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Retraction

Retracted: Bioinformatics Analyses Reveals a Comprehensive Landscape of CXC Chemokine Family Functions in Non-Small Cell Lung Cancer

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope

(2) Discrepancies in the description of the research reported

(3) Discrepancies between the availability of data and the research described

(4) Inappropriate citations

(5) Incoherent, meaningless and/or irrelevant content included in the article

(6) Manipulated or compromised peer review

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

References

[1] H. Tian, L. Wang, Y. Liu et al., "Bioinformatics Analyses Reveals a Comprehensive Landscape of CXC Chemokine Family Functions in Non-Small Cell Lung Cancer," *BioMed Research International*, vol. 2021, Article ID 6686158, 34 pages, 2021.

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Research Article

Bioinformatics Analyses Reveals a Comprehensive Landscape of CXC Chemokine Family Functions in Non-Small Cell Lung Cancer

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Backgrounds. Lung cancer is a major source of tumor-related death each year with non-small cell lung cancer (NSCLC) being a prevalent subtype. The metastasis from NSCLC to the brain usually imposes many neuron disorders. Previous studies have suggested that communications among cancer cells and interstitial cells are essential in tumorigenesis and are influenced by chemokines. In the tumor microenvironment, CXC chemokines can participate in the shifting of immune cells and manage tumor cell condition, thus affecting the progression of cancer and patient destinies. However, the expression and values of CXC chemokine family in NSCLC have not been systematically illustrated using public databases. *Methods*. UALCAN, STRING, ONCOMINE, GeneMANIA, cBioPortal, GEPIA, TISIDB, TRRUST, TIMER, Kaplan-Meier Plotter, and R software were utilized in this study. *Results*. Based on the TIMER and UACLCAN databases, in LUAD patients, the expression levels of CXCL10, CXCL13, and CXCL14 were significantly elevated while the transcriptional levels of CXCL2/3/4/7/12/16 were significantly reduced; in LUSC patients, the expression levels of CXCL6/10/13/14 were significantly elevated while the expression levels of CXCL2/3/4/5/7/11/12/16/17 were significantly reduced. We found remarkable relevance between the pathological stages of LUAD patients and the expressions of CXCL8 (positive) and CXCL17 (negative). Similarly, there are significant correlations between the pathological stages of LUSC patients and the expressions of CXCL1/2/6/17. In LUAD, patients with low expression levels of CXCL1/4/7/8 and patients with high expression levels of CXCL12/14/16 were associated with a significantly better prognosis. But in LUSC, all correlations between chemokines and prognosis are statistically insignificant. Pairwise expression correlation analysis among CXC chemokines shows that there are 7 significant correlations (between CXCL1 and CXCL2, between CXCL1 and CXCL3, between CXCL1 and CXCL8, between CXCL2 and CXCL3, between CXCL4 and CXCL7, between CXCL9 and CXCL10, and between CXCL9 and CXCL11) in LUAD and 4 significant correlations (between CXCL1 and CXCL8, between CXCL2 and CXCL3, between CXCL4 and CXCL7, and between CXCL10 and CXCL11) in LUSC. Significant correlations between the expressions of CXC chemokines and the infiltration of six common types of immune cells were also discovered in both LUAD and LUSC. *Conclusions*. We provided a comprehensive landscape of the CXC chemokine family in LUAD and LUSC using the bioinformatics method and found differences between LUSC and LUAD in the field of CXC chemokines. Our study may help validate and identify known novel immunotherapeutic targets and prognostic biomarkers.

1. Introduction

Annually, about 1.8 million people develop lung cancer, and 1.6 million people die as a result of this disease [1]. Approximately 85% of patients are classified as NSCLC (non-small cell lung cancer), of which the main histological types are adenocarcinoma and squamous cell carcinoma. There are enormous genomic and clinical heterogeneities between LUAD and LUSC [2]. Squamous-cell lung cancer, constituting 25%-30% of NSCLC cases, is usually centrally located and more likely to invade large blood vessels and has rare mutations/alterations which can be targeted from afar, which lead to many challenges in the treatment [3, 4]. Adenocarcinoma of the lung comprises around 40% of all lung cancer and tends to occur in the periphery of the lung. It is easier to identify before it has spread outside of the lungs, so it has better prognosis than LUSC [5]. Many researchers have focused on the therapeutic targets of NSCLC, especially immune checkpoint suppressor and oncogene mutations, and many advancements have been made [6]; however, it is far from sufficient; more therapeutic targets and prognostic biomarkers need to be identified.

Endogenous chemokine ligands and G protein-coupled seven-transmembrane spanning signaling receptors are the main members of the chemokine superfamily; they are chemotactic cytokines that control the shifting and communication cells and tissues [7]. In cancer microenvironment, chemokines are mainly produced by tumor cells and immune cells and play important role in mediating immune cell trafficking and lymphoid tissue development; thus, they are involved in antitumor immunological responses [8]. It has been convinced that chemokines can affect many cancer-related biological processes including tumor angiogenesis, tumorigenesis, progression, and metastasis and even influence patients' clinical outcomes [9–11].

CXC chemokines participate in many biological progresses of malignant disease in several different organs, including the lung [12], breast [13], colorectal [14], and kidney [15]. In lung cancer, CXC chemokines are mainly involved in angiogenesis, immunoangiostasis, and metastases [16, 17]. CXCL1 [18], CXCL5 [19], CXCL8 [20], and CXCL12 [21] are CXC chemokines that have attracted much attention, since they are considered to facilitate lung cancer initiation, development, and metastasis by different mechanisms; however, for some CXC chemokines like CXCL4 [22, 23], CXCL14 [24, 25], and CXCL16 [26, 27], researchers have opposite opinions towards their roles in tumor development.

Generally, previous studies have sporadically characterized the function of some CXC chemokines in NSCLC, but our study is the first to give a comprehensive landscape of CXC chemokines in LUAD and LUSC using public database and bioinformatics skills. Technology development of second-generation gene sequencing and springing up of various databases will accelerate the macrolevel research of the CXC chemokine family in tumors.

Our study conducted a comprehensive and profound bioinformatics analysis of the expression of CXC chemokines in LUAD and LUSC and explored their roles as therapeutic targets and prognostic biomarkers on several credible public databases and R packages, thus offering more evidence to facilitate the clinical routine in selecting new drugs and investigating NSCLC patients' long-term outcome more accurately.

2. Materials and Methods

2.1. ONCOMINE. ONCOMINE (http://www.oncomine.org) is a translational bioinformatics service that provides powerful, genome-wide expression analysis. Datasets in ONCOMINE were collected from public repositories such as Gene Expression Omnibus (GEO) and Array Express by Compendia Bioscience (a prominent cancer bioinformatics company widely used by the pharmaceutical industry to identify novel gene targets for drug discovery and development), and the datasets are composed of microarray data of primary tumors, cell lines, or xenografts [28]. Data were extracted to evaluate the expression of CXC chemokines in lung cancer. In our study, a p value of 0.05, a fold change of 2, and a gene rank in the top 10% were set as the significance thresholds. Student's t test was used to analyze the difference in the expression of CXC chemokines in lung cancer.

2.2. GEPIA. GEPIA (http://gepia.cancer-pku.cn/index.html) is an analysis tool containing RNA sequence expression data of 9736 tumors and 8587 normal tissue samples, which was developed at Peking University. Data in GEPIA are extracted from TCGA and GETx projects using a standard pipeline, and the RNA-Seq datasets GEPIA used are based on the UCSC Xena project (http://xena.ucsc.edu) [29]. In this study, we performed pathological stage analysis with the "Single Gene Analysis" module and correlation analysis with the "Correlation Analysis" module. Multiple gene comparison analysis of CXC chemokines was performed with the "Multiple Gene Comparison" module, using the "LUAD" and "LUSC" datasets. 3D principle component analysis and survival map were performed with the "Dimensionality Reduction" module and "Survival Analysis" module of GEPIA2 (test), respectively. Student's t test was used to generate a p value for expression or pathological stage analysis, and we take 0.05 as the cutoff of the p value.

2.3. UALCAN. UALCAN (http://ualcan.path.uab.edu/) is a comprehensive web resource, providing analyses based on The Cancer Genome Atlas (TCGA) and MET500 cohort data [30]. In our study, expression data for CXC chemokines was obtained using the "Expression Analysis" module and "Methylation Analysis" module of UALCAN and the "LUAD" and "LUSC" datasets. Student's t test was used to generate a p value. We set 0.05 as the p value cutoff.

2.4. cBioPortal. cBioPortal (http://www.cbioportal.org) is a comprehensive web resource, which can visualize and analyze multidimensional cancer genomics data; it stores nonsynonymous mutations, DNA copy-number data, mRNA and microRNA expression data, protein-level and phosphoprotein-level data, DNA methylation data, and deidentified clinical data. The data in cBioPortal was based on TCGA database and a large number of published articles

[31]. Genetic change, coexpression, and the network module of CXC chemokines based on TCGA database were obtained from cBioPortal. A total of 556 LUAD samples (TCGA, Pan-Cancer Atlas) and 487 LUSC (TCGA, Pan-Cancer Atlas) were included. mRNA expression z scores (RNA Seq V2 RSEM) were obtained using a z score threshold of ±2.0.

2.5. GeneMANIA. GeneMANIA (http://www.genemania.org) is a website providing information for genetic interactions and proteins, pathways, coexpression, colocalization, and protein domain similarity of genes; it can find other genes that are related to input genes with a very large set of functional association data, and it relies on several data sources including GEO, BioGRID, EMBL-EBI, Pfam, Ensembl, NCBI, MGI, I2D, InParanoid, and Pathway Commons [32].

2.6. STRING. STRING (https://string-db.org/) is aimed at collecting, scoring, and integrating all publicly available sources of protein-protein interaction (PPI) data and at complementing these with computational predictions of potential functions; it relies on many credible resources including COG, Ensembl, Intact, RefSeq, PubMed, Reactome, DIP, BioGRID, MINT, KEGG, SGD, FlyBase, Swiss-Prot/UniProt, SWISS-MODEL, HUGO, OMIM, NCI/NaturePID, PDB, The Interactive Fly, BioCyc, Gene Ontology, and SIMAP [33]. We conducted a PPI network analysis of differentially expressed CXC chemokines to explore the interactions among them with STRING in July 2020.

2.7. TRRUST. TRRUST (https://www.grnpedia.org/trrust/) is a reliable, intuitive tool for transcriptional regulatory networks based on 11237 articles describing small-scale experimental studies of transcriptional regulations in PubMed. Containing 8444 transcription factor- (TF-) target regulatory relationships of 800 human TFs, the TRRUST database can provide information on how these interactions are regulated [34].

2.8. TIMER. TIMER (https://cistrome.shinyapps.io/timer/) is an online tool that evaluated the infiltration of different immune cells and their clinical impact, and its data is based on samples from TCGA [35]. In our study, the correlation between immune cell and CXC chemokine level was evaluated with "Gene module"; the "Diff Exp" module was applied to show the expression of CXC chemokines among different cancer types; somatic copy number alteration of CXC chemokines was performed with "SCNA" module; and "Survival module" was used to evaluate the correlation among clinical outcome and the infiltration of immune cells and CXC chemokine expression.

2.9. TISIDB. TISIDB (http://cis.hku.hk/TISIDB/) is a gene-based resource exploring interactions between tumor and immune contexture. It is based on several credible resources, including PubMed database, high throughput screening data, RNA, and exome sequencing datasets of patient cohorts with immunotherapy, TCGA, UniProt, GO, DrugBank, etc. TISIDB allows users to interrogate the function of a specific gene in tumor-immune interplay through literature mining and the data analysis of genome-wide screening and high-throughput profiling [36]. In our study, we used the "Chemokine" module of TISIDB to make Spearman expression correlations between 16 CXC chemokines in our research and several common chemokines (including the 16 CXC chemokines themselves in our study) among different types of cancer.

2.10. Kaplan-Meier Plotter. The Kaplan-Meier Plotter-Lung Cancer (https://kmplot.com/analysis/index.php?p=service&cancer=lung) is an online tool that can show how genes influence survival in lung cancer ($n = 3{,}452$). EGA, GEO, and TCGA are included as sources in this database. Initially, the main function of this website is to identify survival biomarkers based on meta-analysis [37]. In our study, 719 lung adenocarcinoma samples and 524 lung squamous-cell carcinoma samples were used to draw the OS survival curve of CXC chemokines in LUAD and LUSC, respectively.

2.11. Statistical Analysis. R software with package "clusterProfiler" was used for GO and KEGG analysis and plotting.

3. Results

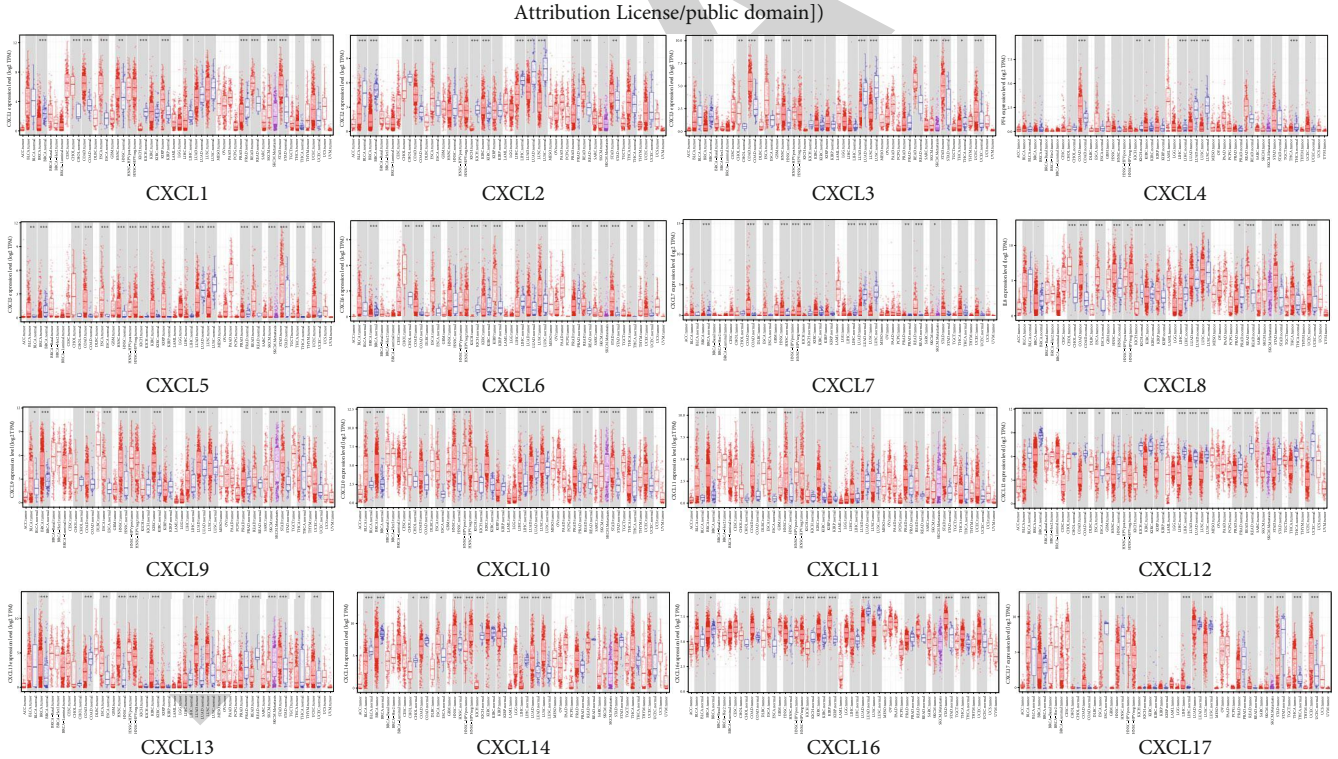
3.1. Aberrant Transcription and Methylation Level of CXC Chemokines in LUAD and LUSC Patients. Sixteen CXC chemokines (not including CXCL15) were retrieved from the ONCOMINE database. We first explored the mRNA expression levels of CXC chemokines in lung cancer and normal lung tissues with ONCOMINE (where LUAD and LUSC are not discriminated). Results are displayed in Figure 1(a). Based on the data from ONCOMINE, the expressions of CXCL14, CXCL13, and CXCL9 in lung cancer tissues were elevated with statistical significance while the transcriptional levels of CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL7, CXCL12, CXCL16, and CXCL17 were significantly reduced in lung cancer vs. normal renal tissue. We also assessed the expression levels of CXC chemokines in cancer vs. normal tissue with TIMER where LUAD and LUSC are divided in Figure 1(b). In LUAD, CXCL9, CXCL10, CXCL13, and CXCL14 were significantly elevated and CXCL2, CXCL3, CXCL4, CXCL5, CXCL7, CXCL12, and CXCL16 were significantly reduced, while in LUSC, CXCL6, CXCL10, CXCL13, and CXCL14 were significantly elevated and CXCL2, CXCL3, CXCL4, CXCL5, CXCL7, CXCL12, CXCL16, and CXCL17 were significantly reduced. For triple verification, we visited the UALCAN database, using its "Expression analysis" module to explore the CXC chemokine transcriptional level in cancer vs. normal tissue in Figure 1(c), and the results are as follows: the transcriptional levels of CXCL2, CXCL3, CXCL4/PF4, CXCL7/PPBP, CXCL11, CXCL12, CXCL16, and CXCL17 were significantly reduced in both LUAD and LUSC tissues; the transcriptional levels of CXCL6, CXCL9, CXCL10, CXCL13, and CXCL14 were significantly elevated in both LUAD and LUSC tissues. For CXCL5, it was significantly reduced in LUSC tissues and reduced in LUAD tissues without statistical significance. From the TIMER and UALCAN databases, CXCL9/10/13/14 were elevated and

