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Identification of Therapeutic Targets and Prognostic Biomarkers Among CC Chemokines in the Pancreatic Adenocarcinoma Microenvironment

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Abstract

Pancreatic adenocarcinoma (PAAD) as one of the most aggressive and lethal malignant tumors is correlated with increased morbidity and mortality. Tumorigenesis, growth, and metastasis are affected by various cytokines. Among them, CC chemokines can modulate the infiltration of immune cells and recruit cancer-associated immune cells, which play an important role in the inhibition of tumor immunity and affect the clinical outcome of cancer patients. However, the therapeutic potential and prognostic value of CC chemokines in PAAD have not yet been elucidated. To do this, we comprehensively explore and analyze large amounts of data on the basis of ONCOMINE database, GEPIA, LinkedOmics, cBioPortal, GeneMANIA, UALCAN, jvenn, DAVID 6.8, TRRUST, TIMER, and TISIDB. We found the transcriptional levels of CCL5/7/13/15/18/19/20 in PAAD tissues were remarkably increased, whereas the transcriptional level of CCL17 was decreased. CCL20 expression had significantly been correlated with the tumor stage of PAAD patients. High expressions of CCL5, CCL7, CCL13, CCL18, and CCL20 were notably correlated with the prognosis of patients. Moreover, patients with CCL18 and CCL19 alterations showed a poor prognosis in both overall survival (OS) and disease-specific survival (DSS), and patients with CCL5 and CCL15 alterations also presented a poor prognosis in OS. The functions of the aberrantly expressed CC chemokines were mainly correlated with the cytokine-cytokine receptor interaction, chemokine signaling pathway, inflammatory response, and immune response. Our study shows that the key transcription factors for CC chemokines are RELA and NF-κB1. We also discovered significant associations between the expression levels of CC chemokines and six infiltrating immune cells including $CD8^+$ T cells, $CD4^+$ T cells, B cells, macrophages, neutrophils, and dendritic cells. Taken together, our study indicated the interaction between CC chemokines and PAAD and clarified the value of CC chemokines as potential therapeutic targets as well as prognostic markers for PAAD.

Keywords: pancreatic adenocarcinoma, CC chemokines, prognosis, cytokines, tumor microenvironment

Introduction

Pancreatic adenocarcinoma (PAAD) with pancreatic ductal adenocarcinoma (PDAC) as the primary pathological type is one of the most aggressive and lethal malignant tumors [1]. PDAC has the dismal 5-year relative survival rate in contrast to all other solid tumor malignancies and is speculated to become a major cause of cancer-related death [2]. This horrible mortality rate is closely related to the

late diagnosis, early metastasis, and treatment resistance in PAAD patients [3]. Less than 20% of patients with PADC can have the tumors surgically removed [2], and surgery does not typically lead to a patient being cured [4,5]. To date, all available treatment methods, such as chemotherapy and radiotherapy, have almost no effect on the clinical course of PAAD. Therefore, a deep and comprehensive understanding of the molecular biology of PAAD is essential for the discovery of therapeutic value and prognostic markers [6].

Chemokines are relatively low-molecular-weight proteins (8-14 kDa), which are divided into four main subfamilies: C, CC, CXC, and CX3C according to their differences in molecular structure [7]. To date, approximately 50 chemokines have been identified, mainly secreted by immune and cancer cells, including stromal cells in the tumor microenvironment [8,9]. They are mainly involved in regulating the progression, differentiation, migration of immune cells, and growth of lymphoid tissues, thereby modulating the anti-tumor immune response in a spatiotemporal manner [10]. In addition, chemokines play a crucial role in wound healing, angiogenesis, and inflammatory responses [11]. The CC (β) subfamily is composed of 28 types of CC motif chemokine ligands with an N-terminal CC domain called CCL1-28 and plays a significant role in immune cells recruitment and tumor formation. The numbers in the symbols of chemokines represent the order of discovery, and all of the above-named chemokines correspond to 10 receptors called CCR1-10 [12,13]. In addition to anti-cancer properties, chemokines also show some cancer-promoting properties in tumor formation [13].

A few studies have already investigated the general expression landscape and function of certain CC motif chemokine ligands in PAAD but whether CC chemokines serve a vital role in the tumor formation, treatment, and prognosis of PAAD is still an urgent issue requiring exploration. In this present study, comprehensive and systematic bioinformatics exploration was conducted to illustrate the expression and alteration of CC chemokines in PAAD and evaluate their potential functions as well as prognostic value based on several large public databases. This study provides a reference for clinicians to choose appropriate therapeutic drugs and prognostic markers for PAAD patients.

Results

CC Chemokines Expression Level in PAAD

We retrieved twenty-four CC chemokines (not including CCL6, CCL9, CCL10, and CCL12) from the ONCOMINE microarray database. First, we discussed the mRNA levels of CC chemokines in PAAD and normal pancreatic tissues. According to data presented in **Figure 1** and **Table 1**, the mRNA levels of CCL5, CCL18, CCL19, and CCL20 in PAAD were remarkably elevated. These data were corresponding with the study of Segara et al. who found a significant upregulation of CCL5 in PAAD (fold change=2.214, *p*=9.86e-4) [14]. Moreover, two datasets revealed that the transcriptional level of CCL18 was up-regulated in PAAD tissues [15,16]. The datasets of Iacobuzio-Donahue, Pei, and Logsdon et al. revealed that the levels of CCL19 and CCL20 were notably higher in PAAD compared with normal tissue samples [16-18].

We then utilized UALCAN to compare the expressions of CC chemokines in PAAD vs normal tissues (CCL6, CCL9, CCL10, and CCL12 were not recognized). The results revealed that the transcriptional levels of CCL7 (*p*=9.69e-05), CCL13 (*p*=3.57e-08), CCL15 (*p*=1.62e-12), CCL18 (*p*=6.94e-08), and CCL20 (*p*=6.81e-04) significantly increased, whereas the transcriptional level of CCL17 (*p*=3.80e-04) decreased (**Figure 2**). According to the relative expression levels of CC chemokines in PAAD through GEPIA, we detected that the relative level of CCL2 was the highest among all the CC chemokines (**Figure 3**). In order to identify the CC chemokines that are actually correlated with the tumor formation, progression, and prognosis of PAAD, we assessed all the aberrantly expressed CC cytokines (CCL5, CCL7, CCL13, CCL15, CCL17, CCL18, CCL19, and CCL20) in PAAD and normal tissues. As a result, CCL2 was excluded from further analysis because

of its similar expression levels in PAAD as well as normal tissues. We then evaluated the relationship between the CC chemokines mentioned above and the tumor stage of PAAD and found that only the CCL20 expression has a significant association with the tumor stage (p=0.004, **Figure 4**). This indicated that the high expression of CCL20 is closely related to tumor progression and might play an important role in the invasiveness and development of PAAD.

Prognostic Features of CC Chemokines in PAAD

We first utilized GEPIA datasets to explore the relationship between aberrantly expressed CC chemokines and clinical prognosis of PAAD. Unfortunately, the results suggested that there was no statistical significance between the aberrantly expressed CC chemokines (CCL5, CCL7, CCL13, CCL15, CCL17, CCL18, CCL19, and CCL20) and the prognosis of patients with PAAD both in overall survival (OS) and disease-free survival (DFS) (**see Supplementary Fig. S1**). However, we analyzed the OS data from LinkedOmics databases and found that high expressions of CCL7, CCL18 and CCL20 were notably correlated with the prognosis of patients (**Figure 5**). Given that the results of the two databases are divergent, Kaplan-Meier Plotter was further leveraged to explore the survival data in PADC. As **Figure 6** depicted, high expressions of CCL5, CCL7, CCL13, CCL18, and CCL20 are relevant to poor OS and upregulations of CCL13, CCL18, and CCL20 also have correlations with poor relapse-free survival (RFS).
In addition, we investigated the potential relationship between genetic alterations of the aberrantly expressed CC chemokines and the clinical outcome of PAAD patients. Patients with CCL5, CCL15, CCL18, and CCL19 mutations had a worse prognosis in aspect of OS and patients with CCL15 and CCL18 mutations had a poor prognosis in disease-specific survival (DSS) compared with the unaltered patients (**see Supplementary Fig. S2**).

