Cancer Research Journal
2022; 10(1): 1-15
http://www.sciencepublishinggroup.com/j/crj
doi: 10.11648/j.crj.20221001.11
ISSN: 2330-8192 (Print); ISSN: 2330-8214 (Online)

Comprehensive Analysis of the Prognosis and Correlations with Immune Infiltration of CXC Chemokine Family Members in Diffuse Large B-cell Lymphoma

Yuli Cao[1], Fenling Zhou[1], Cuilan Deng[2], Gexiu Liu[1, *]

[1]Institute of Hematology, Jinan University, Guangzhou, China

[2]Department of Hematology, The First Affiliated Hospital, Jinan University, Guangzhou, China

Email address:

tliugx@jnu.edu.cn (Gexiu Liu)

[*]Corresponding author

To cite this article:

Yuli Cao, Fenling Zhou, Cuilan Deng, Gexiu Liu. Comprehensive Analysis of the Prognosis and Correlations with Immune Infiltration of CXC Chemokine Family Members in Diffuse Large B-cell Lymphoma. *Cancer Research Journal*. Vol. 10, No. 1, 2022, pp. 1-15.
doi: 10.11648/j.crj.20221001.11

Received: December 21, 2021; **Accepted**: January 13, 2022; **Published**: January 21, 2022

Abstract: *Background:* Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma. The failure rate of treatment for its subsets is still very high. CXC chemokine, secreted by a variety of cells, is a vital component in the immune process. It also participated in the growth, development, and metastasis of tumors. This study aimed to explore the prognosis of CXC chemokines in DLBCL and the relationship with immune infiltration through bioinformatics analysis. *Methods:* We systematically analyzed the expression level and prognostic value of CXC chemokines in DLBCL patients, and the correlation between CXC chemokines and tumor immune infiltration through databases, such as Oncomine, GEO, GEPIA, GeneMANIA, DAVID, HPA, GenomicScape, and TIMER2.0. *Results:* With the comprehensive analysis of different databases, we found that CXC chemokines (CXCL1/2/5/6/7/8/9/10/11/12/13/14) had significantly higher transcription levels in DLBCL patients vs. the control groups. The up-regulation mRNA levels of CXCL1/2/6/7/10/12 were associated with poor prognoses in DLBC patients. Further enrichment analysis of CXC chemokines and their receptors revealed that they were related to the infiltration and metastasis of immune cells. Besides, we found that the expression of CXCL9/10/11 were significantly correlated with tumor-infiltrating lymphocytes (TILs) (B cells, CD8+ T cells, CD4+ T cells, macrophages, Treg cells, and NK cells) and immune checkpoints in DLBCL. *Conclusion:* Our study may provide novel understandings for CXC chemokines as immunotherapeutic targets and prognostic biomarkers in diffuse large B-cell lymphoma through systematic analysis.

Keywords: Diffuse Large B-cell Lymphoma (DLBCL), Immune Infiltration, Prognosis, Biomarker, Bioinformatics Analysis

1. Introduction

Diffuse large B cell lymphoma (DLBCL), a most common type of non-Hodgkin's lymphoma (NHL), accounts for approximately 30-40% of all NHL patients, with three pathological types (ABC, GBC, unclassified) [1, 2]. As for the high failure rate of DLBCL subsets treatment, it has become a trend that the combination of chemotherapy, immunotherapy, and drug target molecules [3]. In recent years, the exploration of DLBCL therapeutic targets, especially immune checkpoint inhibitors, has made some progress [4-6]; however, this is far from adequate so that more therapeutic targets and prognostic biomarkers should be found.

CXC chemokines, as a small molecule secreted protein of approximately 8 to 10 kDa, participate in many biological processes, such as regulating angiogenesis, tumor growth, metastasis, and straightly utilizing an autocrine or paracrine method to stimulate tumor proliferation [7, 8]. Some studies have reported that the expression of CXC chemokines and their receptors in DLBCL patients were related to worse outcomes [9-11].

Although some previous studies have found that high expression levels of CXC chemokines and their receptors were related to the survival and prognosis of DLBCL patients, CXC chemokines have not been systematically studied. With the maturity and cost reduction of the second-generation high

throughput gene sequencing technology, the comprehensive analysis of CXC chemokines becomes possible.

Based on the integrated analysis of various databases, the expression of CXC chemokines in DLBCL were distinctly elevator. Meanwhile, it is found that their high expression had a certain correlation with the poor survival of DLBCL patients. We further found that they were related to immune function through the biological enrichment of CXC chemokines and co-expressed genes. In addition, we had an in-depth understanding of the relationship between CXC chemokines and immune infiltrating cells or various immune markers. The above results indicated that CXC chemokines with high transcription levels might be considered as the prognosis and immune infiltration markers of DLBCL. They could help clinicians design more reasonable treatment plans and further more confidently predict the treatment effects of patients. The flowchart of this research was shown in Figure 1.

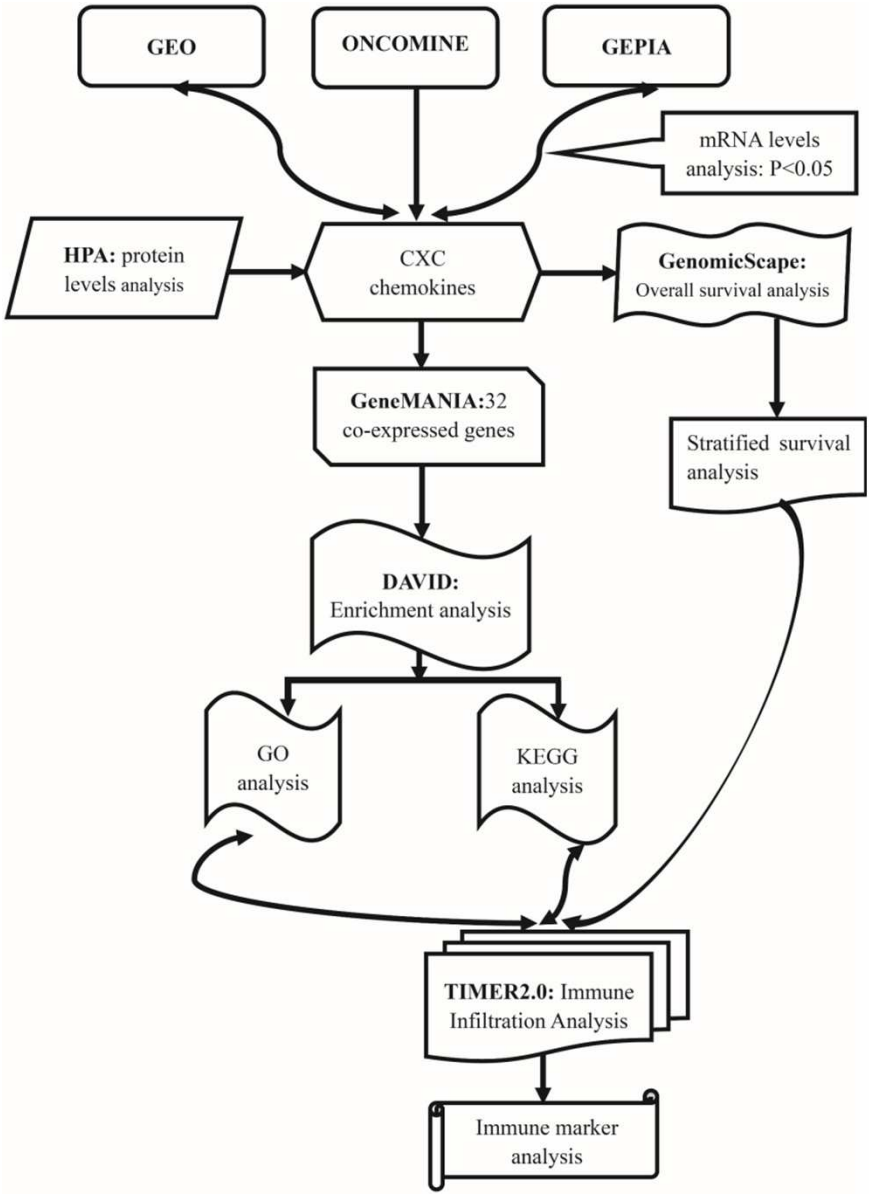

Figure 1. *Flow diagram of this study.*

2. Materials and Methods

2.1. Gene Expression Omnibus

Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) is an integrated, public genomics database that stores three types of data: gene expression datasets, original series and platforms [12]. To further identify mRNA levels of the CXC chemokines, the gene expression profiles of GSE56315 [13], based on GPL570 Platform [(HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array], were downloaded. Microarray data of GSE56315 includes a total of 55 DLBCL and 33 non-cancer samples. Then, with defined P-value cutoffs of <0.05, the differentially expressed CXC chemokines were screened out

based on the SangerBox website, (http://sangerbox.com/Tool), as a simple visualization platform based on the R package, which was used for analyzing the data obtained.

2.2. ONCOMINE Database

ONCOMINE (www.oncomine.org) is a powerful data mining platform, aim to analyze the transcription level of the genome-wide and understand the expression differences of various diseases [14]. The mRNA levels of the CXC family in DLBCL were analyzed using ONCOMINE databases. To evaluate the expression of CXC family in DLBCL, the threshold fold change was set as 1.0, and the critical value of P-value was defined as 0.05.

2.3. GEPIA Dataset

Based on the Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects, the GEPIA database, developed by Zhang Laboratory of Peking University, contains high-throughput sequencing data of 9,736 tumors and 8,587 normal samples [15]. Using multiple gene comparison sections, we assessed the relative transcription levels of CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7 (PPBP), CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL16, and CXCL17. Based on the "Correlation" module, we explored the correlation between different immune checkpoints and the CXC family.

2.4. GeneMANIA

GeneMANIA (http://www.genemania.org), a flexible and user-friendly web interface, could provide the protein interaction network, gene co-expression, similar protein domains, co-localization, and involved pathways of studied genes [16].

2.5. DAVID 6.8 Database

The Database for Annotation, Visualization, and Integrated Discovery (DAVIDv6.8; https://david.ncifcrf.gov/) was used to annotate and analyze numerous genes [17]. We performed the enrichment analysis of the CXC family and the top 20 strongly associated genes, including two aspects: Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Among them, GO was composed of biological process (BP), molecular function (MF), and cellular component (CC). Threshold conditions for GO terms and KEGG pathways were set at $P < 0.05$.

2.6. The HPA Database

The Human Protein Atlas (HPA, http://www.proteinatlas.org) is an interactive database involving the human transcriptome, proteome, and metabolic functions of different tissues and organs [18]. Based on the HPA database, translational-level validation of the CXC family in DLBC tissues by immunohistochemistry was carried out.