Analysis type by cancer	Cancer vs. normal CXCL1		Cancer vs. normal CXCL2		Cancer vs. normal CXCL3		Cancer vs. normal CXCL4		Cancer vs. normal CXCL5		Cancer vs. normal CXCL6		Cancer vs. normal CXCL7		Cancer vs. normal CXCL8		Cancer vs. normal CXCL9		Cancer vs. normal CXCL10		Cancer vs. normal CXCL11		Cancer vs. normal CXCL12		Cancer vs. normal CXCL13		Cancer vs. normal CXCL14		Cancer vs. normal CXCL16		Cancer vs. normal CXCL17	
Bladder cancer			1	2		1					1	2			1		2		2					5	1							
Brain and CNS cancer	5				1			1	1		1	1			4	1	1		2		1	1		4	1		1	2	8			
Breast cancer		15		27	1	9		4		7	1	5		4			15		16		12		2	16	2		1	8	2	2	1	
Cervical cancer	3				1				1		1				4		2		2		2			1	2			3	1			
Colorectal cancer	21		14	1	23		4		10		7		2		19		1	1	4		12			8		5		2	5		3	
Esophageal cancer	7		4		4		2				3		1		5		1		1		1		1					1	1			2
Gastric cancer	4								1	1					4		1				1			4				7	1			8
Head and neck cancer	10	1	3		3				1		2				5		6		7		6			3	4		2	1				2
Kidney cancer	1	3				2		2	2	2	1			7			4		4		3			7	1	2		1	3			
Leukemia		9	1	3	1	3	1	10	1				2	8	1	3	1					1	1	3	1			2		1		
Liver cancer	1	1		6					1		4			1	2		4	1	5		3		1	4				5				
Lung cancer		2		12		6		10		2				11			2							3	7		4		4		1	
Lymphoma			3	2				1			1					5	21		16		7		9		13	1	8		3			
Melanoma	1								1	1					1		1		1					1				2				
Myeloma	1			1		1		1		1						1								2								
Other cancer	3		2	2	1	1			3		1		2	1	6		6	1	11		1			2	3		2	1	3	2	1	
Ovarian cancer	1					1									1				1		1			1	2		2					
Pancreatic cancer			2		2				5		1		1		3		1		1					3	1		1		2		1	
Prostate cancer		1		1								1		1	1		1				1		2	1		2	4					
Sarcoma		1		7					1	3		1		2			2		4		2		2	2		2		8			1	
Significant unique analyses	58	33	30	64	37	24	7	29	8	16	24	10	8	34	57	10	72	3	76		53	2	18	70	38	12	25	42	29	9	6	14
Total unique analyses	447		440		407		433		392		409		440		464		452		435		392		458		413		385		296		253	

1 5 10 10 5 1

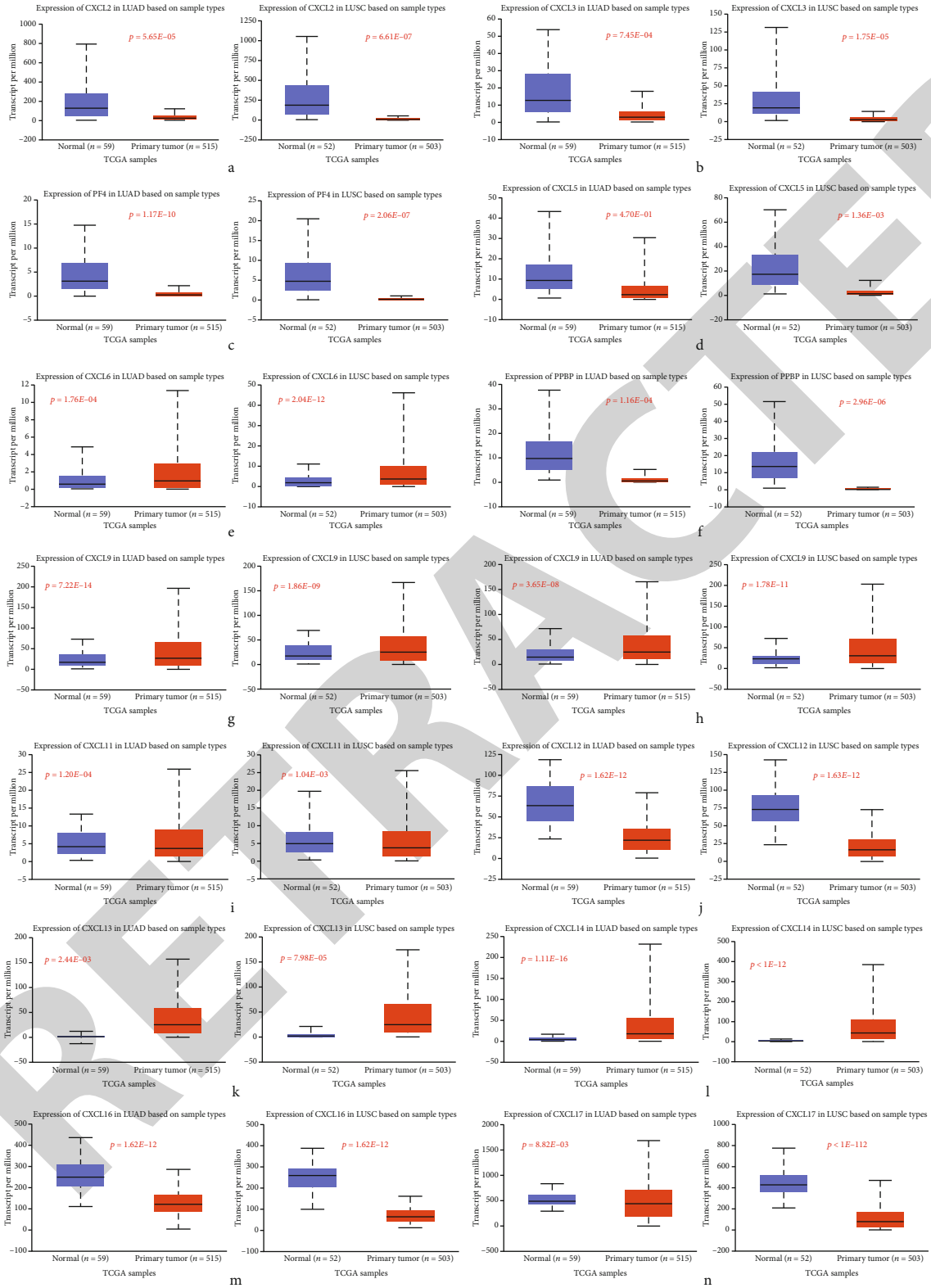
%

(a) mRNA levels of CXC chemokines in lung cancer (ONCOMINE). The figure shows the numbers of datasets with statistically significant mRNA overexpression (red) or downregulated expression (blue) of CXC chemokines (reproduced from Zeng et al. 2020 [under the Creative Commons Attribution License/public domain])



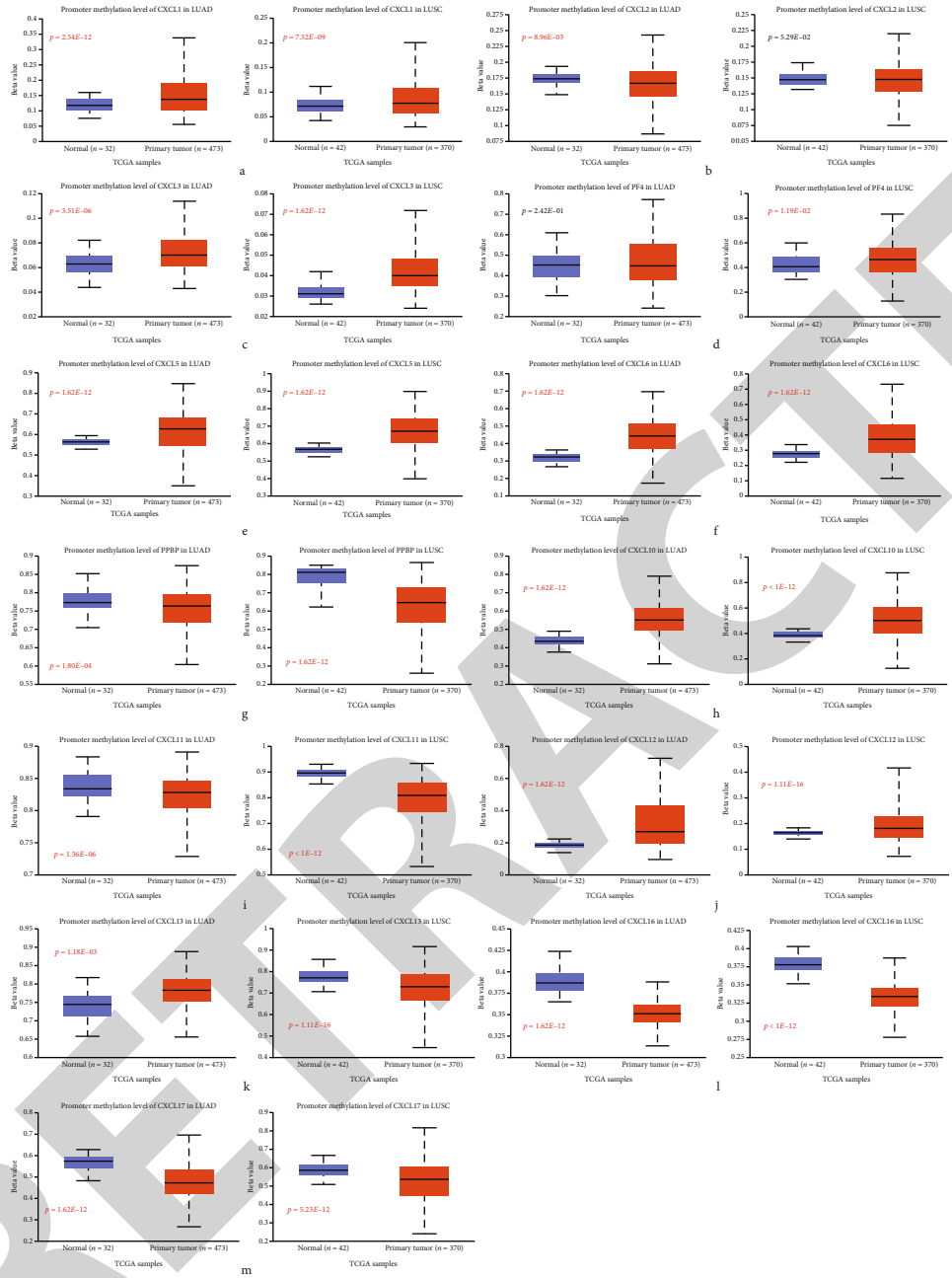
(b) Differential expression between tumor and adjacent normal tissues for CXC chemokines across all TCGA tumors types (TIMER); statistical significance was evaluated using the Wilcoxon test. Columns are gray when normal data are available

Figure 1: Continued.

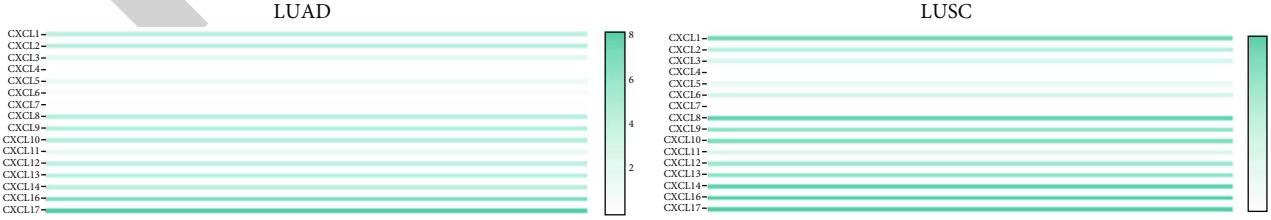


(c) The transcription of CXC chemokines in LUAD and LUSC (UALCAN): (A) CXCL2, (B) CXCL3, (C) CXCL4, (D) CXCL5, (E) CXCL6, (F) CXCL7, (G) CXCL9, (H) CXCL10, (I) CXCL11, (J) CXCL12, (K) CXCL13, (L) CXCL14, (M) CXCL16, and (N) CXCL17

Figure 1: Continued.



(d) The methylation level of CXC chemokines in LUAD and LUSC (UALCAN). (A) CXCL1 (B) CXCL2 (C) CXCL3 (D) CXCL4 (E) CXCL5 (F) CXCL6 (G) CXCL7 (H) CXCL10 (I) CXCL11 (J) CXCL12 (K) CXCL13 (L) CXCL16 (M) CXCL17



(e) The relative level of CXC chemokines in LUAD and LUSC

Figure 1: Expression of the CXC chemokine family in lung cancer.

CXCL2/3/4/7/12/16 were decreased in LUAD with significance and CXCL6/10/13/14 were elevated and CXCL2/3/4/5/7/12/16/17 were reduced in LUSC with significance.

In the UALCAN database, except for the expression level, we also explored the methylation level of CXC chemokines in LUAD and LUSC since epigenetic modifications are on the spotlight of tumor research; the results are presented in Figure 1(d). The methylation levels of CXCL7/PPBP, CXCL11, CXCL16, and CXCL17 were significantly reduced in both LUAD and LUSC tissues; the methylation levels of CXCL1, CXCL3, CXCL5, CXCL6, CXCL10, and CXCL12 were significantly elevated in both LUAD and LUSC tissues; the methylation level of CXCL2 was significantly decreased in LUAD, but its data is not significant in LUSC; the methylation level of CXCL4/PF4 was only significantly higher in LUSC. For CXCL13, its methylation level is significantly elevated in LUAD, but is significantly decreased in LUSC, which is the opposite trend in LUAD and LUSC.

We also compared the relative expression levels of CXC chemokines in LUAD and LUSC tissues using GEPIA in Figure 1(e) and found that among all CXC chemokines we evaluated, the relative level of CXCL17 was the highest in LUAD; those of CXCL1, CXCL8, CXCL10, CXCL14, CXCL16, and CXCL17 were higher in LUSC. We evaluated all the 16 CXC chemokines so that we could identify additional CXC chemokines associated with tumorigenesis, development, and clinical outcome in LUAD and LUSC.

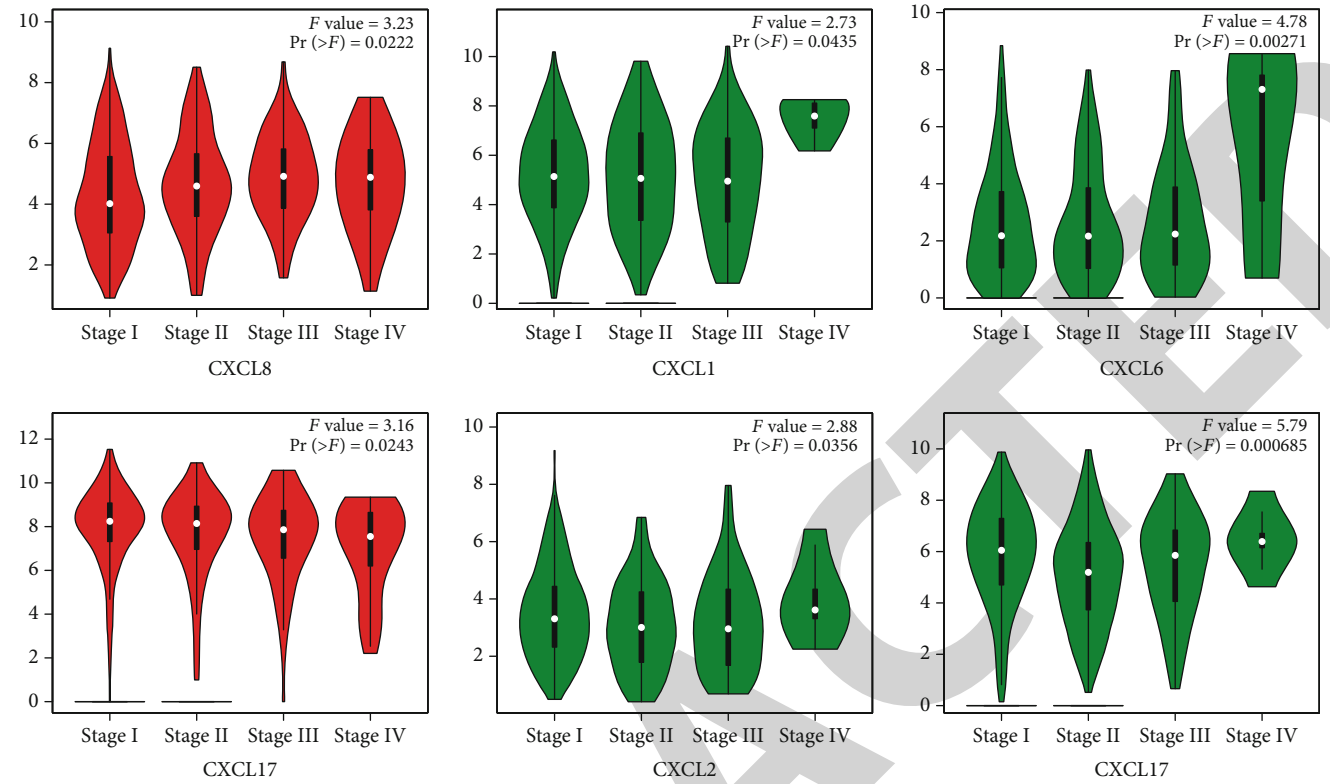
3.2. The Prognostic Value of CXC Chemokines in Patients with LUAD and LUSC. We firstly assessed the correlation between the CXC chemokines and the pathological stage of LUAD and LUSC patients in Figure 2(a). In LUAD, significant associations were found between the expressions of CXCL8 ($p = 0.022$) and CXCL17 ($p = 0.024$). As the tumor progressed, the expression of CXCL8 increased and the expression of CXCL17 decreased. In LUSC, we found a significant correlation between the expressions of CXCL1 ($p = 0.044$), CXCL2 ($p = 0.036$), CXCL6 ($p = 0.003$), and CXCL17 ($p \leq 0.001$), and as the tumor progressed to stage IV, the expression levels of CXCL1 and CXCL6 increased positively.

To evaluate the value of CXC chemokines in the progression of LUAD and LUSC, we assessed the correlation between CXC chemokines and clinical outcome using Kaplan-Meier Plotter-Lung Cancer. OS (overall survival) curves are presented. In LUAD (Figure 2(b)), patients with low expression levels of CXCL1 ($p = 0.024$), CXCL4 ($p = 0.043$), CXCL7 ($p = 0.034$), and CXCL8 ($p = 0.008$) were significantly associated with longer OS; patients with high expression levels of CXCL12 ($p \leq 0.001$), CXCL14 ($p = 0.007$), and CXCL16 ($p = 0.003$) were significantly associated with longer OS. In LUSC (Figure 2(c)), all of the 16 CXC chemokines in our study have no statistical significance correlation with clinical outcome, so we displayed the OS curve of the CXC chemokines that are significant in LUAD to make a comparison.

