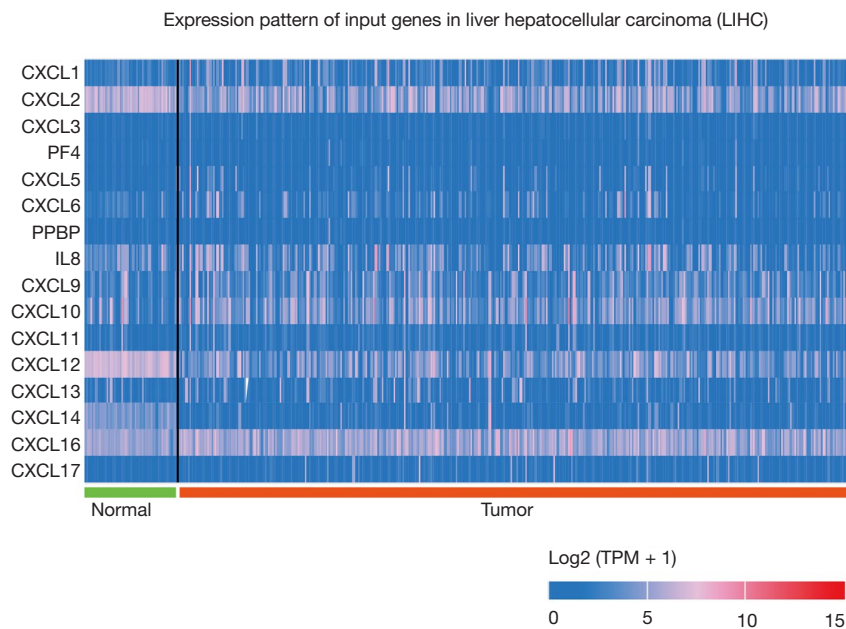
Figure 3 Heatmap of CXC chemokines between HCC and normal tissues (UALCAN). HCC, hepatocellular carcinoma. TPM, transcripts per million.

signaling pathway (*Figure 5*).

The prognostic value of CXC chemokines in patients with HCC

The association between the expression levels of CXC chemokines and overall survival was analyzed using Kaplan-Meier plotter database. *Figure 6* illustrates 16 overall survival curves associated with CXC chemokines based on the most significant cut-off value. The analysis revealed that the high mRNA expression levels of CXCL1, CXCL3, CXCL5, CXCL6 and CXCL8 were correlated with poor overall survival, whereas high mRNA expression levels of CXCL2, CXCL4, CXCL7, CXCL9, CXCL10 and CXCL12 were correlated with better overall survival.

The interaction between CXC chemokines and ten cancer pathways

The association between CXC chemokines and activity of ten cancer pathways was analyzed using the GSCALite online tool (*Figure 7*). The analysis revealed that the DNA damage response and hormone AR signaling pathways were inhibited, whereas apoptosis, EMT and Ras/MAPK signaling pathways were activated.

The association between CXC chemokines and immune cell infiltration

The association between CXC chemokines and infiltration of immune cells within HCC microenvironments was further evaluated using data from TIMER database (*Figure 8*). Results of the analysis demonstrated that the expression levels of CXCL1, CXCL3, CXCL5, CXCL6, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14 and CXCL16 were positively correlated with six immune cell subsets. The expression levels of CXCL2 and CXCL7 were positively correlated with neutrophils. The expression level of CXCL4 was positively correlated with both macrophages and neutrophils. The expression level of CXCL17 was positively correlated with both B cells and CD4+ T cells.