2.7. GenomicScape

GenomicScape (http://genomicscape.com/) was established based on data obtained from Gene Expression Omnibus (GEO), which could analyze the correlation between the expression levels of various cancers and the prognosis of their patient [19]. Through the survival analysis module, twelve individuals of the CXC chemokines were entered into GenomicScape, respectively, to analyze their prognostic value in DLBC patients. Then, we can obtain Kaplan–Meier survival plots with hazard ratios (HR) and log-rank p-value. The statistical threshold was regarded as $P<0.05$.

2.8. TIMER2.0 Database Analysis

TIMER2.0 (Tumor IMmune Estimation Resource 2.0; http://timer.cistrome.org/) is an intuitive web server that provides comprehensive exploration and visualization functions of gene-tumor-immune interactions. Based on the validation of pathological estimates, the TIMER2.0 database can systematically analyze tumor immunology, the composition and abundance of immune cells in different tumors, and clinical characteristics. Tumor purity was utilized for the correction of Spearman-based correlation analysis [20]. Moreover, we screened out subtype-based biomarkers to further analyze the relationship between the CXC family and immune infiltration.

2.9. Statistical Analysis

Based on the R language, the default t-test and Wilcoxon analysis were adopted to screen for the differentially expressed gene by the SangerBox website. The differential expression of genes in ONCOMINE database was analyzed by t-test. The spearman algorithm was utilized for gene expression correlation analysis in the GEPIA database. The log-rank test was used for Kaplan-Meier survival. The Spearman correlation analysis was utilized to explore the relationship between the CXC family and immune infiltration in TIMER2.0. The fold change revealed the ratio of the expression levels between DLBCL samples and normal lymph nodes. Generally, the screening threshold of |log2FC| was set as 1, and greater than 1 were differentially expressed genes. A p-value < 0.05 was defined as being statistically different.

3. Results

3.1. Validation for mRNA Levels of CXC Chemokines Family in Multi-databases

We utilized the ONCOMINE database to detect mRNA levels of 16 CXC chemokines (excluding CXCL15) in DLBC compared with normal tissues. The results were shown in Table 1. The transcriptional levels of CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, and CXCL16 were significantly elevated in patients, while CXCL17 did not differ significantly.

To verify the mRNA expression of CXC chemokines, we

downloaded the GSE56315 dataset from the GEO database. The gene expression profile analysis demonstrated that the transcript levels of CXCL1/2/5/6/7/8/9/10/11/12/13/14/17 were more ascendant in DLBCL patients than in normal lymph node tissues (Figure 2A).

In additional, we analyzed the relative mRNA levels between CXC chemokines in DLBCL patients. Among the CXC family members we identified, the relative transcript level of CXCL9 was the highest vs. other CXC chemokines (Figure 2B).

Ultimately, we excluded CXCL3, CXCL4, CXCL16, and CXCL17 from further analysis since their expression was no significant difference between DLBC samples and normal samples.

Table 1. *The mRNA levels of CXC chemokines in DLBC tissues (ONCOMINE).*

GENE	Datatype	Type	P-value	t-Test	Fold Change	References
CXCL1	mRNA	DLBC vs normal	2.15E-11	10.498	1.984	[21]
	mRNA	DLBC vs normal	3.00E-03	2.887	1.496	[22]
CXCL2	mRNA	DLBC vs normal	1.50E-02	3.853	3.005	[23]
	mRNA	DLBC vs normal	2.00E-03	3.816	1.628	[21]
	mRNA	DLBC vs normal	4.40E-02	1.772	1.371	[24]
	mRNA	DLBC vs normal	1.73E-06	5.101	2.544	[22]
	mRNA	DLBC vs normal	3.00E-03	3.398	1.260	[25]
CXCL3	mRNA	DLBC vs normal	4.50E-02	1.732	1.450	[26]
	mRNA	DLBC vs normal	7.00E-03	2.875	1.174	[25]
	mRNA	DLBC vs normal	2.00E-03	3.107	1.343	[22]
CXCL4	mRNA	DLBC vs normal	8.01E-04	3.334	1.438	[22]
CXCL5	mRNA	DLBC vs normal	5.86E-07	5.443	1.107	[22]
CXCL6	mRNA	DLBC vs normal	2.40E-02	2.030	1.393	[22]
CXCL7	mRNA	DLBC vs normal	6.16E-04	3.456	1.890	[22]
CXCL8	mRNA	DLBC vs normal	3.00E-02	2.049	1.112	[25]
CXCL9	mRNA	DLBC vs normal	2.00E-03	5.659	9.957	[23]
	mRNA	DLBC vs normal	3.86E-16	14.862	17.389	[24]
	mRNA	DLBC vs normal	1.44E-08	7.017	19.907	[26]
	mRNA	DLBC vs normal	6.91E-09	15.964	6.797	[21]
	mRNA	DLBC vs normal	6.12E-31	23.643	54.026	[22]
	mRNA	DLBC vs normal	6.37E-04	5.642	24.803	[27]
	mRNA	DLBC vs normal	3.27E-05	6.522	19.134	[25]
CXCL10	mRNA	DLBC vs normal	8.61E-09	7.348	5.390	[24]
	mRNA	DLBC vs normal	6.66E-08	13.703	5.656	[21]
	mRNA	DLBC vs normal	9.08E-07	5.484	8.586	[26]
	mRNA	DLBC vs normal	8.76E-27	19.120	22.362	[22]
	mRNA	DLBC vs normal	1.00E-03	5.144	7.699	[27]
CXCL11	mRNA	DLBC vs normal	3.09E-08	6.825	15.820	[26]
	mRNA	DLBC vs normal	1.38E-19	15.464	14.345	[22]
	mRNA	DLBC vs normal	1.00E-03	4.238	1.576	[21]
	mRNA	DLBC vs normal	3.00E-03	3.423	1.469	[25]
CXCL12	mRNA	DLBC vs normal	1.03E-05	5.044	2.438	[23]
	mRNA	DLBC vs normal	1.66E-06	6.258	3.254	[24]
	mRNA	DLBC vs normal	7.11E-05	5.805	1.645	[21]
	mRNA	DLBC vs normal	1.74E-15	11.315	3.485	[22]
	mRNA	DLBC vs normal	1.40E-02	2.503	1.239	[25]
CXCL13	mRNA	DLBC vs normal	1.68E-11	15.445	8.487	[21]
	mRNA	DLBC vs normal	2.33E-10	7.874	6.352	[24]
	mRNA	DLBC vs normal	4.36E-09	6.802	10.064	[26]
	mRNA	DLBC vs normal	1.09E-12	9.726	17.816	[22]
	mRNA	DLBC vs normal	3.19E-04	4.852	4.685	[25]
CXCL14	mRNA	DLBC vs normal	5.00E-03	3.576	4.734	[27]
	mRNA	DLBC vs normal	2.59E-11	8.320	5.168	[22]
	mRNA	DLBC vs normal	2.54E-04	4.969	2.930	[25]
CXCL16	mRNA	DLBC vs normal	2.39E-18	12.583	3.107	[22]
	mRNA	DLBC vs normal	2.80E-02	2.194	1.546	[22]
	mRNA	DLBC vs normal	6.00E-03	3.005	1.867	[25]

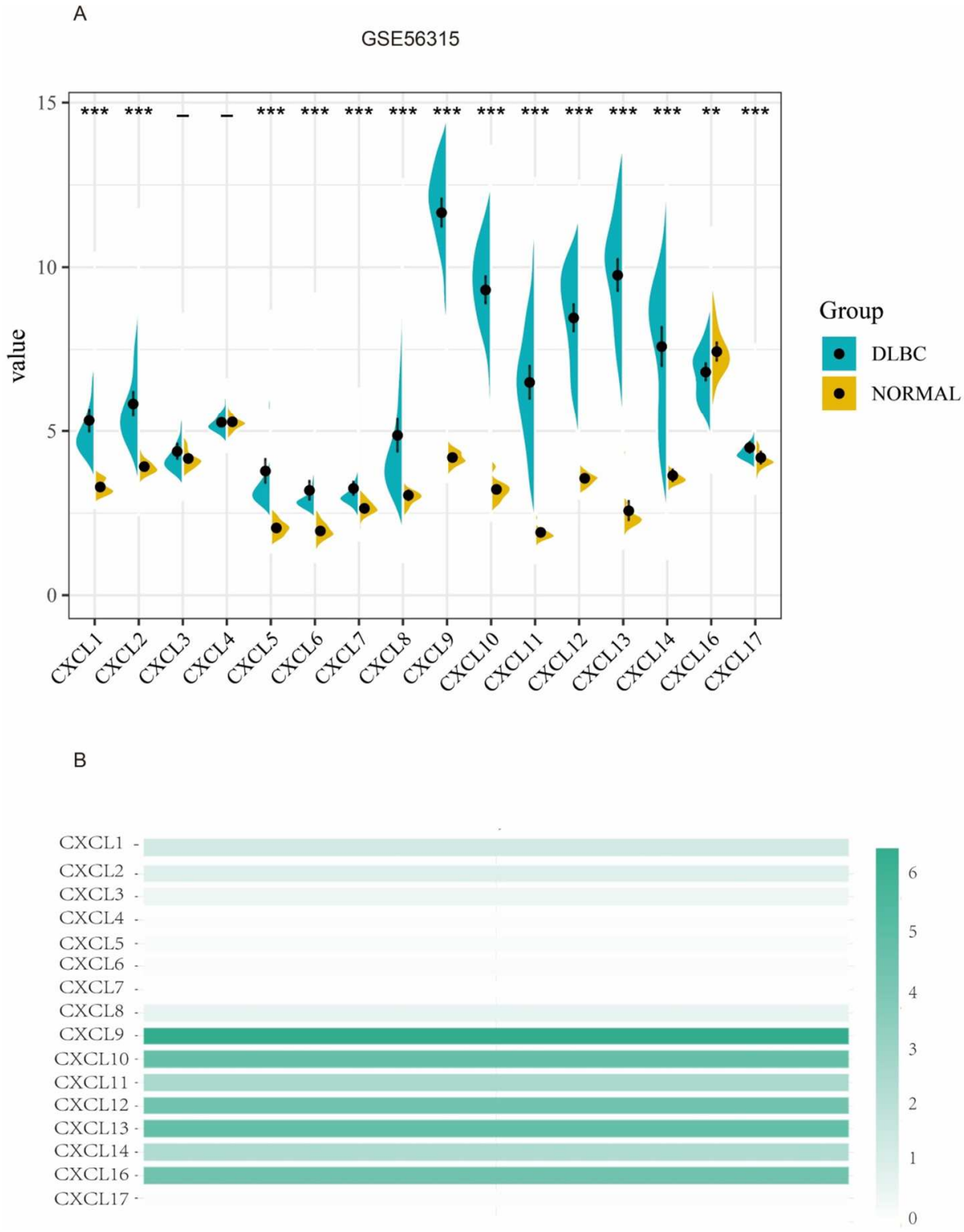

Figure 2. *The expression of CXC chemokines in DLBCL. (A) Violin illustration of CXC chemokine expression in DLBCL. Blue violin, tumor; Yellow violin, normal tissue. *p<0.05, **p<0.01, ***p<0.001, -p>0.05. (B) The relative transcript levels of CXC chemokines in DLBCL. The darker the color, the higher the expression. DLBCL: Diffuse large B cell lymphoma.*

3.2. Protein Levels of CXC Chemokines Family in the HPA Databases

In the HPA database analysis, CXCL8, CXCL11, CXCL12, and CXCL13 were significantly higher in the DLBC tissues than in normal lymph nodes, while the protein levels of CXCL5, CXCL7, CXCL14 in DLBC and the control groups were not detected (Figure 3). There were no related DLBC samples for CXCL1, CXCL2, CXCL6, CXCL9, and CXCL10.