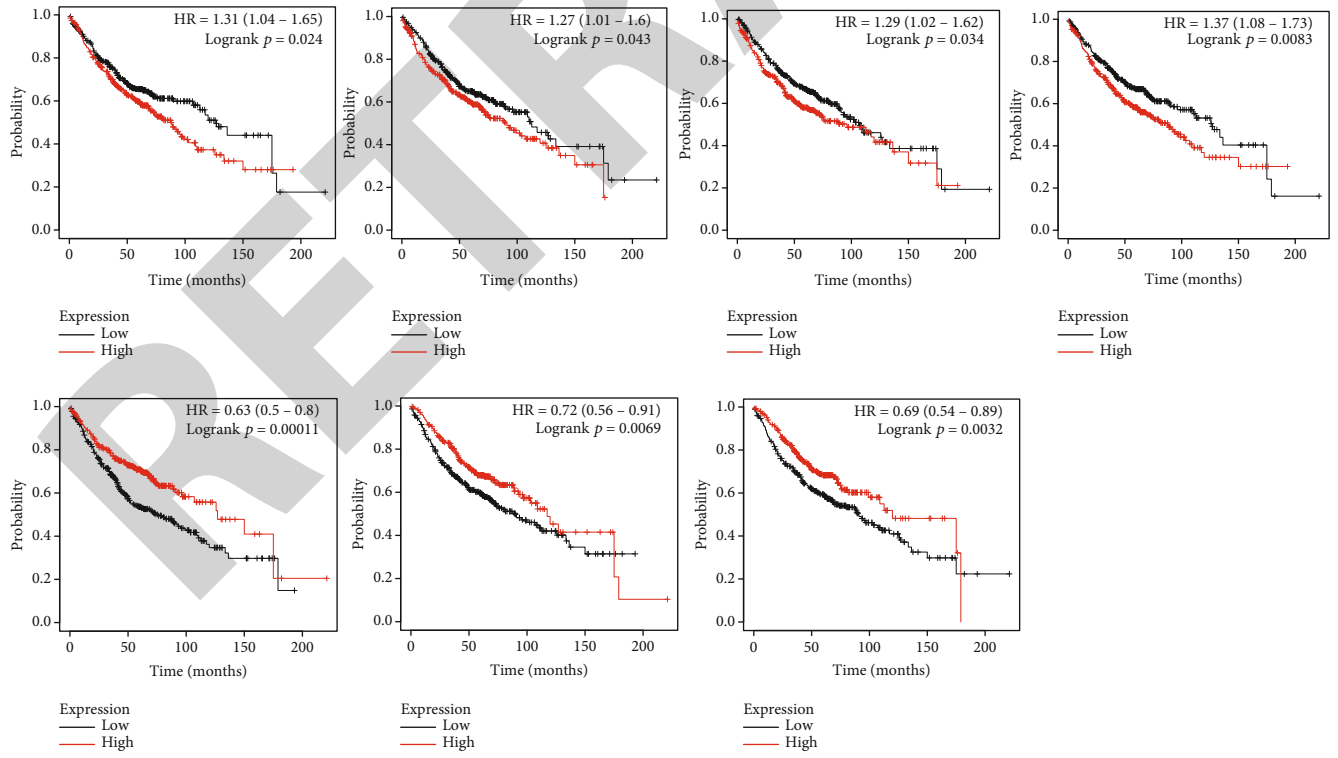
We also conducted a survival map using GEPIA2.0, which displayed the survival contribution of different CXC chemokines in multiple cancer types, including LUAD and LUSC (Figure 2(d).

We conducted a comprehensive analysis of the molecular characteristics of CXC chemokines. Using the cBioPortal database, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL16, and CXCL17 were mutated 4, 5, 5, 4, 4, 6, 5, 4, 6, 5, 5, 5, 6, 6, 5, and 6% of the queried LUAD samples, respectively (Figure 3(a)), and they were mutated in 7, 6, 7, 6, 7, 9, 7, 5, 7, 6, 6, 4, 6, 5, 6, and 7% of the queried LUSC samples, respectively (Figure 3(a)). Enhanced/declined mRNA expression was the most common change in these samples. Next, we conducted a correlation heat map of CXC chemokines in different types of cancer, including LUAD and LUSC using the "Chemokine" module of the TISIDB database (Figure 3(b)); blank areas in CXCL4 and CXCL17 were due to the data vacancy of these two CXC chemokines in TISIDB. There was a moderate to high correlation among CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, and CXCL8 and another moderate to high correlation among CXCL9, CXCL10, CXCL11, and CXCL13. Using the "Correlation Analysis" module of GEPIA, we conducted gene-to-gene correlation analysis among CXC chemokines (Figure 3(c)). Under the standard "p value < 0.05 and R value ≥ 0.6," in LUAD, we found significant expression correlation between these pairs: CXCL1 and CXCL2 ($R = 0.6$), CXC1 and CXCL3 ($R = 0.69$), CXCL1 and CXCL8 ($R = 0.7$), CXCL2 and CXCL3 ($R = 0.79$), CXCL4 and CXCL7 ($R = 0.91$), CXCL9 and CXCL10 ($R = 0.6$), CXCL9 and CXCL11 ($R = 0.62$), CXCL10 and CXCL11 ($p < 0.05$; $R = 0.82$); in LUSC, we found significant expression correlation between these couples: CXCL1 and CXCL8 ($R = 0.68$), CXC2 and CXCL3 ($R = 0.79$), CXCL4 and CXCL7 ($R = 0.92$), and CXCL10 and CXCL11 ($R = 0.72$). Moreover, we made a PPI network analysis of CXC chemokines in humans with STRING in July 2020 to explore the potential interactions among them. As expected, several nodes (16) and several edges (111) were obtained in the PPI network (Figure 3(d)), and the PPI enrichment p value <1.0e-16. The function of these CXC chemokines was associated with the chemokine signaling pathway and the inflammatory reaction. Outcomes of GeneMANIA also showed that the functions of these CXC chemokines were mainly related to chemotaxis and several chemokine activities such as chemokine receptor binding process; it showed 20 related genes and 1512 total links (Figure 3(e)).

3.3. Function Enrichment and Pathway Analysis of CXC Chemokines in Patients with LUAD and LUSC. R software with package "clusterProfiler" was applied to analyze the functions of differentially expressed CXC chemokines and their neighboring genes in LUAD and LUSC, respectively (Figure 4). Figure 4(a) shows the top 10 most highly enriched GO items in LUAD. Among the 10 most highly enriched functions in the BP category, chemokine-mediated signaling pathway, response to chemokine, cellar response to chemokine, and cell chemotaxis were associated with the tumorigenesis and progression of LUAD. The external side of the plasma membrane, focal adhesion, and cell-substrate junction were

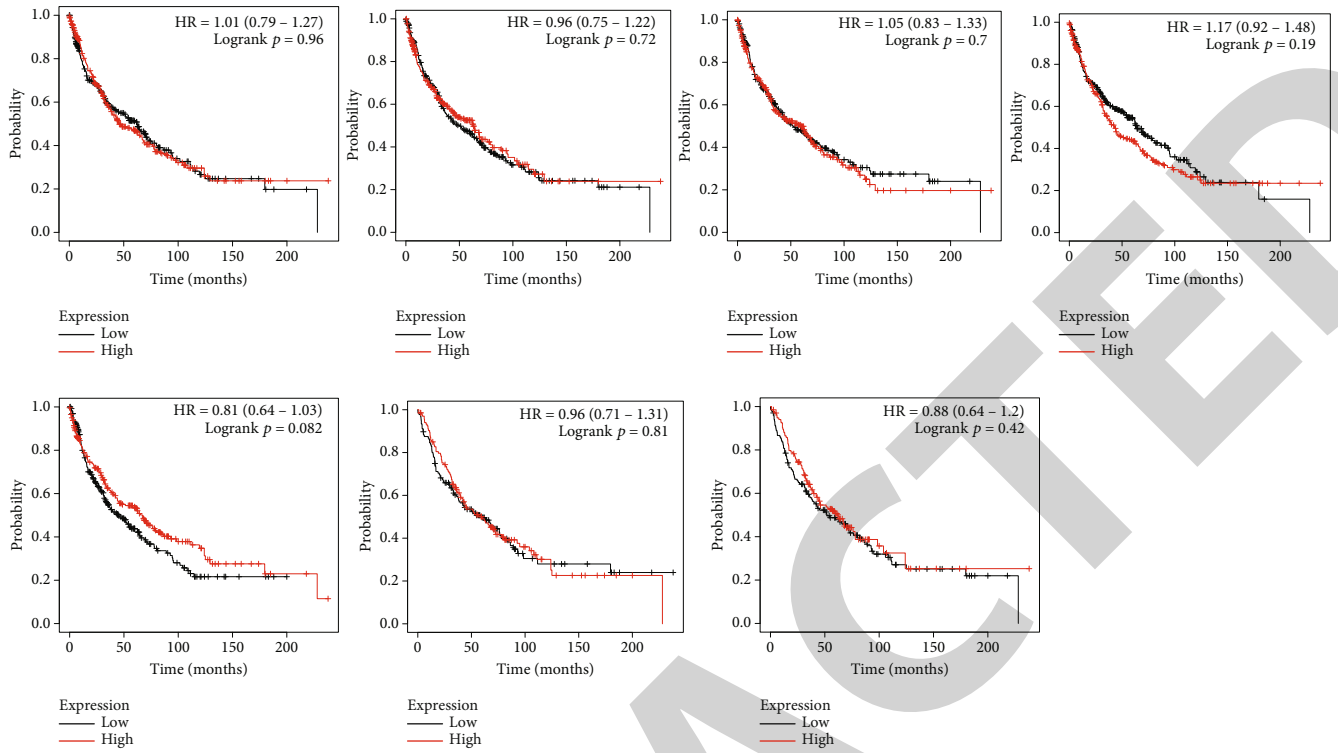


(a) Correlation between different expressed CXC chemokines and the pathological stage of LUAD (red color) and LUSC (green color) patients (GEPIA); $^{*}p < 0.05$

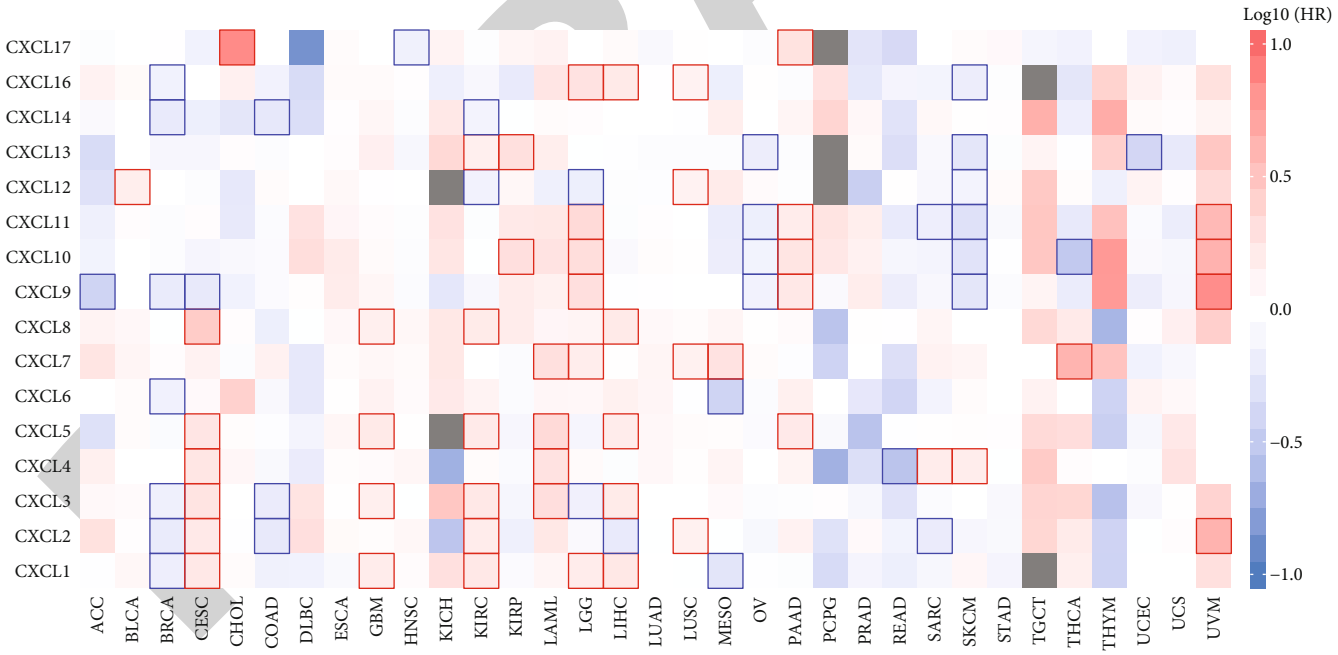


(b) The overall survival curve of different expressed CXC chemokines in LUAD patients. Upper from left to right are CXCL1, CXCL4, CXCL7, and CXCL8; lower from left to right are CXCL12, CXCL14, and CXCL16; $^{*}p < 0.05$

Figure 2: Continued.



(c) The overall survival curve of different expressed CXC chemokines in LUSC patients. Upper from left to right are CXCL1, CXCL4, CXCL7, and CXCL8; lower from left to right are CXCL12, CXCL14, and CXCL16

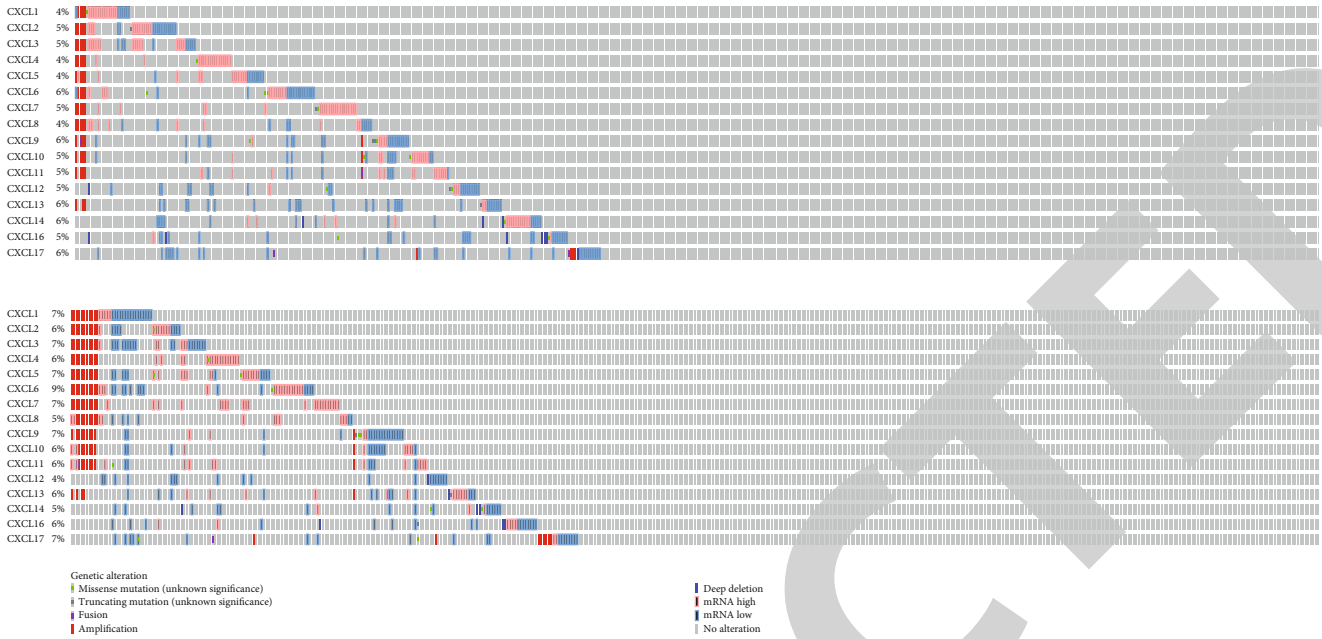


(d) Survival map, which showed the survival contribution of different CXC chemokines in multiple cancer types, estimated using the Mantel-Cox test (GEPIA)

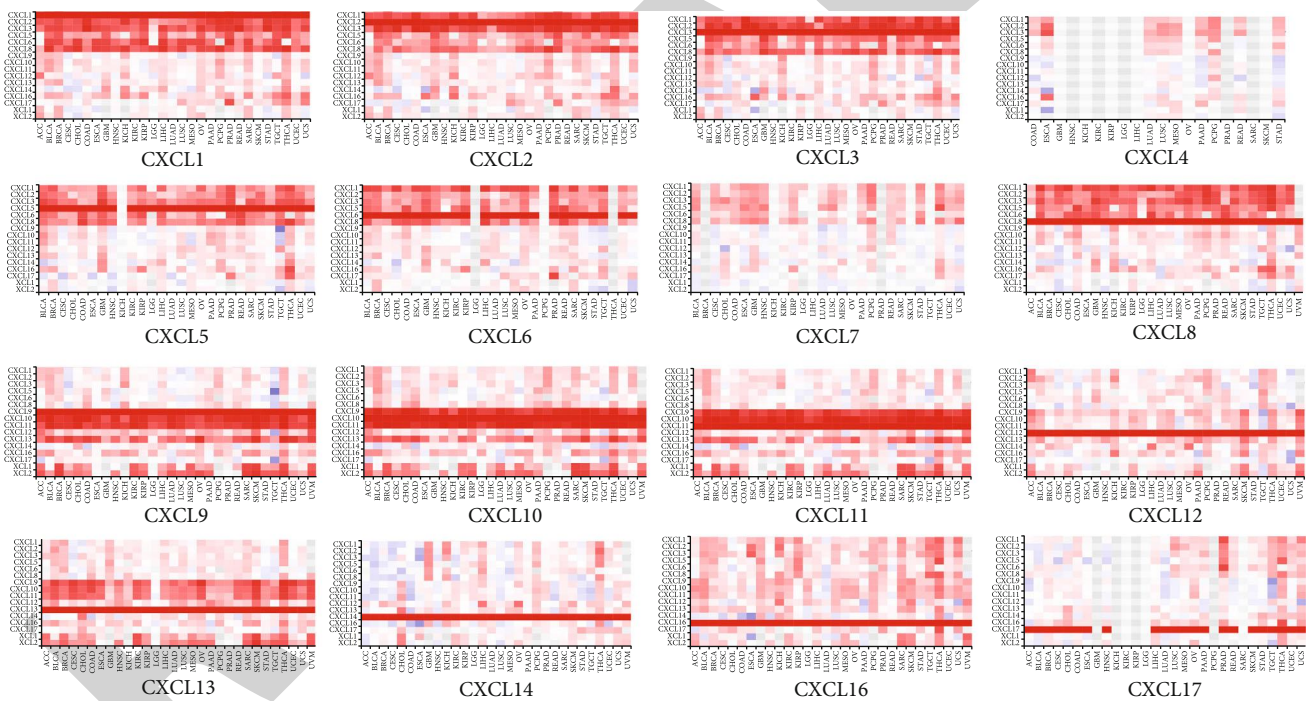
Figure 2: Prognostic value of different expressed CXC chemokines in lung cancer.

the 3 most highly enriched items in the CC category. In the molecular function part, the CXC chemokines and their neighboring genes were mainly enriched in chemokine receptor binding, chemokine activity, G protein-coupled receptor binding, cytokine receptor binding, and cytokine activity.