Furthermore, the associations between the six immune cell subsets and CXC chemokines, and clinical outcomes of patients with HCC were analyzed using the Cox proportional hazard model. The analysis revealed that B cells, macrophages, dendritic cells, CXCL2, CXCL6, CXCL8 and CXCL12 can significantly affect the clinical outcomes of patients with HCC (*Table 2*).
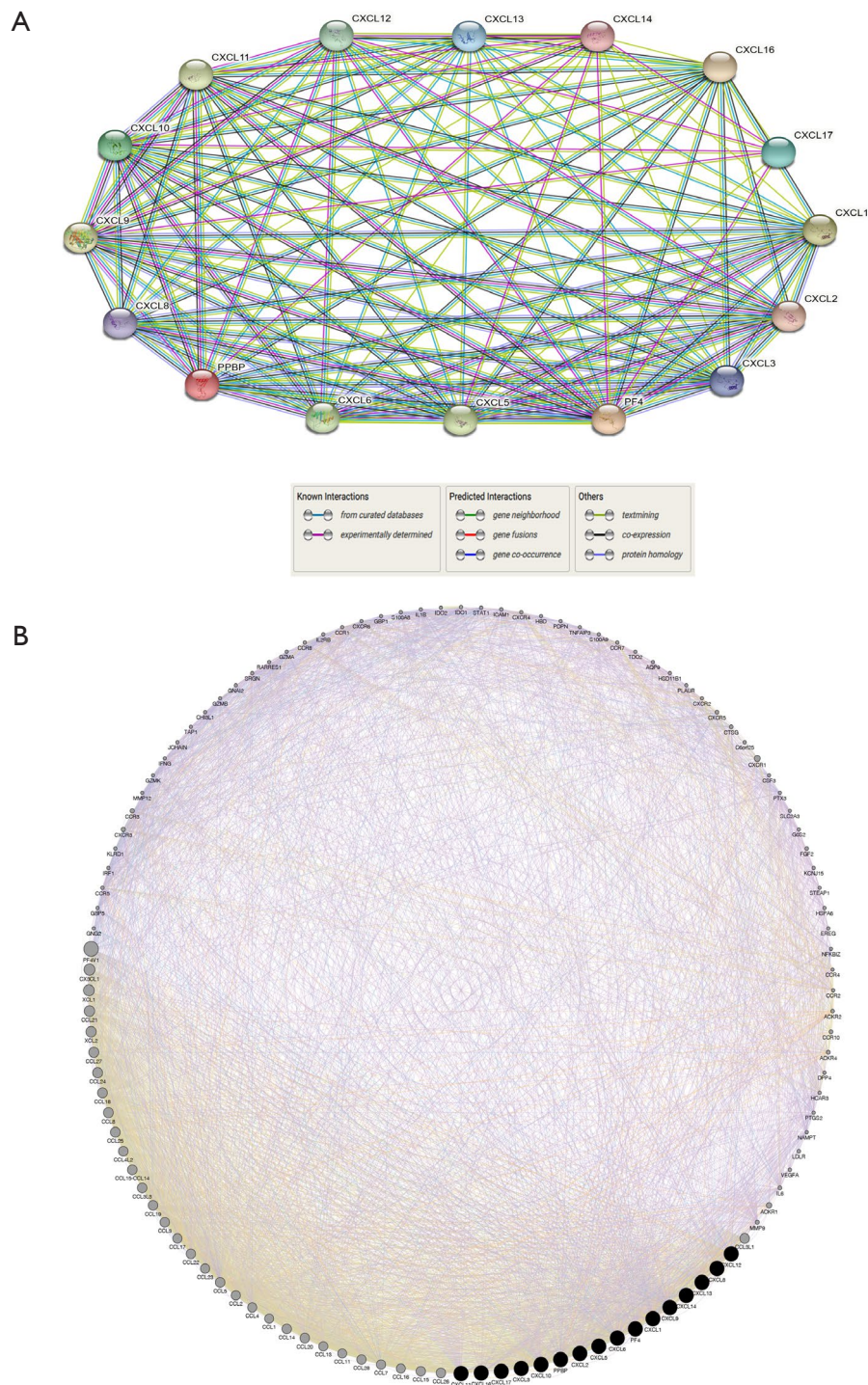
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Figure 4 Protein-protein interaction network of CXC chemokines and identification of CXC chemokines-related genes (STRING and GeneMinia). (A) protein-protein interaction network of CXC chemokines. Each network node represents the protein produced by a single, protein-coding gene. Edges represent protein-protein association. (B) Gene-gene interaction