Gene Alteration and Interaction of CC Chemokines in PAAD

In view of the correlation between some CC chemokine mutations and clinical prognosis, we explored their mutation locations and mutation levels in detail through the TCGA datasets. The results showed that CCL5, CCL7, CCL13, CCL15, CCL17, CCL18, CCL19, and CCL20 were altered in 6, 4, 8, 9, 8, 8, 9, and 9% of PAAD samples, respectively (**Figure 7a**).
PPI network analysis was performed with STRING to analyze the potential interactions and connections among the aberrantly expressed CC chemokines and we obtained 8 nodes and 16 edges (**Figure 8a**). We also screened 50 experimentally verified proteins that bind to aberrantly expressed CC chemokines (CCL5, CCL7, CCL13, CCL15, CCL17, CCL18, CCL19, and CCL20) and identified the functions of these CC chemokines in chemokine signaling pathways and inflammatory responses (**Figure 7b**). Similarly, **Figure 7c** also reveals that the functions of aberrantly expressed CC chemokines are mainly correlated with cell chemotaxis, chemokine receptor binding, chemokine activity, and cytokine activity.

Functional Enrichment Analysis of CC Chemokines in PAAD

We used GEPIA to obtain the top 50 genes similar to every aberrantly expressed CC chemokine (CCL5, CCL7, CCL13, CCL15, CCL17, CCL18, CCL19, and CCL20) in the TCGA database. Theoretically, the number of genes should be 400. After excluding duplicated genes, 366 genes were identified. Among them, the CCL17 and CCL20 groups had a similar gene, Metazoa-SRP (**Figure 8b**). CCL7, CCL13, and CCL18 groups have three common and similar genes, namely ALOX5AP, TREM1, and CD300E (**Figure 8d**). There is also a similar gene in common called FCGR3A between the CCL7 and CCL13 groups, whereas the CCL7 and CCL18 groups have 24 similar genes (**Figure 8d**). The CCL5 and CCL19 groups have two similar genes in common, namely ARHGAP30 and

SLAMF1 (**Figure 8e**). Simultaneously, we created Venn diagrams between similar genes and 50 experimentally verified genes of interacting proteins. We found the same gene between the CCL17 group and the genes of the interacting proteins as CCL22 (**Figure 8c**). The CCL5 group and genes of interacting proteins have two common similar genes, namely, CCR5 and XCL2 (**Figure 8e**). The common gene of the CCL19 group and interacting proteins was CCR7 (**Figure 8e**). In addition, we combined 366 similar genes with 50 genes of interacting proteins and utilized DAVID 6.8 for GO and KEGG enrichment analysis.

In GO enrichment analysis, extracellular area, integral composition of the plasma membrane, extracellular space, external side of plasma membrane, plasma membrane were the top five enriched items in the CC (**Figure 9a)**. Furthermore, BP showed that the chemokine-mediated signaling pathway, immune response, chemotaxis, inflammatory response, regulation of immune response, neutrophil chemotaxis, cell-cell signaling, adaptive immune response, cell chemotaxis, and innate immune response were correlated with the invasiveness and development of PAAD (**Figure 9a**). As for MF, genes similar to aberrantly expressed CC chemokines and interacting genes were primarily enriched in chemokine activity, CC chemokine receptor activity, and chemokine receptor activity (**Figure 9a**).

In KEGG enrichment analysis, the top 10 pathways that were remarkably related to the development of PAAD were cytokine-cytokine receptor interaction, chemokine signaling pathway, osteoclast differentiation, rheumatoid arthritis, phagosome, hematopoietic cell lineage, malaria, NK cell-mediated cytotoxicity, NF-κB signaling pathway, and *Staphylococcus aureus* infection (**Figure 9b**).

Transcription Factor Targets of Patients with PAAD

Since there was a significant difference in the expression of CC chemokines in PAAD and normal tissues, we used TRRUST to explore possible transcription factor targets of aberrantly expressed CC chemokines. There were two invalid query genes in TRRUST, CCL18, and CCL19. As expected, two transcription factors (RELA and NF-κB1) were related to the modulation of CC chemokines (**Table 2**). Therefore, RELA and NF-κB1 are the key transcription factor targets for CCL5, CCL13, CCL19, and CCL20 and potentially exert various functions in PAAD.

Correlation between Immune Cells and CC Chemokines in PAAD

We utilized the TIMER online tool to systematically explore the association between the aberrantly expressed CC chemokines and infiltrating immune cells, because CC chemokines facilitate inflammation initiation and play a vital role in the immune microenvironment, crucially affecting the clinical prognosis of PAAD patients. **Figure 10** shows the relationship between eight aberrantly expressed chemokines and six kinds of immune cells and indicates that all the aberrantly expressed CC chemokines was positively correlated with one or more immune cells. **Figure 10a** shows a positive association between CCL5 expression and six kinds of infiltrating immune cells including B cells (p=2.67e-10), $CD8^+$ T cells (p=3.31e-10), $CD4^+$ T cells (p=3.31e-11), macrophages (p=5.14e-09), neutrophils (p=6.58e-14), and dendritic cell (p=8.66e-21). CCL7 expression was associated with macrophage (p=1.22e-03), neutrophils (p=4.04e-07), and dendritic cells infiltration (p=3.76e-09, **Figure 10b**). Similarly, CCL13 expression was positively relevant to five infiltrating immune cells, namely B cells (p=2.32e-02), $CD8^+$ T cells (p=1.39e-04), macrophages (p=1.61e-06), neutrophils (p=1.14e-11), and dendritic cells (p=8.54e-18, **Figure 10c**). **Figure 10d** reveals that CCL15 expression was merely associated with B cell infiltration (p=1.33e-03). CCL17 expression also had correlation with six kinds of infiltrating immune cells, B cells (p=1.55e-04), $CD4^+$ T cells (p=1.22e-09), $CD8^+$ T cells (p=4.07e-04), macrophages (p=9.04e-05), neutrophils (p=4.89e-10), and dendritic cells (p=2.32e-09, **Figure 10e**). CCL18 expression and the infiltration of $CD4^+$ T cells (p=4.97e-05),

macrophages (p=5.18e-07), neutrophils (p=2.59e-12), and dendritic cells (p=2.51e-15, **Figure 10f**) were positively correlated. Analogously, the expression of CCL19 has a positive correlation with six kinds of immune cells including B cells (p=1.57e-08), $CD8^+$ T cells (p=3.77e-04), $CD4^+$ T cells (p=2.35e-18), macrophages (p=3.50e-05), neutrophils (p=2.94e-07), and dendritic cells (p=1.27e-07) were shown in **Figure 10g**. **Figure 10h** suggests that CCL20 expression and neutrophil infiltration (p=1.33e-02) were positively correlated.