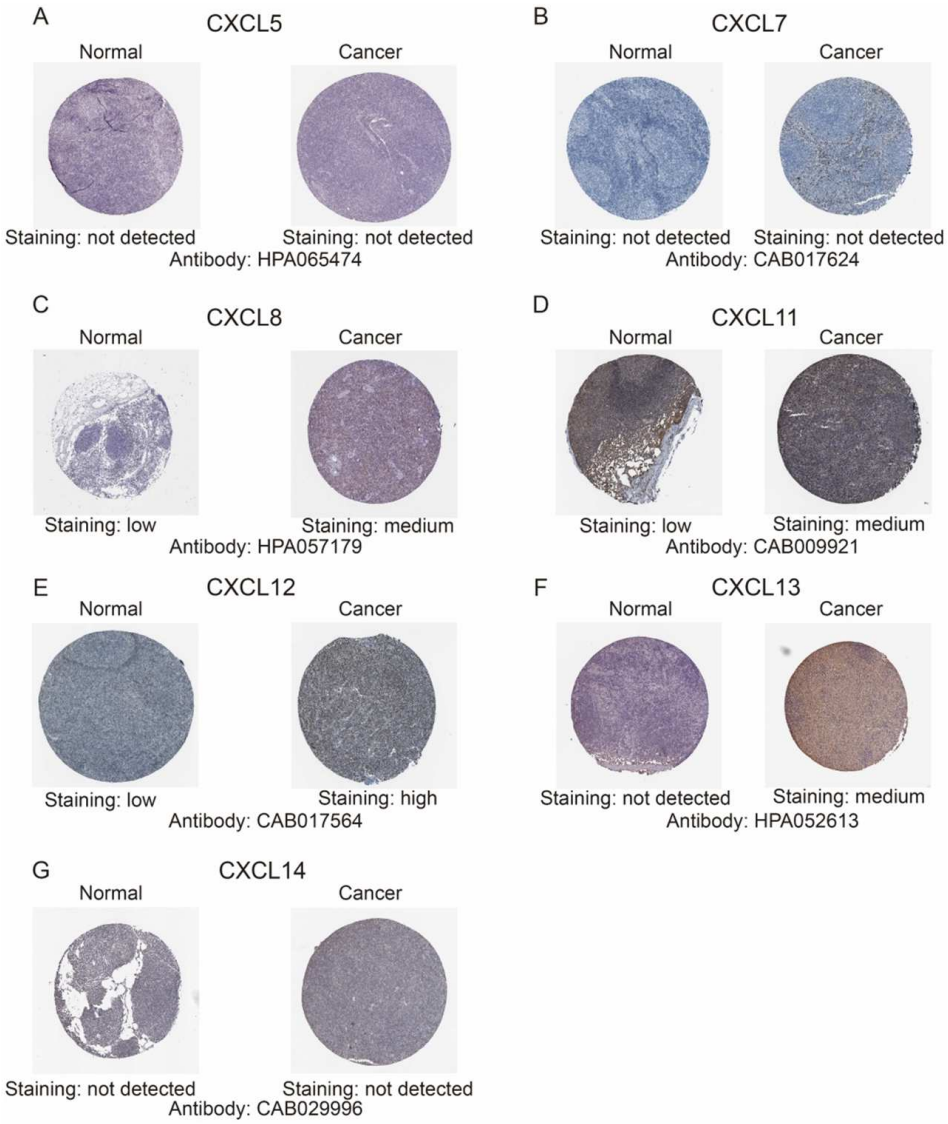

Figure 3. *Representative immunohistochemistry images of CXC chemokines in DLBCL tissues and normal lymph nodes (Human Protein Atlas). (A) CXCL5; (B) CXCL7; (C) CXCL8; (D) CXCL11; (E) CXCL12; (F) CXCL13; (G) CXCL14. DLBCL, Diffuse large B cell lymphoma.*

3.3. Functional Enrichment and Co-expression Analysis of CXC Chemokines in DLBCL

We explored the potential correlation analysis of twelve CXC chemokines in DLBCL, as shown in Figure 4A. Red color represented positive correlations, while blue color represented negative correlations. As a result, the correlation between CXC chemokines could be roughly divided into three grades: low to moderate correlation (CXCL5-8); moderate to high correlation (CXCL1/2/14); high correlation (CXCL9-11).

Based on the GeneMANIA, we revealed that the function of these differentially expressed CXC chemokines (CXCL1-2/CXCL5-14) were primarily involved in chemokine activity, chemokine receptor binding, cellular response to chemokine, and response to chemokines (Figure 4D).

To determine the biological function of the CXC family and the top 20 genes with strong correlations, we analyzed the GO annotation function and KEGG pathway using DAVID 6.8, with a threshold of $P< 0.05$. The GO enrichment analysis results showed that the top 15 categories of GO terms for CXC chemokines included six bioprocesses (BP), three categories of cell components (CC), and six molecular functions (MF). The GO term of “chemokine-mediated signaling pathway” was the most significant enrichment for the BP category. In the CC category, “extracellular space” was the highest enrichment term. The GO term of “chemokine activity” was the most important of the MF category (Figure 4B). A total of 14 KEGG pathways were significantly enriched with a P-value <0.05, as shown in Figure 4C. As to the KEGG pathway analysis, it was found that these genes were mainly involved in chemokine signaling pathway, cytokine-cytokine receptor interaction, rheumatoid arthritis, Toll-like receptor signaling pathway, and TNF signaling pathway. The results revealed that these genes may play important roles in the occurrence and development of DLBCL.

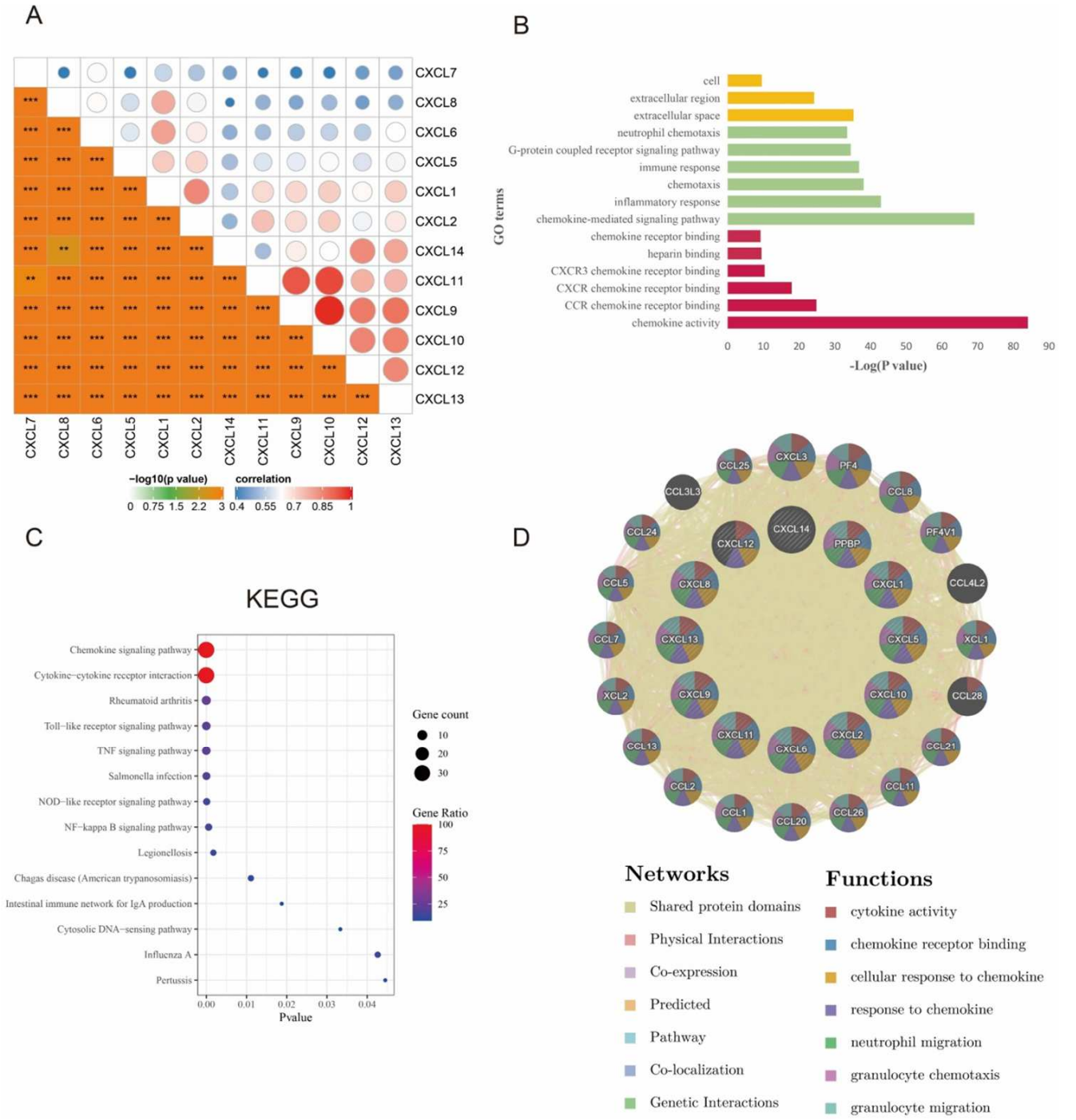

Figure 4. *Functional enrichment, neighbor gene network, and interaction analyses of differently expressed CXC chemokines in DLBC patients. (A) Correlation analysis of differently expressed CXC chemokines in DLBCL. Red color represented positive correlations, while blue color represented negative correlations. *p<0.05, **p<0.01, ***p<0.001, -p>0.05 (B) The top 15 categories of GO terms. Yellow bar graph, CC; Green bar graph, BP; Red bar graph, MF. (C) The top 14 enriched KEGG pathways. (D) Protein-protein interaction network of differently expressed CXC chemokines. BP, bioprocesses; CC, cell components; MF, molecular functions; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DLBCL, Diffuse large B cell lymphoma.*

3.4. The Overexpression of CXC Chemokines Predicts Prognosis in DLBCL Patients

We evaluated the correlation between overexpressed CXC chemokines and clinical outcomes using GenomicScape. The survival curve analysis showed that the high mRNA levels of CXCL1/2/6/7/10/12 were associated with worse overall survival (OS), and CXCL9/13/14 were inter-related with better OS in DLBCL patients. However, the expression of CXCL5/CXCL11 were not associated with the prognosis of DLBCL patients (Figure 5). There were no related DLBCL samples for CXCL8.