network of CXC chemokines and their related top 100 genes. The top 100 related genes meet these criteria: co-expression, co-localization, genetic interactions, pathway, physical interactions, predicted, and shared protein domains.
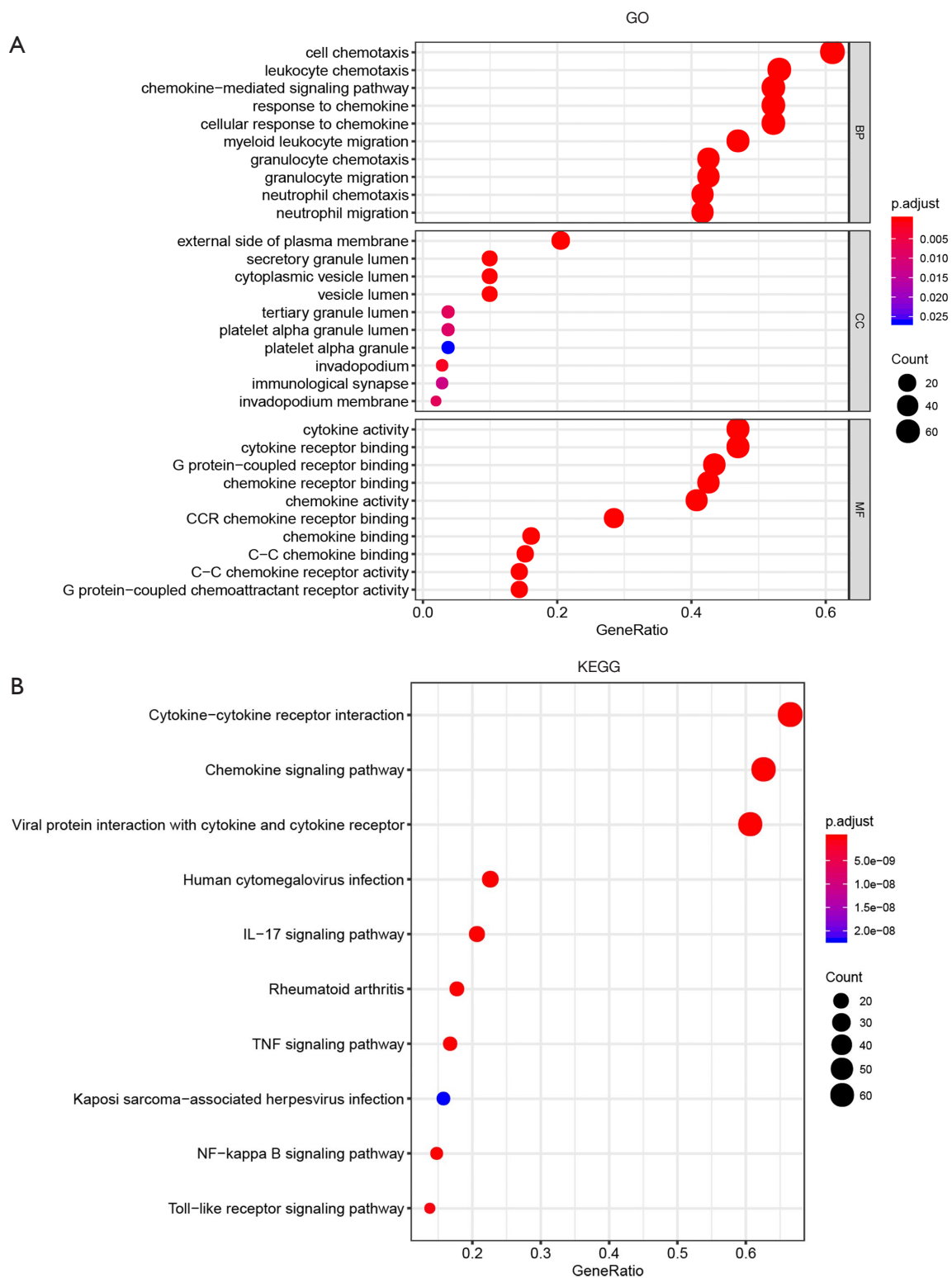
Figure 5 Top 10 enrichment function and pathway terms of CXC chemokines and their related genes in hepatocellular carcinoma (DAVID). (A) Gene Ontology (GO) analysis including three categories: biological process (BP), cellular component (CC) and molecular function (MF). (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.
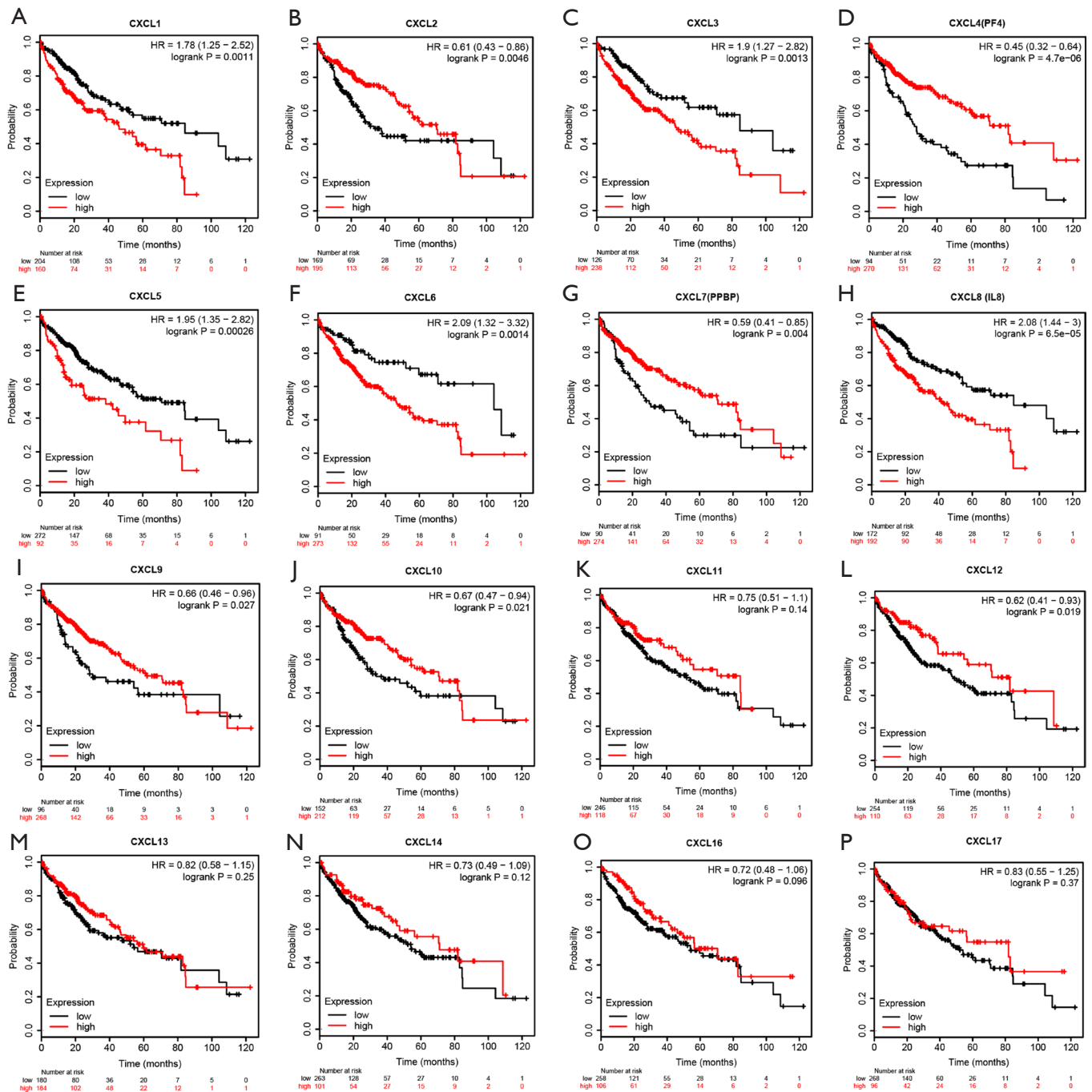
Figure 6 Association between CXC chemokines and overall survival in patients with hepatocellular carcinoma (Kaplan-Meier plotter). The high mRNA expression levels