TISIDB was further leveraged to verify the relationship between CC chemokines and immune cells using spearman correlation test and we found that CCL5, CCL13, CCL17, CCL18, and CCL19 expressions were positively relevant to the 6 types of immune cells (**see Supplementary Fig. S3**). CCL7 expression was associated with $CD4^+$ T cells, dendritic cells, macrophages and neutrophils. CCL15 expression was merely related to $CD8^+$ T cells and CCL20 was correlated with $CD4^+$ T cells, dendritic cells, and neutrophils (**see Supplementary Fig. S3**).

We also explored the relationship between aberrantly expressed CC chemokines and infiltrating immune cells on the basis of the Cox proportional hazard model. As shown in **Table 3,** B cells (p =0.008), $CD4^+$ T cells (p=0.020), dendritic cells (p=0.005), and CCL5 (p=0.027) were significantly related to the clinical outcomes of PAAD patients.

Discussion

Chemokines are correlated with various human diseases, including chronic inflammation, immune dysfunction, tumorigenesis, and metastasis. CC chemokines play a crucial role in tumor formation, angiogenesis, metastasis as well as tumor immunity, especially in the tumor microenvironment [19]. CC chemokines not only exert direct effects on tumor cells but also indirectly participate in tumor immunity by regulating various pathways. To further validate chemokines and their receptors as therapeutic targets or prognostic markers, it is essential to fully understand the biological mechanisms and functions of CC chemokines [20].

First, we explored the expression level of CC chemokines and their relationship with the tumor stage in patients with PAAD. We discovered that eight genes were aberrantly expressed in PAAD tissues in contrast to normal tissues (upregulation of CCL5/7/13/15/18/19/20 and downregulation of CCL17). In addition, the expression of CCL20 increased as the tumors progressed. Just as the study of Liu et al. demonstrated that the expression level of CCL20 was up-regulated in PAAD tissues and could induce the invasiveness of pancreatic cancer cells [21]. We further discovered that high expressions of CCL7, CCL18, and CCL20 were notably related to the prognosis of PAAD patients. Besides, Meng et al. found that CCL18 expression in tumor cells was associated with lymph node metastasis, pathological stage, and overall survival in a study of sixty-two PDAC patients, indicating that serum CCL18 levels may become potential biomarkers to diagnose and predict survival rate of PDAC [22]. PAAD patients with CCL15 and CCL18 alterations had a poor prognosis in both OS and DSS, whereas patients with CCL5 and CCL19 alterations only showed a poor prognosis in OS. The above results indicate that aberrantly expressed CC chemokines serve a crucial role in PAAD.

We also explored gene alterations and interacting proteins of the aberrantly expressed CC chemokines in PAAD. Varieties of cytokines are involved and play a synergistic role in the tumor formation and progression of PAAD, which lay the foundation for further functional enrichment analysis.

We then explored genes that were similar to the aberrantly expressed CC chemokines. Subsequently, we investigated the functions of aberrantly expressed CC chemokines using GO and KEGG pathway enrichment analysis. Not surprisingly, the functions of these genes were mainly associated with the cytokine-cytokine receptor interaction, chemokine signaling pathway, inflammatory response, and immune response. Some recent explorations clarified the role of chemokines in regulating inflammation, modulating tumor pathogenesis, facilitating tumor progression, and subsequent

metastasis [23-26]. The above results indicated that the aberrantly expressed CC chemokines might be involved in the occurrence and progression of PAAD and become a potential treatment target.
We then tried to confirm the transcription factor targets of the aberrantly expressed CC chemokines and discovered that RELA and NF-κB1 were key transcription factors. RELA phosphorylation participates in disease progression by regulating NF-κB, especially in inflammatory diseases and cancers [27]. NF-κB1 exerts an inhibitory effect on the initiation and progression of various tumors as an inhibitor of inflammation and cancer [28,29]. Geismann et al. proved that the activity of the RelA-containing NF-κB signaling pathway was in charge of TRAIL-mediated CCL20 expression in PDAC cell lines, emphasizing that the TRAIL-RelA-CCL20 pathway in PDAC cells recruited immune cells through a paracrine mechanism [30]. Another study manifested that CCL18 up-regulated the expression of VCAM-1 in PDAC cells by activating NF-κB signal transduction, which proves that CCL18-positive TAMs play a crucial role in the malignant progression of PDAC by activating NF-κB signaling [31]. Our conclusions provide additional data for the complex correlation among the PAAD, CC chemokines, and NF-κB signaling pathways.
Chemokines regulate the localization and migration of immune cells [9]. Tumor immune response is a complex process indispensable of immune cells, cytokines, and surrounding conditions in the tumor microenvironment [32]. A study has confirmed that M2 macrophages in PAAD are associated with early metastasis, tumor recurrence, and ultimately reduced OS [33]. Another study showed that PDAC patients with fewer regulatory T cells in tumors and higher $CD8^+$ T cells around the tumors were correlated with long-term survival [34]. Certain DC subgroups can not only promote the proliferation of Tregs but also inhibit $CD8^+$ T cells, which is conducive to the establishment of an immunosuppressive microenvironment for tumor metastasis [35,36]. In addition, mutations in the KRAS gene promote aggregation of B cells, and certain B cells producing IL-35 can stimulate the progression of PAAD [37]. Increasing integrative evidence suggests that tumor-infiltrating immune cells may participate in tumor progression as well as recurrence and the immune microenvironment as an essential determinant significantly affects immunotherapy effect and clinical prognosis [38,39]. In our present study, the aberrantly expressed CC chemokines were distinctly related to six types of infiltrating immune cells (B cells, $CD8^+$T cells, $CD4^+$T cells, macrophages, neutrophils, and dendritic cells), manifesting that CC chemokines could reflect the immune status of PAAD patients to some extent.
In conclusion, there were some limitations to our analysis, including the small sample size in the database when we analyzed the association between the aberrantly expressed CC chemokines and the clinical prognosis of PAAD patients, the number and proportion of genes recognized by DAVID 6.8, and the limited number of genes involved in each pathway. However, our results still explored new insights into the interaction between CC chemokines and PAAD and various therapeutic targets and tumor markers for patients with PAAD, which predict the clinical outcome of PAAD patients more accurately.

Methods

ONCOMINE

ONCOMINE is a publicly accessible cancer database, which serves as a relatively comprehensive data-mining platform facilitating the discovery of meaningful clues and information from genome-wide expression analyses [40]. Data were queried and visualized to annotate the expression of CC chemokines in PAAD. The expression level of CC chemokines between tumor tissues and their respective normal tissues in PAAD was calculated by Student's t-test.

Gene Expression Profiling and Interactive Analysis (GEPIA)

GEPIA is a web-based platform delivering fast and customizable analyzing functions [41]. We used the "Single Gene Analysis" module of the gene analysis software to analyze the aberrant expression level of tumors and normal tissues and performed a pathological staging analysis as well as a related prognostic assessment of CC chemokines. We facilitated the "Multiple Gene Comparison" module of the universal gene analysis software and the "PAAD" dataset to perform the multi-gene comparative analysis of CC chemokines. We utilized the student's t-test was to judge the expression level and the correlation with the pathological stage. The Kaplan-Meier curve was used for the prognostic analysis. We also detected similar genes in CC chemokines using GEPIA.

UALCAN

UALCAN is an easily accessible and comprehensive network tool that furnishes a convenient way to accurately obtain cancer-related data based on the The Cancer Genome Atlas (TCGA) and MET500 cohort data [42]. The database was used to find the transcriptional level of CC chemokines in normal vs primary tumor tissues using the "Expression Analysis" module and "PAAD" dataset.