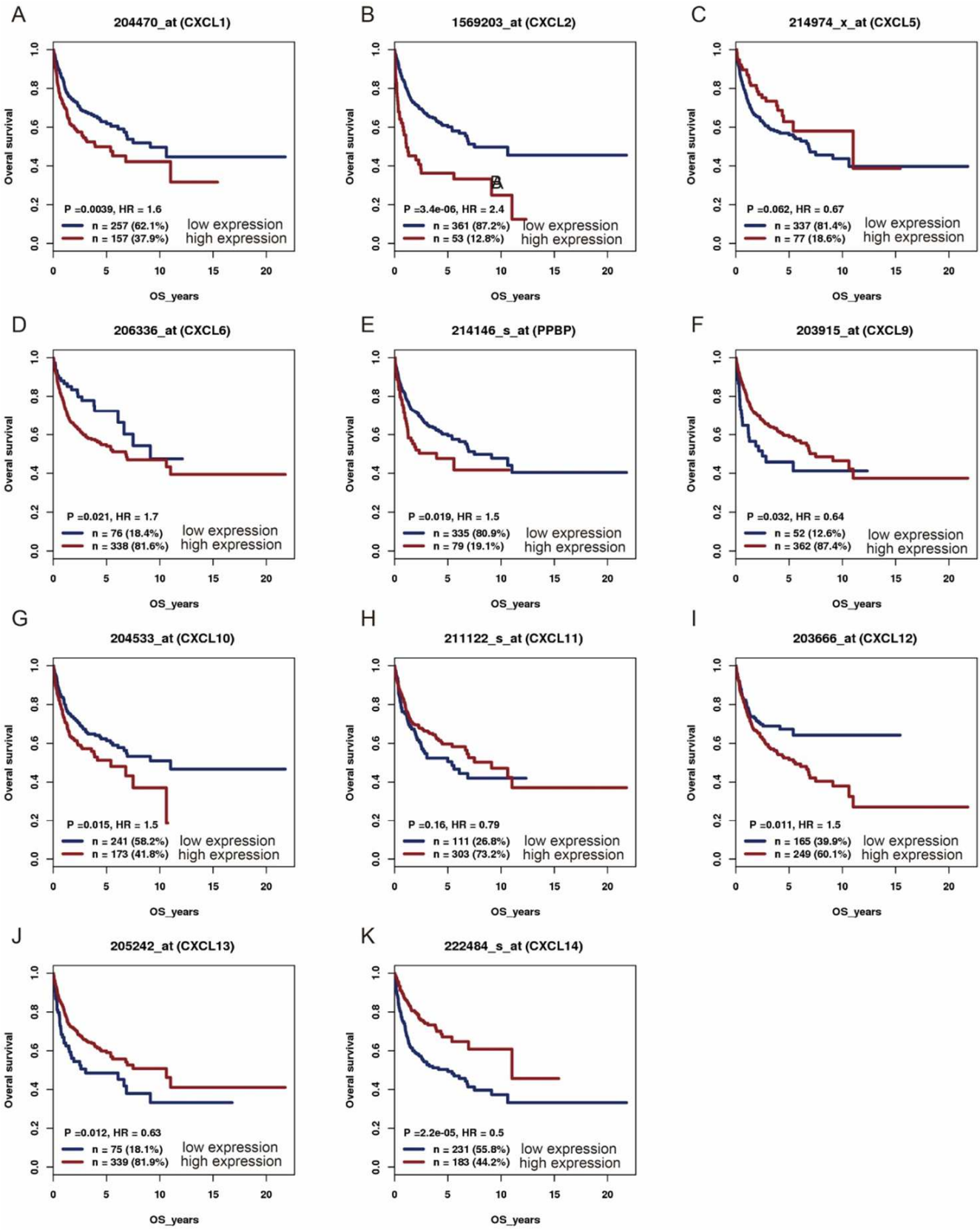

Figure 5. *The survival curve analysis of CXC chemokines mRNA expression in DLBCL patients (GenomicScape). (A) CXCL1; (B) CXCL2; (C) CXCL5; (D) CXCL6; (E) PPBP; (F) CXCL9; (G) CXCL10; (H) CXCL11; (I) CXCL12; (J) CXCL13; (K) CXCL14. The log-rank statistics were used to calculate P-values. OS, overall survival; HR, hazard ratio; DLBCL, Diffuse large B cell lymphoma.*

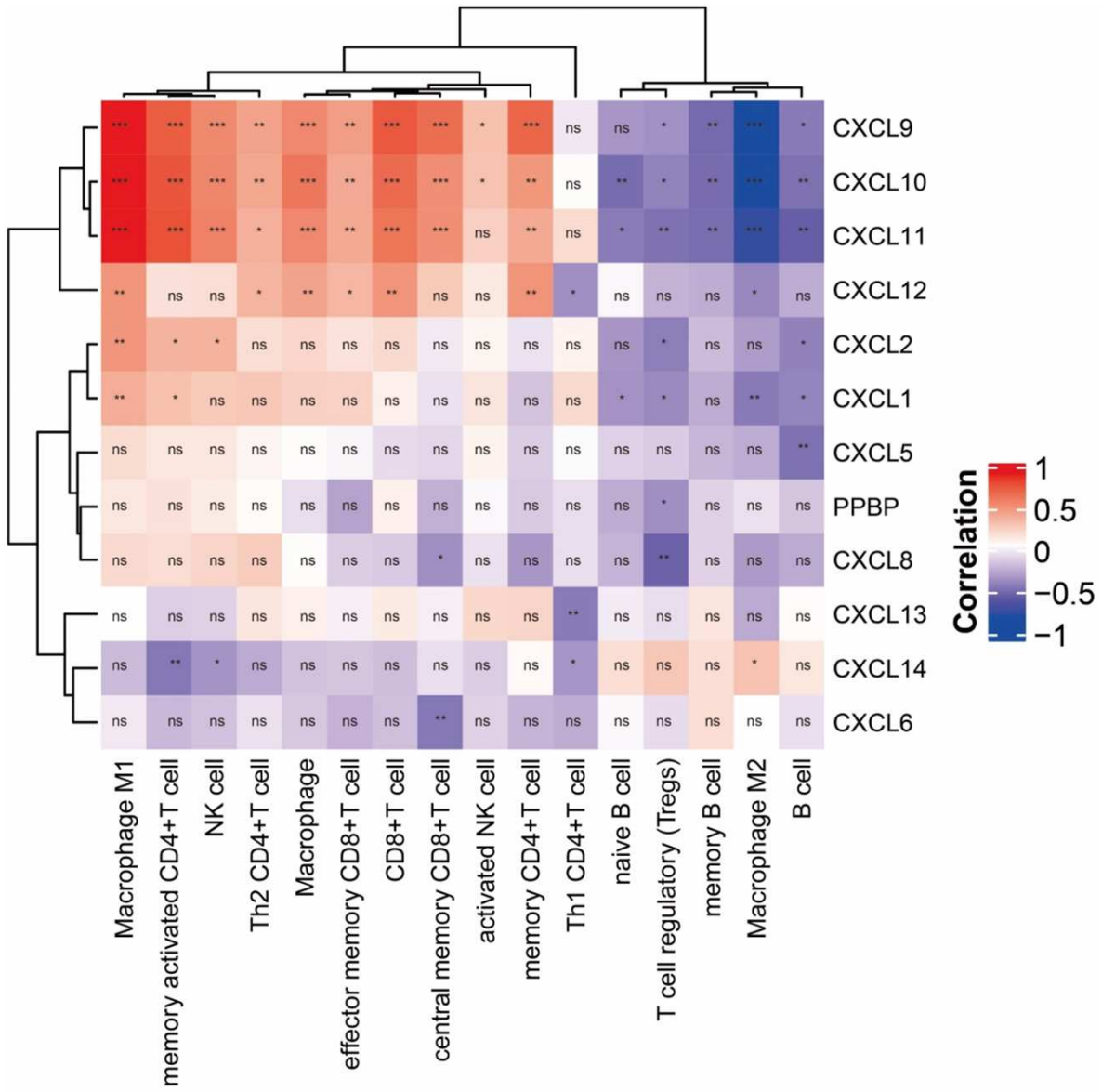

Figure 6. *The heatmap analysis between the expression levels of CXC chemokines and tumor-immune infiltration (TIMER2.0). The correlation coefficient was shown in Fig. ns, no statistical significance; *p < 0.05; **p < 0.01; ***p < 0.001.*

3.5. Immune Cell Infiltration of CXC Chemokines in Patients with DLBCL

Chemokines play a vital role in cancer-related inflammation. Thereby they, directly and indirectly, affect the proliferation and invasion characteristics of tumor cells. Elucidating their role will further promote our understanding of the pathophysiological process of tumor, and will help innovate potential anti-cancer strategies [28]. Some researchers have clarified that CXCL16 may promote the rapid growth of lung cancer cells by regulating the NF-κB pathway, and even increase its invasive ability [11]. By the TIMER2.0, we tried to analyze the correlation between the expression of CXC family and tumor immune infiltration in DLBCL patients.