of (A) CXCL1, (C) CXCL3, (E) CXCL5, (F) CXCL6, and (H) CXCL8 were associated with poor overall survival, whereas high mRNA expression levels of (B) CXCL2, (D) CXCL4, (G) CXCL7, (I) CXCL9, (J) CXCL10, and (L) CXCL12 were associated with better overall survival. Besides, the expression levels of five genes, including (K) CXCL11, (M) CXCL13, (N) CXCL14, (O) CXCL16, and (P) CXCL17, do not significantly correlate with the overall survival.
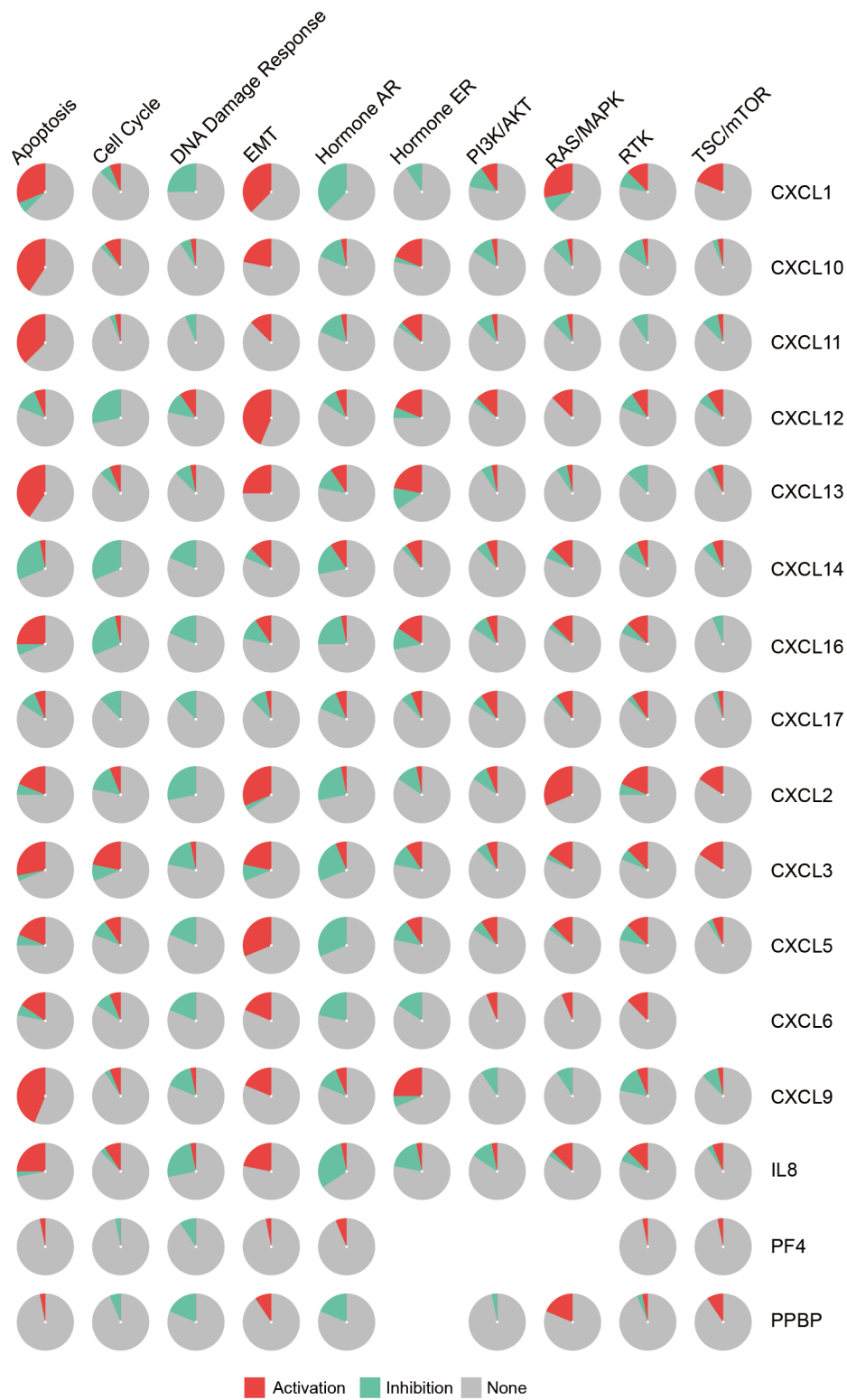
Figure 7 The association between CXC chemokines and activity of ten cancer pathways (GSCALite). Red indicates the activation percentage of CXC chemokines to a pathway. Green indicates the inhibition percentage of CXC chemokines to a pathway. Grey indicates the percentage of CXC chemokines that have no effect on a pathway.