LinkedOmics

LinkedOmics is a unique and convenient platform containing cancer multi-omics data from TCGA project, which provides the opportunity for clinicians to acquire and analyze data [43]. The "LinkFinder" module was utilized to annotate mRNA sequencing data and clinical data of PAAD patients to determine the association between the differentially expressed CC chemokines and prognosis of patients.

cBioPortal

cBioPortal is a prevalent public database and multidimensional research platform that allows private instances and extensions [44]. The genetic mutations of CC chemokines in 183 pancreatic adenocarcinoma samples (TCGA, provisional) were obtained from cBioPortal. We also analyzed overall survival curves and disease-specific survival curves between the altered and unaltered CC chemokine groups, using samples with mutation and CAN data (175 patients/samples).

STRING

STRING was developed to integrate a protein-protein interaction (PPI) network of available co-expressed genes and predict potential functions based on computational analysis [45]. A PPI network with CC chemokines was constructed to analyze and explore their physical as well as functional interactions and connections.

GeneMANIA

GeneMANIA is a user-friendly and multifunctional prediction server that provides information on gene functions and prioritizes genes for functional assays [46]. We also used GeneMANIA to create a PPI network with aberrantly expressed CC chemokines to illustrate their interactions and indicate their predictive value.

jvenn

jvenn is an interactive Venn diagram viewer that can perform cross-analysis and compare the correlation and interaction among different genes [47]. We created Venn diagrams to find the common

genes among the top 50 genes similar to every aberrantly expressed CC chemokine and 50 experimentally verified genes of interacting proteins.

David 6.8

DAVID 6.8, a high-throughput and integrated data-mining platform, primarily offered typical batch analysis and functional annotation [48]. In this study, the Gene Ontology (GO) enrichment analysis, including biological processes (BP), cellular components (CC), and molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of similar genes of CC chemokines and genes of interacting proteins were extracted from DAVID 6.8.

TRRUST

TRRUST is an expanded reference online tool for human transcriptional regulatory interactions, which can show how various interaction relationships regulate information and how transcriptional regulation is involved in human diseases [49]. We used the "find key regulators for query genes" module to explore probable transcriptional factor targets of aberrantly expressed CC chemokines.

Tumor Immune Estimation Resource (TIMER)

TIMER is a reliable and comprehensive web server that utilizes expression profile data of RNA-Seq to systematically excavate tumor-infiltrating immune subsets and tumor-immune connections. TIMER elucidated infiltration information regarding six kinds of immune cells (B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells) [50]. "Gene module" and "Survival module" were separately applied to detect the association between the expression of CC chemokines and immune cells as well as the correlation between clinical prognosis and immune cells in PAAD.

TISIDB

TISIDB is a user-friendly web portal designed to comprehensively investigate interactions between various tumors and immune system [51]. The "lymphocyte" module was applied to verify the association between CC chemokines and immune cells based on spearman correlation test.

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Conflict 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.

Author Contributions

XL designed the study and writing the manuscript. QZ, TM, CM, JG, and Zhang CP contributed to performed data analysis work. ZT and XL revised the article. All authors read and approved the final manuscript.

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Data Availability Statement

The datasets analyzed for this study can be found in the Oncomine, GEPIA, UALCAN and cBioPortal web resources, and requests to further access to datasets can be directed to 332379225@qq.com.

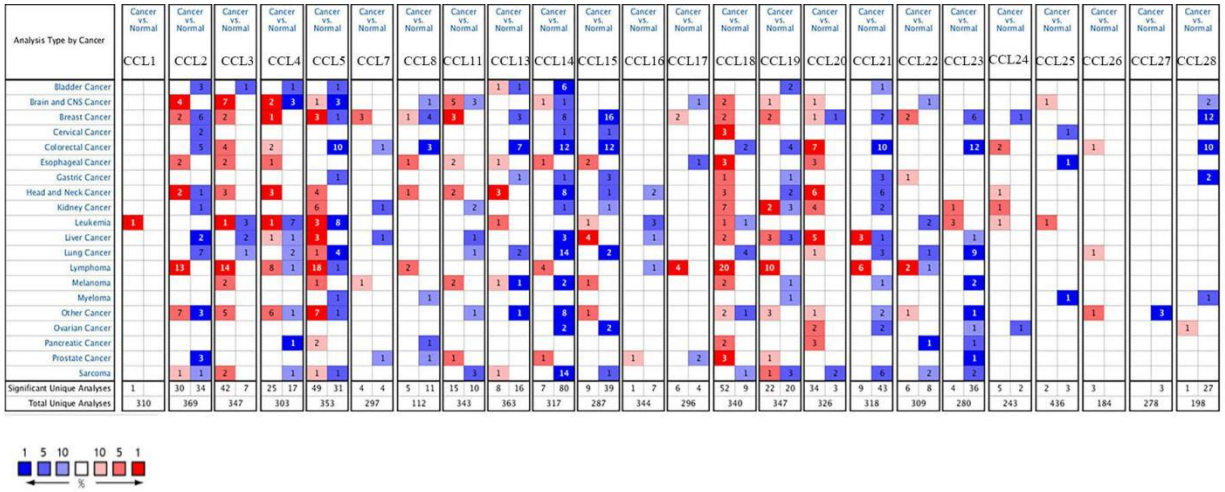

FIGURE 1 | mRNA levels of CC chemokines in PAAD (ONCOMINE). The figure shows the numbers of datasets with statistically significant mRNA over-expression (red) or downregulated expression (blue) of CC chemokines.

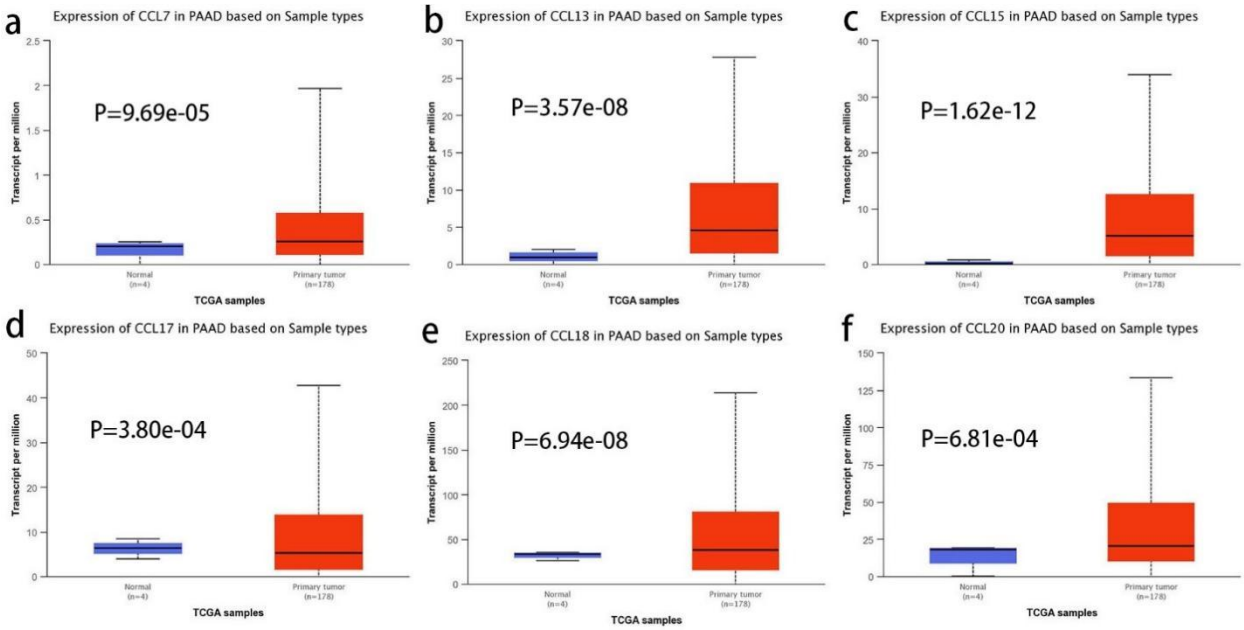

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FIGURE 2 | The transcription of CC chemokines in PAAD (UALCAN). The transcriptional levels of (a)
479 CCL7, (b) CCL13, (c) CCL15, (e) CCL18 and (f) CCL20 in PAAD tissues were significantly elevated while the transcriptional levels of (d) CCL17 were significantly reduced. The p value was set at 0.05.