Figure 4(b) showed the KEGG pathway analyses of the CXC chemokine in LUAD. As expected, among the top 30 KEGG pathways, CXC chemokines were mainly enriched in the chemokine signaling pathway, viral protein interaction with cytokine and cytokine receptor, cytokine-cytokine receptor interaction, Kaposi sarcoma-associated herpesvirus

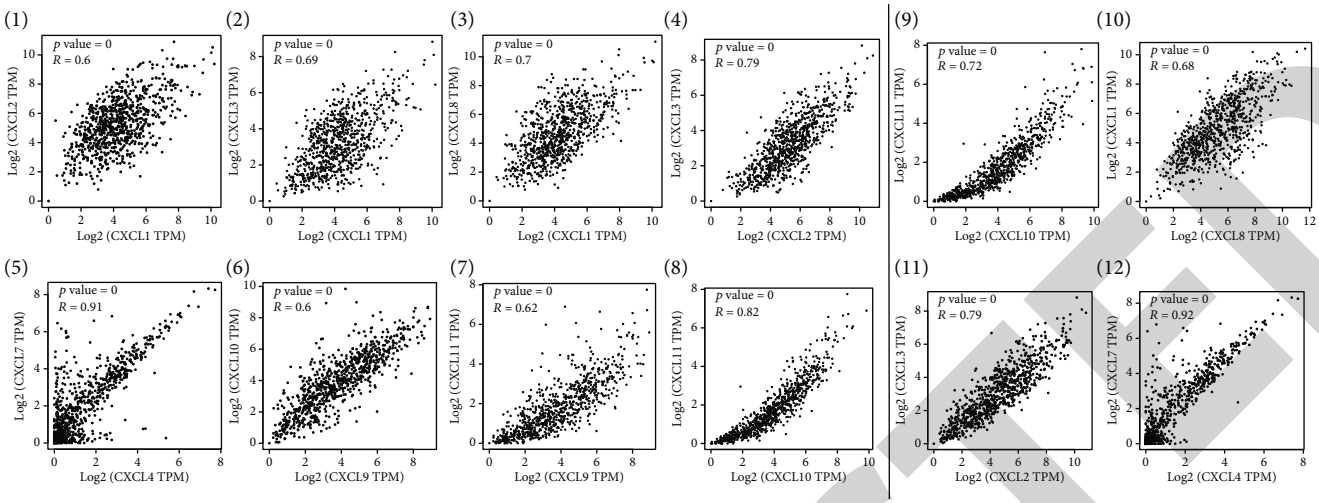


(a) Summary of alterations in CXC chemokines in LUAD (upper) and LUSC (lower) (cBioPortal)

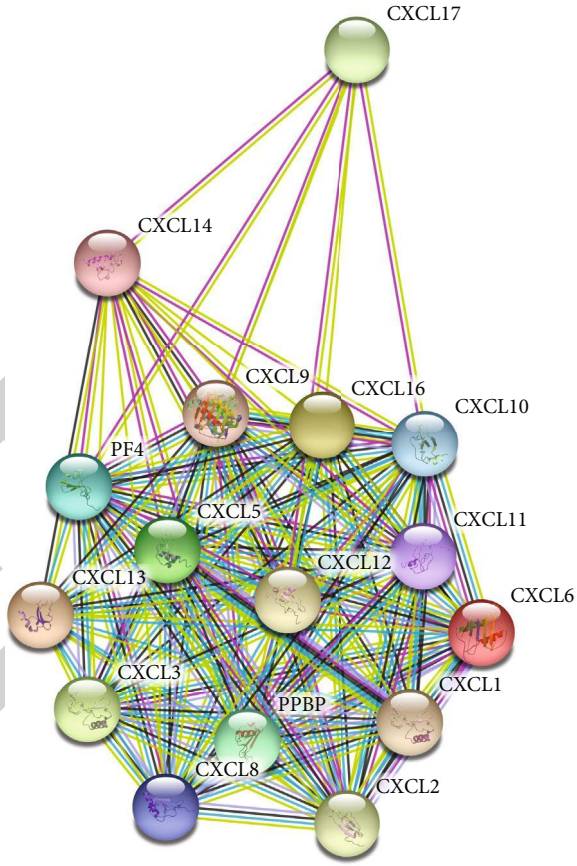


(b) Correlation heat map of different expressed CXC chemokines in different types of cancer, including LUAD and LUSC (TISIDB)

Figure 3: Continued.

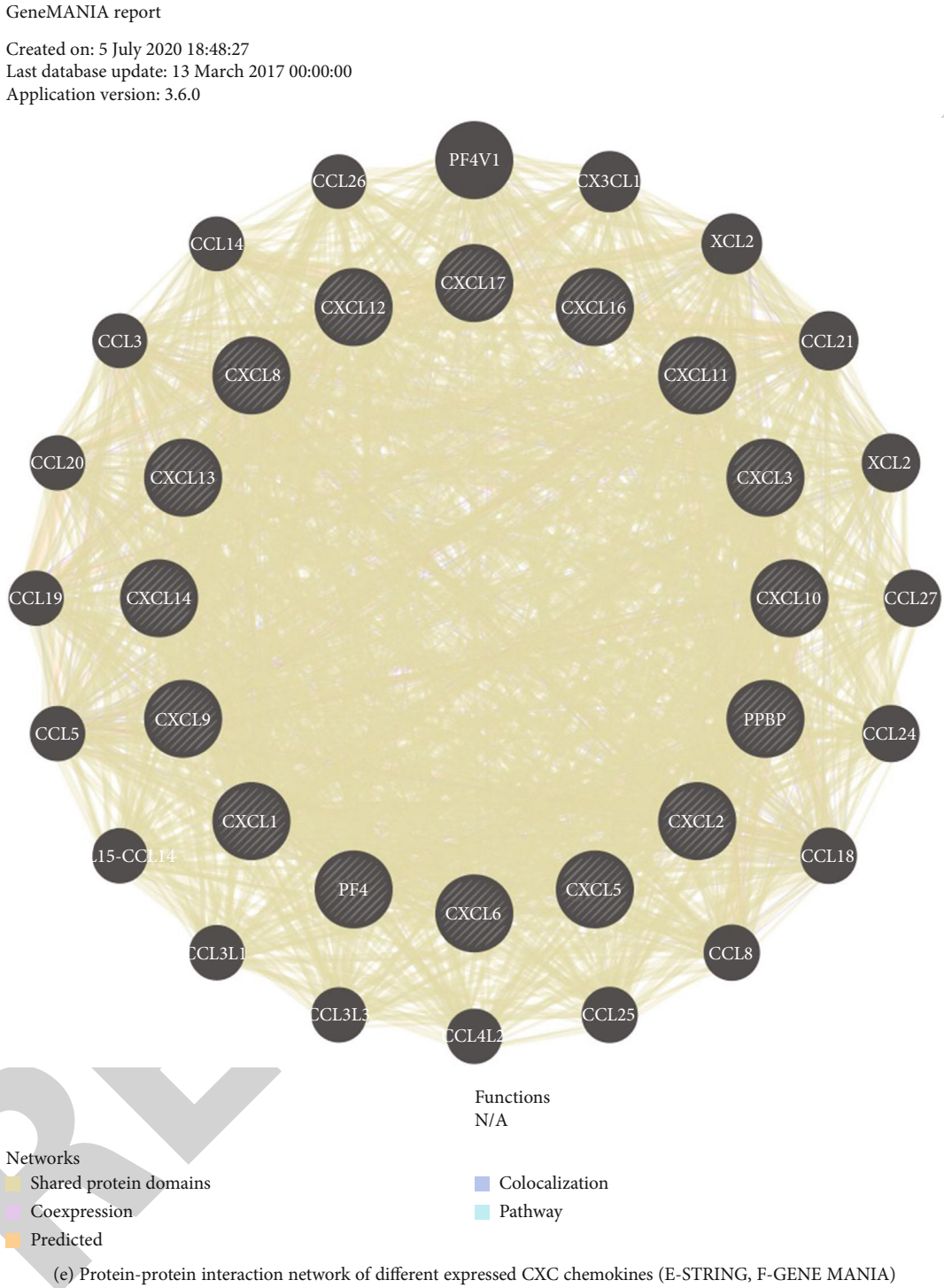


(c) Correlation analysis between different CXC chemokines with statistical significance ($p < 0.05$) in LUAD and LUSC (GEPIA). (1) to (8) were analyses in LUAD patients; (9) to (12) were analyses in LUSC patients



(d) Protein-protein interaction network of different expressed CXC chemokines (E-STRING, F-GENE MANIA)

Figure 3: Continued.



(e) Protein-protein interaction network of different expressed CXC chemokines (E-STRING, F-GENE MANIA)

Figure 3: Genetic alteration, interaction analyses, and neighbor gene network of different expressed CXC chemokines in LUAD and LUSC patients.

infection, and human cytomegalovirus infection. Figure 4(c) displayed the GO enrichment analysis in LUSC. The top 10 items are cell chemotaxis, chemokine-mediated signaling pathway, response to chemokine, cellular response to chemokine, leukocyte migration, myeloid leukocyte migration, positive regulation of chemotaxis, granulocyte chemotaxis, and positive regulation for leukocyte chemotaxis. The external side of the plasma membrane, focal adhesion, and cell-substrate junction were the 3 most highly enriched items in the CC category. In the molecular function category, the CXC chemokines and their neighboring genes were mainly enriched in G protein-coupled receptor binding, cytokine

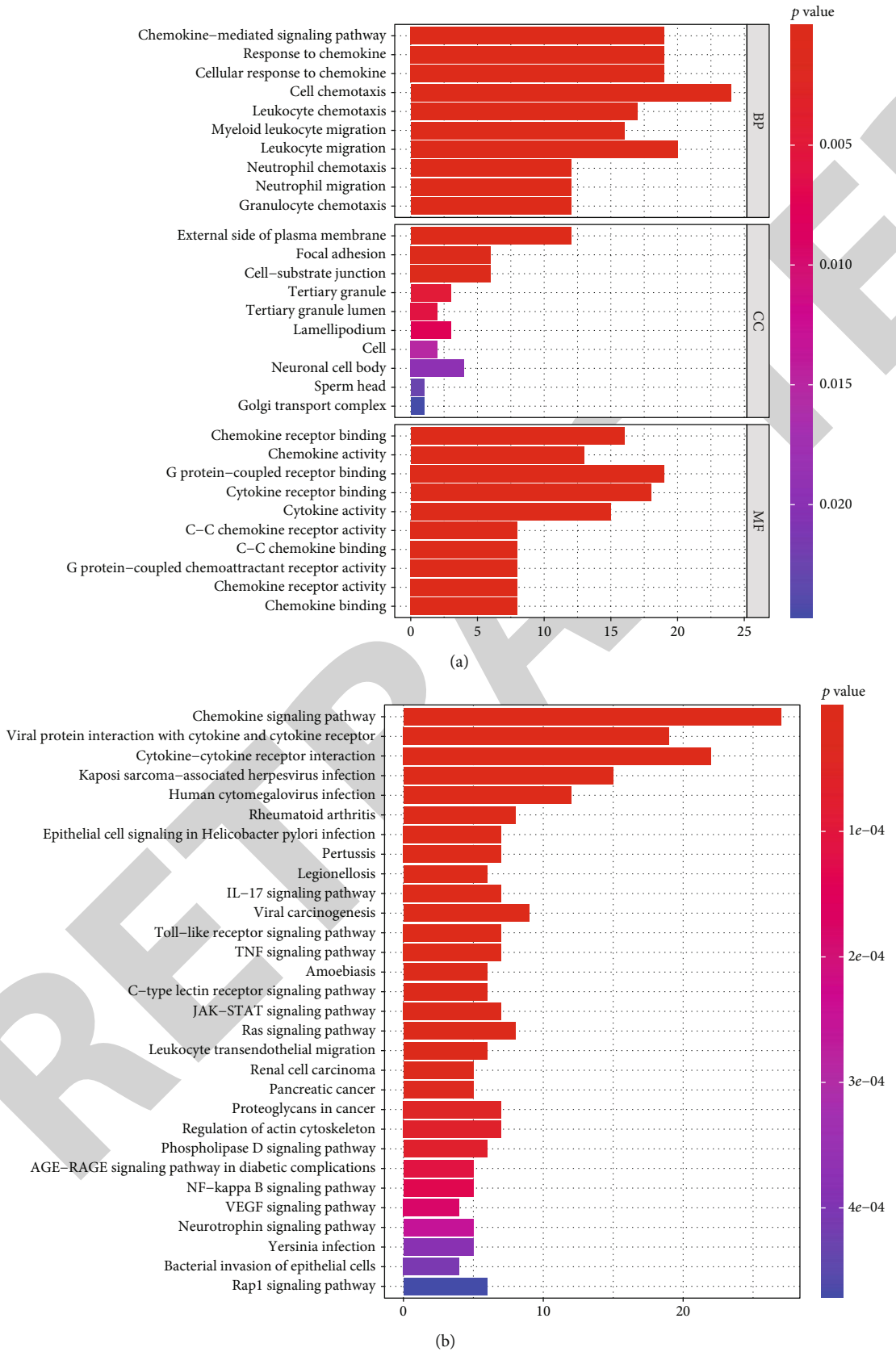


Figure 4: Continued.

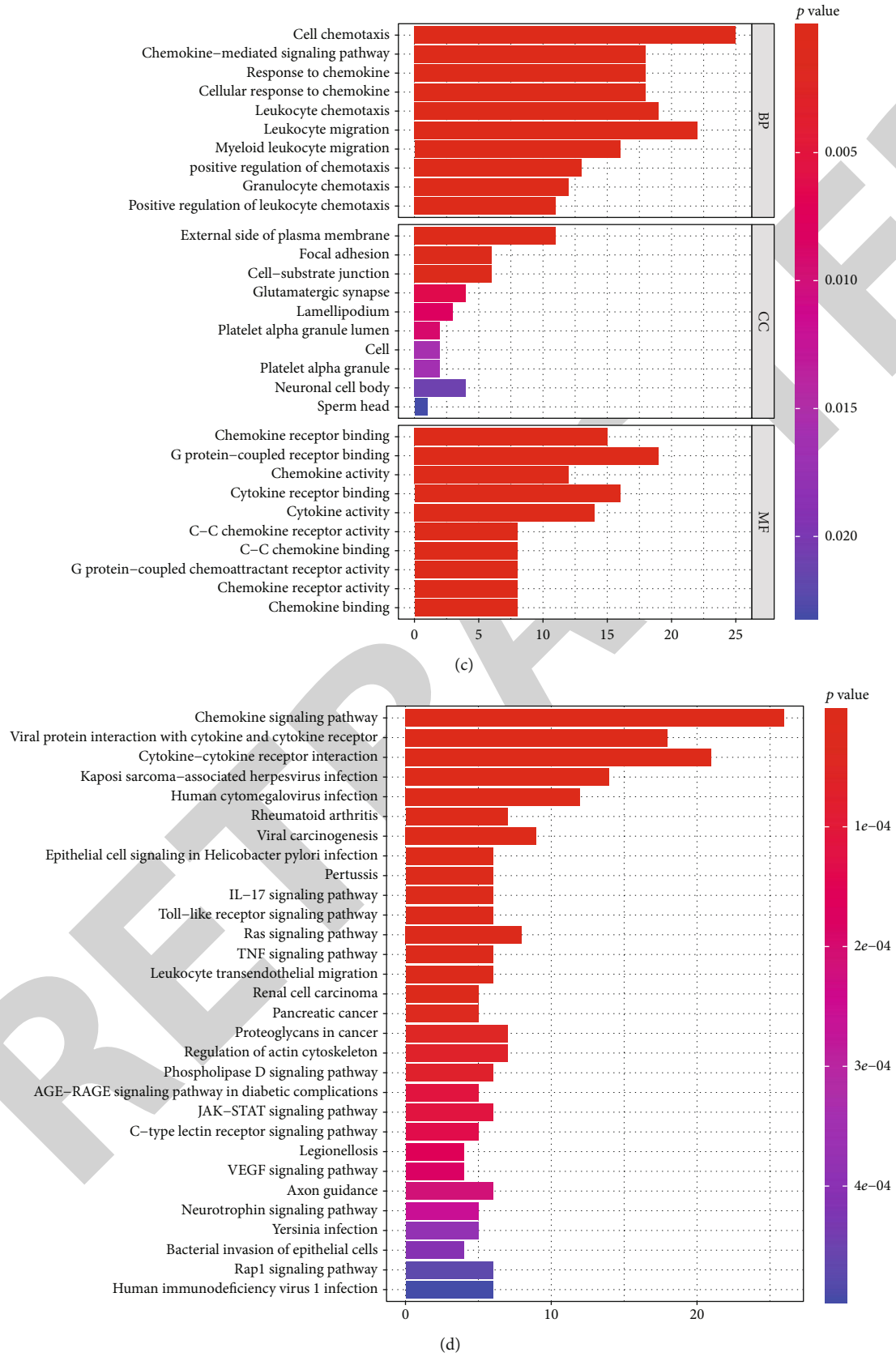


Figure 4: Bar plots of GO enrichment analysis in cellular component terms, biological process terms, and molecular function terms and KEGG enriched terms of different expressed CXC chemokines in LUAD and LUSC: (a, b) LUAD; (c, d) LUSC.

Table 1: Key regulated factor of CXC chemokines in human (TRRUST).

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

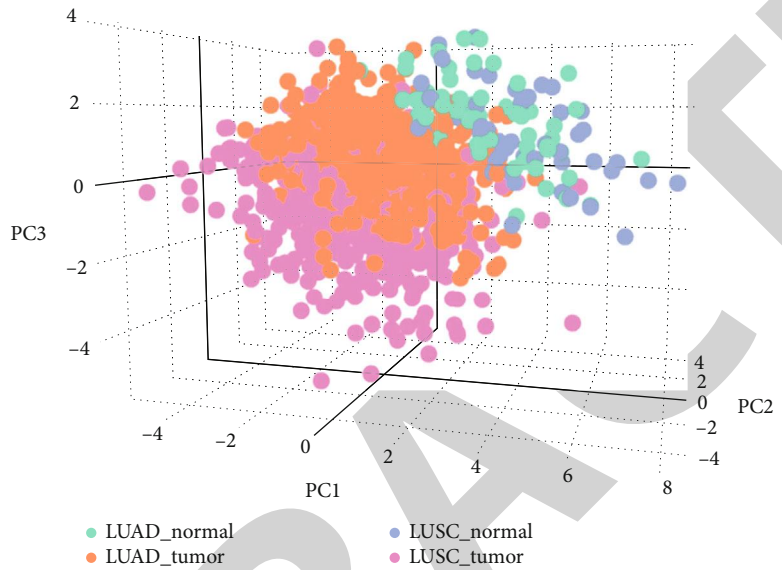
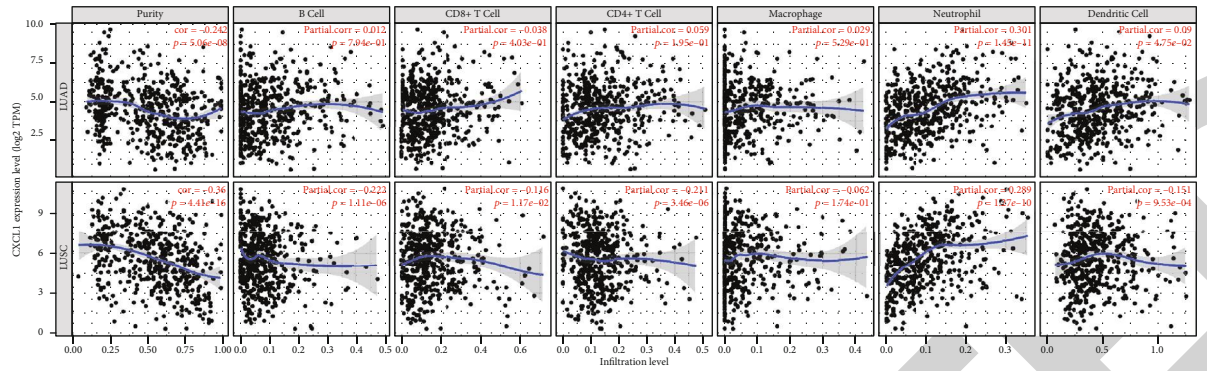


Figure 5: PCA (principal component analysis) on samples from TCGA LUAD and TCGA LUSC based on their expression of CXC chemokines (GEPIA2.0).