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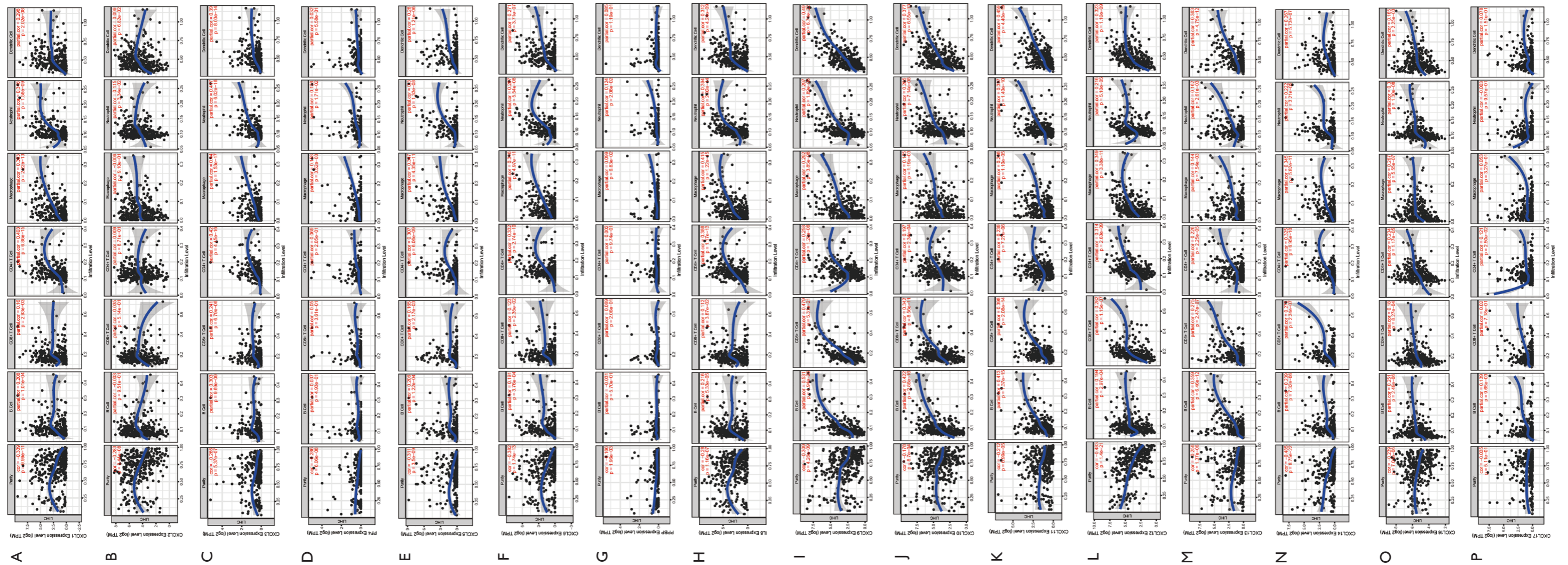Wang et al. CXC chemokines in the HCC