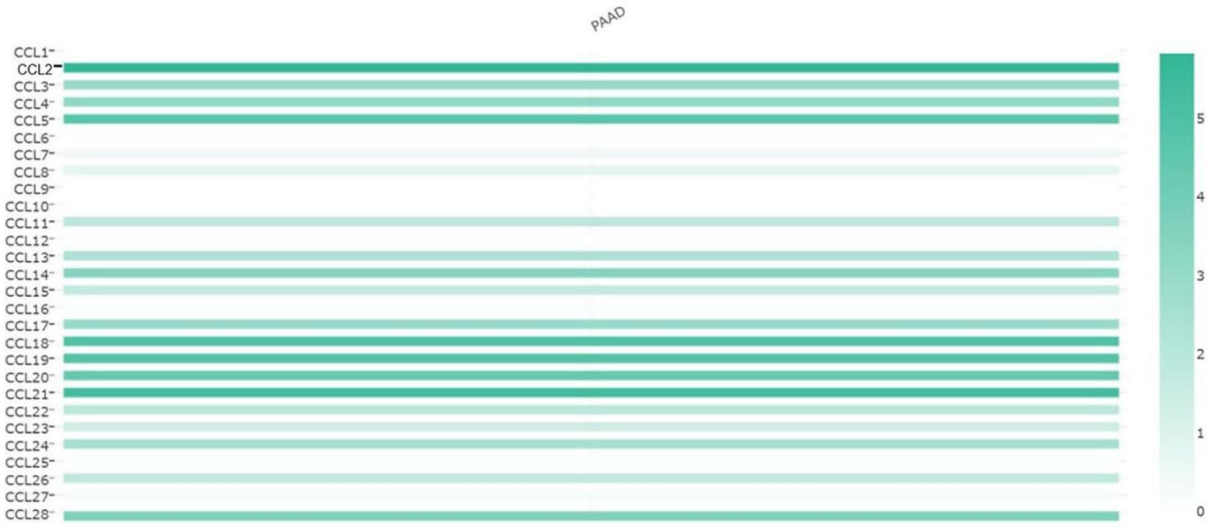

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FIGURE 3 | The relative level of CC chemokines in PAAD (GEPIA).

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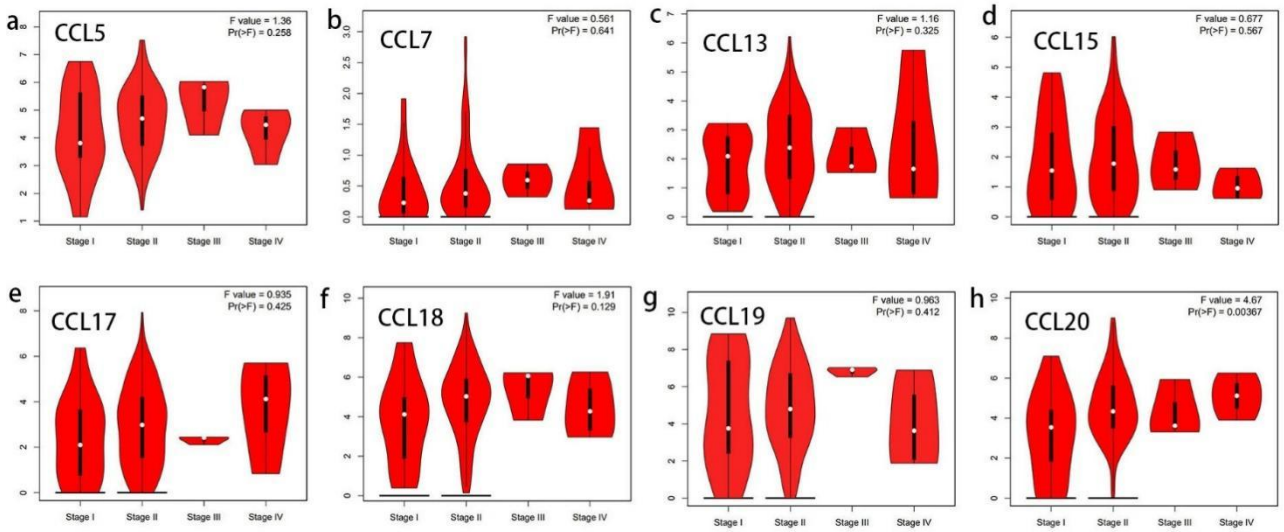

FIGURE 4 | Correlation between different expressed CC chemokines and the pathological stage of PAAD patients (GEPIA). (a) CCL5, (b) CCL7, (c) CCL13, (d) CCL15, (e) CCL17, (f) CCL18, (g) CCL19 and (h) CCL20. The p value was set at 0.05.

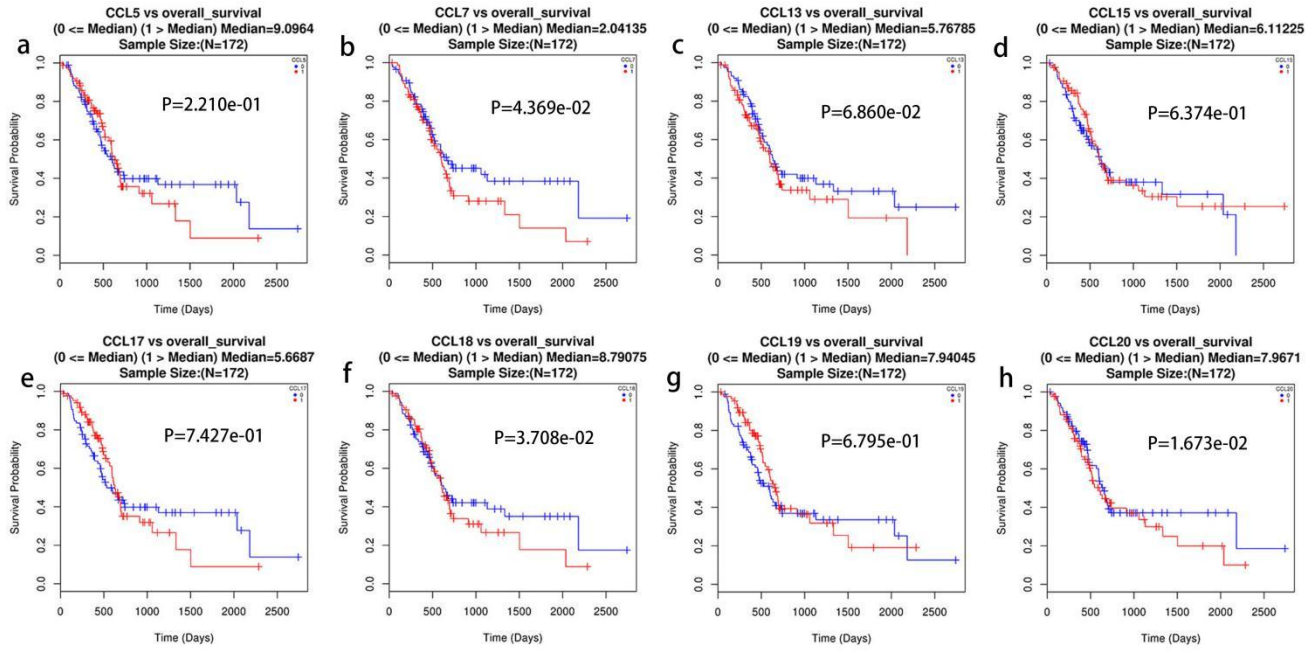

FIGURE 5 | The prognostic value of CC chemokines in PAAD patients in the overall survival curves (LinkedOmics). The overall survival curves of (a) CCL5, (b) CCL7, (c) CCL13, (d) CCL15, (e) CCL17, (f) CCL18, (g) CCL19 and (h) CCL20 in PAAD.

