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Comprehensive analysis of the clinical and biological significances for chemokine CXCL3 in cholangiocarcinoma

Hongyue Ren, MD[a], Xiaofan Yang, MD[b], Wenrong Hou, MD[b], Jiarong Meng, BD[c], Deqing Luo, MD[d], Chunbin Zhang, PhD[a,e,*]

Abstract

Cholangiocarcinoma (CHOL) is a race malignant cancer arising from bile duct epithelial cells in clinical practice. C-X-C motif chemokine ligand 3 (CXCL3) is a member of chemokines family, which participates in the pathogenesis of various tumors. However, the association between CXCL3 and CHOL is unclear. This present study was to assess the role of CXCL3 expression in the progress of CHOL. TIMER, GEPIA, UALCAN, GSCA, LinkedOmics, Metascape and STRING databases were performed to evaluate the clinical and biological significances for CXCL3 with CHOL patients including expression, clinicopathological factors, immune cell infiltration, GO enrichment and KEGG pathway analyses, as well as PPI network analysis. The immunohistochemistry analysis of tissue microarray was conducted to detect the protein expression level, subcellular localization, clinicopathological factors and prognosis of CXCL3 in CHOL. The mRNA and protein expression levels of CXCL3 were markedly increased in CHOL tissues. The overexpression of CXCL3 was strongly associated with maximum tumor diameter of patients with CHOL. Additionally, there were negative correlations between the expression of CXCL3 and monocyte as well as Th17. Low infiltration of neutrophil indicated significantly shorter cumulative survival in CHOL patients. And CXCL3 was significantly associated with arm-level deletion of $CD8^+$ T cell. Furthermore, functional network analysis suggested that CXCL3 and its associated genes were mainly enriched for chemotaxis, secretory granule membrane, cytokine activity and IL-17 signaling pathway. CXCL3 might potentially participate in the carcinogenesis of CHOL, which provided a direction for future research on the mechanism of CXCL3 in CHOL.

Abbreviations: CHOL = cholangiocarcinoma, CXCL3 = C-X-C motif chemokine ligand 3, CXCR2 = CXC chemokine receptor 2, DFS = disease-free survival, OS = overall survival, TCGA = the cancer genome atlas.

Keywords: cholangiocarcinoma, CXCL3, immune infiltration, prognosis

1. Introduction

Cholangiocarcinoma (CHOL) is rare but aggressive tumor, which can emerge at any point of the biliary tree. Due to the different anatomical locations, CHOL can be divided into 3 subtypes: intrahepatic CHOL, periportal CHOL and distal CHOL.[1] In the world wide, the incidence and mortality rates of CHOL are constantly increasing with an incidence rate of 0.3 to 6/100,000 and a mortality rate of 1 to 6/100,000 inhabitants per year.[2] Due to the silent clinical course, lack of biomarkers, hidden anatomical location, and highly desmoplastic and paucicellular nature, CHOL is known as insidious onset and difficult treatment.[3] Early CHOL is mostly asymptomatic, resulting

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Institute Research Ethics Committee of Zhangzhou Health Vocational College.

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[a] Basic Medical College, Zhangzhou Health Vocational College, Zhangzhou, Fujian Province, China, [b] Basic Medical College, Jiamusi University, Jiamusi, Heilongjiang Province, China, [c] Department of Pathology, Dongnan Hospital of Xiamen University, School of Medicine, Xiamen University, Zhangzhou, Fujian Province, China, [d] Department of Orthopaedic Surgery, Dongnan Hospital of Xiamen University, School of Medicine, Xiamen University, Zhangzhou, Fujian Province, China, [e] Medical Technology College, Zhangzhou Health Vocational College, Zhangzhou, Fujian Province, China.

** Correspondence: Chunbin Zhang, Medical Technology College/Collaborative Innovation Center for Translation Medical Testing and Application Technology, Zhangzhou Health Vocational College, Zhangzhou 363000, Fujian Province, China (e-mail: zhangcb@jmsu.edu.cn).*

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in a high detection rate of diagnosis at an advanced stage. Surgical resection is the main curative option for CHOL, but it is no longer feasible for advanced patients.[4] Other systemic therapies, traditional chemotherapy and target therapies are currently available choices for unresectable CHOL patients, but effective treatment biomarkers have not yet been identified.[3]