Figure 8 The correlation between CXC chemokines and immune cell infiltration abundance (TIMER). The x-axis represents the abundance of immune cell infiltration. The y-axis represents the expression levels of CXC chemokines. The blue line represents the trend of expression level. The scatter represents the distribution of the expression value for each sample.

Table 2 The Cox proportional hazard model of CXC chemokines and six immune cells in hepatocellular carcinoma (TIMER)

Factor	coef	HR	95% CI_low	95% CI_up	P value	sig
B cell	−9.198	0.000	0.000	0.146	0.013	*
CD8_Tcell	−5.388	0.005	0.000	1.095	0.054	
CD4_Tcell	−3.968	0.019	0.000	15.100	0.245	
Macrophage	7.093	1,203.394	5.072	285,492.940	0.011	*
Neutrophil	−4.923	0.007	0.000	5,375.505	0.475	
Dendritic	6.610	742.556	16.357	33,710.562	0.001	**
CXCL1	0.130	1.139	0.928	1.397	0.213	
CXCL2	−0.226	0.798	0.699	0.911	0.001	**
CXCL3	−0.393	0.675	0.434	1.050	0.081	
PF4	−0.058	0.944	0.631	1.412	0.778	
CXCL5	0.068	1.071	0.880	1.302	0.493	
CXCL6	−0.219	0.803	0.676	0.954	0.013	*
PPBP	−0.095	0.909	0.484	1.710	0.768	
IL8	0.292	1.340	1.126	1.594	0.001	**
CXCL9	−0.174	0.840	0.671	1.051	0.127	
CXCL10	−0.002	0.998	0.797	1.249	0.984	
CXCL11	0.039	1.040	0.758	1.427	0.810	
CXCL12	−0.173	0.841	0.725	0.975	0.022	*
CXCL13	0.080	1.083	0.960	1.223	0.195	
CXCL14	−0.003	0.997	0.822	1.210	0.978	
CXCL16	−0.058	0.943	0.790	1.126	0.517	
CXCL17	0.142	1.153	0.994	1.336	0.059	

*P<0.05, **P<0.01.

Meta-analysis of external multiple gene sets for validating TCGA data

Three GSE datasets were integrated (a total of 221 samples) and meta-analysis performed using NetworkAnalyst online tool to validate mRNA expression of CXC chemokines in HCC and normal tissues. The meta-analysis revealed that the mRNA expression levels of CXCL10 and CXCL11 were significantly upregulated, whereas the mRNA expression levels of CXCL2, CXCL4, CXCL6, CXCL7, CXCL12 and CXCL14 were significantly downregulated. These results were consistent with results obtained from TCGA database (*Table 3*).

Discussion

The analysis of CXC chemokines in the HCC microenvironment has become more convenient and accurate with the establishment of several online databases. The present study explored the mRNA expression levels of CXC chemokines in HCC and normal tissues, and performed functional enrichment analyses for a total of 116 genes. Subsequently, the association between CXC chemokines and activity of ten cancer pathways was analyzed, and the correction between CXC chemokines and infiltration of six immune cell subsets was evaluated. Additionally, three GSE datasets were integrated as

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Table 3 The different expression levels of CXC chemokines between hepatocellular carcinoma and normal tissues by meta-analysis (NetworkAnalyst)

Entrez ID	Gene	Type	CombinedTstat	CombinedPval
2920	CXCL2	Hepatocellular carcinoma vs. Normal	−115.71	0
5196	CXCL4	Hepatocellular carcinoma vs. Normal	−62.788	7.23E-11
6372	CXCL6	Hepatocellular carcinoma vs. Normal	−28.223	1.88E-04
5473	CXCL7	Hepatocellular carcinoma vs. Normal	−78.391	7.25E-14
3627	CXCL10	Hepatocellular carcinoma vs. Normal	25.257	6.12E-04
6373	CXCL11	Hepatocellular carcinoma vs. Normal	29.874	9.70E-05
6387	CXCL12	Hepatocellular carcinoma vs. Normal	−165.13	0
9547	CXCL14	Hepatocellular carcinoma vs. Normal	−237.31	0

external data to validate mRNA expression levels of CXC chemokines.

CXCL1 was initially cloned from fibroblasts (33). Cao *et al.* demonstrated that the expression of CXCL1 significantly increased in HCC tissues compared to normal tissues, and high expression of CXCL1 could promote proliferation and invasion of HCC cells through the NF-kB-dependent pathway, which was associated with poor overall survival in comparison with low expression (34). Previous studies have demonstrated that the mechanisms of CXCL1 that induced tumor cell invitation were associated with stimulating the expression of epidermal growth factor (EGF) and increasing the rate of angiogenesis (35,36). The present study revealed that the expression of CXCL1 decreased in HCC tissues, and high expression of CXCL1 positively correlated with poor prognosis in patients with HCC.

CXCL2 is secreted by macrophages, endothelial, epithelial and tumor cells (37). Peng *et al.* revealed that monocyte-derived CXCL2 was responsible for the recruitment and survival of neutrophils in HCC environment, subsequently enhancing progression of HCC (15). Moreover, miRNA-532-5p can act as a tumor suppressor by downregulating the expression of its target, CXCL2 mRNA (38). By contrast, Ding *et al.* identified CXCL2 as a tumor suppressor for the first time, and

established that CXCL2 was downregulated in HCC tissues compared to adjacent normal tissues, and upregulated CXCL2 indicated better overall survival, which is consistent with the findings of this study (39).