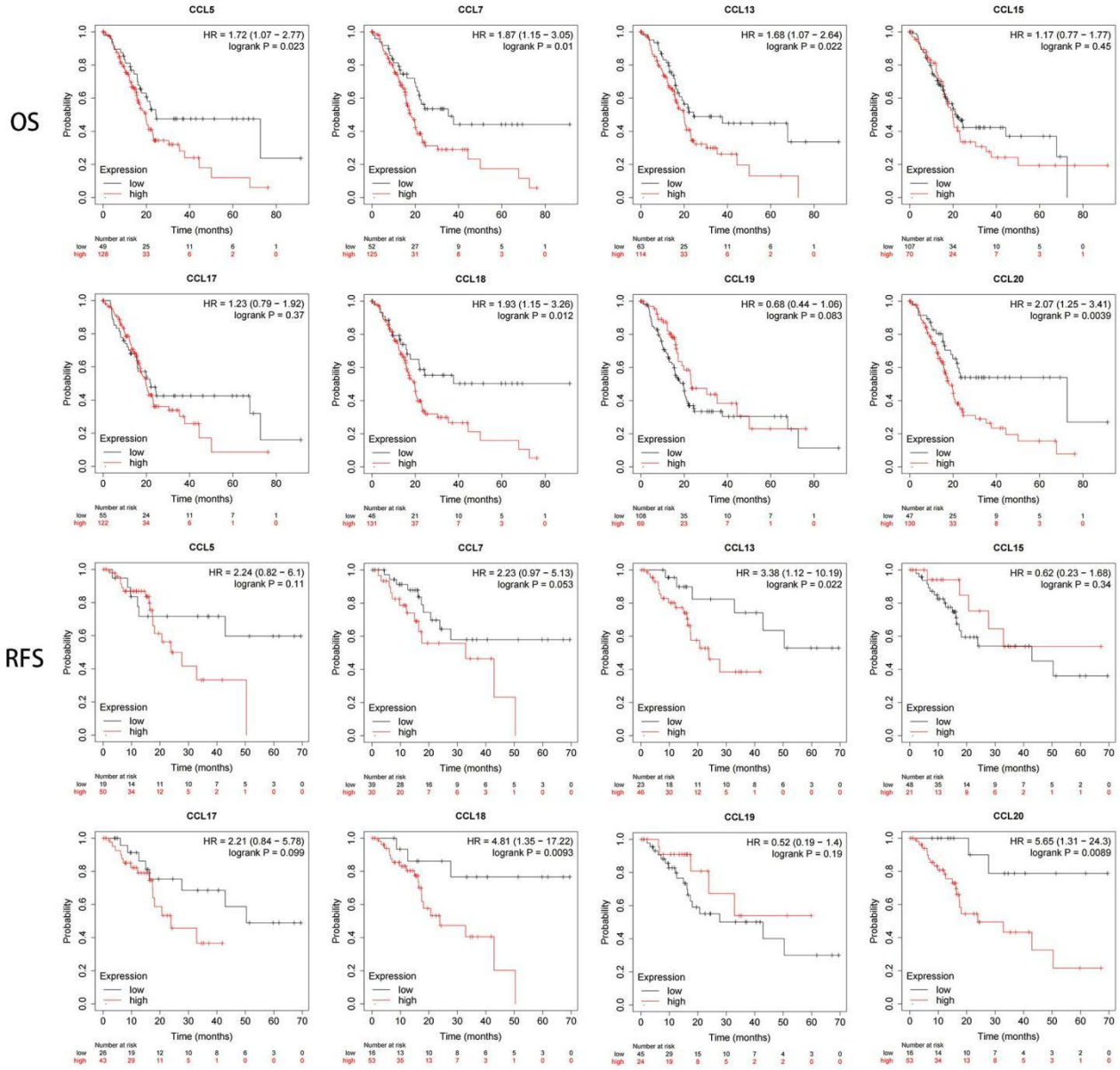

FIGURE 6 | The prognostic value of CC chemokines in PDAC patients in overall survival and relapse-free survival curves (Kaplan-Meier plotter).

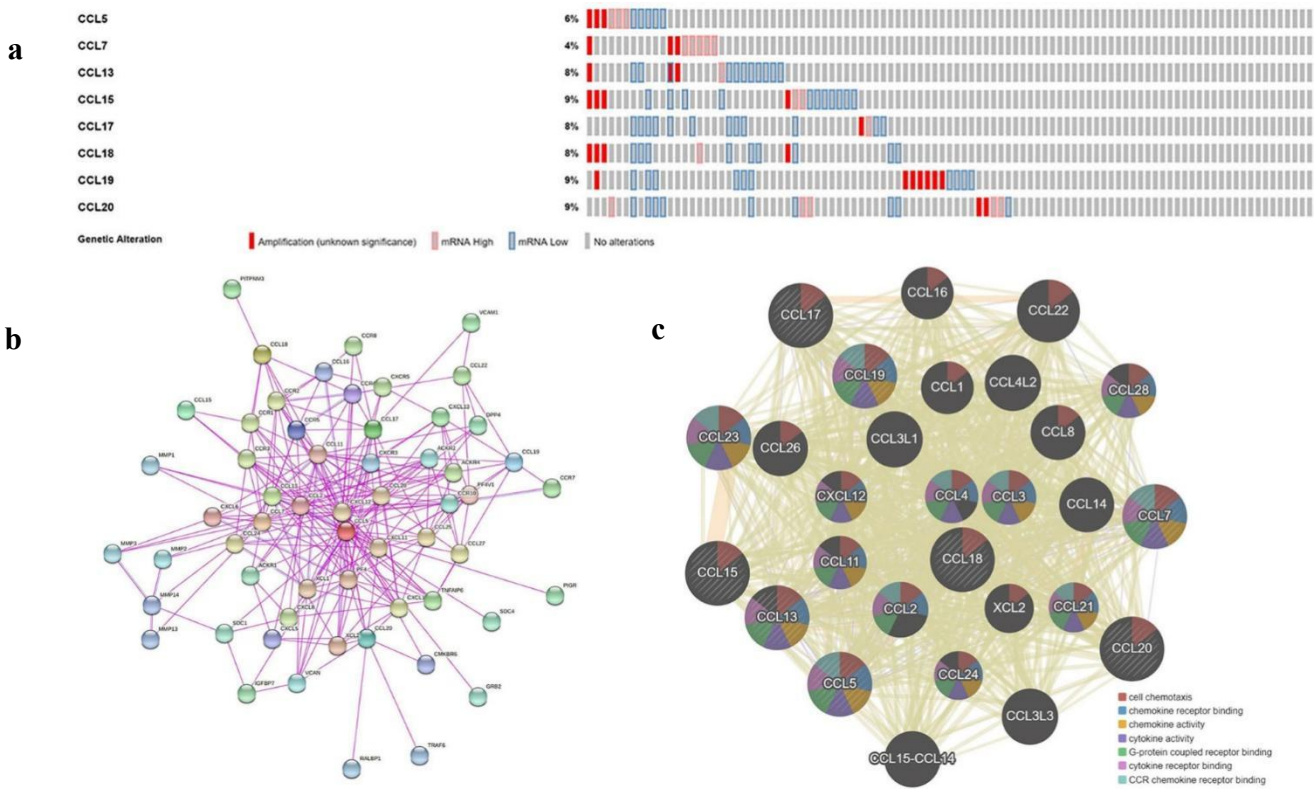

FIGURE 7 | (a) Summary of alterations in different expressed CC chemokines in PAAD (cBioPortal). (b) The correlation between different expressed CC chemokines and 50 interacted proteins in PAAD (STRING). (c) Protein–protein interaction network of different expressed CC chemokines (GeneMANIA).