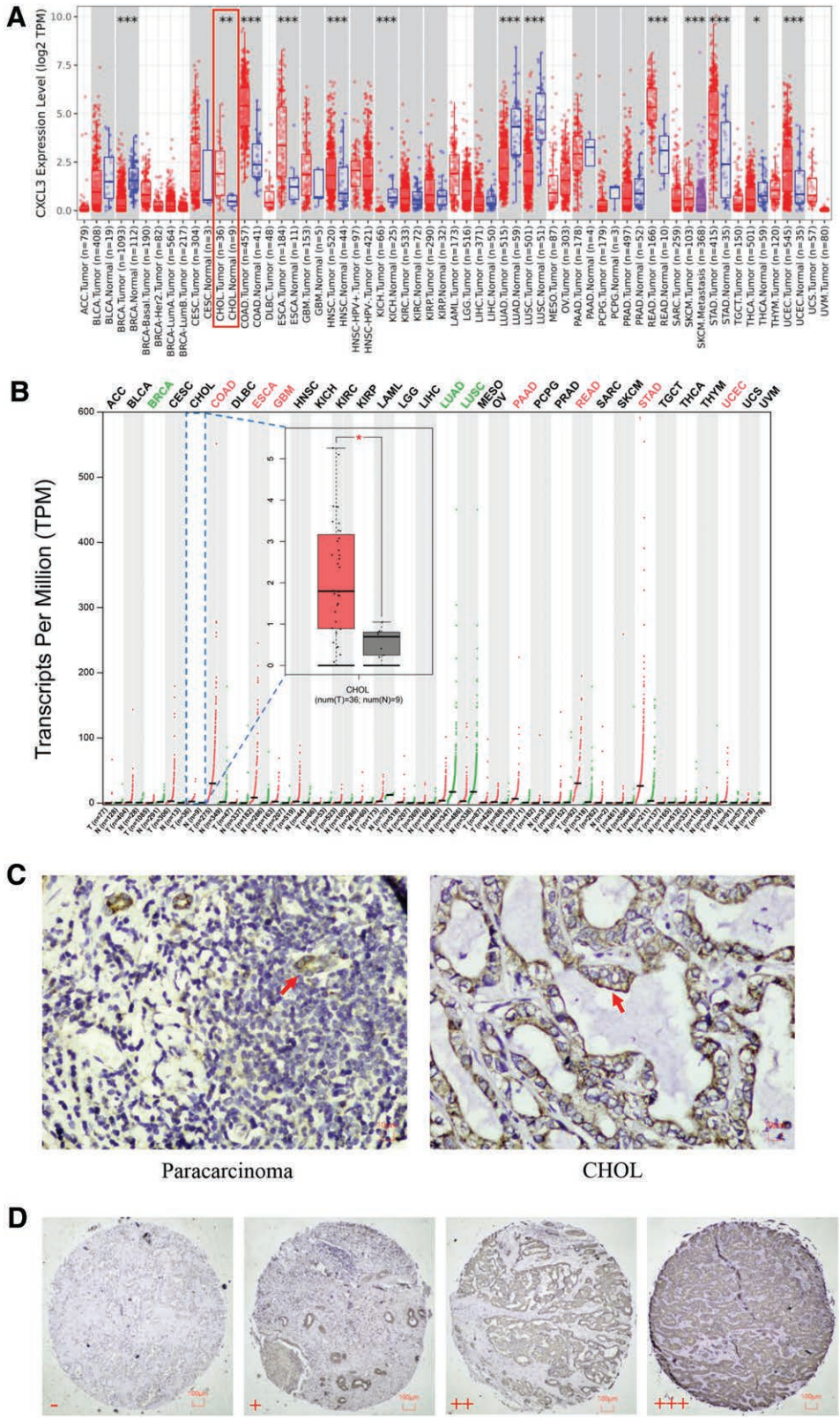

Figure 1. Differential expression of CXCL3 in CHOL patients. (A) The mRNA expression of *CXCL3* in the TIMER database. (B) The mRNA expression of *CXCL3* in the GEPIA database. (C) IHC analysis of CXCL3 protein in CHOL tissue microarray. Left red arrow, normal bile duct tissue; Right red arrow, CHOL tissue. Zoom: 400×. (D) Four grades of CXCL3 staining intensity in CHOL according to their different positive rates of CXCL3 expression. Zoom: 100×. **P* < .05, ***P* < .01, ****P* < .001. CHOL = cholangiocarcinoma, CXCL3 = C-X-C motif chemokine ligand 3.

Table 1

Distribution of CXCL3 classifications in CCA and para carcinoma tissues.

Tissue type	Number	CXCL3 stain grades				X2	P
		–	+	++	+++		
Tumor	60	4	18	21	17	12.64	.0055
Paracarcinoma	60	10	31	12	7		

CXCL3 = C-X-C motif chemokine ligand 3.

Consequently, CHOL patients have very poor prognosis with a 5-year overall survival of <10%.[5] As such, it is necessary to identify novel and reliable biomarkers that can be employed in the clinical screening, diagnosis, treatment and prognosis of CHOL patients.

Chemokines play a key role in the occurrence and development of tumors, such as inflammation, angiogenesis, and tumor metastasis.[6] According to the conservative motif at the N-terminus, chemokines are divided into 4 subfamilies including CXC (α), CC (β), CX3C (γ), and C (δ).[7] As a member of chemokines family, C-X-C motif chemokine ligand 3 (CXCL3) is encoded by the human *GRO* gene, which locates within the chromosomal region of 4q13.3. CXCL3 binds to its receptor, CXC chemokine receptor 2 (CXCR2), and CXCL3/CXCR2 axis has been implicated in the process of various tumors, for example, head and neck squamous cell carcinoma,[8] oral squamous cell carcinoma,[9] pancreatic ductal adenocarcinoma[10] and colon adenocarcinoma.[11] However, the relationship between CXCL3 and CHOL has not been clarified yet. Hence, our research question is to identify the effect of CXCL3 on CHOL patients in order to further elucidate the pathogenic process of CXCL3 in CHOL.

In this present study, we performed a comprehensive analysis of the clinical and biological significance for CXCL3 in CHOL, which might provide a theoretical basis for discovering the potential diagnostic and therapeutic target against CHOL.

2. Materials and methods

2.1. Reagents

Antibody against CXCL3 was purchased from ImmunoWay Biotechnology Company (TX). Citric acid buffer, second antibody and DAB detection kit were from Maixin Biotech (Fuzhou, China). Tissue microarray of human CHOL was obtained from Superbiotek (Shanghai, China).[12]

2.2. Clinical samples and immunohistochemistry (IHC)

Tissue microarray (LVC1202) included 60 pairs of CHOL specimens and paracarcinoma tissues. The inclusion and exclusion criteria for patient selection were as follow: patients without receiving neoadjuvant treatment before primary surgery, patients with survival time, paired patients with CHOL and paracarcinoma specimens, patients informed and consented the following study. The clinicopathological factors were summarized in Supplemental Table 1, http://links.lww.com/MD/L858 including age, sex, maximum tumor diameter, tumor number, differentiation, TNM stage, vascular invasion, satellite lesion, lymph node metastasis, tumor recurrence, CA199, CEA, AFP, HBsAg, overall survival (OS) and disease-free survival (DFS). IHC was conducted as previously described.[13] The CHOL tissue microarray section was de-paraffinized and rehydrated in graded alcohols. After epitope retrieval in 0.01 M citric acid buffer (pH 6.0) at 100°C for 2 minutes, the tissue microarray section was treated with 3% H_2O_2 for 10 minutes for blockage of endogenous peroxidase activity. Consequently, the CHOL tissue microarray section was reacted with primary antibody to CXCL3 (YT2075, 1:100) for 1h at room temperature. After incubation with second antibody, the CHOL tissue microarray section was detected with DAB detection kit. Finally, hematoxylin was performed for the nucleus counterstain at room temperature.

2.3. Gene expression profiling interactive analysis (GEPIA)

GEPIA (http://gepia.cancer-pku.cn/index.html) is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9736 tumors and 8587 normal samples from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression projects, using a standard processing pipeline.[14] We used GEPIA to evaluate the expression and prognostic value of CXCL3 in 36 CHOL patients. Prognostic analysis was conducted using Kaplan–Meier curves. In data analysis, the median CXCL3 expression was used as a cutoff value to classify groups.

2.4. The University of Alabama at Birmingham cancer data (UALCAN)

UALCAN (http://ualcan.path.uab.edu/analysis.html) is a comprehensive web resource, which provides analyses from TCGA and MET500 cohort data.[15] In this study, UALCAN was applied to analyze the expression, clinicopathological factors and prognostic value of CXCL3 in CHOL. The results were analyzed online and *P* value cutoff was set at .05.

2.5. Tumor immune estimation resource (TIMER)

TIMER database (http://timer.cistrome.org/) provides analyses for 10,897 tumors from 32 cancer types including 6 tumor-infiltrating immune subsets.[16] We performed TIMER to assess the expression, immune cell infiltration and correlation analysis of CXCL3 in patients with CHOL. The median CXCL3 expression was severed as a cutoff value, and the difference at a *P* value < .05 was considered significant.

2.6. Gene set cancer analysis (GSCA)

GSCA (http://bioinfo.life.hust.edu.cn/GSCA) is an integrated platform for genomic, pharmacogenomic, and immunogenomic gene set cancer analysis.[17] In this study, GSCA was conducted to evaluate the relationship between immune cell infiltration of CHOL and CXCL3. The color of the bubbles showed the degree of correlation. In detail, darker red or blue represented positive or negative correlation, respectively. The size of the bubble indicated the degree of significance, while the point raised by the black contour coil demonstrated a False discovery rate (FDR) value of <0.05.

