Integrative Analysis of Gene Expression Based on Multi-Omics Databases Identified CXC Chemokines as Immune Prognostic Biomarkers of Rectal Adenocarcinoma

Xiaoshuai Wang
The Seventh Affiliated Hospital Sun Yat-sen University
Zhiyang Hu
Sun Yat-sen University Zhongshan School of Medicine
Zefeng Du
Sun Yat-sen University Zhongshan School of Medicine
Dongchun Hong
Sun Yat-sen University Cancer Center
Haoyang Huang
Sun Yat-sen University Zhongshan School of Medicine
Yueyin Han
China-Japan Friendship Hospital
Yingdong Hou
Sun Yat-sen University Zhongshan School of Medicine
Haoqian Feng
Sun Yat-sen University Zhongshan School of Medicine
Tianyu Chen
The Third Affiliated Hospital of Southern Medical University
Zhicheng Xue (xuezc@sysucc.org.cn)
Sun Yat-sen University Zhongshan School of Medicine

Research

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Abstract

Background: Rectal adenocarcinoma (READ) is one of the most frequent malignancies with a high recurrence rate. CXC chemokines, as indispensable components of the immune system, are considered broadly involving in tumorigenesis by orchestrating the immune cell chemotaxis and thus affect the prognosis of READ patients. However, the values of CXC chemokines as prognostic biomarkers and potential regulatory mechanisms for READ remain unclear.

Results: The expression levels of *CXCL3, CXCL12,* and *CXCL13* were aberrant in both TCGA and ONCOMINE databases. Lower expression of *CXCL3* and *CXCL13* predicted poor survival of READ patients. Additionally, both *CXCL3* and *CXCL13* were associated with several clinicopathological features. *CXCL3* and *CXCL13* expressions were significantly correlated with the tumor infiltration levels of immune cells in READ tissue. CeRNA networks of mRNA-miRNA-lncRNA were constructed to reveal the potential mechanisms that regulated the expressions of *CXCL3* and *CXCL13*. Furthermore, GSEA revealed the association between immune-related pathways and *CXCL3* as well as *CXCL13*.

Conclusions: *CXCL3* and *CXCL13* could be valuable prognostic biomarkers in READ. *CXCL3/miR-425-5p/chr22-38_28785274–29006793.1* was identified as the most potential ceRNA network in READ. Our results might provide novel insights in READ immunotherapy.

Highlights

- Expression of *CXCL3*, *CXCL12*, and *CXCL13* are aberrant in rectal adenocarcinoma.
- Lower *CXCL*3 and *CXCL13* expressions predict poorer survival of rectal adenocarcinoma patients.
- *CXCL3/miR-425-5p/chr22-38_28785274–1* might be the most potential ceRNA network in rectal adenocarcinoma.
- *CXCL3* and *CXCL13* might be the key therapeutic targets and prognostic biomarkers for rectal adenocarcinoma.

1. Background

Rectal adenocarcinoma (READ) is one of the most frequent malignancies with a high recurrence rate, with estimated 400,000 new cases every year around the world(1). Although great progress in standard treatments for locally advanced rectal cancer, there are still a considerable amount of patients with local or distant recurrence(2). Recently, immunotherapy, especially immune checkpoint blockade therapy has emerged as a novel strategy to treat different types of tumors, including colon cancer and rectal cancer(3). Immunotherapy has great potential for treating patients with READ, but the therapeutic targets and the corresponding mechanisms remain unclear.

Chemokines are subordinate to the family of low-molecular-weight heparin-binding chemotactic cytokines, which induce immunocyte migration(4). They are currently classified into four subfamilies

based on the N-terminal cysteine (C) motifs: C, CC, CXC, and CX_3C, in which X represents any amino acid(5, 6). In-depth studies on cancer immunotherapy suggested that CXC chemokines can modulate anti-tumor immunological responses by regulating tumor immune infiltrates of specific immunocytes such as $CD8^+$ T cells and $CD4^+$ T cells, which regulate the outcome of immunotherapy(7, 8). A total of sixteen CXC chemokines (*CXCL1* - *CXCL17*,excluding *CXCL15*) have been discovered, and they might be key circuits regulating tumor immunosuppression via mediating effector T cell trafficking(7, 9). The expression of CXC chemokines may also predict the prognosis of patients with different kinds of tumors. For instance, higher *CXCL9*, *CXCL10*, and *CXCL13* predict better overall survival of melanoma patients(10), while breast cancer patients with decreased expression of *CXCL8* presented with better outcomes(11). Although several studies have suggested the prognostic and therapeutic values of CXC chemokines in rectal cancer(12–14), comprehensive analysis of CXC chemokines with sufficient samples are yet to be performed.

In this study, we presented an integrative bioinformatics analysis of multi-omics data including expressions, gene alterations, immune infiltrates, and functional analyses of CXC chemokines in READ (Fig. 1). We evaluated the potential of sixteen CXC chemokines as prognostic biomarkers for READ. We also explored the association between CXC chemokines and immune infiltrates as well as the underlying mechanisms. These data might illustrate the prognostic and immunotherapeutic values of CXC chemokines in READ treatment.