As shown in Figure 6, tumor purity has been used to correct the correlation analysis based on Spearman. Notably, we observed that CXCL9, CXCL10, and CXCL11 showed a consistent correlation with immune cell infiltration, of which they showed strongly positive correlations with central memory CD8+ T cell, effector memory CD8+ T cell, CD8+ T cell, memory activated CD4+ T cell, memory CD4+ T cell, Th2 CD4+ T cell, Macrophage M1, Macrophage, NK cell; and strongly negatively relevance to memory B cell, B cell, T cell regulatory (Tregs), Macrophage M2. But these genes were not significant for Th1 CD4+ T cells. In addition, there was a positive correlation between CXCL1 expression and the infiltration of memory activated CD4+ T cell, Macrophage M1, and a negative interrelation between the expression of CXCL1 and the infiltration of naive B cell, B cell, T cell regulatory (Tregs), Macrophage M2. The expression level of CXCL2 was positively related to the infiltration of memory activated CD4+ T cell, Macrophage M1, NK cell, and negatively relevant to the infiltration of B cell and T cell regulatory (Tregs). CXCL5 expression only was negatively pertinent to the infiltration of B cells. Interestingly, we

discovered that CXCL6, PPBP, and CXCL8 just were negatively associated with central memory CD8+ T cell and T cell regulatory (Tregs). There was a positive interrelation between the expression of CXCL12 and the infiltration of effector memory CD8+ T cell, CD8+ T cell, memory CD4+ T cell, Th2 CD4+ T cell, Macrophage M1, Macrophage, and a negative relevance to Th1 CD4+ T cell/Macrophage M2. CXCL13 expression only was negatively associated with the infiltration of Th1 CD4+ T cell. CXCL14 expression were negatively correlated with infiltration of the three immune cell types (memory activated CD4+ T cell, Th1 CD4+ T cell, NK cell), and positively correlated with infiltration of Macrophage M2. The results indicated that the expression levels of CXCL9/10/11 were related to a variety of immune cells in DLBCL.

3.6. Correlation Between Distinct CXCLs Expression and Biomarkers of Immune Cells in DLBCL

We further investigated the relationship between CXCLs and various immune markers of different immune cells, including T cells, B cells, monocytes, tumor-associated macrophage (TAMs), M0/M1/M2 macrophages, neutrophils, dendritic cells, a series of helper T cells, and Tex (T cell Exhaustion) using TIMER 2.0. The results indicated that the expression levels of CXCL9/10/11 were significantly correlated with most biomarkers of diverse immune cells in DLBCL and its molecular subtypes. Besides, as shown in Table 2, CXCL1/2/5/6/8/12/13/14/PPBP were associated with immune-infiltration markers in the tumor microenvironment.

Table 2. *The correlation analysis between immune markers and CXC chemokines (TIMER2.0).*

Immune cells	Gene markers	Purity		CXCL1		CXCL2		CXCL5		CXCL6		CXCL7		CXCL8	
		rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
CD8+T cell	CD8A	-0.54	***	0.05	0.77	0.18	0.26	-0.02	0.88	-0.26	0.09	-0.12	0.45	-0.14	0.39
	CD8B	-0.41	***	0.12	0.47	0.12	0.44	0.05	0.76	-0.25	0.11	-0.13	0.42	-0.10	0.54
T cell	CD3D	-0.71	**	-0.15	0.37	-0.21	0.18	-0.20	0.21	-0.27	0.08	-0.19	0.23	-0.50	**
	CD3E	-0.75	***	-0.05	0.76	-0.04	0.81	-0.11	0.49	-0.23	0.15	-0.29	0.07	-0.40	*
	CD2	-0.74	***	-0.06	0.72	-0.08	0.62	-0.15	0.36	-0.33	*	-0.25	0.11	-0.36	*
B cell	CD19	0.15	0.36	-0.29	0.06	-0.18	0.26	-0.33	*	0.01	0.94	0.03	0.85	-0.10	0.52
	CD79A	0.03	0.84	-0.32	*	-0.27	0.09	-0.18	0.27	0.09	0.59	-0.09	0.58	-0.13	0.43
monocyte	CD86	-0.39	*	0.10	0.52	-0.13	0.42	-0.04	0.81	-0.02	0.92	-0.03	0.87	0.21	0.20
	CD115 (CSF1R)	-0.51	***	0.26	0.09	0.16	0.31	0.06	0.70	-0.12	0.46	-0.12	0.46	0.12	0.46
	CCL2	-0.25	0.11	0.61	***	0.52	***	0.26	0.10	0.34	*	0.19	0.23	0.46	**
TAM	CD68	-0.41	**	0.31	0.05	0.22	0.17	0.18	0.26	-0.06	0.69	-0.04	0.82	0.27	0.08
	IL10	-0.21	0.18	0.28	0.08	0.30	0.06	0.05	0.76	-0.20	0.20	0.14	0.39	0.15	0.36
M1 Macrophage	INOS (NOS2)	-0.20	0.22	0.23	0.14	0.47	**	0.41	**	0.12	0.46	0.51	**	0.17	0.29
	IRF5	-0.26	0.10	-0.11	0.51	0.08	0.63	-0.25	0.11	-0.10	0.52	0.08	0.60	-0.16	0.33
	COX2 (PTGS2)	-0.32	*	0.44	**	0.55	**	0.51	**	0.23	0.15	0.35	*	0.45	**
M2 Macrophage	CD163	-0.08	0.60	0.31	0.05	0.40	**	0.11	0.49	-0.07	0.64	0.13	0.42	0.29	0.06
	VSIG4	-0.16	0.32	0.36	*	0.45	**	0.15	0.34	-0.05	0.75	0.18	0.26	0.30	0.05
	MS4A4A	-0.20	0.20	0.31	*	0.40	*	0.00	1.00	0.05	0.77	0.19	0.22	0.29	0.07
Neutrophils	CD66b (CEACAM8)	-0.27	0.08	-0.10	0.53	-0.08	0.60	-0.03	0.83	0.07	0.65	0.16	0.31	0.17	0.27
	CD11b (ITGAM)	-0.31	*	0.17	0.29	0.08	0.63	0.16	0.31	0.15	0.35	-0.02	0.91	0.34	*
NK cell	CCR7	-0.50	***	-0.05	0.75	0.07	0.65	-0.06	0.73	-0.06	0.70	0.01	0.94	-0.16	0.32
	KIR2DL1	-0.35	*	0.24	0.13	0.24	0.13	0.11	0.49	0.05	0.73	-0.04	0.81	0.03	0.85
	KIR2DL3	-0.42	**	0.18	0.25	0.15	0.34	-0.01	0.93	0.03	0.85	0.03	0.83	0.15	0.34
	KIR2DL4	-0.21	0.19	0.22	0.16	0.20	0.22	0.00	0.98	-0.15	0.35	0.11	0.51	0.27	0.09
	KIR3DL1	-0.29	0.07	0.12	0.46	-0.14	0.40	-0.08	0.63	-0.06	0.70	-0.23	0.15	0.05	0.75
	KIR3DL2	-0.61	***	-0.06	0.72	0.14	0.38	0.03	0.83	-0.06	0.72	0.15	0.35	-0.06	0.70
	KIR3DL3	-0.12	0.46	0.22	0.17	0.12	0.47	-0.05	0.75	0.06	0.73	-0.10	0.54	-0.05	0.75
	KIR2DS4	-0.24	0.13	-0.04	0.80	0.13	0.41	-0.02	0.92	-0.19	0.24	-0.06	0.71	-0.01	0.93
Dendritic cell	HLA-DPB1	-0.21	0.19	-0.35	*	-0.41	**	-0.27	0.08	-0.15	0.34	-0.26	0.11	-0.28	0.07
	HLA-DQB1	-0.16	0.31	-0.21	0.19	-0.12	0.47	0.12	0.44	0.13	0.43	-0.25	0.12	-0.13	0.42
	HLA-DRA	-0.20	0.22	-0.30	0.06	-0.13	0.42	-0.09	0.59	0.02	0.91	-0.17	0.29	-0.20	0.21
	HLA-DPA1	-0.30	0.05	-0.34	*	-0.30	0.06	-0.16	0.32	-0.15	0.35	-0.22	0.17	-0.33	*
	BCDA-1 (CD1C)	-0.03	0.87	-0.08	0.60	0.04	0.82	-0.15	0.36	0.33	*	0.05	0.76	0.10	0.53
	BDCA-4 (NRP1)	-0.26	0.09	0.13	0.43	0.39	*	0.03	0.85	-0.05	0.74	0.31	*	0.27	0.09
	CD11c (ITGAX)	-0.53	***	-0.04	0.79	-0.08	0.63	0.04	0.80	0.25	0.11	-0.15	0.35	0.09	0.57
Th1	TBX21	-0.71	***	0.36	*	0.27	0.09	-0.01	0.95	-0.11	0.48	0.00	0.99	0.01	0.95
	STAT1	-0.45	**	0.23	0.15	0.18	0.25	0.11	0.49	-0.12	0.47	-0.10	0.55	0.04	0.79
	STAT4	-0.73	***	-0.05	0.77	0.00	0.98	-0.21	0.19	-0.26	0.10	0.20	0.21	-0.21	0.20
	IFN-g (IFNG)	-0.54	***	0.30	0.05	0.32	*	0.10	0.54	-0.18	0.25	-0.03	0.85	0.02	0.89
	TNF-a (TNF)	-0.33	*	-0.08	0.61	-0.06	0.71	-0.14	0.39	-0.27	0.09	-0.13	0.40	-0.17	0.29
Th2	GATA3	-0.69	***	-0.12	0.45	-0.11	0.49	-0.20	0.21	-0.26	0.09	-0.25	0.11	-0.43	**
	STAT6	0.07	0.68	-0.01	0.95	0.14	0.38	-0.11	0.48	-0.08	0.63	0.03	0.85	0.09	0.57
	STAT5A	-0.42	**	-0.10	0.52	-0.15	0.36	-0.08	0.62	-0.26	0.10	-0.23	0.15	-0.02	0.91
	IL13	-0.30	0.06	-0.18	0.26	-0.10	0.52	0.08	0.63	-0.05	0.78	0.02	0.91	0.08	0.62
Tfh	BCL6	0.18	0.25	-0.32	*	-0.44	**	-0.34	*	-0.05	0.74	-0.05	0.74	-0.09	0.57
	IL21	-0.41	**	-0.05	0.78	-0.10	0.55	-0.03	0.84	-0.11	0.49	-0.33	*	-0.22	0.18