2.7. LinkedOmics

LinkedOmics (http://www.linkedomics.org) is publicly available database, which contains multi-omics data and

clinical data for 32 cancer types and a total of 11,158 patients from TCGA project.[18] The co-expressed genes of CXCL3 were obtained from LinkedOmics platform in this study. The results were analyzed using Pearson correlation coefficient, and showed via heat maps as well as volcano plots.

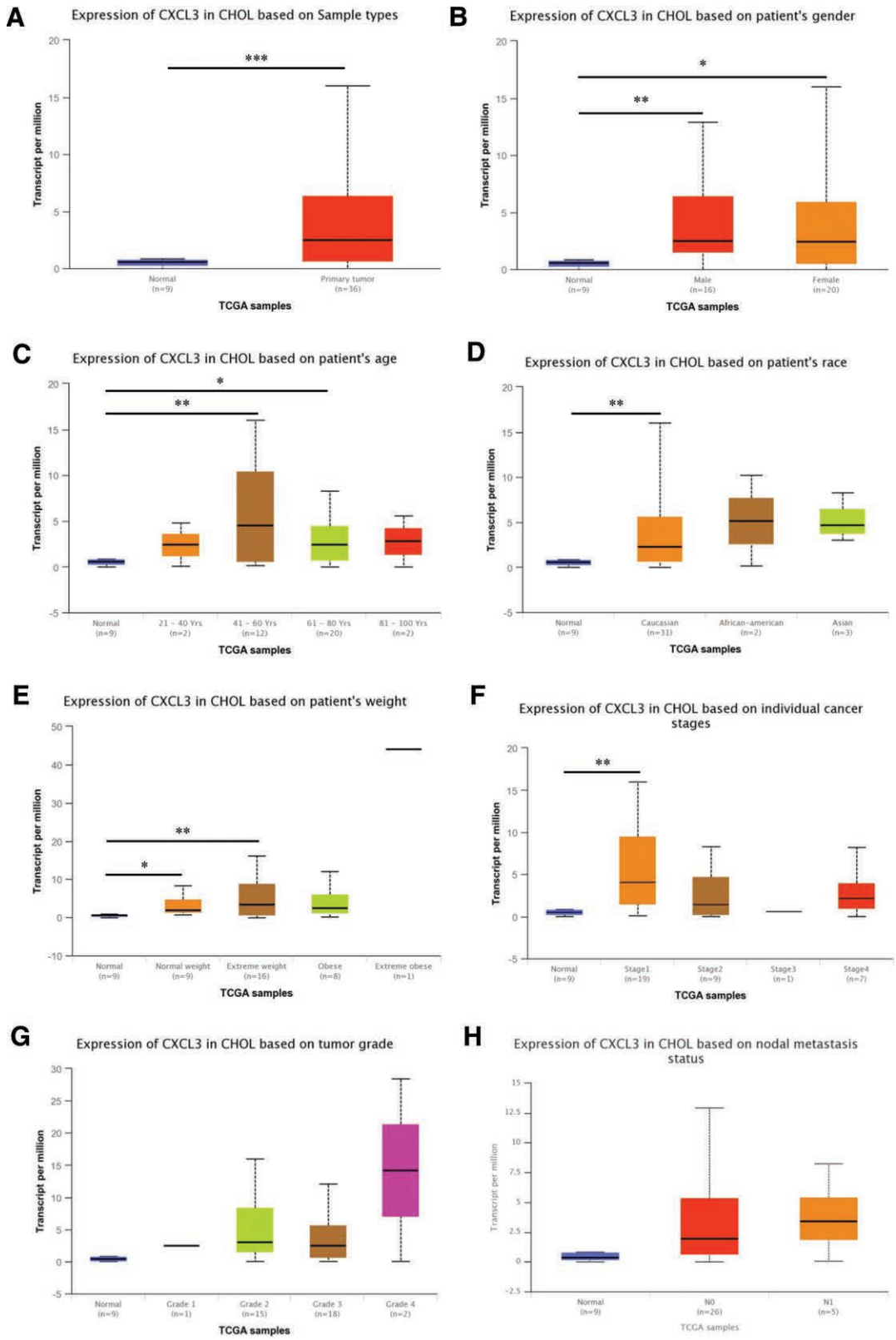

Figure 2. Clinicopathological factors related with *CXCL3* mRNA expression in CHOL patients using UALCAN database. (A) The mRNA expression of *CXCL3* in the UALCAN database. Relative expression of *CXCL3* between CHOL and normal bile duct tissues in subgroup analysis based on gender (B), age (C), race (D), patient weight (E), individual cancer stage (F), tumor grade (G) and nodal metastasis status (H). **P* < .05, ***P* < .01. CHOL = cholangiocarcinoma, CXCL3 = C-X-C motif chemokine ligand 3.

2.8. Metascape

Metascape (http://metascape.org) is an effective and efficient tool, which can provide a comprehensive gene list annotation and analysis resource for experimental biologists.[19] In our study, Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and visualization were performed using the Metascape network. The screening conditions of Min overlap and Min Enrichment were 3 and 1.5, respectively. The P value < .01 was considered significant.

2.9. Search tool for the retrieval of interacting genes (STRING)

The STRING database (https://string-db.org/) is a powerful platform, which can systematically collect and integrate protein-protein interactions (PPI) both physical interactions as well as functional associations.[20] We performed PPI network of CXCL3 in CHOL using STRING database. Furthermore, the PPI network was constructed by Cytoscape software (version 3.9.1) and identified the key hub genes. The cluster analysis performed Cytoscape plug-in molecular complex detection technology (MCODE) with default parameters as follows: K-core = 2, degree cutoff = 2, max depth = 100, and node score cutoff = 0.2.

2.10. Statistical analysis

Statistical analyses of experimental correlation were analyzed using GraphPad Prism 9 (San Diego, CA) and SPSS 27.0 software (SPSS Inc., Chicago, IL). The expression of CXCL3 between CHOL and paracarcinoma tissues were

Table 2

Correlations of CXCL3 expression with clinicopathological factors in CCA.