CXCL3 is an encoding growth factor protein that binds to CXCR2 and acts as a chemoattractant for neutrophils. Han *et al.* established that the mRNA and protein levels of CXCL3 were upregulated in HCC tissues compared to adjacent normal tissues in nude mice models (40). Similarly, Zhang *et al.* reported that the high expression of CXCL3 indicated poor prognosis, which enhanced maintenance and tumorigenesis of hepatocellular stem cells via phosphorylation of the ERK1/2 pathway (41). Results of this study indicated that the high expression of CXCL3 was associated with poor prognosis.

CXCL5 is believed to recruit neutrophils, promote angiogenesis and remodel connective tissues by binding to CXCR2. Moreover, CXCL5 participates in a positive feedback loop between tumor-associated neutrophils (TANs) and HCC stem-like cells induced by TANs (16). Zhou *et al.* demonstrated that the expression of CXCL5 increased in HCC tissues compared to adjacent normal tissues, and high expression of CXCL5 promoted the progression of HCC by activating the PI3K-Akt and ERK1/2 pathways (42). Results of the present study indicated that the expression of CXCL5 decreased in HCC tissues, and high expression

of CXCL5 positively correlated with poor prognosis in patients with HCC.

CXCL6, which functions as an angiogenic gene, is also a chemotactic for neutrophils in HCC. In our research, *Table 1* indicated that the expression level of CXCL6 was increased in HCC tissues compared with adjacent normal tissues. This finding was from a small sample size. On the contrary, based on TCGA database (N=374) and integrated three GEO datasets (*Table 3*) (N=221), we found that CXCL6 was decreased in HCC tissues compared with adjacent normal tissues. This conclusion was from a large sample size. Additionally, according to information from the TCGA database, *Table 2* indicated that CXCL6 was an independent protective factor (HR =0.803) of OS in patients with HCC. Therefore, from sample size perspective, we think that CXCL6 may be decreased in HCC tissues compared with adjacent normal tissues. This conclusion was in accordance with a previous study. Zhao *et al.* demonstrated that circ-HOMER1 facilitated the growth and aggressiveness of HCC by enhancing the inhibition of miR-1322 on CXCL6 (43). However, Kaplan-Meier analysis in *Figure 6* showed that increased expression of CXCL6 positively correlated with poor prognosis of patients with HCC, which caused confusing among researchers and clinicians. Although Tian *et al.* reported that HIF-1α induced migration and invasion of HCC cells in a CXCL6-dependent manner, and that high expression of CXCL6 was positively correlated with poor prognosis (44). For this inconsistent finding in our study, we consider that this is primary due to the different choices of cut-off value. In this study, we chose the most significant cut-off value not median value to perform Kaplan-Meier analysis, which leaded to inconsistent results. Therefore, the relationship between the expression level of CXCL6 and prognosis of HCC warrants further research.

CXCL8 is a chemotactic for neutrophils and is secreted by macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells, and fibroblasts. A study by Yin *et al.* revealed that tumor associated macrophages (TAMs) triggered growth and metastasis of HCC cells by expressing high levels of CXCL8 (45). Sun *et al.* reported that the expression of CXCL8 was upregulated in highly metastatic HCC cell lines compared to low metastatic cell lines, and that CXCL8 promoted invasion of HCC cells through activation of the PI3K/Akt pathway (46). Huang *et al.* explored the association between long non-coding RNAs (lncRNAs) and proliferation, and invasion of HCC cells (47). The study demonstrated that the lncRNA,

MALAT1 can upregulate the expression of CXCL6 and CXCL8 to stimulate HCC progression. In this study, we established that high expression of CXCL8 positively correlated with poor survival in patients with HCC.

CXCL9, which binds to CXCR3, is a chemoattractant for lymphocytes. Ding *et al.* demonstrated that the CXCL9-CXCR3 axis can promote metastasis of HCC cells by activating the ERK1/2-MMP2/MMP9 pathway (48). Moreover, a previous study revealed that high expression of CXCL9 was positively correlated with poor prognosis in patients with HCC (49). Additionally, Liu *et al.* reported that CXCR3+ B cells induced M2b macrophage polarization resulting in early recurrence of HCC, in which recruitment and maturation were associated with high expression of CXCL9 stimulated by proinflammatory IL-17+ cells (50). By contrast, the present study demonstrates that high expression of CXCL9 is positively correlated with better prognosis in patients with HCC.

CXCL10 can activate monocytes, natural killer cells and T-cell migration by binding to CXCR3. Huang *et al.* established that the expression of circular RNA, circMET increased in HCC tissues compared to adjacent normal tissues, which subsequently induced immunosuppression within the HCC microenvironment through miR-30-5p/Snail/DPP4/CXCL10 axis (14). Li *et al.* reported that IL-25-induced M2 macrophages activated EMT in HCC cells, which was associated with secretion of CXCL10 and phosphorylation of ERK (51). Ren *et al.* demonstrated that upregulated CXCL10 enhanced invasion and metastasis of HCC cells by activating MMP-2 expression (52). The present study established that the high expression of CXCL10 is associated with better prognosis in patients with HCC.