Immune cells	Gene markers	Purity		CXCL1		CXCL2		CXCL5		CXCL6		CXCL7		CXCL8	
		rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Th17	STAT3	-0.28	0.08	0.23	0.15	0.36	*	0.13	0.42	-0.01	0.95	0.19	0.25	0.30	0.05
	IL17A	-0.51	***	0.21	0.19	0.13	0.43	0.22	0.16	0.28	0.07	-0.05	0.76	-0.01	0.95
Treg	FOXP3	-0.63	***	-0.30	0.06	-0.01	0.96	0.18	0.25	0.03	0.85	-0.04	0.80	-0.19	0.25
	CCR8	-0.48	**	-0.24	0.13	-0.01	0.96	0.10	0.52	0.02	0.91	0.08	0.60	0.03	0.84
	STAT5B	-0.32	*	-0.11	0.48	0.10	0.55	-0.12	0.46	-0.14	0.37	-0.08	0.60	-0.08	0.60
Tex	TGFβ (TGFB1)	-0.52	***	0.08	0.64	0.12	0.45	-0.10	0.53	-0.08	0.63	0.10	0.53	0.13	0.42
	PD-1 (PDCD1)	-0.53	***	-0.08	0.62	-0.15	0.35	-0.14	0.40	-0.08	0.64	-0.15	0.36	-0.16	0.33
	CTLA4	-0.70	***	-0.13	0.42	-0.21	0.19	-0.10	0.53	-0.14	0.37	-0.14	0.37	-0.30	0.06
	LAG3	-0.56	***	0.34	*	0.22	0.16	-0.01	0.93	-0.09	0.58	-0.09	0.56	-0.03	0.87
	TIM-3 (HAVCR2)	-0.39	*	0.39	*	0.31	*	0.18	0.25	-0.10	0.52	0.13	0.43	0.20	0.22
	GZMB	-0.24	0.12	0.32	*	0.35	*	0.16	0.30	-0.24	0.13	0.05	0.73	0.08	0.60
	PDL1 (CD274)	-0.37	*	0.21	0.20	0.30	0.06	0.09	0.57	-0.15	0.36	0.08	0.60	0.16	0.33
	TIGIT	-0.43	**	-0.12	0.46	-0.06	0.70	-0.10	0.54	-0.23	0.14	-0.13	0.43	-0.32	*
	PVR (CD155)	-0.44	***	0.37	*	0.30	0.06	0.18	0.26	-0.05	0.76	0.23	0.15	0.31	*
	PVRL2 (CD112)	-0.43	**	0.38	*	0.29	0.06	0.30	0.06	-0.01	0.93	0.15	0.36	0.39	*
	PVRL3 (CD113)	-0.51	***	0.05	0.75	0.07	0.69	0.10	0.55	0.13	0.40	0.10	0.52	0.15	0.36
	DNAM-1 (CD226)	-0.66	***	0.04	0.78	0.33	*	-0.02	0.91	0.10	0.55	0.16	0.33	-0.01	0.94
	CD96	-0.70	***	-0.05	0.76	0.03	0.87	-0.19	0.24	-0.31	0.05	0.08	0.63	-0.26	0.10

Table 2. *Continued.*

Immune cells	Gene markers	Purity		CXCL9		CXCL10		CXCL11		CXCL12		CXCL13		CXCL14	
		rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
CD8+T cell	CD8A	-0.54	***	0.67	***	0.55	***	0.56	***	0.37	*	-0.02	0.90	-0.05	0.73
	CD8B	-0.41	***	0.36	*	0.27	0.09	0.31	*	0.18	0.25	-0.13	0.43	0.08	0.62
T cell	CD3D	-0.71	**	0.23	0.16	0.12	0.46	0.11	0.50	0.27	0.09	0.12	0.47	0.20	0.21
	CD3E	-0.75	***	0.63	***	0.45	**	0.42	**	0.44	**	0.08	0.62	0.13	0.41
	CD2	-0.74	***	0.63	***	0.48	**	0.45	**	0.48	**	0.08	0.61	0.06	0.69
B cell	CD19	0.15	0.36	-0.36	*	-0.27	0.09	-0.35	*	-0.09	0.57	-0.07	0.66	0.15	0.35
	CD79A	0.03	0.84	-0.07	0.66	-0.16	0.31	-0.17	0.28	0.28	0.08	0.01	0.94	0.21	0.18
monocyte	CD86	-0.39	*	0.25	0.12	0.23	0.15	0.23	0.15	0.37	*	0.16	0.31	-0.10	0.52
	CD115 (CSF1R)	-0.51	***	0.59	***	0.52	***	0.44	**	0.51	***	0.32	*	-0.02	0.88
TAM	CCL2	-0.25	0.11	0.47	**	0.45	**	0.46	**	0.41	**	0.11	0.49	-0.04	0.80
	CD68	-0.41	**	0.62	***	0.67	***	0.63	***	0.48	**	0.16	0.31	-0.21	0.18
	IL10	-0.21	0.18	0.66	***	0.64	***	0.64	***	0.36	*	0.22	0.16	-0.25	0.11
M1 Macrophage	INOS (NOS2)	-0.20	0.22	0.17	0.30	0.25	0.11	0.26	0.10	-0.08	0.62	0.18	0.27	-0.03	0.87
	IRF5	-0.26	0.10	0.35	*	0.28	0.08	0.27	0.09	0.23	0.15	0.20	0.21	0.08	0.62
	COX2 (PTGS2)	-0.32	*	0.31	*	0.32	*	0.32	*	0.04	0.80	0.06	0.69	-0.15	0.35
M2 Macrophage	CD163	-0.08	0.60	0.60	***	0.71	***	0.67	***	0.51	***	0.01	0.93	-0.44	**
	VSIG4	-0.16	0.32	0.62	***	0.74	***	0.65	***	0.47	**	0.12	0.47	-0.37	*
	MS4A4A	-0.20	0.20	0.41	**	0.47	**	0.45	**	0.57	***	-0.04	0.81	-0.32	*
Neutrophils	CD66b (CEACAM8)	-0.27	0.08	-0.20	0.20	-0.04	0.79	-0.03	0.83	-0.05	0.76	-0.15	0.34	-0.35	*
	CD11b (ITGAM)	-0.31	*	0.20	0.21	0.22	0.17	0.19	0.23	0.19	0.23	0.23	0.15	-0.13	0.40
	CCR7	-0.50	***	0.31	*	0.24	0.13	0.24	0.13	0.09	0.57	-0.08	0.63	-0.22	0.18
NK cell	KIR2DL1	-0.35	*	0.24	0.12	0.18	0.25	0.14	0.37	-0.01	0.95	0.38	*	0.22	0.17
	KIR2DL3	-0.42	**	0.13	0.43	0.08	0.62	0.15	0.34	0.05	0.75	-0.02	0.90	0.19	0.24
	KIR2DL4	-0.21	0.19	0.30	0.06	0.32	*	0.34	*	0.09	0.59	-0.17	0.28	-0.32	*
	KIR3DL1	-0.29	0.07	0.41	**	0.36	*	0.34	*	0.17	0.29	0.05	0.74	0.01	0.95
	KIR3DL2	-0.61	***	0.21	0.18	0.16	0.31	0.18	0.27	-0.03	0.86	-0.11	0.51	0.01	0.97
	KIR3DL3	-0.12	0.46	0.26	0.10	0.19	0.24	0.21	0.19	0.05	0.74	0.10	0.55	0.12	0.46
	KIR2DS4	-0.24	0.13	0.31	0.05	0.24	0.13	0.21	0.19	0.07	0.65	0.07	0.66	0.09	0.57
Dendritic cell	HLA-DPB1	-0.21	0.19	0.04	0.80	-0.03	0.85	-0.06	0.71	0.22	0.18	-0.10	0.52	0.14	0.37
	HLA-DQB1	-0.16	0.31	0.13	0.41	0.07	0.68	-0.04	0.81	0.28	0.08	-0.13	0.41	0.03	0.86
	HLA-DRA	-0.20	0.22	0.22	0.17	0.05	0.74	-0.02	0.89	0.33	*	-0.04	0.80	0.09	0.59
	HLA-DPA1	-0.30	0.05	0.34	*	0.15	0.37	0.10	0.55	0.36	*	0.03	0.87	0.15	0.34
	BCDA-1 (CD1C)	-0.03	0.87	-0.02	0.88	0.01	0.93	0.08	0.63	0.22	0.17	0.01	0.97	0.27	0.09
	BDCA-4 (NRP1)	-0.26	0.09	0.51	***	0.50	***	0.51	***	0.42	**	-0.07	0.67	-0.28	0.08
	CD11c (ITGAX)	-0.53	***	0.05	0.75	-0.02	0.89	-0.06	0.71	0.01	0.95	0.27	0.09	0.19	0.24
Th1	TBX21	-0.71	***	0.61	***	0.61	***	0.61	***	0.33	*	0.03	0.87	-0.21	0.18
	STAT1	-0.45	**	0.87	***	0.76	***	0.74	***	0.43	**	0.10	0.55	-0.26	0.11
	STAT4	-0.73	***	0.51	***	0.43	**	0.39	*	0.46	**	0.10	0.54	0.17	0.30
	IFN-g (IFNG)	-0.54	***	0.87	***	0.79	***	0.76	***	0.47	**	0.13	0.41	-0.23	0.14
	TNF-a (TNF)	-0.33	*	0.28	0.08	0.17	0.30	0.17	0.30	0.26	0.09	0.38	*	0.17	0.28

Immune cells	Gene markers	Purity		CXCL9		CXCL10		CXCL11		CXCL12		CXCL13		CXCL14	
		rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Th2	GATA3	-0.69	***	0.58	***	0.37	*	0.34	*	0.46	**	0.10	0.52	0.22	0.18
	STAT6	0.07	0.68	0.52	***	0.34	*	0.34	*	0.46	**	0.04	0.81	-0.17	0.28
	STAT5A	-0.42	**	0.17	0.30	0.08	0.64	0.06	0.72	0.03	0.83	-0.08	0.64	-0.30	0.05
	IL13	-0.30	0.06	0.02	0.92	-0.04	0.78	-0.06	0.73	-0.18	0.25	0.00	1.00	-0.05	0.74
Tfh	BCL6	0.18	0.25	-0.15	0.36	-0.22	0.16	-0.29	0.07	0.22	0.16	0.10	0.52	0.20	0.22
	IL21	-0.41	**	0.54	***	0.35	*	0.28	0.08	0.19	0.25	0.15	0.36	0.03	0.87
Th17	STAT3	-0.28	0.08	0.66	***	0.58	***	0.55	***	0.38	*	0.20	0.22	-0.25	0.12
	IL17A	-0.51	***	0.20	0.21	0.17	0.28	0.00	0.98	-0.04	0.80	0.24	0.13	0.00	0.98
Treg	FOXP3	-0.63	***	0.20	0.21	0.05	0.75	-0.01	0.93	0.12	0.46	0.03	0.84	0.09	0.59
	CCR8	-0.48	**	0.37	*	0.23	0.15	0.20	0.20	0.34	*	0.01	0.95	-0.13	0.43
	STAT5B	-0.32	*	0.62	***	0.43	**	0.36	*	0.45	**	0.10	0.53	-0.12	0.45
Tex	TGFβ (TGFB1)	-0.52	***	0.55	***	0.40	**	0.44	**	0.51	***	0.17	0.28	-0.10	0.52
	PD-1 (PDCD1)	-0.53	***	0.30	0.05	0.20	0.21	0.17	0.28	0.25	0.12	-0.09	0.59	0.12	0.47
	CTLA4	-0.70	***	0.31	*	0.24	0.14	0.22	0.17	0.20	0.21	0.10	0.53	0.19	0.23
	LAG3	-0.56	***	0.59	***	0.57	***	0.59	***	0.27	0.09	-0.08	0.62	-0.18	0.25
	TIM-3 (HAVCR2)	-0.39	*	0.78	***	0.74	***	0.70	***	0.54	***	0.18	0.27	-0.10	0.55
	GZMB	-0.24	0.12	0.67	***	0.70	***	0.72	***	0.19	0.22	-0.11	0.49	-0.34	*
	PDL1 (CD274)	-0.37	*	0.81	***	0.72	***	0.72	***	0.37	*	0.17	0.30	-0.26	0.11
	TIGIT	-0.43	**	0.34	*	0.22	0.17	0.17	0.29	0.33	*	0.07	0.67	0.39	*
	PVR (CD155)	-0.44	***	0.57	***	0.59	***	0.59	***	0.29	0.07	0.30	0.06	-0.04	0.82
	PVRL2 (CD112)	-0.43	**	0.40	**	0.47	**	0.46	**	0.24	0.13	0.22	0.17	-0.09	0.58
	PVRL3 (CD113)	-0.51	***	0.42	**	0.33	*	0.31	*	0.27	0.09	0.18	0.26	0.01	0.97
	DNAM-1 (CD226)	-0.66	***	0.55	***	0.44	**	0.42	**	0.30	0.06	0.09	0.58	0.08	0.63
	CD96	-0.70	***	0.58	***	0.44	**	0.37	*	0.46	**	0.32	*	0.24	0.13