Features	Number	CXCL3		χ^2	P
		Low (%)	High (%)		
Age				0.6287	.4278
<60	34	11 (18.3)	23 (38.3)		
≥60	26	11 (18.3)	15 (25.0)		
Sex				0.6238	.4297
Female	23	7 (11.7)	16 (26.7)		
Male	37	15 (25.0)	22 (36.7)		
Maximum tumor diameter				4.3390	**.0372***
≤5 cm	25	13 (21.7)	12 (20.0)		
>5 cm	35	9 (15.0)	26 (43.3)		
Tumor number				0.2486	.6181
1	47	18 (30.0)	29 (48.3)		
≥2	13	4 (6.7)	9 (15.0)		
Differentiation				2.981	.2253
Moderate	46	19 (31.7)	27 (45.0)		
Moderate-poor	9	1 (1.7)	8 (13.3)		
Poor	5	2 (3.3)	3 (5.0)		
TNM stage				3.194	.3627
I	24	9 (15.0)	15 (25.0)		
II	10	4 (6.7)	6 (10.0)		
III	12	2 (3.3)	10 (16.7)		
IV	14	7 (11.7)	7 (11.7)		
Vascular invasion				0.9513	.3294
No	51	20 (33.3)	31 (51.7)		
Yes	9	2 (3.3)	7 (11.7)		
Satellite lesion				0.0028	.9581
No	52	19 (31.7)	33 (55.0)		
Yes	8	3 (5.0)	5 (8.3)		
Lymph node metastasis				0.6432	.4225
No	47	16 (26.7)	31 (51.7)		
Yes	13	6 (10)	7 (11.7)		
Tumor recurrence				0.0718	.7888
No	12	4 (6.7)	8 (13.3)		
Yes	48	18 (30.0)	30 (50.0)		
CA199 (U/mL)#				0.008	.9778
<37	24	9 (15.3)	15 (25.4)		
≥37	35	13 (22.0)	22 (37.3)		
CEA (μg/L)				0.4019	.5261
<5	25	8 (13.3)	17 (28.3)		
≥5	35	14 (23.3)	21 (35.0)		
AFP (μg/L)				0.2758	.5995
<25	51	18 (30.0)	33 (55.0)		
≥25	9	4 (6.7)	5 (8.3)		
HBsAg#				1.930	.1648
Negative	40	16 (29.0)	24 (43.6)		
Positive	15	3 (5.5)	12 (21.8)		

CXCL3 = C-X-C motif chemokine ligand 3.

* A P value of <.05 was considered statistically significant.

The data of a few patients were not available.

calculated using Student *t* test. The correlations between CXCL3 and clinicopathological factors of CHOL were evaluated using Pearson χ^2 test. Kaplan–Meier method and log-rank tests were used to construct survival curves and to assess differences between groups, respectively. Cox proportional hazards regression models were applied to investigate the significance of prognostic factors. *P* values <.05 were considered statistically significant.

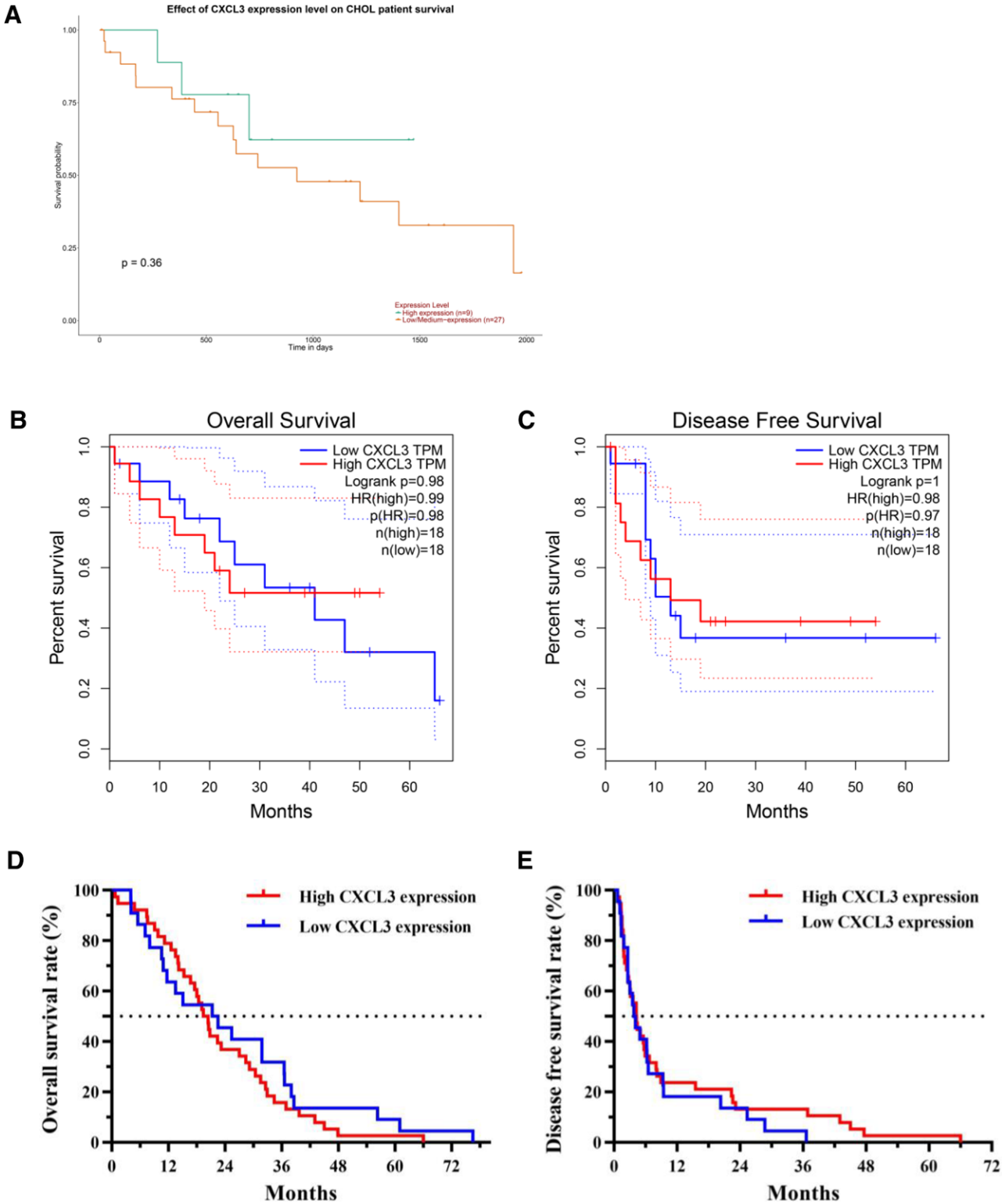

Figure 3. Survival analysis of CXCL3 expression in CHOL patients. (A) The prognostic value of *CXCL3* in patients with CHOL using UALCAN database. Survival analyses of OS (B) and DFS (C) for *CXCL3* expression in CHOL patients using GEPIA database. Associations between CXCL3 expression and OS (D) as well as DFS (E) in 60 patients with CHOL by the Kaplan–Meier method. CHOL = cholangiocarcinoma, CXCL3 = C-X-C motif chemokine ligand 3, DFS = disease-free survival, OS = overall survival.