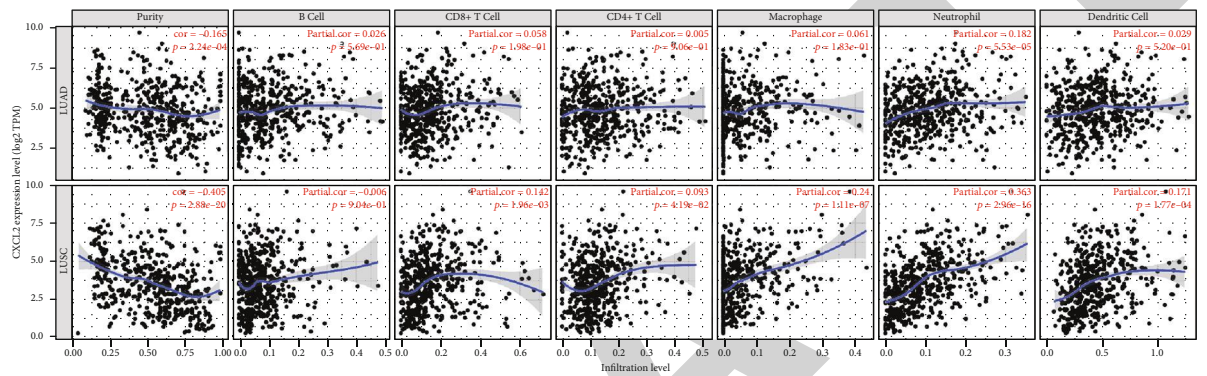
receptor binding, and chemokine receptor binding. Figure 4(d) is the KEGG pathway analyses of the CXC chemokine in LUSC where CXC chemokines were mainly enriched in the chemokine signaling pathway, cytokine-cytokine receptor interaction, and viral protein interaction with the cytokine and cytokine receptor.

3.4. Transcription Factor Targets and Principal Component Analysis of CXC Chemokines in Patients with LUAD and LUSC. Since there is significant difference in the expressions of CXC chemokines in LUAD/LUSC vs. normal tissue, we explored possible transcription factor targets of the CXC chemokines using the TRRUST database in Table 1. CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL12, CXCL13, CXCL14, CXCL16, and CXCL17 were included in TRRUST. Three transcription factors (RELA, NFKB1, and SP1) were found to be related to the regulation of CXC chemokines. RELA and NFKB1 were the key transcription factors for CXCL1, CXCL2, CXCL5, CXCL8, CXCL10, and CXCL12. SP1 was the key transcription factor for CXCL1, CXCL5, and CXCL14. Figure 5 showed the 3D PCA (principal component analysis) on samples from the TCGA tumor and TCGA normal, including LUAD, LUAD normal, LUSC, and LUSC normal, based on their expression of CXC chemokines using the GEPIA2.0 database.

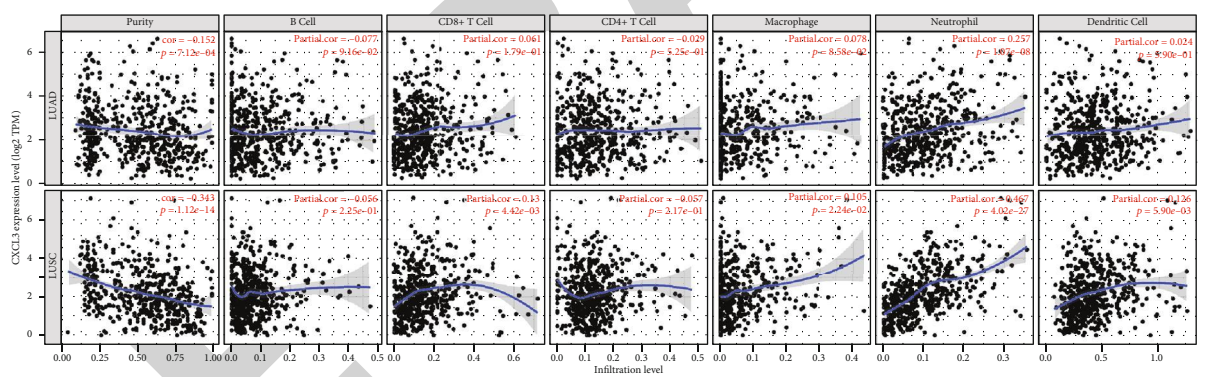
3.5. Immune Cell Infiltration Levels among Tumors with Different SCNA of CXC Chemokines in Patients with LUAD and LUSC. CXC chemokines are part of immune cell infiltration and inflammatory responses and so can affect the clinical outcome of LUAD and LUSC patients. Therefore, we explored the correlation between CXC chemokines and immune cell infiltration with the TIMER database. The correlation maps are displayed from CXCL1, CXCL2 to CXCL17 in Figure 6. To extract the key information more easily, we made summaries for Figure 6 in Tables 2 and 3, which are for LUAD and LUSC, respectively. CXCL9, CXCL10, CXCL11, CXCL12, and CXCL16 were chemokines that were positively correlated with all 6 types of immune cells in both LUAD and LUSC. In LUAD, expressions of CXCL4, CXCL5, CXCL6, CXCL7, and CXCL8 were negatively correlated with $CD4^+$ T cells. CXCL5, CXCL7, CXCL8, and CXCL17 were chemokines that had both positive and negative associations among different immune cells. In LUSC, all 16 CXC chemokines (except CXCL17) had positive or negative correlation with neutrophil cells, and CXCL13 was positively associated with all 6 types of immune cell. We also evaluated the correlation of CXC chemokines and immune cell infiltration (Tables 4(a) and 4(b)). The Cox proportional hazard model was adopted. B cells ($p < 0.01$) was significantly associated with the clinical outcome of LUAD patients (Table 4(a)). In LUSC patients, CXCL2 ($p < 0.05$), CXCL8 ($p < 0.05$), and



(a)



(b)



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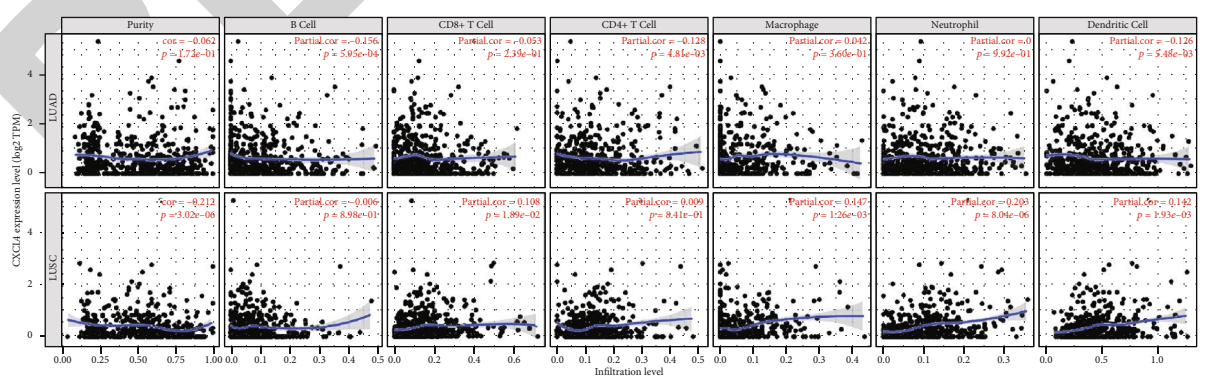
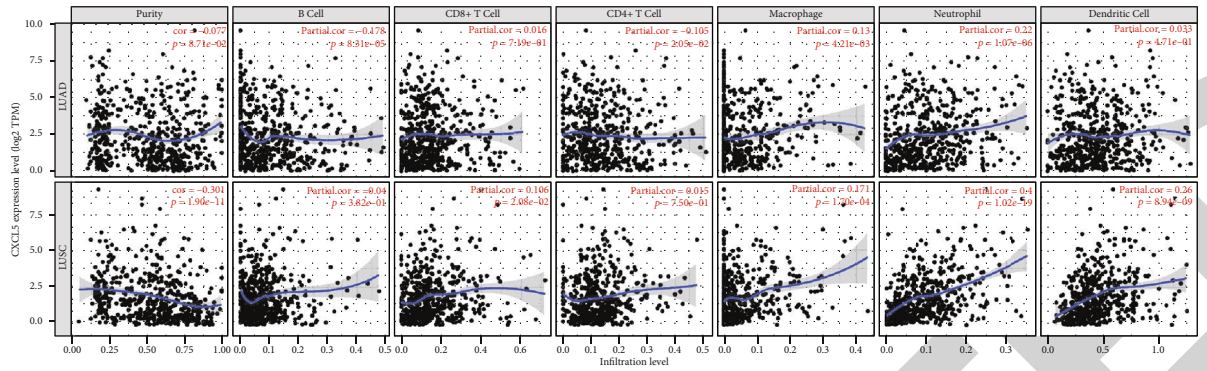


(d)

Figure 6: Continued.



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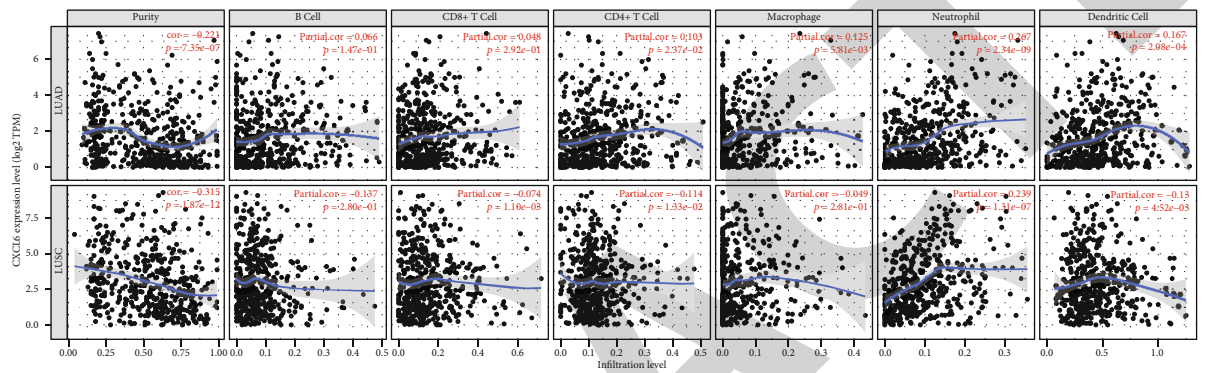

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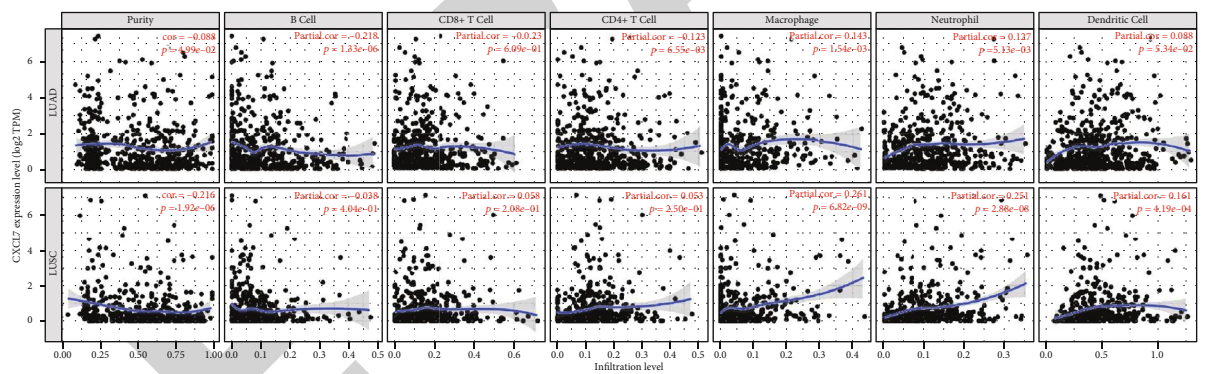

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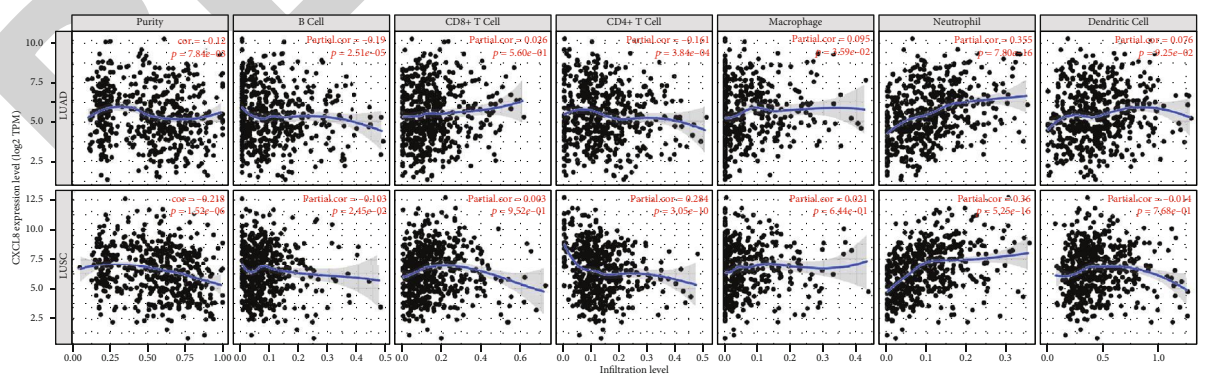

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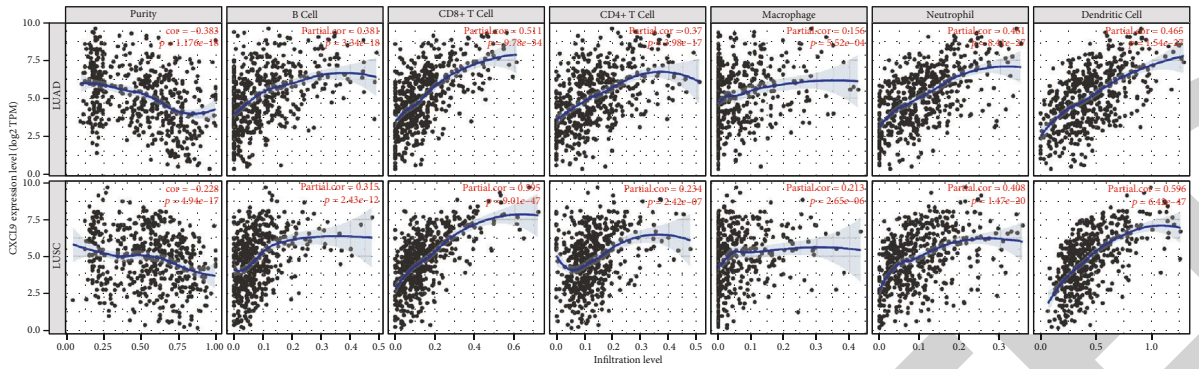
Figure 6: Continued.



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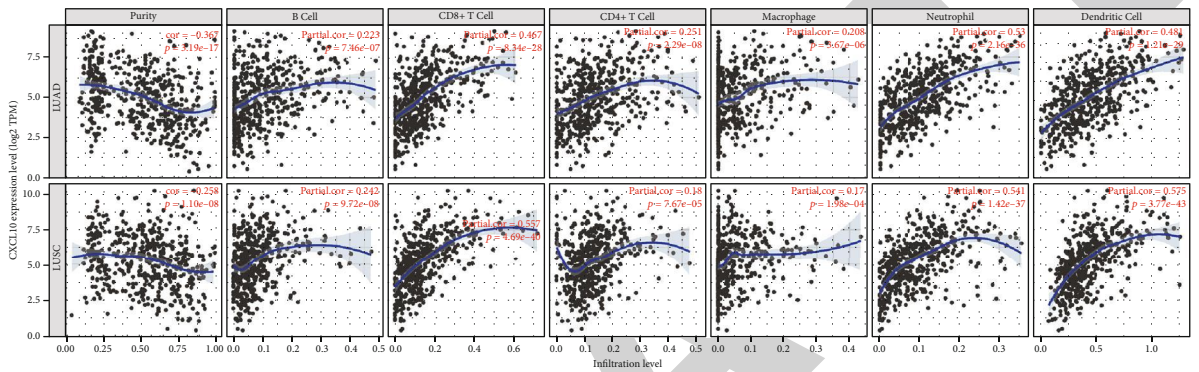

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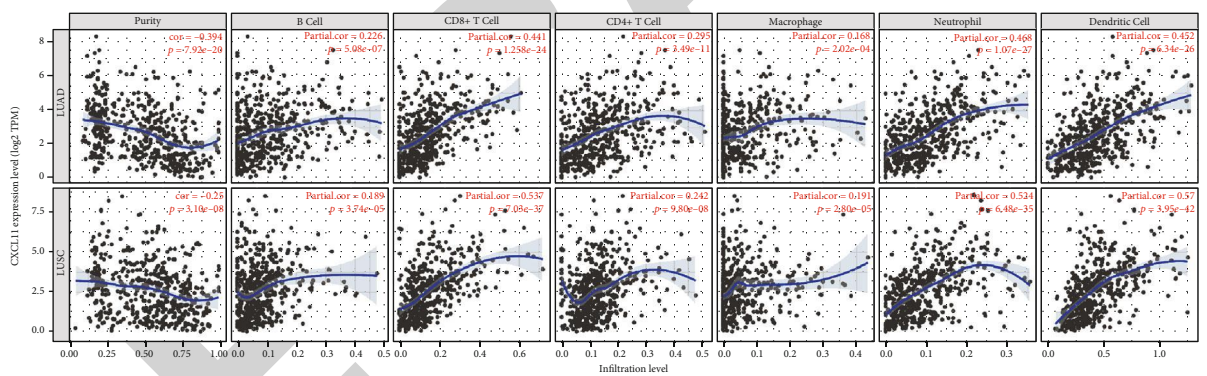