*p<0.05, **p<0.01, ***p<0.001.

3.7. Relationship Between CXCL9/10/11 and Immune Checkpoints of Tex/Deplete NK Cells in DLBCL

The anti-tumor effect of immune checkpoint inhibitors (ICIs) depends on the high expression levels of immune checkpoint in tumor cells. Thus, we explored the relationship between the recently popular immune checkpoints of Tex/deplete NK cells and the expression of CXCL9/10/11 using the TIMER 2.0. We found that increased expression of CXCL9/10/11 were strongly related to high expression of LAG3, TIM-3, GZMB, PDL1, PVR, PVRL2, PVRL3, DNAM-1, and CD96 in DLBCL (Table 2). Through the GEPIA dataset, we further verified results that were basically consistent with TIMER 2.0 (Table 3).

Table 3. *The correlation between immune checkpoints of Tex/deplete NK cells and CXCL9/10/11 (GEPIA).*

Gene markers	CXCL9		CXCL10		CXCL11	
	R	P	R	P	R	P
PD-1 (PDCD1)	0.55	***	0.46	**	0.39	**
CTLA4	0.63	***	0.56	***	0.48	***
LAG3	0.69	***	0.68	***	0.66	***
TIM-3 (HAVCR2)	0.84	0	0.83	0	0.79	0
GZMB	0.62	***	0.69	***	0.71	***
PDL1 (CD274)	0.71	***	0.73	***	0.72	***
TIGIT	0.59	***	0.43	**	0.36	*
PVR (CD155)	0.66	***	0.68	***	0.64	***
PVRL2 (CD112)	0.36	*	0.44	**	0.39	**
PVRL3 (CD113)	0.47	***	0.51	***	0.55	***
DNAM-1 (CD226)	0.69	***	0.60	***	0.55	***
CD96	0.59	***	0.55	***	0.46	**

*p<0.05, **p<0.01, ***p<0.001.

4. Discussion

Chemokines, a type of soluble cytokines with low molecular mass (8 –15 kDa), are involved in cell proliferation, differentiation, and survival, especially the migration of immune cells. Chemokines are related to many human diseases such as chronic inflammation, immune dysfunction, and cancer metastasis [29]. The interaction between tumor cells and stromal cells may have a certain impact on the expression mechanism of chemokines in different cell types, thereby providing favorable conditions for the growth, invasion and metastasis of tumor cells. Accumulating evidence indicate that CXC chemokines play a vital role in tumorigenesis and tumor metastasis [9, 30]. However, the specific immune mechanism of the CXC chemokines in DLBCL is still unclear. In this paper, the correlation between CXC chemokines and immune-infiltration in DLBCL was

integratively analyzed through multiple database resources.

We first explored the mRNA expression level of CXC chemokines in DLBCL through multi-databases. The results indicated that most genes of CXCs, including CXC1-2, and CXC5-14, were significantly up-regulated in DLBCL compared with normal tissue. Indeed, the abnormal expression of CXCs in DLBCL patients has been reported [31, 32]. Moreover, we found that mRNA level of CXCL9 was the highest among CXC chemokines in DLBCL. The HPA analysis also demonstrated that the significantly higher protein levels of CXCL8 and CXCL11-13 were found in DLBCL tissues. Due to insufficient DLBCL tissues of immunohistochemical results in the Human Protein Atlas, the sample size should be further expanded and follow-up validation experiments should be performed.

In this study, we found that the higher expression of CXCL1, CXCL2, CXCL6, CXCL7, CXCL10, and CXCL12 were related to the worse prognosis of DLBCL patients, and the higher mRNA level of CXCL9, CXCL13, CXCL14 were significantly associated with better overall survival. Stephen M. A et al clarified that elevated serum level of CXCL10 was associated with an increase in the possibility of disease recurrence and a decrease in survival rate in patients with DLBCL [33]. However, previous studies on the relationship between the expression levels of CXC chemokines and overall survival in DLBCL patients are still far from sufficient.

Next, we explored the co-expression relationship between the expression levels of 12 CXC chemokines by the GEO database, and there was the highest correlation among CXCL9/10/11. Some literature has reported that CXCR3 is a co-selective receptor of CXCL9/10/11, and the CXCL9, 10, 11/CXCR3 axis participates in the regulation of immune cell migration, differentiation, and activation, thereby inhibiting tumors [34, 35]. From the results of the enrichment analysis, we discovered that they were significantly associated with chemokine activity, chemokine receptor binding, cellular response to chemokine, and response to chemokine. By the GeneMANIA database, we explored the protein-protein interaction network of CXC chemokines as well as the top relative 20 genes. Through the enrichment analysis of the 32 genes, we found that these genes were chiefly involved in the chemotaxis and inflammation of cytokines. CXC chemokines, as angiostatic chemokines, were predominantly relevant to chemokine signaling pathways and cytokine-cytokine receptor interactions. It has been reported that chemokine signaling pathways play a critical role in various cancers, including proliferation, senescence, angiogenesis, immune escape, and invasion of tumor cells [36-39].

DChimeric antigen receptor (CAR) T-cell therapy has greatly changed the treatment pattern of DLBCL. It is already in randomized phase III clinical trials and may become a breakthrough in the treatment of DLBCL patients in the future [4, 40]. In this paper, the results revealed that CXC chemokines played a vital role in the immune infiltration of DLBCL. In particular, CXCL9/10/11 has a positive correlation with most immune cells. Therefore, combining these inhibitors with immunotherapy may be a promising treatment strategy for DLBCL patients. The high transcription level of CXCL9/10/11 had a strong correlation with some highly expressed immune checkpoint markers on Tex/deplete NK cells, such as PD1/PD-L1, CTLA4, LAG3, TIM-3, GZMB, CD155, CD112, CD113, CD226, and CD96. In recent years, the suppression therapy of immune checkpoints has become a breakthrough point for refractory/relapsed malignant tumors. Some scholars have found that the combined inhibition of PD1/PD-L1, PD1/CTLA4, PD1/TIM-3, and PD1/LAG3 can significantly inhibit cytokines in response to malignant neoplasm therapy [5, 41-44]. T cell immunoglobulin and ITIM domain (TIGIT), as an inhibitory receptor, is an emerging cancer immunotherapy target. It can be combined with its ligand CD155/CD112 to inhibit the production of IFNγ, TNFα, and IL2 by T cells in tumors, and combined with PD1/PD-L1 to treat patients with advanced solid malignant tumors. [6, 45, 46]. To some extent, all results demonstrated the correlation between the high expression of CXC chemokines and immune infiltration in DLBCL. In the future, it may become a breakthrough in the treatment of refractory/relapsed DLBCL patients.

This study has some limitations because of the prediction results without verification by experiments. It is necessary to further validate the predicted results at the cellular and animal levels by different methods, including reverse transcription polymerase chain reaction (RT-PCR), western blotting, immunohistochemistry, flow cytometry, etc.

5. Conclusion

In summary, through systematic analysis, we found that the high expression of CXCL1/2/6/7/10/12 were pertinent to poorer survival of DLBCL patients, and CXCL9/10/11 were positively correlated with most of the infiltrating immune cells. In addition, we hope that the combination of these genes with immune markers of Tex/deplete NK cells could promote the upgrading of DLBCL treatment regimens and reduce unnecessary side effects, thereby helping clinicians more accurately grasp the survival of DLBCL patients. Overall, these findings indicated that these CXC chemokines and their related immune checkpoints may be potential targets for immunotherapy in patients with DLBCL.

Data Availability

The data of this study are attached on the manuscript.

Funding

Open Access funding provided by the National Science Fund Subsidized Project (81270568).

Conflict of Interest Statement

The authors declare that they have no competing interests.

Authors' Contributions

(I) Conception and design: Gexiu Liu, Yuli Cao, Fenling Zhou; (II) Administrative support: Gexiu Liu; (III) Provision of study materials or patients: Gexiu Liu; (IV) Collection and assembly of data: Yuli Cao, Fenling Zhou, Cuilan Deng; (V) Data analysis and interpretation: Gexiu Liu, Yuli Cao, Cuilan Deng; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

References

[1] Li S, Young KH, Medeiros LJ. Diffuse large B-cell lymphoma. Pathology 2018, 50 (1): 74-87.

[2] N. NV. In vivo modeling of diffuse large B cell lymphoma (DLBCL) with the myeloid differentiation primary response gene 88 (MYD88) L265P mutation. Translational Cancer Research 2016, 5 (S4).

[3] Thieblemont C, Bernard S, Meignan M, Molina T. Optimizing initial therapy in DLBCL. Best Pract Res Clin Haematol 2018, 31 (3): 199-208.

[4] Kersten MJ, Spanjaart AM, Thieblemont C. CD19-directed CAR T-cell therapy in B-cell NHL. Curr Opin Oncol 2020, 32 (5): 408-17.