3. Results

3.1. Differential expression of CXCL3 in CHOL patients

To explore the role of CXCL3 in CHOL, we initially analyzed its expression value using TIMER database. As shown in Figure 1A, *CXCL3* transcription level was differentially expressed in pan-cancer. The expression level of *CXCL3* was significantly higher in CHOL tissues than in normal tissues. The result of GEPIA database also indicated that *CXCL3* was overexpressed in CHOL tissues (Fig. 1B). We further verified the protein expression level of CXCL3 in CHOL using a tissue microarray including a cohort of 60 CHOL patients. The IHC result showed that CXCL3 was strongly elevated in CHOL tissues, but was weakly stained in paracarcinoma tissues (Fig. 1C). Moreover, CXCL3 mainly existed in the cytoplasm. To further detect the expression level of CXCL3 in above 2 tissues, the staining intensity of CXCL3 was divided into low (– to +) or high (++ to +++) groups (Fig. 1D). As shown in Table 1, the high expression of CXCL3 in CHOL tissues was 38 cases (63.3%), which was much higher than that in paracarcinoma tissues (19 cases, 31.7%).

3.2. Association of CXCL3 expression with clinicopathological factors in CHOL patients

The relationships between CXCL3 expression and clinicopathological factors in CHOL patients were analyzed using UALCAN database and CHOL microarray. The result of UALCAN database showed that the *CXCL3* expression was significantly higher in primary tumor tissues than normal bile duct tissue samples (Fig. 2A). The transcription level of *CXCL3* was elevated in CHOL patients comparing with healthy people in some subgroups including male, female, 21 to 60 years old, Caucasian, normal weight, extreme weight, and stage 1 (Fig. 2B–F). There were no obvious differences between CHOL and normal bile duct tissues in subgroup analysis based on tumor grade and nodal metastasis (Fig. 2G–H). In addition, clinicopathological analysis of CHOL microarray revealed that overexpression of CXCL3 was strongly correlated with maximum tumor diameter of patients with CHOL, but was not related with age, sex, tumor number, differentiation, TNM stage, vascular invasion, satellite lesion, lymph node metastasis, tumor recurrence, CA199, CEA, AFP and HBsAg of CHOL patients (Table 2).

Table 3

Univariate and multivariate Cox regression analyses for OS in CCA.

	Univariate analysis			Multivariate analysis		
Variables	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age (<60 vs ≥60)	0.933	0.547–1.590	.798	0.968	0.468–2.003	.930
Sex (Female vs Male)	1.044	0.609–1.789	.876	0.858	0.375–1.964	.717
Maximum tumor diameter (cm) (≤5 vs >5)	0.697	0.404–1.202	.194	0.499	0.210–1.183	.114
Tumor number (1 vs ≥2)	0.502	0.255–0.986	**.045***	0.488	0.109–2.192	.349
Differentiation (moderate vs moderate-poor/poor)	1.146	0.614–2.137	.669	1.644	0.680–3.978	.270
TNM stage (I/II vs III/IV)	0.039	0.014–0.109	**<.001***	0.109	0.029–0.407	**.001***
Vascular invasion (no vs yes)	0.717	0.346–1.486	.371	0.615	0.245–1.547	.302
Satellite lesion (no vs yes)	0.703	0.313–1.579	.393	1.390	0.265–7.291	.697
Lymph node metastasis (no vs yes)	0.110	0.047–0.260	**<.001***	0.152	0.046–0.504	**.002***
Tumor recurrence (no vs yes)	0.600	0.313–1.148	.123	0.678	0.234–1.965	.475
CXCL3 expression (low vs high)	0.718	0.410–1.257	.247	0.540	0.242–1.206	.133
CA199 (U/mL) (<37 vs ≥37)	0.583	0.338–1.004	.052	0.732	0.364–1.473	.382
CEA (μg/L) (<5 vs ≥5)	1.179	0.688–2.022	.550	1.408	0.604–3.280	.428
AFP (μg/L) (<25 vs ≥25)	0.472	0.216–1.031	.060	0.737	0.198–2.752	.650
HBsAg (negative vs positive)	0.681	0.367–1.262	.222	1.134	0.501–2.568	.763

CI = confidence interval, CXCL3 = C-X-C motif chemokine ligand 3, HR = hazard ratio, OS = overall survival.

* A *P* value of <.05 was considered statistically significant.

Table 4

Univariate and multivariate Cox regression analyses for DFS in CCA.

	Univariate analysis			Multivariate analysis		
Variables	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age (<60 vs ≥ 60)	0.915	0.539–1.554	.742	0.822	0.399–1.697	.597
Sex (Female vs Male)	0.618	0.354–1.080	.091	0.572	0.271–1.211	.145
Maximum tumor diameter (cm) (≤ 5 vs > 5)	0.844	0.495–1.437	.532	1.185	0.520–2.702	.686
Tumor number (1 vs ≥ 2)	0.812	0.417–1.581	.540	3.145	0.834–11.858	.091
Differentiation (Moderate vs Moderate-Poor/Poor)	0.731	0.382–1.397	.343	0.734	0.304–1.771	.491
TNM stage (I/II vs III/IV)	0.206	0.103–0.412	**<.001***	0.307	0.108–0.878	**.028***
Vascular invasion (No vs Yes)	0.681	0.332–1.398	.296	0.539	0.221–1.315	.175
Satellite lesion (No vs Yes)	0.894	0.398–2.010	.787	0.604	0.143–2.546	.492
Lymph node metastasis (No vs Yes)	0.214	0.098–0.467	**<.001***	0.473	0.159–1.406	.178
Tumor recurrence (No vs Yes)	0.133	0.053–0.336	**<.001***	0.081	0.020–0.327	**<.001***
CXCL3 expression (Low vs High)	1.103	0.632–1.926	.730	0.894	0.411–1.947	.778
CA199 (U/ml) (< 37 vs ≥ 37)	0.728	0.425–1.248	.249	0.707	0.368–1.357	.297
CEA (μg/l) (< 5 vs ≥ 5)	1.426	0.817–2.486	.211	0.722	0.299–1.745	.470
AFP (μg/l) (< 25 vs ≥ 25)	0.734	0.345–1.560	.421	0.513	0.157–1.676	.269
HBsAg (Negative vs Positive)	0.722	0.388–1.346	.306	0.859	0.389–1.898	.707

CI = confidence interval, CXCL3 = C-X-C motif chemokine ligand 3, DFS = disease-free survival, HR = hazard ratio.

* A *P* value of <.05 was considered statistically significant.