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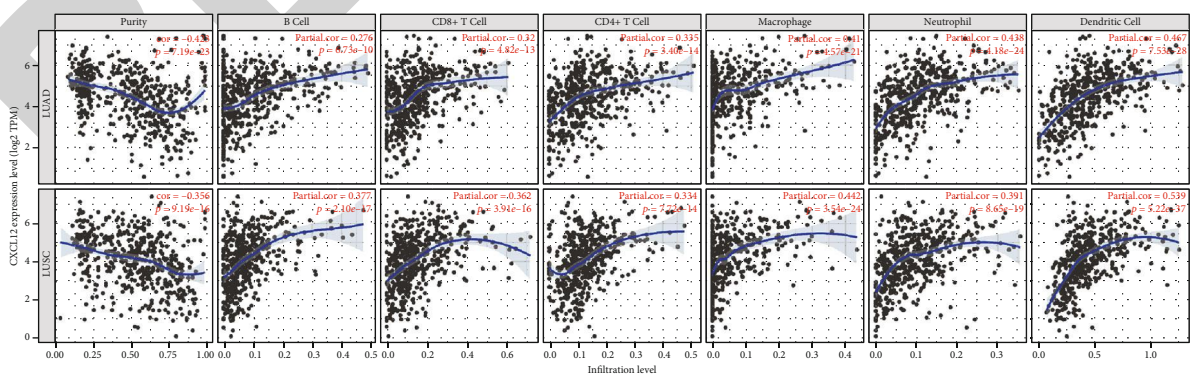
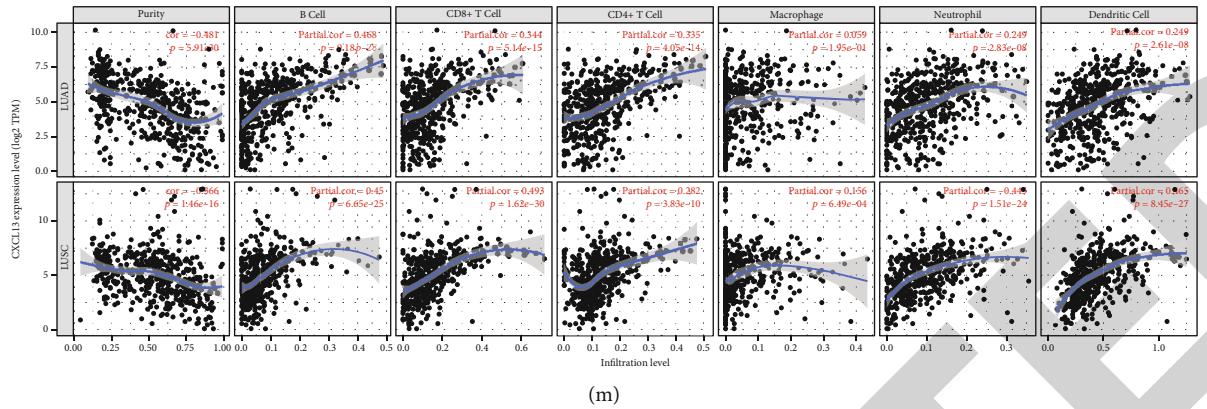
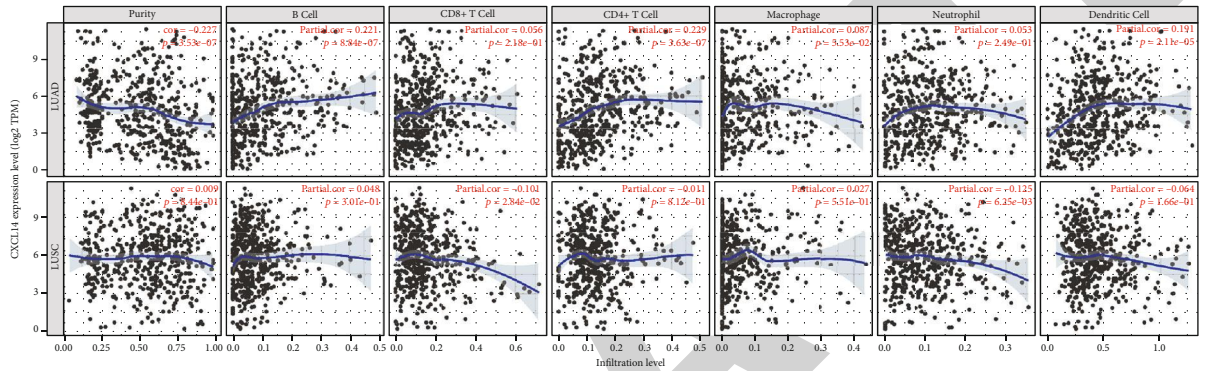


(l)

Figure 6: Continued.



(m)



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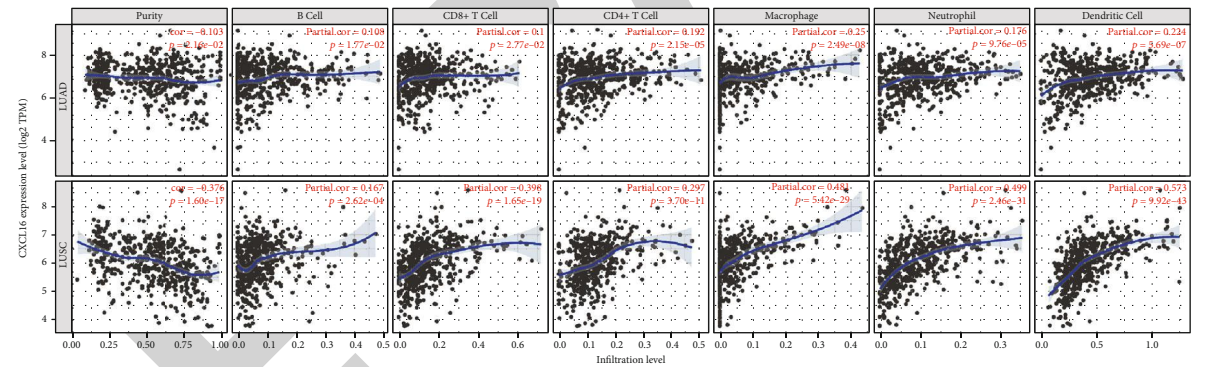
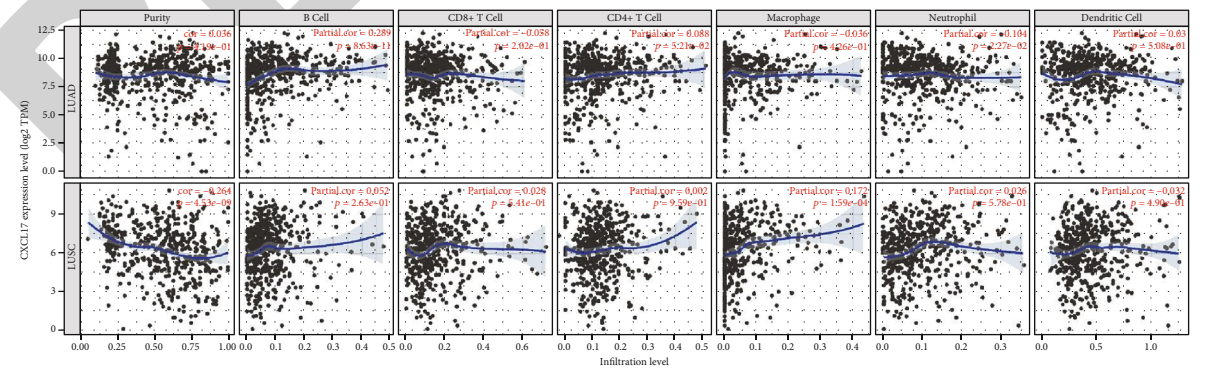


(o)



(p)

Figure 6: The correlation between different expressed CXC chemokines and immune cell infiltration in LUAD and LUSC patients (TIMER). The correlation between the abundance of immune cell and the expression of (a) CXCL1, (b) CXCL2, (c) CXCL3, (d) CXCL4, (e) CXCL5, (f) CXCL6, (g) CXCL7, (h) CXCL8, (i) CXCL9, (j) CXCL10, (k) CXCL11, (l) CXCL12, (m) CXCL13, (n) CXCL14, (o) CXCL16, and (p) CXCL7 in LUAD and LUSC patients.

Table 2: Summary of LUAD data in Figure 6.

	B cell	CD8+ cell	CD4+ cell	Macrophage	Neutrophil	Dendritic cell
CXCL1					*	*
CXCL2					*	
CXCL3						
CXCL4	**		**			**
CXCL5	**		**	*	*	
CXCL6			**	**	**	**
CXCL7	**		**	*	*	
CXCL8	**		**	*	*	
CXCL9	*	*	*	*	*	*
CXCL10	*	*	*	*	*	*
CXCL11	*	*	*	*	*	*
CXCL12	*	*	*	*	*	*
CXCL13	*	*	*		*	*
CXCL14	*		*			*
CXCL16	*	*	*	*	*	*
CXCL17	*				**	

*$p < 0.05$, positively correlated; **$p < 0.05$, negatively correlated.

Table 3: Summary of LUSC data in Figure 6.

	B cell	CD8+ cell	CD4+ cell	Macrophage	Neutrophil	Dendritic cell
CXCL1	*	*	*		*	*
CXCL2		*	*	*	*	*
CXCL3		*		*	*	*
CXCL4		**		**	**	**
CXCL5		**		*	*	*
CXCL6	**		**		**	**
CXCL7				*	*	*
CXCL8	**		**		*	
CXCL9	*	*	*	*	*	*
CXCL10	*	*	*	*	*	*
CXCL11	*	*	*	*	*	*
CXCL12	*	*	*	*	*	*
CXCL13	*	*	*	*	*	*
CXCL14		*			*	
CXCL16	*	*	*	*	*	*
CXCL17				*		

*$p < 0.05$, positively correlated; **$p < 0.05$, negatively correlated.

CXCL13 ($p < 0.05$) were significantly associated with the clinical outcome.

At last, we conducted the comparison of tumor infiltration levels among tumors with different SCNAs (somatic copy number alterations) for CXC chemokines in LUAD and LUSC samples (Figure 7) using the TIMER database. From the results, we found that in both LUAD and LUSC patients, all of the SCNA types that are significantly correlated with immune cells are (1) arm-level deletion, (2) arm-level gain, and (3) high amplification. In LUAD, all 16 CXC chemokines have a significant part in $CD4^+$ T cells (in which SCNA types are mainly arm-level depletion) and neutrophil cells (in which SCNA types are mainly high amplification). In LUSC, all 16 CXC chemokines have a significant part in $CD4^+$ T cells, macrophage cells, and neutrophil cells; predominant SCNA types of the three types are the

Table 4: The cox proportional hazard model of CXC chemokines and six tumor-infiltrating immune cells in LUAD and LUSC patients (TIMER): (a) LUAD; (b) LUSC.

(a)

	Coef	HR	95% CI_l	95% CI_u	*p* value	Sig
B_cell	-4.563	0.010	0.001	0.163	0.001	**
CD8_T cell	0.412	1.510	0.171	13.339	0.711	
CD4_T cell	2.862	17.503	0.715	428.624	0.079	
Macrophage	0.052	1.054	0.066	16.919	0.971	
Neutrophil	-2.638	0.072	0.000	11.525	0.309	
Dendritic	0.224	1.251	0.281	5.565	0.769	
CXCL1	-0.039	0.962	0.813	1.138	0.651	
CXCL2	-0.096	0.908	0.768	1.074	0.261	
CXCL3	0.231	1.259	0.978	1.622	0.074	
CXCL4	-0.107	0.898	0.742	1.088	0.274	
CXCL5	0.039	1.040	0.937	1.154	0.463	
CXCL6	-0.001	0.999	0.863	1.156	0.984	
CXCL7	-0.022	0.978	0.861	1.112	0.738	
CXCL8	0.068	1.071	0.919	1.247	0.382	
CXCL9	-0.008	0.992	0.789	1.248	0.948	
CXCL10	-0.172	0.842	0.592	1.197	0.339	
CXCL11	0.238	1.269	0.964	1.670	0.089	
CXCL12	-0.047	0.954	0.810	1.123	0.571	
CXCL13	-0.010	0.990	0.884	1.108	0.857	
CXCL14	0.043	1.044	0.976	1.117	0.210	
CXCL16	-0.047	0.954	0.770	1.182	0.666	
CXCL17	-0.074	0.929	0.855	1.009	0.079	

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

(b)

	Coef	HR	95% CI_l	95% CI_u	*p* value	Sig
B_cell	1.631	5.110	0.341	76.487	0.237	
CD8_T cell	0.627	1.873	0.266	13.192	0.529	
CD4_T cell	2.269	9.674	0.628	149.081	0.104	
Macrophage	-2.730	0.065	0.004	1.010	0.051	
Neutrophil	1.133	3.105	0.037	263.329	0.617	
Dendritic	0.626	1.870	0.404	8.653	0.423	
CXCL1	-0.109	0.896	0.779	1.031	0.127	
CXCL2	0.180	1.197	1.012	1.415	0.035	*
CXCL3	-0.174	0.840	0.654	1.078	0.172	
CXCL4	0.015	1.015	0.739	1.394	0.927	
CXCL5	-0.007	0.993	0.872	1.132	0.921	
CXCL6	-0.047	0.954	0.860	1.059	0.378	
CXCL7	0.095	1.099	0.936	1.291	0.249	
CXCL8	0.162	1.176	1.036	1.336	0.012	*
CXCL9	-0.165	0.848	0.718	1.002	0.053	
CXCL10	0.097	1.102	0.841	1.445	0.481	

Table 4: Continued.

	Coef	HR	95% CI_l	95% CI_u	p value	Sig
CXCL11	-0.050	0.951	0.755	1.197	0.667	
CXCL12	0.011	1.011	0.887	1.152	0.866	
CXCL13	-0.111	0.895	0.806	0.994	0.038	*
CXCL14	0.011	1.011	0.946	1.080	0.753	
CXCL16	-0.005	0.995	0.766	1.293	0.970	
CXCL17	-0.059	0.943	0.862	1.032	0.202	

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

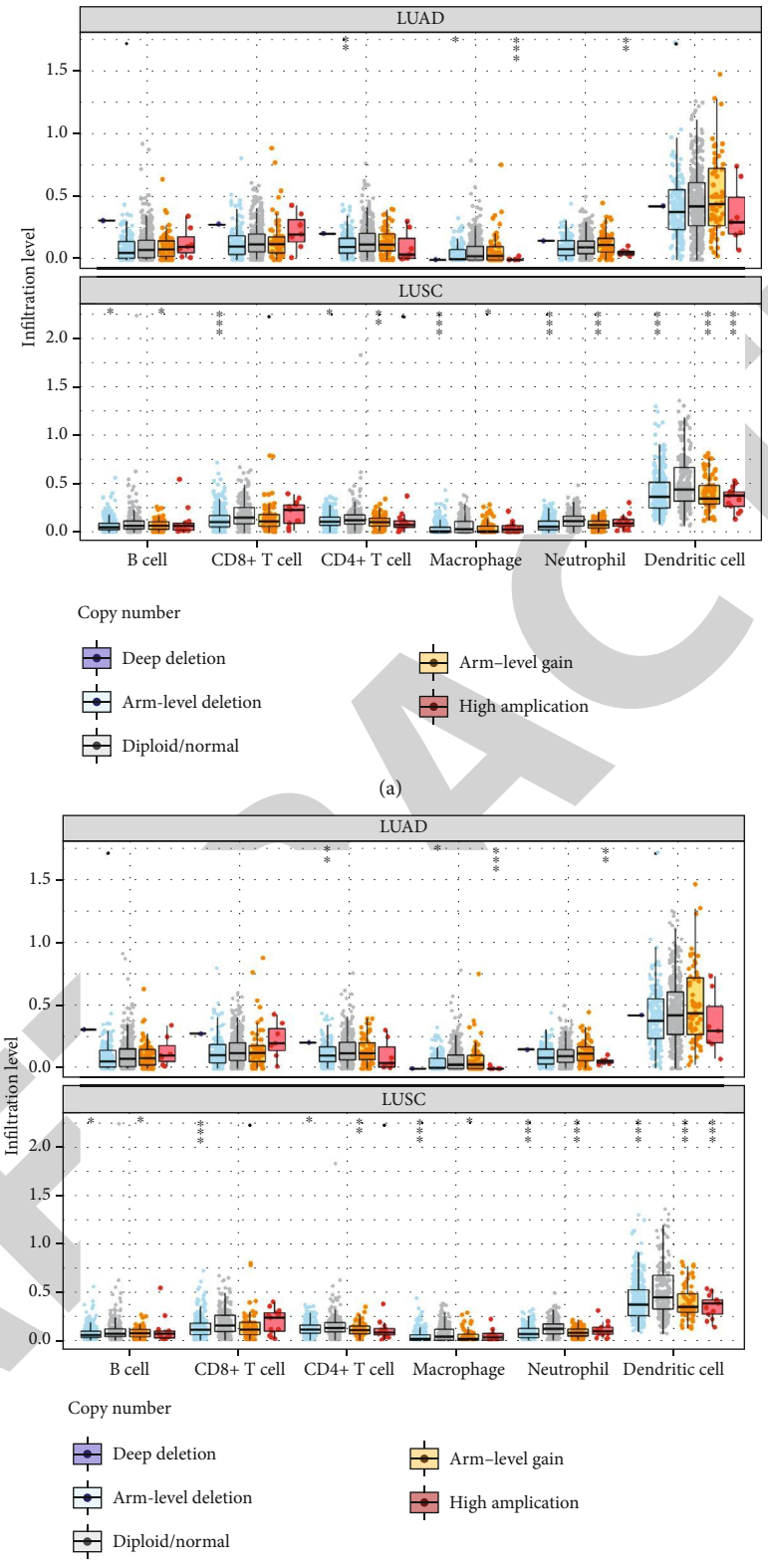
same—arm-level alterations. In LUAD samples, CXC14 are only chemokines of which SCNA is significantly correlated with $CD8^+$ T cells among 16 CXC chemokines.

4. Discussion

CXC chemokines and their receptors were initially identified as important regulators in inflammatory response [10]. In cancer, accumulative evidence has proven that CXC chemokines are critical in tumor initiation, angiogenesis, and progression [7], and with the rapid development of tumor immunology, the correlation among CXC chemokines, tumor microenvironment, and cancer immunotherapy have been reported [8, 11].