CXCL11 is a dominant ligand for CXCR3, and is a chemoattractant for T-cells. Zhang *et al.* reported that the high expression of CXCL10 promoted acquisition and maintenance of the properties of HCC stem cells by binding to CXCR3, which was associated with activation of the ERK1/2 pathway (18). Monnier *et al.* indicated that mRNA and protein expression levels of CXCL10 in HCC tissues were higher than those in adjacent normal tissues, resulting in the growth and metastasis of HCC cells (53). In addition, CXCL11 facilitated M2b macrophage polarization and caused early recurrence in patients with HCC (50).

CXCL12 is a ligand for CXCR4 and is responsible for inflammatory responses. Tsai *et al.* established that CXCL12 activated by SOX4 can modulate neovascularization and cause distant metastasis of HCC (17). Yang *et al.* also

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demonstrated that the CXCL12-CXCR4 axis participated in angiogenesis of HCC, and was positively correlated with poor survival of patients with HCC (54). Lu *et al.* reported that high expression of miR-342 could suppress the Wnt/β-catenin pathway by inhibiting CXCL12 expression, which subsequently inhibited proliferation and metastasis of HCC cells (55). In this study, we established that the expression of CXCL12 decreased in HCC tissues, and high expression of CXCL12 positively correlated with better prognosis in patients with HCC.

CXCL13, which is a chemoattractant for B lymphocytes, is strongly expressed in the spleen, lymph nodes, and Peyer's patches. Li *et al.* reported that the serum levels of CXCL13 were significantly higher in patients with HCC compared to healthy controls, and that the serum level of CXCL13 was positively correlated with large tumor size, HCC metastasis and advanced stage of HCC (56). Similarly, a previous study demonstrated that high mRNA and serum levels of CXCL13 contributed to the progression of HCC by activating the Wnt/β-catenin pathway (57).

CXCL14 is a chemoattractant for monocytes and has been identified as an essential tumor suppressor in HCC (58). Two previous studies have indicated that the expression of CXCL14 decreased in HCC tissues compared to adjacent normal tissues, and that high expression of CXCL14 can inhibit proliferation and invasion of HCC cells, which is consistent with the findings of the present study (59,60).

CXCL16 is a ligand for CXCR6, which plays a role in tumorigenesis. Two previous studies have indicated that the expression of CXCL16-CXCR6 axis increased in HCC tissues compared to adjacent normal tissues, which was associated with early recurrence and poor survival of patients with HCC (61,62). In addition, Cai *et al.* reported that the expression of CXCL16 was upregulated in M2-polarized macrophages, which triggered migration and invasion of HCC cells (63). Results of this study also revealed that the expression of CXCL16 increased in HCC tissues.

CXCL17 is a chemoattractant for monocytes and dendritic cells, and it promotes tumorigenesis by inducing angiogenic activity. Previous studies have revealed that the expression of CXCL17 increased in HCC tissues compared to adjacent normal tissues (64,65). This may play a role in promoting progression of HCC in patients. Furthermore, Wang *et al.* established that CXCL17 could regulate proliferation, metastasis and autophagy of HCC cells via the LKB1-AMPK axis (66). Results obtained in this study on the expression of CXCL16 in HCC tissues are consistent with those of previous studies.

The roles of CXC chemokines in the occurrence and progression of HCC was analyzed using several online databases. These databases can be used to analyze several aspects conveniently including mRNA expression levels, survival of patients, activity of cancer pathways, and infiltration of immune cells. Nevertheless, we identified disparities with reference to mRNA levels and prognostic values for certain CXC chemokines after reviewing and comparing previous studies. The contrasting findings observed in CXC chemokines may be associated with several reasons. First, different clinical samples and experimental conditions may result in the difference. Second, these online analysis tools used in this study may adopt different data cleaning methods. Third, different RNA sequencing technologies could lead to inconsistent findings. Therefore, further clinical trials are required to validate the potential roles and mechanisms of the CXC chemokines evaluated by this study. This study provides a better understanding of the roles of CXC chemokines in HCC and the information will help clinicians to predict prognosis and select suitable therapies for patients with HCC.

Conclusions

This study has demonstrated that CXC chemokines can modulate the interactions between immune and HCC cells in a tumor microenvironment. This consequently influences the prognosis and therapy of patients with HCC. Analysis of differential expression levels of CXC chemokines between HCC and adjacent normal tissues revealed that high mRNA expression levels of CXCL1/3/5/6/8 were associated with poor overall survival, whereas high mRNA expression levels of CXCL2/4/7/9/10/12 were associated with better overall survival. Analysis of the association between CXC chemokines and activity of cancer pathways indicated that the DNA damage response and hormone AR signaling pathways were inhibited, whereas apoptosis, EMT and Ras/MAPK signaling pathways were activated. In addition, the expressions of CXC chemokines were positively correlated with infiltration of six types of immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells).

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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