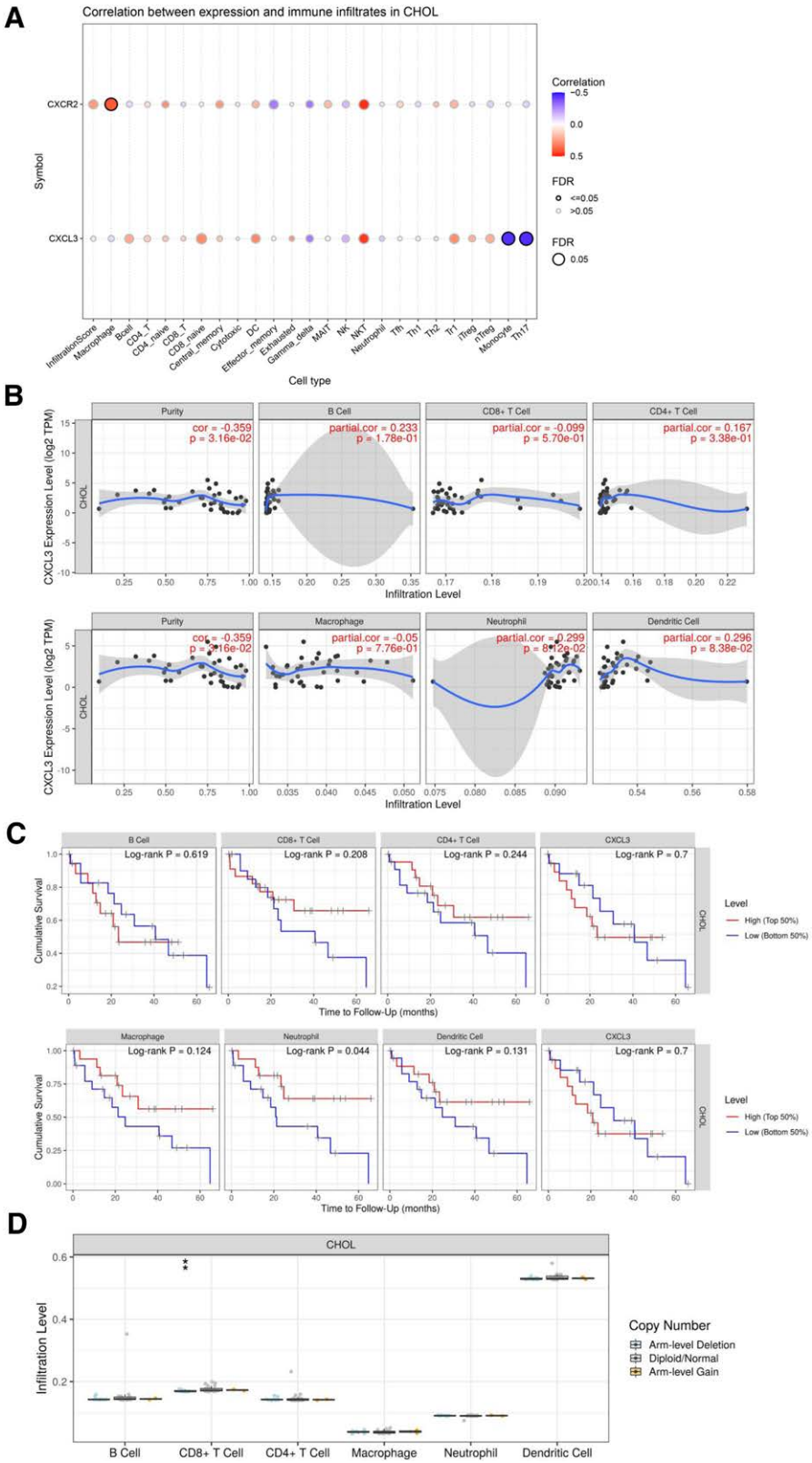

Figure 4. Association between CXCL3 expression and immune infiltration in CHOL. (A) The relationships between CXCL3 as well as CXCR2 and immune cell infiltration of CHOL using the GSCA database. (B) The potential immunological associations between CXCL3 expression and tumor-infiltrating immune cells using TIMER database. (C) The prognostic values of 6 immune infiltrating cells in patients with CHOL using TIMER database. (D) The relationships of the SCNA of CXCL and the infiltration levels of 6 immune cells using TIMER database. $^{**}P < .01$. CHOL = cholangiocarcinoma, CXCL3 = C-X-C motif chemokine ligand 3, CXCR2 = CXC chemokine receptor 2.