In NSCLC, much attention has been paid to the functions of some CXC chemokines. For example, the CXCL1 paracrine network was identified to be linked with cancer chemoresistance and metastasis in 2012 [18], and in 2020, researchers found that CXCL1 was an unfavorable prognosis factor negatively regulated by DACH1 in NSCLC using immunohistochemistry staining [38]. In 2004, researchers found that COX-2 (cyclooxygenase-2) contributes to the progression of NSCLC tumorigenesis by enhancing the expression of angiogenic chemokines CXCL8 and CXCL5 [39], both of which can contribute to lung cancer progression. As for the cancer treatment, combination of CXCL9 or CXCL10 with cisplatin improves therapeutic efficacy in solid tumors [40, 41]. Changes in the serum level of CXCL8 can reflect and predict the response to anti-PD-1 therapy in NSCLC [42]. Although the CXC chemokine family in lung cancer has been studied for years, there are still unknown corners and even controversies about several CXC chemokines. For instance, researchers have opposite views about the roles of CXCL4 [22, 43], CXCL14 [24, 44], and CXCL16 [27, 45], and we know little about the positions of CXCL2, CXCL6, and CXCL13 in NSCLC. Generally, although many researchers have studied the function of some CXC chemokines singly and have put up solid evidence, the comprehensive landscape of CXC chemokines and their behavior in LUAD and LUSC distinctly have not been well-portrayed yet.

Firstly, we explored the expression and methylation of CXC chemokines in 3 database and their correlation with pathological stages in LUAD and LUSC. In the ONCOMINE database where lung cancer types are not discriminated, we found that 12 genes were differentially expressed in lung cancer compared with normal tissue (upregulation of CXCL9/13/14; downregulation of CXCL1/2/3/4/5/7/12/16/17). And in the TIMER and UALCAN databases, where LUAD and LUSC are distinguished, we found that 9 genes were differentially expressed in LUAD compared with normal tissue (upregulation of CXCL10/13/14; downregulation of CXCL2/3/4/7/12/16), and in LUSC, 13 genes were differentially expressed compared with normal tissue (upregulation of CXCL6/10/13/14; downregulation of CXCL2/3/4/5/7/11/12/16/17). That is the whole expression condition of CXC chemokines in lung cancer. In the UALCAN database where we conducted the methylation analysis of CXC chemokines, we found that methylation levels of CXC1/3/5/6/10/12 were significantly elevated, and methylation levels of CXCL7/11/16/17 were significantly reduced in both LUAD and LUSC. Suzuki et al. reported that aberrant methylation of CXCL12 in NSCLC is associated with poor prognosis [46], which agrees with our research to some degree and inspires us that the methylation level of other CXC chemokines may be involved with tumor prognosis. Then, we explore the association between CXC chemokines and pathological stages and the clinical prognosis. We found that in LUAD, expression of CXCL8 increased and expression of CXCL17 decreased as the tumor progressed, and in LUSC, the expressions of CXCL1 and CXCL6 increased considerably when the tumor progressed to stage IV. These results imply that CXCL1/6/8/17 may predict the stage or tendency of tumors. In the UALCAN database, we found that LUAD patients with low expression of CXCL1/4/7/8 and LUAD patients with high expression of CXCL12/14/16 were significantly associated with better OS (overall survival), while no CXC chemokines in LUSC have statistical significance. These data demonstrate that differently expressed CXC chemokines may be important in LUAD. Yu et al. has identified CXCL1 as an unfavorable prognosis factor in NSCLC [38], which highly agrees with our results, and many researchers have believed that CXCL8 was associated with tumor progression, angiogenesis, and relapse for a long time [47]; therefore, our results may pave the way for CXCL8 to be an adverse prognosis factor in LUAD, even NSCLC. These data also suggest that in LUAD, CXCL4/7 may be adverse prognosis factors while CXCL12/14/16 may be favorable prognosis factors. As mentioned before, there are opposite views about the roles of CXCL4, CXCL14, and CXCL16 in NSCLC; our data gives more evidence on these controversies. So far, we do not know enough about CXCL7, and these data offer new information. For CXCL12, it has been believed that CXCL12 and its receptor, CXCR4, formed the CXC12/CXCR4 axis, and the axis was believed to contribute to tumor progression and metastasis in LUAD [21, 48], but in our study, LUAD patients with high



(a)

(b)

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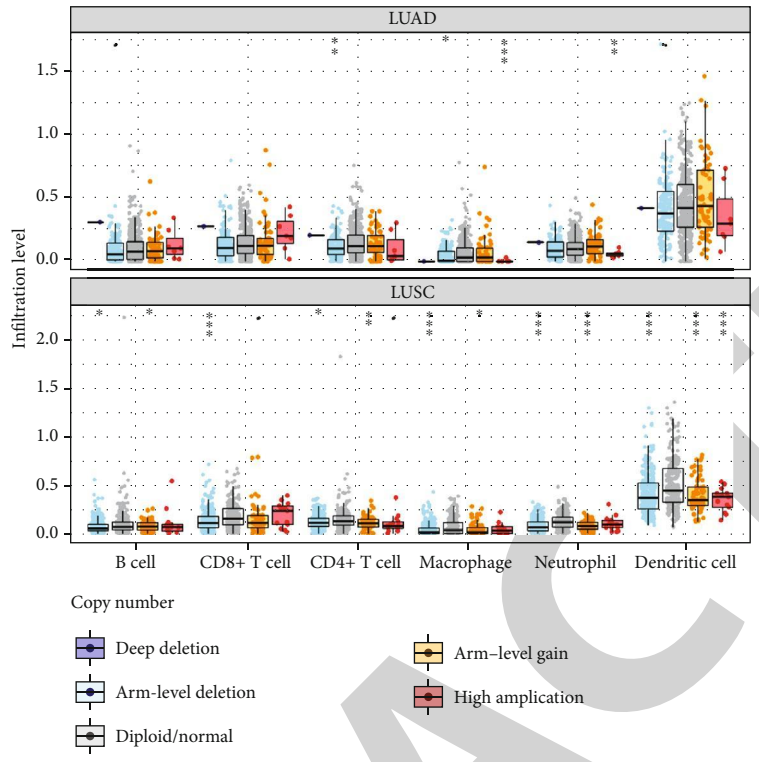

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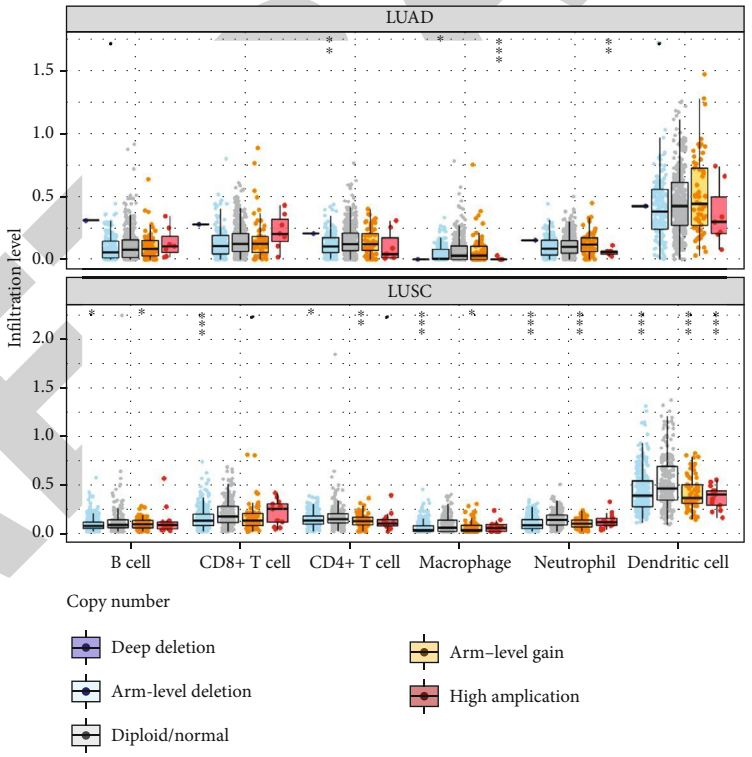

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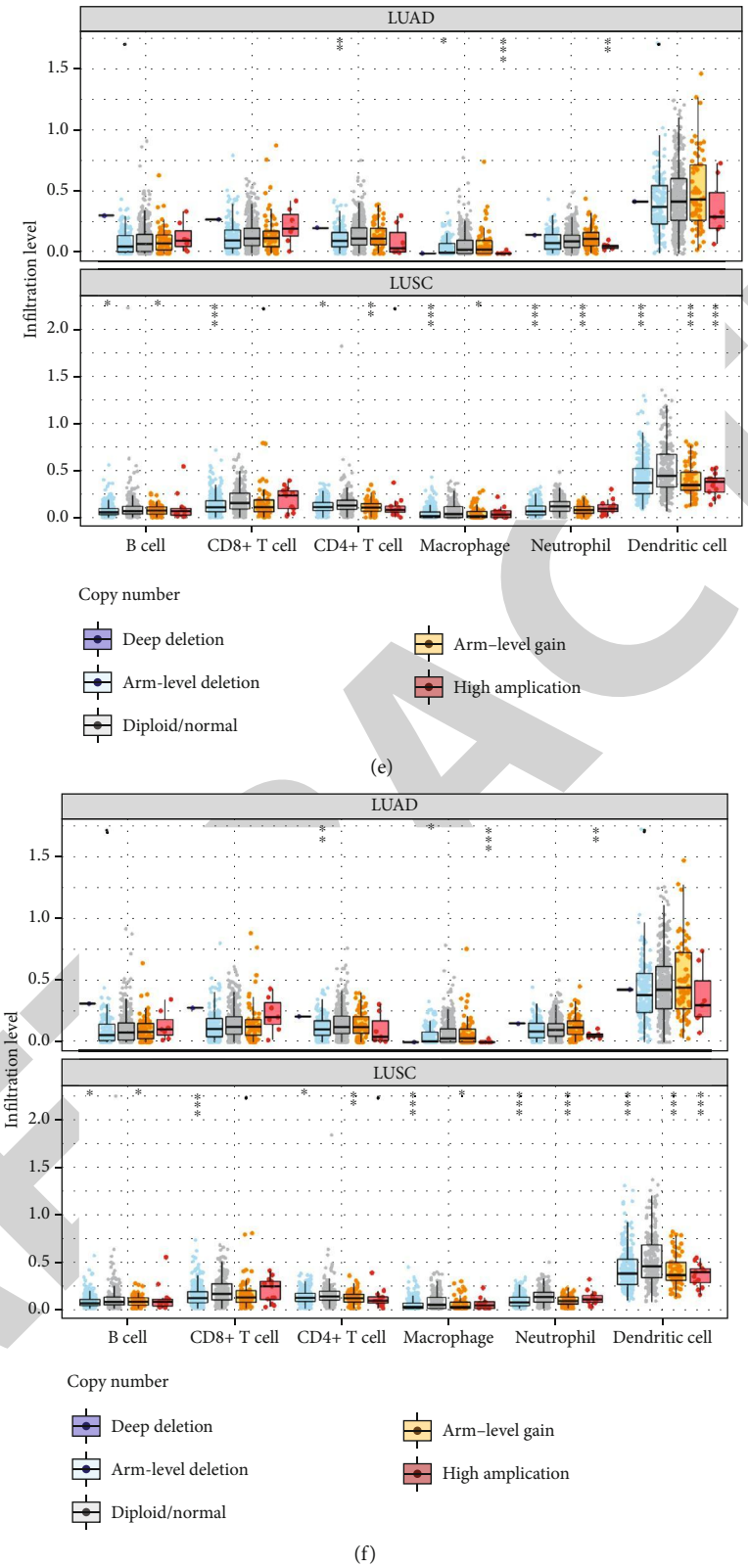

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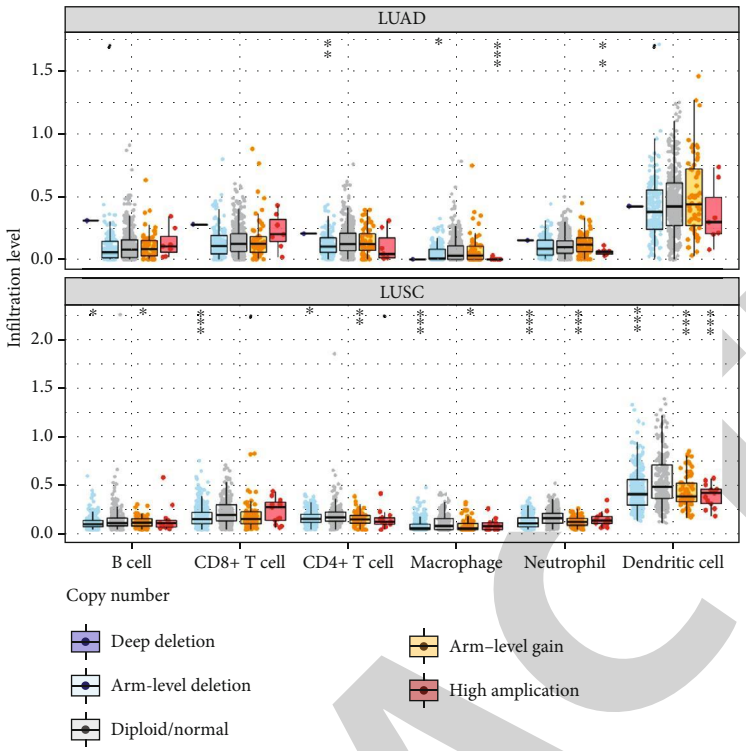

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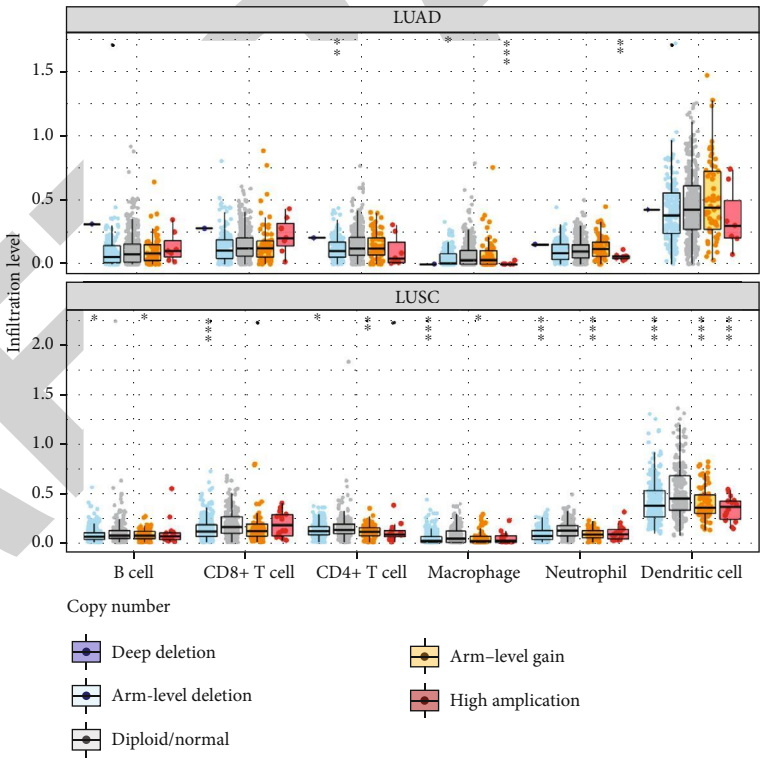

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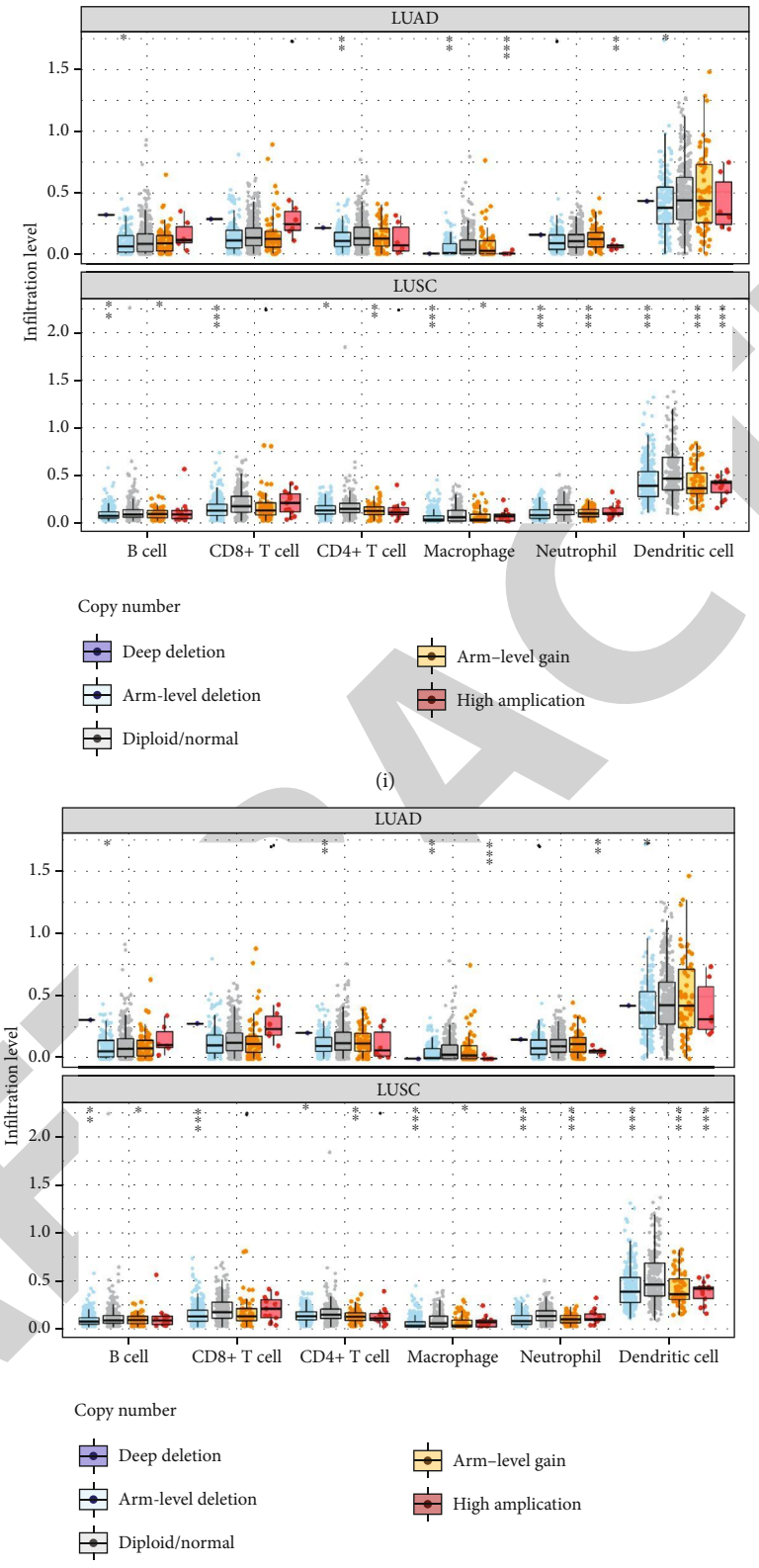

(i)