[5] Xie W, Medeiros LJ, Li S, Yin CC, Khoury JD, Xu J. PD-1/PD-L1 Pathway and Its Blockade in Patients with Classic Hodgkin Lymphoma and Non-Hodgkin Large-Cell Lymphomas. Curr Hematol Malig Rep 2020, 15 (4): 372-81.

[6] Josefsson SE, Beiske K, Blaker YN, Førsund MS, Holte H, Østenstad B, Kimby E, Köksal H, Wälchli S, Bai B, Smeland EB, Levy R, Kolstad A, Huse K, Myklebust JH. TIGIT and PD-1 Mark Intratumoral T Cells with Reduced Effector Function in B-cell Non-Hodgkin Lymphoma. Cancer Immunol Res 2019, 7 (3): 355-62.

[7] Cabrero-de Las Heras S, Martínez-Balibrea E. CXC family of chemokines as prognostic or predictive biomarkers and possible drug targets in colorectal cancer. World J Gastroenterol 2018, 24 (42): 4738-49.

[8] Zeng Q, Sun S, Li Y, Li X, Li Z, Liang H. Identification of Therapeutic Targets and Prognostic Biomarkers Among CXC Chemokines in the Renal Cell Carcinoma Microenvironment. Front Oncol 2019, 9: 1555.

[9] Du H, Gao L, Luan J, Zhang H, Xiao T. C-X-C Chemokine Receptor 4 in Diffuse Large B Cell Lymphoma: Achievements and Challenges. Acta Haematol 2019, 142 (2): 64-70.

[10] Charbonneau B, Wang AH, Maurer MJ, Asmann YW, Zent CS, Link BK, Ansell SM, Weiner GJ, Ozsan N, Feldman AL, Witzig TE, Cunningham JM, Dogan A, Habermann TM, Slager SL, Novak AJ, Cerhan JR. CXCR5 polymorphisms in non-Hodgkin lymphoma risk and prognosis. Cancer Immunol Immunother 2013, 62 (9): 1475-84.

[11] Liang K, Liu Y, Eer D, Liu J, Yang F, Hu K. High CXC Chemokine Ligand 16 (CXCL16) Expression Promotes Proliferation and Metastasis of Lung Cancer via Regulating the NF-κB Pathway. Med Sci Monit 2018, 24: 405-11.

[12] Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013, 41: D991-D5.

[13] Dybkær K, Bøgsted M, Falgreen S, Bødker JS, Kjeldsen MK, Schmitz A, Bilgrau AE, Xu-Monette ZY, Li L, Bergkvist KS, Laursen MB, Rodrigo-Domingo M, Marques SC, Rasmussen SB, Nyegaard M, Gaihede M, Møller MB, Samworth RJ, Shah RD, Johansen P, El-Galaly TC, Young KH, Johnsen HE. Diffuse large B-cell lymphoma classification system that associates normal B-cell subset phenotypes with prognosis. J Clin Oncol 2015, 33 (12): 1379-88.

[14] Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A, Chinnaiyan AM. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 2004, 6 (1): 1-6.

[15] Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017, 45 (W1): W98-W102.

[16] Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montojo J, Shao Q, Wright G, Bader GD, Morris Q. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 2010, 38: W214-W20.

[17] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009, 4 (1): 44-57.

[18] Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA-K, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist P-H, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Pontén F. Proteomics. Tissue-based map of the human proteome. Science 2015, 347 (6220): 1260419.

[19] Kassambara A, Rème T, Jourdan M, Fest T, Hose D, Tarte K, Klein B. GenomicScape: an easy-to-use web tool for gene expression data analysis. Application to investigate the molecular events in the differentiation of B cells into plasma cells. PLoS Comput Biol 2015, 11 (1): e1004077.

[20] Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020, 48 (W1): W509-W14.

[21] Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM, Hurt EM, Zhao H, Averett L, Yang L, Wilson WH, Jaffe ES, Simon R, Klausner RD, Powell J, Duffey PL, Longo DL, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Montserrat E, López-Guillermo A, Grogan TM, Miller TP, LeBlanc M, Ott G, Kvaloy S, Delabie J, Holte H, Krajci P, Stokke T, Staudt LM. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002, 346 (25): 1937-47.

[22] Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, Shen Q, Bertoni F, Ponzoni M, Scandurra M, Califano A, Bhagat G, Chadburn A, Dalla-Favera R, Pasqualucci L. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature 2009, 459 (7247): 717-21.

[23] Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO, Staudt LM. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000, 403 (6769): 503-11.

[24] Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, Yang L, Pickeral OK, Rassenti LZ, Powell J, Botstein D, Byrd JC, Grever MR, Cheson BD, Chiorazzi N, Wilson WH, Kipps TJ, Brown PO, Staudt LM. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001, 194 (11): 1639-47.

[25] Brune V, Tiacci E, Pfeil I, Döring C, Eckerle S, van Noesel CJM, Klapper W, Falini B, von Heydebreck A, Metzler D, Bräuninger A, Hansmann M-L, Küppers R. Origin and pathogenesis of nodular lymphocyte-predominant Hodgkin lymphoma as revealed by global gene expression analysis. J Exp Med 2008, 205 (10): 2251-68.

[26] Basso K, Margolin AA, Stolovitzky G, Klein U, Dalla-Favera R, Califano A. Reverse engineering of regulatory networks in human B cells. Nat Genet 2005, 37 (4): 382-90.

[27] Storz MN, van de Rijn M, Kim YH, Mraz-Gernhard S, Hoppe RT, Kohler S. Gene expression profiles of cutaneous B cell lymphoma. J Invest Dermatol 2003, 120 (5): 865-70.

[28] Amedei A, Prisco D, D' Elios MM. The use of cytokines and chemokines in the cancer immunotherapy. Recent Pat Anticancer Drug Discov 2013, 8 (2): 126-42.

[29] Bikfalvi A, Billottet C. The CC and CXC chemokines: major regulators of tumor progression and the tumor microenvironment. Am J Physiol Cell Physiol 2020, 318 (3): C542-C54.

[30] Susek KH, Karvouni M, Alici E, Lundqvist A. The Role of CXC Chemokine Receptors 1-4 on Immune Cells in the Tumor Microenvironment. Front Immunol 2018, 9: 2159.

[31] Hong JY, Ryu KJ, Lee JY, Park C, Ko YH, Kim WS, Kim SJ. Serum level of CXCL10 is associated with inflammatory prognostic biomarkers in patients with diffuse large B-cell lymphoma. Hematol Oncol 2017, 35 (4): 480-6.

[32] Manfroi B, McKee T, Mayol JF, Tabruyn S, Moret S, Villiers C, Righini C, Dyer M, Callanan M, Schneider P, Tzankov A, Matthes T, Sturm N, Huard B. CXCL-8/IL8 Produced by Diffuse Large B-cell Lymphomas Recruits Neutrophils Expressing a Proliferation-Inducing Ligand APRIL. Cancer Res 2017, 77 (5): 1097-107.

[33] Ansell SM, Maurer MJ, Ziesmer SC, Slager SL, Habermann TM, Link BK, Witzig TE, Macon WR, Dogan A, Cerhan JR, Novak AJ. Elevated pretreatment serum levels of interferon-inducible protein-10 (CXCL10) predict disease relapse and prognosis in diffuse large B-cell lymphoma patients. Am J Hematol 2012, 87 (9): 865-9.

[34] Koper OM, Kamińska J, Sawicki K, Kemona H. CXCL9, CXCL10, CXCL11, and their receptor (CXCR3) in neuroinflammation and neurodegeneration. Adv Clin Exp Med 2018, 27 (6): 849-56.

[35] Tokunaga R, Zhang W, Naseem M, Puccini A, Berger MD, Soni S, McSkane M, Baba H, Lenz H-J. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy. Cancer Treat Rev 2018, 63: 40-7.

[36] Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, Sagrinati C, Mazzinghi B, Orlando C, Maggi E, Marra F, Romagnani S, Serio M, Romagnani P. 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 2003, 197 (11): 1537-49.

[37] Lim SY, Yuzhalin AE, Gordon-Weeks AN, Muschel RJ. Targeting the CCL2-CCR2 signaling axis in cancer metastasis. Oncotarget 2016, 7 (19): 28697-710.

[38] Roy I, Getschman AE, Volkman BF, Dwinell MB. Exploiting agonist biased signaling of chemokines to target cancer. Mol Carcinog 2017, 56 (3): 804-13.

[39] Sarvaiya PJ, Guo D, Ulasov I, Gabikian P, Lesniak MS. Chemokines in tumor progression and metastasis. Oncotarget 2013, 4 (12): 2171-85.

[40] Sermer D, Brentjens R. CAR T-cell therapy: Full speed ahead. Hematol Oncol 2019, 37 Suppl 1: 95-100.

[41] Xu-Monette ZY, Xiao M, Au Q, Padmanabhan R, Xu B, Hoe N, Rodríguez-Perales S, Torres-Ruiz R, Manyam GC, Visco C, Miao Y, Tan X, Zhang H, Tzankov A, Wang J, Dybkær K, Tam W, You H, Bhagat G, Hsi ED, Ponzoni M, Ferreri AJM, Møller MB, Piris MA, van Krieken JH, Winter JN, Westin JR, Pham LV, Medeiros LJ, Rassidakis GZ, Li Y, Freeman GJ, Young KH. Immune Profiling and Quantitative Analysis Decipher the Clinical Role of Immune-Checkpoint Expression in the Tumor Immune Microenvironment of DLBCL. Cancer Immunol Res 2019, 7 (4): 644-57.

[42] Zhang L, Du H, Xiao T-w, Liu J-z, Liu G-z, Wang J-x, Li G-y, Wang L-x. Prognostic value of PD-1 and TIM-3 on CD3+ T cells from diffuse large B-cell lymphoma. Biomed Pharmacother 2015, 75: 83-7.

[43] Zhang T, Ren T, Song Z, Zhao J, Jiao L, Zhang Z, He J, Liu X, Qiu L, Li L, Zhou S, Meng B, Zhai Q, Ren X, Qian Z, Wang X, Zhang H. Genetic Mutations of Tim-3 Ligand and Exhausted Tim-3 CD8 T Cells and Survival in Diffuse Large B Cell Lymphoma. J Immunol Res 2020: 6968595.

[44] Liu Y, Guo X, Zhan L, Wang L, Wang X, Jiang M. LAG3 and PD1 Regulate CD8+ T Cell in Diffuse Large B-cell Lymphoma Patients. Comput Math Methods Med 2021: 4468140.

[45] Harjunpää H, Guillerey C. TIGIT as an emerging immune checkpoint. Clin Exp Immunol 2020, 200 (2): 108-19.

[46] Chauvin J-M, Zarour HM. TIGIT in cancer immunotherapy. J Immunother Cancer 2020, 8 (2): e000957.