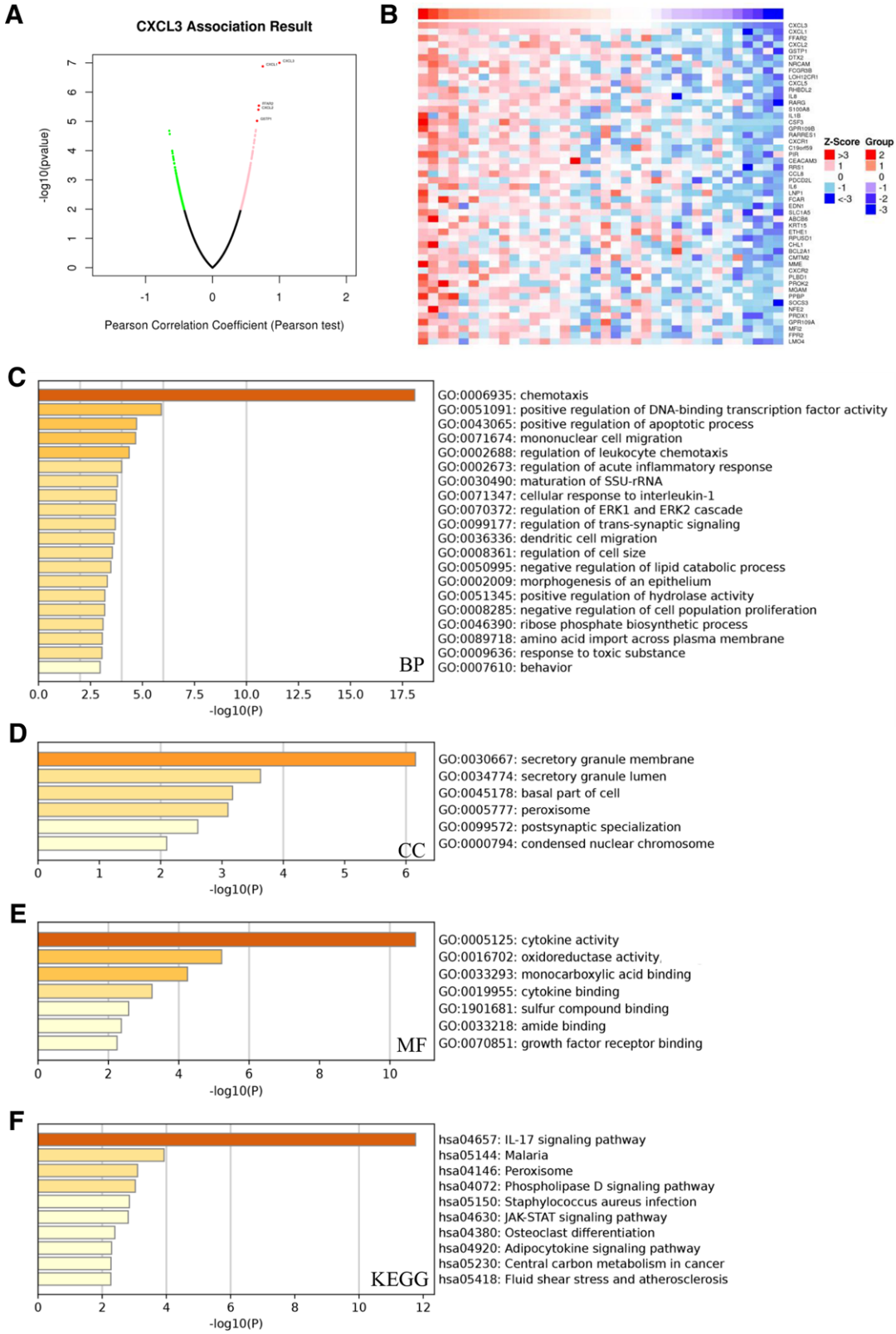

Figure 5. Function enrichment analysis of CXCL3 and its co-expressed genes in patients with CHOL. (A) Volcano plot analysis of co-expressed genes related with CXCL3 in CHOL using LinkedOmics. (B) The top 50 positively related genes of CXCL3 using LinkedOmics. GO enrichment analyses of BP (C), CC (D) and MF (E) of CXCL3 and its co-expressed genes using Metascape. (F) KEGG pathway analysis of CXCL3 and its co-expressed genes using Metascape. BP = biological process, CC = cellular composition, CHOL = cholangiocarcinoma, CXCL3 = C-X-C motif chemokine ligand 3, MF = molecular function.

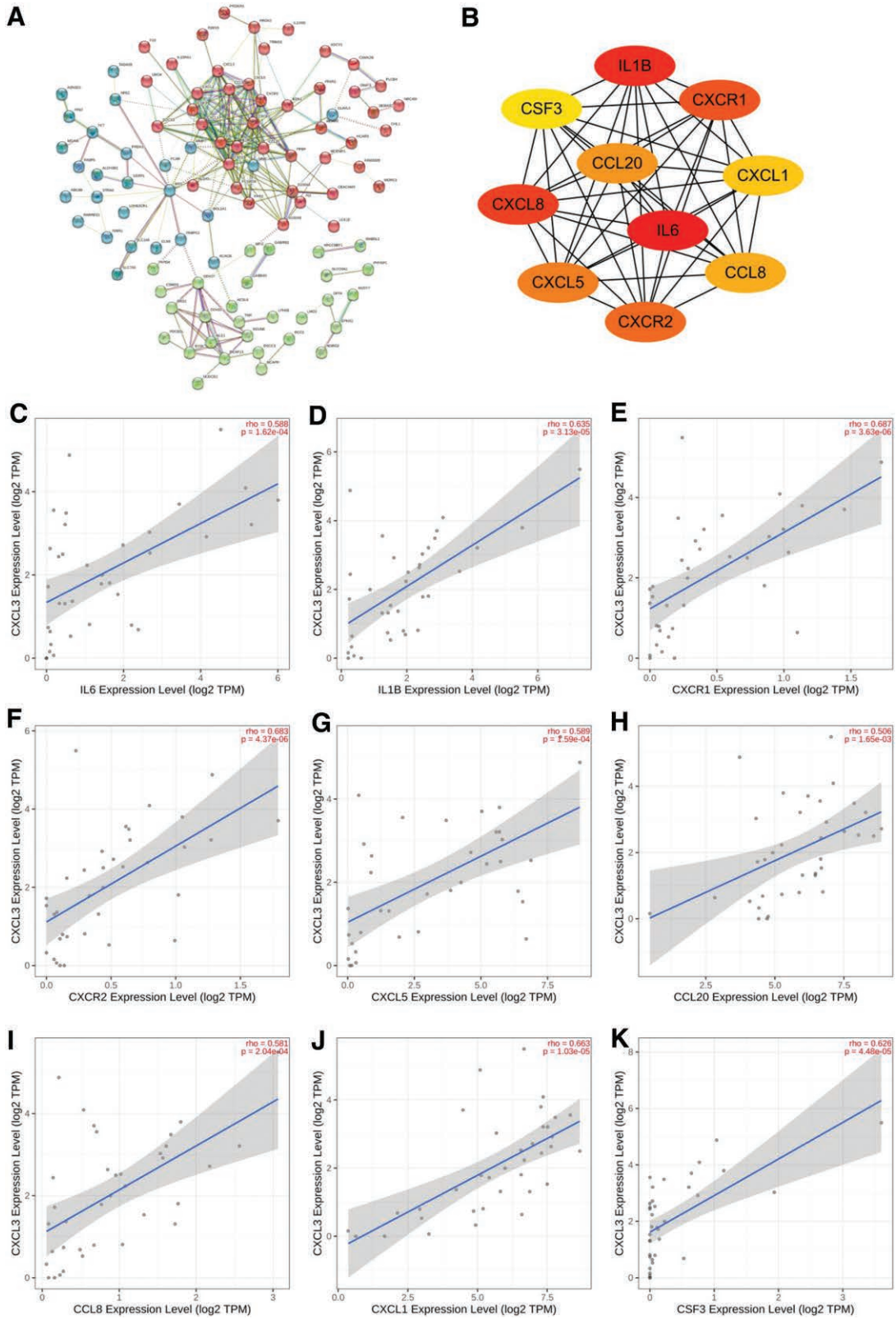

Figure 6. Protein-protein interaction (PPI) network analysis of CXCL3 in CHOL patients. (A) The PPI network analysis of CXCL3 in CHOL using the STRING protein interaction database. (B) The top 10 hub targets related with CXCL3 using the CytoScape. (C–K) The correlation of CXCL3 and the above top 10 hub targets in CHOL patients using TIMER database. CHOL = cholangiocarcinoma, CXCL3 = C-X-C motif chemokine ligand 3.

3.3. Prognostic value of CXCL3 in CHOL patients

The prognostic value of CXCL3 in patients with CHOL was evaluated using UALCAN database, GEPIA database and CHOL microarray. As shown in Figure 3A, *CXCL3* expression had no effect on the survival of CHOL patients, which was analyzed through UALCAN database. Moreover, the result of GEPIA database revealed that there were no obvious differences of OS and DFS between high and low *CXCL3* groups

(Fig. 3B–C). Correspondingly, our result of Kaplan–Meier survival analysis also demonstrated no significant impacts on OS and DFS in CHOL patients for the expression of CXCL3 (Fig. 3D–E). To further investigate the independent prognostic risk for DFS and OS of CHOL patients, univariate and multivariate Cox regression analyses were conducted in this study. As shown in Table 3, univariate analysis supported that the OS of 60 patients with CHOL were related with tumor number, TNM stage and lymph node metastasis. And multivariate analysis indicated that TNM stage and lymph node metastasis may be independent prognostic markers for OS of CHOL patients. In addition, the DFS of CHOL patients were associated with TNM stage, lymph node metastasis and tumor recurrence via univariate analysis. Multivariate analysis suggested that TNM stage and tumor recurrence may be independent prognostic markers for DFS of CHOL patients (Table 4).