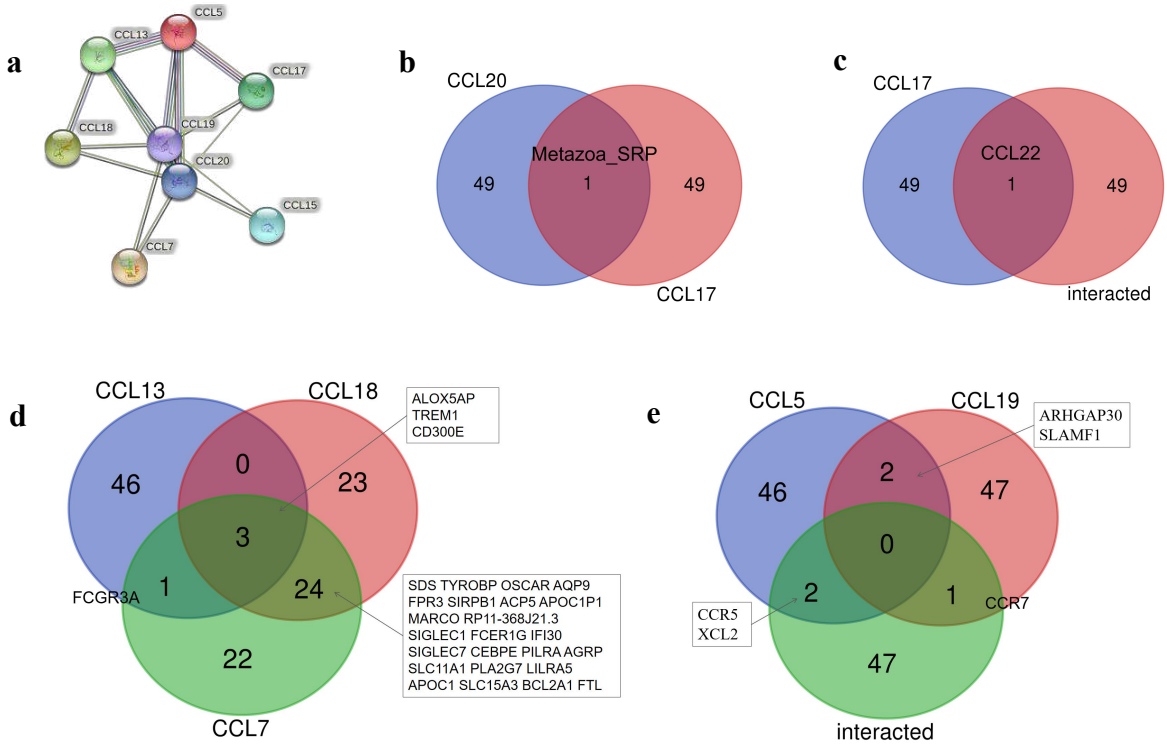

FIGURE 8 | (a) Protein–protein interaction network of different expressed CC chemokines (STRING). (b) Venn diagrams of genes associated with CCL17 and CCL20. (c) Venn diagrams of genes associated with CCL7 and differentially expressed CC-interacted proteins. (d) Venn diagrams of genes associated with CCL7, CCL13 and CCL18. (e) Venn diagrams of genes associated with CCL5, CCL19 and differentially expressed CC-interacted proteins (Venn).

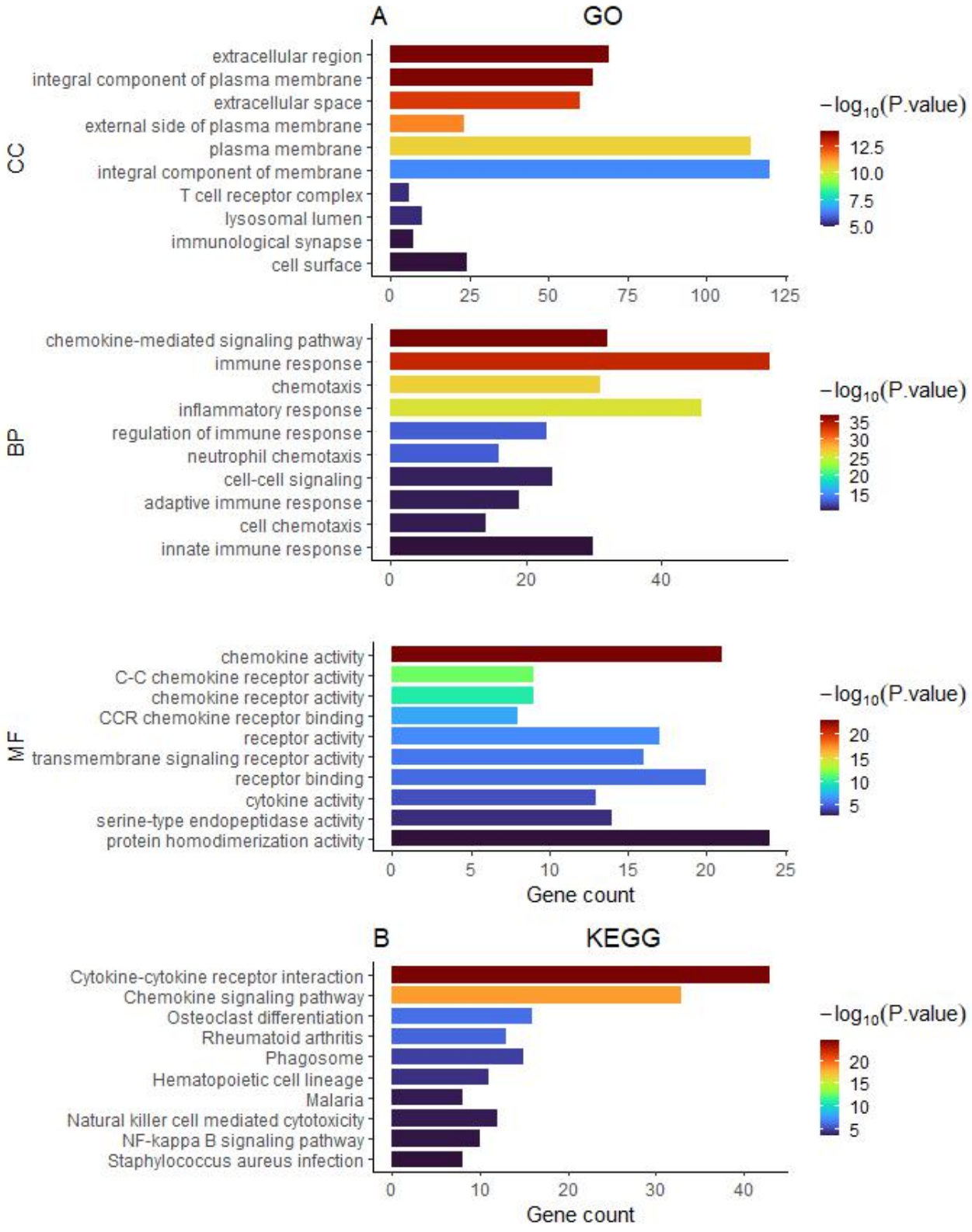

FIGURE 9 | The enrichment analysis of genes related different expressed CC chemokines and 50 interacted protein genes in PAAD (David 6.8). (a) Bar plot of GO enrichment in cellular component terms, biological process terms and molecular function terms. (b) Bar plot of KEGG enriched terms.