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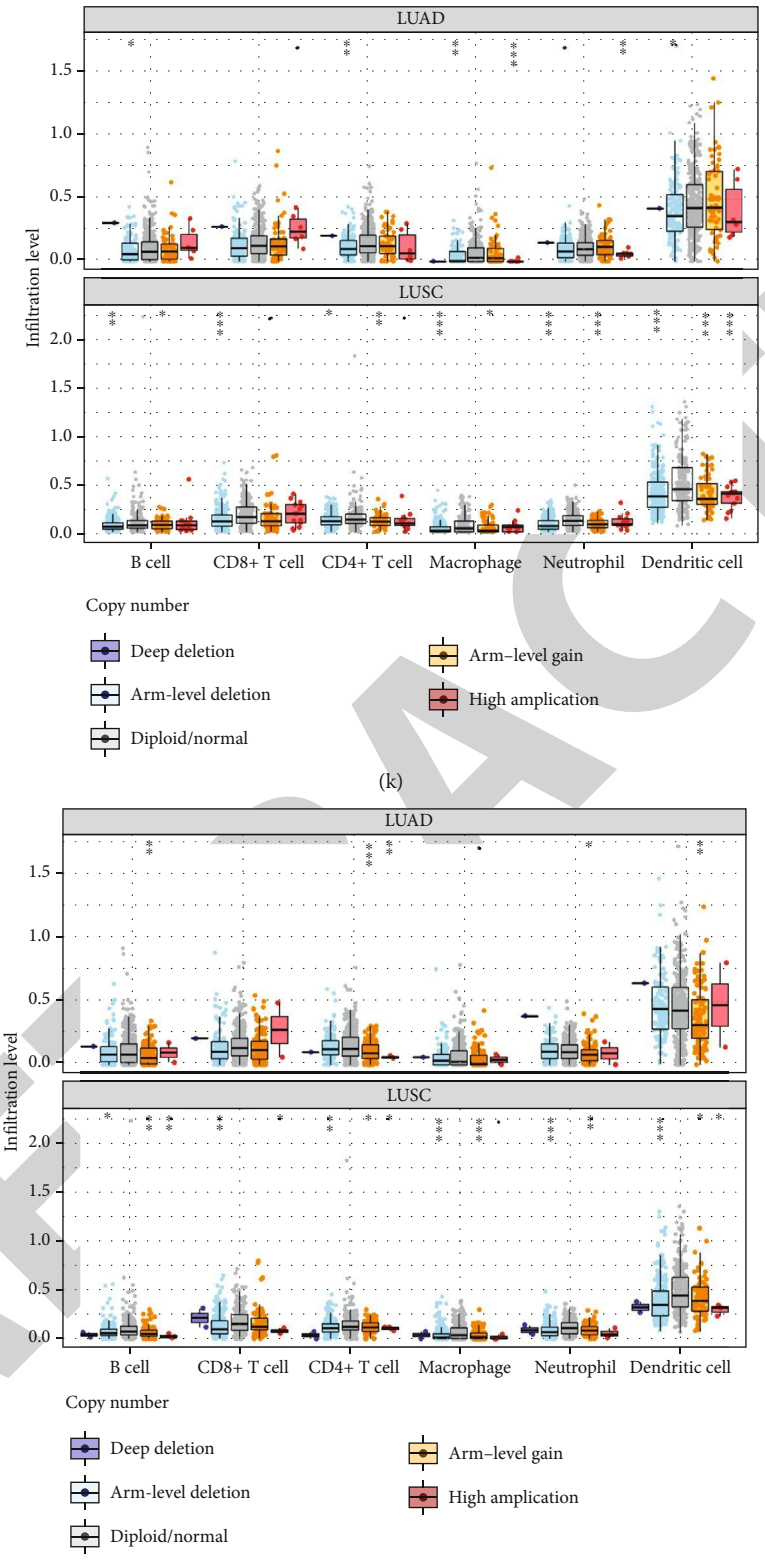

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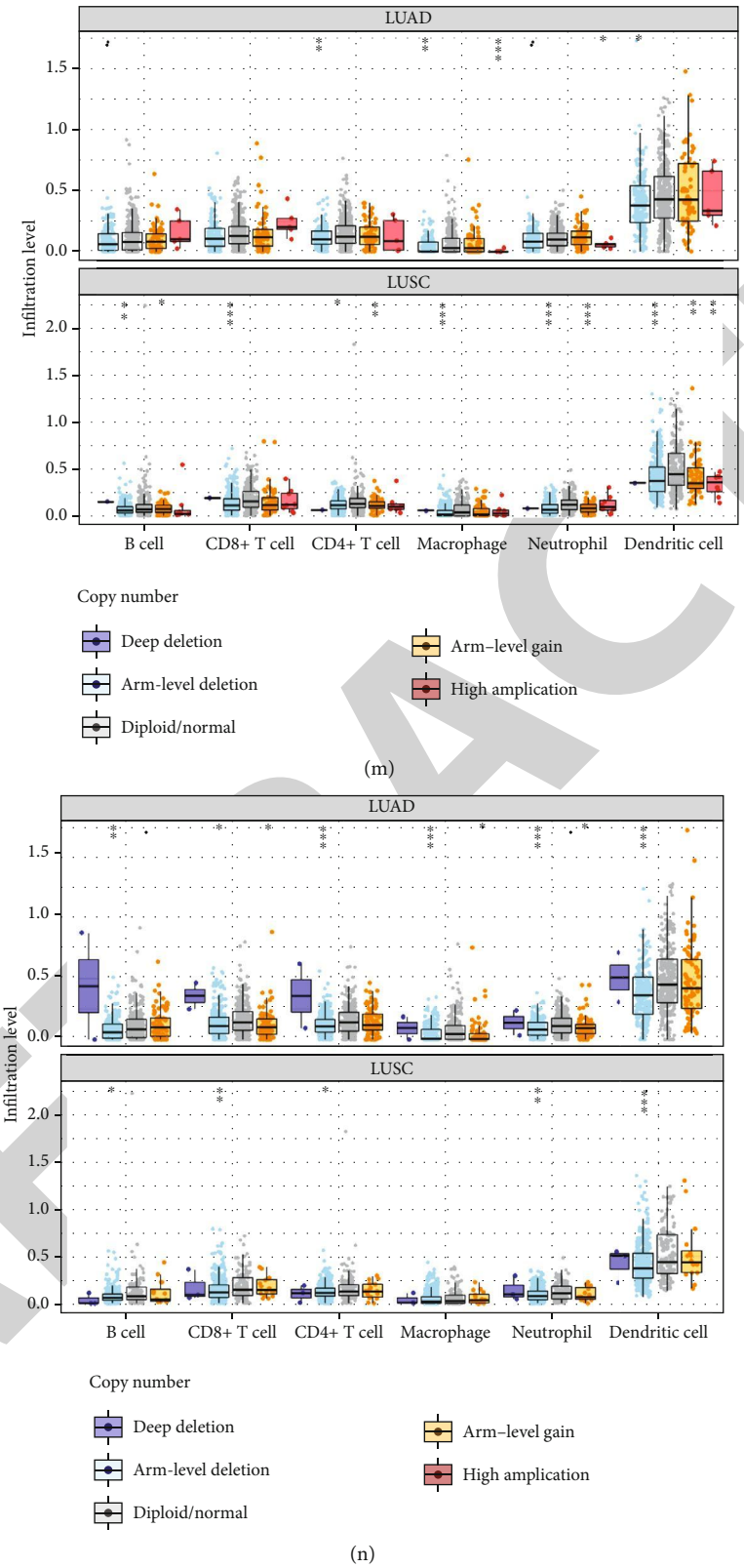

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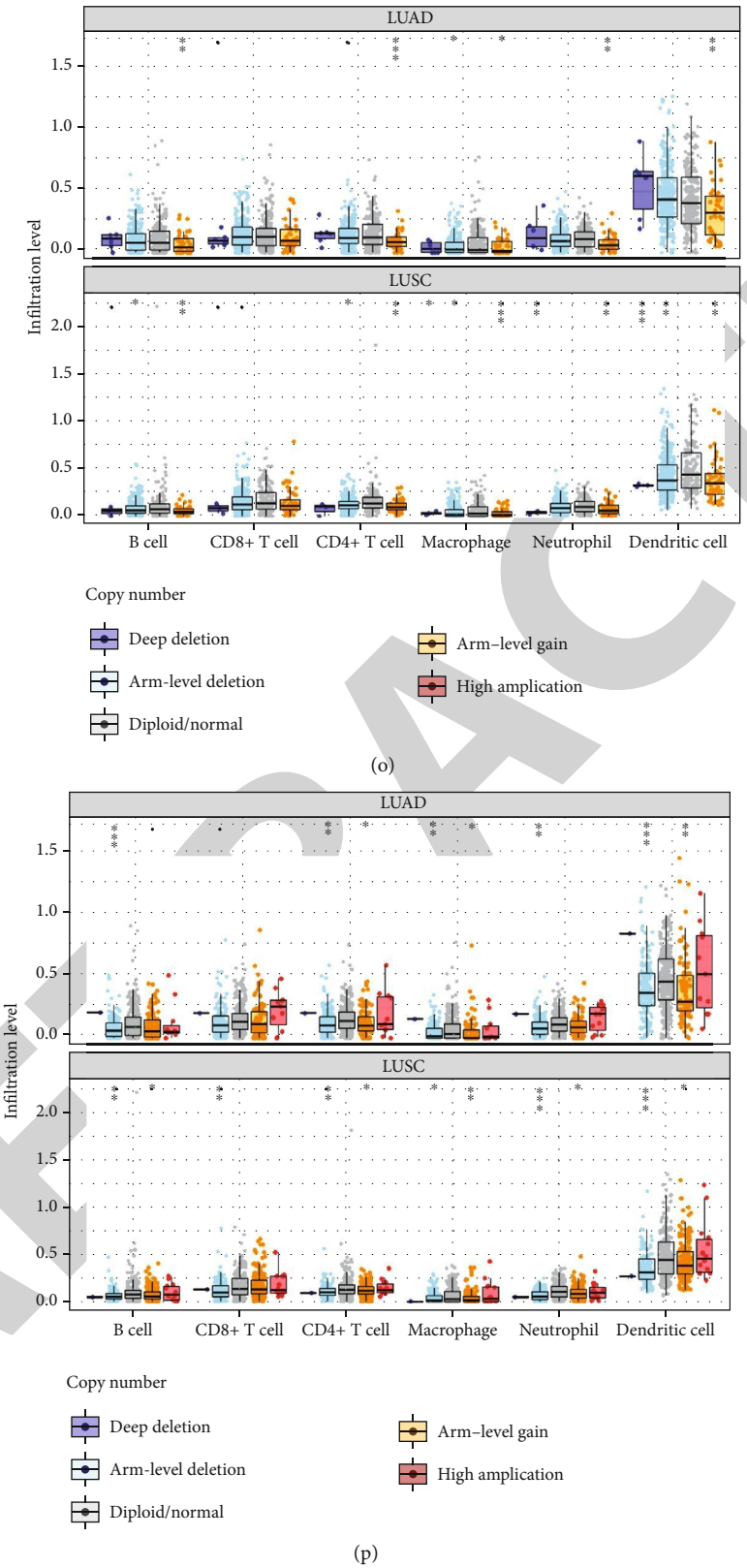

Figure 7: Comparison of tumor infiltration levels among tumors with different SCNAs (somatic copy number alterations) for CXC chemokines in LUAD and LUSC samples. SCNAs are defined by GISTIC2.0. Box plots are presented to show the distributions of each immune subset at each copy number status in LUAD and LUSC patients. The infiltration level for each SCNA category is compared with the normal using a two-sided Wilcoxon rank-sum test: (a) CXCL1, (b) CXCL2, (c) CXCL3, (d) CXCL4, (e) CXCL5, (f) CXCL6, (g) CXCL7, (h) CXCL8, (i) CXCL9, (j) CXCL10, (k) CXCL11, (l) CXCL12, (m) CXCL13, (n) CXCL14, (o) CXCL16, and (p) CXCL7.

CXCL12 expression were associated with better OS significantly; the reasons that lie behind these two contrary findings need further exploration. From the survival map, we found that CXCL2/7/12/16 made significant contribution to the survival in LUSC, and no CXC chemokines were significant in LUAD, which suggest that we expect more research on the roles of CXC chemokines in LUSC.

In lung cancer, since several chemokines were differentially expressed, we explored their molecular traits in LUAD and LUSC. Frequent genetic alterations are found in CXC chemokines, and the elevated/reduced mRNA expression was the type that had the most alteration in both LUAD and LUSC. Tumor initiation progression of lung cancer is complex and intricate, and genetic alteration plays a significant role in this story [49]. From correlation heat maps and gene-to-gene correlation analysis, we found tight corrections among these 16 CXC chemokines in both LUAD and LUSC, implying that these cytokines work synergistically in tumor initiation and progression. In the past 5 years, researchers have identified that CXCL1, CXCL5, and CXCL8 acted as tumor promoters in LUAD or NSCLC, and all of them could be antagonized by DACH1 (the human Dachshund homologue 1) [20, 38, 50], so we speculated if there were any correlations among them. This speculation was partly validated in our study, since we found significant correlations between CXCL1 and CXC8 in both LUAD and LUSC patients. Tight association between CXCL9 and CXCL10 in LUAD was found, and as we mentioned above, combination of CXCL9 or CXCL10 with cisplatin improves therapeutic efficacy in solid tumors [40, 41]; we have reason to believe that there may be a close relation between the CXCL9 and CXCL10. Similarly, Wang et al. found that combination of CXCL10 and CXCL11 led to synergistic antitumor effects [51], and correlations with statistical significance were found between CXCL10 and CXCL11 in both LUAD and LUSC; therefore, we can also speculate the unusual correlation between these two chemokines. As for the driver gene of NSCLC, Luppi et al. and Tsai et al. identified CXCL8 [52] and CXCL12 [53] which worked together with the EGFR gene in the progression of NSCLC, respectively; however, no significant association was12 and EGFR/12 and EGFR (Supplementary Figure 1).

Next, we focused on the function of CXC chemokines using GO enrichment analysis and KEGG pathway enrichment analysis. Not surprisingly, we found that the functions of these genes are mainly related to the chemokine signaling pathway, cytokine-cytokine receptor interactions, and viral protein interaction with cytokine and cytokine receptor in both LUAD and LUSC samples. It has been identified that chemokine signaling pathways are important in the progression, metastasis angiogenesis, senescence, epithelial-mesenchymal transition, and immune evasion of various cancers [54–56]. The roles of cytokine-cytokine receptor interactions are also pivotal in several tumor-associated biological processes [21, 57]. Viral protein can interact with the cytokine and cytokine receptor to affect and even subvert the function of the cytokine network and to regulate the immune response, which is important in cancer [58–60]. Generally, these GO and KEGG results suggest that the CXC chemokines are potential drug therapeutic targets in LUAD and LUSC.

The PCA analysis on samples from LUAD and LUSC samples in TCGA based on their expression of CXC chemokines reflects the ability of all the 16 CXC chemokines in differentiating the LUAD tumor/normal and LUSC tumor/-normal patients; from the 3D figure, we found that CXC chemokines did enable to make these discriminations. We also characterized the transcription factor targets for the 16 CXC chemokines and found that RELA, NFKB1, and SP1 may be the key transcription factors of CXC chemokines. Zeng et al. made the same conclusion without study about this [15], but we included more CXC chemokines in our study. RELA plays a pivotal role in regulating oncogene-induced senescence in preneoplastic lesions [61], is essential to link smoke-induced inflammation with lung cancer growth, and participates in the activation of Wnt/beta-signaling in tumors [62]. NFKB1 is identified as an inhibitor of tumors and inflammatory response; by reducing the aberrant activation of the NF-κB signaling pathway, it negatively regulated the tumorigenesis and progression of several types of cancers [63]. NKKB1 also interfered with diverse complex immunological progresses, associated with many autoinflammatory disorders [64]. SP1 is overexpressed in many cancers and implicated in inflammation, genomic instability, and epigenetic silencing [65]; it has also been reported to be a target in cancer chemotherapy [66]. In NSCL, SP1 could promote cancer progression by interacting with lncRNA LINCo1234 and OTUB1 [67].

The chemokine system can orchestrate the immune cell migration and position them properly in a spatiotemporal manner [68]. In NSCLC, accumulating evidence shows that immune cell infiltration could affect tumor initiation, progression, and prognosis and could be important determinants of response to immunotherapies [69–71]. In our study, we found a significant correlation between the expression of CXC chemokines and the infiltration of the six immune cell types, B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells, indicating that CXC chemokines not only are prognostic indicators but may also reflect the immune status. Gao et al. reported that CXCL11 could promote $CD8^+$ T cell infiltration in NSCLC [72], which is consistent with our findings in this study in that we found that CXCL11 was significantly correlated with $CD8^+$ T cell infiltration in both LUAD and LUSC.

Moreover, we explored the correlation between aneuploidy and the infiltration of the six immune cell types. In cancer, chromosomal instability underpins much of intratumor heterogeneity and can drive phenotypic adaptation during tumor evolution [73]; meanwhile, tumor aneuploidy is correlated with markers in immune evasion and indicates reduced response to immunotherapy [74]. Copy number alterations have been reported as risk factors, predictors, and even drivers of tumor progress and poor clinical outcome in various cancer types [75–78]. In our study, we found a significant association between the SCNA of CXC chemokines and the infiltration of the six immune cell types and therefore offer more information for the exploration of relationship between SCNA and CXC chemokines in lung cancer.

Generally, differences existed in many aspects between LUAD and LUSC; they have a distinct expression pattern in

several CXC chemokines, but it is hard to find a clear law to distinguish them, which might be due to the fact that our study was purely based on bioinformatics analysis; therefore, it was not effective enough to do the distinction, which also might be due to the fact that both LUAD and LUSC belong to non-small cell lung cancer; therefore, the disparity between them is just not so obvious.

Our study is the first to portray the overall view of CXC chemokines in non-small lung cancer using several public databases and bioinformatics analysis, which is our biggest highlight and main limitation. Therefore, independent cohort and in vitro or in vivo research should be performed to validate our results to a large extent. In conclusion, we hope that our results offer a comprehensive landscape and new insight of CXC chemokines in LUAD and LUSC, thus provide more information for the development of new immunotherapy medicine, facilitate the clinical routine of selecting drugs, and help the clinicians predict the prognosis of patients more accurately.

Data Availability

The datasets analyzed for this study can be found in the ONCOMINE, GEPIA, UALCAN, Kaplan-Meier Plotter, and cBioPortal web resources, and requests to further access to datasets can be directed to tianhepumc@163.com.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

JH and CL designed the study and revised the manuscript. HT, LW, YL, FT, and QX performed data analysis work and aided in writing the manuscript. YW, YZ, TF, SG, and BZ designed the study and assisted in writing the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

Figure S1: correlation analysis between CXC8/12 and EGFR expression chemokines: LUAD (upper); LUSC (lower). *(Supplementary Materials)*

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