3.4. Immune cell infiltration of CXCL3 in patients with CHOL

We analyzed the relationship between CXCL3 and immune cell infiltration of CHOL using the GSCA and TIMER database. As shown in Figure 4A, there were negative correlations between the expression of CXCL3 and monocyte as well as Th17 using GSCA database. As the receptor of CXCL3, CXCR2 expression was positively associated with macrophage. However, the result of TIMER database revealed that the expression of CXCL3 in CHOL was not statistically correlated with 6 immune infiltrating cells including B cell, CD8 T cell, CD4 T cell, macrophage, neutrophil, and dendritic cell (Fig. 4B, Supplemental Table 2, http://links.lww.com/MD/L859). We further explored the prognostic value of 6 immune infiltrating cells in patients with CHOL through TIMER database. Interestingly, low infiltration of neutrophil indicated significantly shorter cumulative survival in CHOL patients (Fig. 4C). In addition, somatic copy number alteration (SCNA) of the CXCL3 in CHOL was investigated using TIMER database. As shown in Figure 4D, CXCL3 was significantly associated with arm-level deletion of $CD8^+$ T cell.

3.5. Function enrichment analysis of CXCL3 in patients with CHOL

We screened 153 co-expressed genes of CXCL3 in CHOL using LinkedOmics (Fig. 5A). The top 50 positively related genes were shown in Figure 5B including CXCL1, FFAR2, CXCL2 and so on. Next, GO enrichment and KEGG pathway analyses were conducted using Metascape based on the above 153 related genes. The GO enrichment analyses were divided into 3 functional groups: biological process (BP), cellular composition (CC), and molecular function (MF). In the BP category, CXCL3 and its associated genes were mainly enriched for chemotaxis, positive regulation of DNA-binding transcription factor activity, and positive regulation of apoptotic process (Fig. 5C). In the CC category, secretory granule membrane, secretory granule lumen, basal part of cell, peroxisome, postsynaptic specialization and condensed nuclear chromosome were related to CXCL3 and its associated genes (Fig. 5D). Most highly enriched GO items which found in MF category were mainly cytokine activity, oxidoreductase activity and monocarboxylic acid binding (Fig. 5E). As for KEGG pathway analysis, CXCL3 and its associated genes were mainly enriched for IL-17 signaling pathway, malaria and peroxisome (Fig. 5F).

3.6. Protein-protein interaction (PPI) network analysis of CXCL3 in CHOL patients

As shown in Figure 6A, the STRING database was used to establish the PPI networks analysis of CXCL3 in patients with CHOL. Moreover, we used the CytoScape to evaluate the top 10 hub targets including IL6, IL1B, CXCL8, CXCR1, CXCR2, CXCL5, CCL20, CCL8, CXCL1 and CSF3 (Fig. 6B). The correlation of CXCL3 and the above top 10 hub targets in CHOL patients were further verified using TIMER database. As shown in Figure 6C to K, CXCL3 expression in CHOL was significantly related with the expression of IL6, IL1B, CXCR1, CXCR2, CXCL5, CCL20, CCL8, CXCL1 and CSF3, which was consistent with the result of CytoScape.

4. Discussion

CHOL, also known as biliary tract cancer, is an uncommon adenocarcinoma occurring in the intra- and extrahepatic biliary system. The patient with CHOL is often diagnosed at an advanced stage, when potentially curative surgical treatments are not suitable. Some risk factors related CHOL have been recognized including alcohol consumption, smoking and hepatitis B/C virus infection. It is worth noting that CXCL3 is upregulated in a variety of digestive tumors, such as colorectal cancer,[21] gastric cancer,[22] and hepatocellular carcinoma.[23] Whereas, the role of CXCL3 in the progression of CHOL remains unclear.

In this study, we found that the mRNA expression of CXCL3 was markedly increased in CHOL tissues using TIMER, GEPIA, and UALCAN database. CXCL3 protein was strongly elevated in CHOL tissues comparing with paracarcinoma tissues. Moreover, overexpression of CXCL3 was strongly associated with maximum tumor diameter of patients with CHOL, but was not related with age, sex, tumor number, differentiation, TNM stage, vascular invasion, satellite lesion, lymph node metastasis, tumor recurrence, CA199, CEA, AFP and HBsAg of CHOL patients. In addition, CXCL3 expression had no effect on the survival of CHOL patients, which was evaluated through UALCAN database, GEPIA database and CHOL microarray.

Currently, evidence has indicated that CXCL3 could serve as tumor marker to participate the occurrence and development of cancers.[24] For example, Overexpression of CXCL3 can enhance the oncogenic potential of prostate cancer.[25] Moreover, CXCL3 mediates prostate cancer cells proliferation and migration through autocrine/paracrine pathways.[26] In addition, CXCL3 overexpression affects the malignant behavior of uterine cervical cancer and oral squamous cell carcinoma via the MAPK signaling pathway.[9,27] In breast cancer, adipocyte-derived CXCL3, binding to its specific receptor CXCR2, promotes metastasis of breast cancer cells via the FAK signaling pathway.[28] However, the potential function of CXCL3 in CHOL is undemonstrated.

In the present study, we identified that there were negative correlations between the expression of CXCL3 and monocyte as well as Th17 using GSCA database. Low infiltration of neutrophil indicated significantly shorter cumulative survival in CHOL patients. And CXCL3 was significantly associated with arm-level deletion of $CD8^+$ T cell. Furthermore, GO enrichment and KEGG pathway analyses indicated that CXCL3 and its associated genes were mainly enriched for chemotaxis, secretory granule membrane, cytokine activity and IL-17 signaling pathway. The STRING database was used to establish the PPI networks analysis of CXCL3 in CHOL patients. The top 10 hub targets of CXCL3 in CHOL included IL6, IL1B, CXCL8, CXCR1, CXCR2, CXCL5, CCL20, CCL8, CXCL1 and CSF3, which were significantly related with the expression of CXCL3.

Although there are interesting discoveries identified by these results, our study also has some limitations. First, the detailed effect and mechanism of CXCL3 in CHOL will be the focus of our research work in the future. Second, due to the limited number of patients with CHOL in databases and tissue microarray, the study will be validated with a larger sample size. Third, the research was performed a retrospective method, and needed for further prospective validation.

In summary, we performed comprehensive analysis of the clinical and biological significances for CXCL3 with CHOL patients including expression, clinicopathological factors, immune cell infiltration, GO enrichment and KEGG pathway analyses, as well as PPI network analysis. These findings suggested that CXCL3 may potentially participate in the carcinogenesis of CHOL, which provided a novel therapeutic strategy for CHOL. In addition, CXCL3 could serve as a tumor marker of CHOL, which showed the potential clinical implications of CXCL3 in CHOL, suggesting CXCL3 may be further utilized in CHOL diagnosis or treatment.

Author contributions

Conceptualization: Hongyue Ren, Chunbin Zhang.
Data curation: Xiaofan Yang, Wenrong Hou.
Formal analysis: Jiarong Meng, Deqing Luo.
Methodology: Hongyue Ren, Deqing Luo.
Writing – original draft: Hongyue Ren, Deqing Luo.
Writing – review & editing: Chunbin Zhang.

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