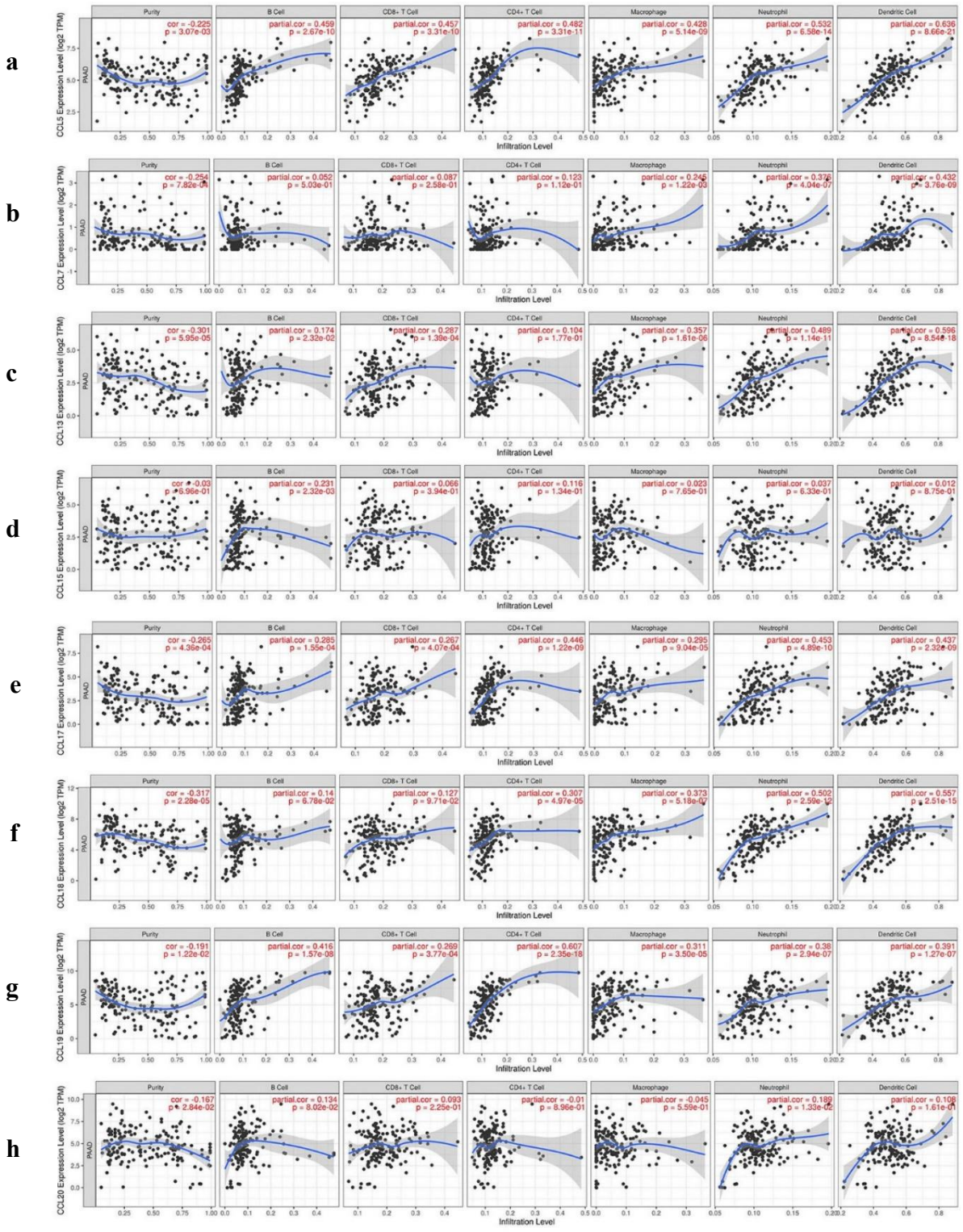

FIGURE 10 | The correlation between different expressed CC chemokines and immune cell infiltration (TIMER). The correlation between the abundance of immune cell and the expression of (a) CCL5, (b) CCL7, (c) CCL13, (d) CCL15, (e) CCL17, (f) CCL18, (g) CCL19 and (h) CCL20 in PAAD.

TABLE 1 | The mRNA levels of CC chemokines in different types of PAAD tissues and normal pancreatic tissues at transcriptome level (ONCOMINE).

CC	Type	Fold change	P-value	t-test	References
CCL5	Pancreatic adenocarcinoma	2.214	9.86E-4	3.885	（14）
CCL18	Pancreatic ductal adenocarcinoma	8.610	0.005	3.015	（15）
	Pancreatic adenocarcinoma	4.180	5.89E-4	4.236	（16）
CCL19	Pancreatic adenocarcinoma	10.370	4.05E-8	6.688	（17）
	Pancreatic adenocarcinoma	3.339	3.64E-4	5.287	（18）
	Pancreatic adenocarcinoma	8.629	0.004	3.804	（16）
CCL20	Pancreatic adenocarcinoma	10.370	4.05E-8	6.688	（17）
	Pancreatic adenocarcinoma	3.339	3.64E-4	5.287	（18）
	Pancreatic adenocarcinoma	8.629	0.004	3.804	（16）

TABLE 2 | Key regulated factor of CC chemokines in PAAD (TRRUST).

Key TF	Description	Regulated gene	P value	FDR
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	CCL5, CCL13, CCL19, CCL20	4.22e-06	4.33e-06
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	CCL5, CCL13, CCL19, CCL20	4.33e-06	4.33e-06

554 **TABLE 3** |The cox proportional hazard model of CC chemokines and six tumor-infiltrating immune cells in PAAD
555 (TIMER).

	coef	HR	95%CI_l	95%CI_u	p.value	sig
B_cell	8.428	4574.794	9.102	2.299340e+06	0.008	**
CD8_Tcell	3.280	26.588	0.018	3.823432e+04	0.377	
CD4_Tcell	-10.204	0.000	0.000	0.203	0.020	*
Macrophage	-4.342	0.013	0.000	9.643	0.198	
Neutrophil	14.900	2958167.905	0.412	2.123689e+13	0.064	·
Dendritic	-6.623	0.001	0.000	0.132	0.005	**
CCL5	0.367	1.444	1.042	2.001	0.027	*
CCL7	0.324	1.383	0.912	2.097	0.127	
CCL13	0.033	1.034	0.849	1.259	0.740	
CCL15	-0.010	0.990	0.864	1.136	0.891	
CCL17	-0.024	0.976	0.783	1.217	0.830	
CCL18	0.126	1.134	0.956	1.346	0.149	
CCL19	-0.125	0.883	0.756	1.030	0.114	
CCL20	0.029	1.030	0.901	1.178	0.667	

556 ·P < 0.1, *P < 0.05, **P<0.01

Supplementary Files

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