2. Results

2.1. Identification of differentially expressed CXC chemokines

Multiple databases were utilized to demonstrate the expression patterns of CXC chemokines. We first explored the expression levels of sixteen CXC chemokines using 131 rectal adenocarcinoma (READ) samples and normal rectum tissues from the TCGA-READ dataset with complete clinicopathological information. *CXCL3* and *CXCL16* were upregulated, while *CXCL12* and *CXCL13* were downregulated in READ tissues compared with normal tissues (Figs. 2A-2D). We then assessed the expression of CXC chemokines in READ and normal tissues using the ONCOMINE database (Table 1 and Additional file 1: Table S1). Based on the data from ONCOMINE, nine CXC chemokines (*CXCL1*, *CXCL2*, *CXCL3*, *CXCL5*, *CXCL6*, *CXCL8*, *CXCL10*, *CXCL11*, and *CXCL17*) were upregulated, while three CXC chemokines (*CXCL9*, *CXCL12*, and *CXCL13*) were downregulated in READ samples. The expression patterns of *CXCL3*, *CXCL12*, and *CXCL13* were consistent in both TCGA and ONCOMINE (Fig. 2E). We also compared the relative expression levels of sixteen CXC chemokines (*CXCL15* was not included as the data were not available) using GEPIA (Additional file 2: Figure S1).

2.2. Genetic alteration analysis, co-expression analysis, and protein-protein interaction (PPI) network construction

A comprehensive analysis of the molecular characteristics of *CXCL3*, *CXCL12*, and *CXCL13* was performed. We first analyzed the genetic alteration of these CXC chemokines. As a result, mutations and CNVs were rarely found in these chemokines in READ. Only *CXCL13* was altered in 0.6% of the 155 READ samples, respectively (Fig. S1B). Only one patient was found to have a missense mutation of *CXCL13*. As for CNVs, a total of 44, 27, and 45 patients had shallow deletions of *CXCL3*, *CXCL12*, and *CXCL13*, respectively (Fig. 3A). Only six, four, and six patients had *CXCL3*, *CXCL12*, and *CXCL13* gain, respectively. Co-expression analysis also found a significant correlation between *CXCL12* and *CXCL13* expression.

We then constructed a PPI network and extended it using STRING to predict the potential interactions among *CXCL3*, *CXCL12*, and *CXCL13* (Fig. 4A). The biological processes of them had a strong association with the chemokine-mediated signaling pathway, positive regulation of leukocyte chemotaxis, and cell chemotaxis. Besides, we utilized cBioPortal to predict neighbor genes that are frequently altered along with *CXCL3*, *CXCL12*, and *CXCL13* in READ, identifying the top 50 most frequently altered neighbor genes. These genes, along with *CXCL3*, *CXCL12*, and *CXCL13*, were used for PPI network construction via STRING. As nine of the neighbor genes did not functionally relate to other genes, the presented PPI network only consisted of 44 genes (Fig. 4B). This network was also related to several biological functions, including regulation of immune system process, immune response, and positive regulation of immune system process. An extended interactive network of *CXCL3*, *CXCL12*, and *CXCL13* was also constructed using GeneMANIA (Fig. 4C).

Furthermore, Metascape was applied to build networks according to the enriched pathways of *CXCL3*, *CXCL12*, *CXCL13*, and the 41 adjacent genes. The biological processes of them associated with T cell activation, chemokine signaling pathway, and cell adhesion molecules (Figs. 5A-B). Metascape also showed six modules constructed by MCODE1 consisted of *CXCL3*, *CXCL12*, *CXCL13*, *CXCL9*, *CCL5*, and *CXCR6* (Fig. 5C).

2.3. The prognostic value of CXC chemokines in READ patients

To assess the prognostic value of *CXCL3*, *CXCL12*, and *CXCL13* in READ patients, we explored the correlation between the expression of these CXC chemokines and the overall survival of READ patients using the TCGA dataset. Kaplan-Meier curves showed that at median cutoff, lower expression levels of *CXCL13* ($P = 0.021$) were associated with poor survival (Fig. 6). On the other hand, lower *CXCL3* ($P = 0.006$) and *CXCL13* ($P = 0.001$) levels correlated with worse outcome at best cutoff. Hence, we analyzed the correlation between clinicopathological features and expression levels of *CXCL3* and *CXCL13*, respectively (Table 2). Lower *CXCL3* expression was significantly correlated with a more advanced pathological stage ($P = 0.007$), T stage ($P = 0.036$), and N stage ($P = 0.035$). Lower *CXCL13* expression was associated with an advanced pathological stage ($P < 0.001$).

2.4. Correlation between immune infiltrates and CXCL3 as well as CXCL13

We used TIMER database to explore the association between immune cell infiltration and *CXCL3* as well as *CXCL13* (Fig. 7). Expression levels of *CXCL3* were negatively correlated with macrophage infiltration (Cor. = -0.344, $P < 0.001$) and were positively related to neutrophil infiltration (Cor. = 0.229, $P = 0.007$). On the other hand, *CXCL13* levels were negatively correlated with tumor purity (Cor. = -0.484, $P < 0.001$) and were positively associated with the infiltration of B cell (Cor. = 0.4, $P < 0.001$), CD8 + T cell (Cor. = 0.422, $P < 0.001$), neutrophil (Cor. = 0.296, $P < 0.001$), and dendritic cell (Cor. = 0.373, $P < 0.001$). CNV of *CXCL13* was also positively correlated with macrophage infiltration.

2.5. CeRNA networks construction & gene set enrichment analysis of CXCL3 and CXCL13

To further explore the potential mechanism underlying the dysregulation of *CXCL3* and *CXCL13*, we constructed mRNA-miRNA-lncRNA ceRNA networks using DIANA tools based on the theory of ceRNA. For the *CXCL3* network, a total of 12 miRNAs and 14 lncRNAs were predicted (Fig. 8A). As for the *CXCL13* network, a total of eight miRNAs and 22 lncRNAs were involved (Fig. 8B). As for these miRNAs, *miR-425-5p* was the unique oncogene reported in colorectal cancer (CRC)(30, 31). LncRNA *chr22-38_28785274–29006793.1* was predicted as the ceRNA of *miR-425-5p*. We ascertained that the *CXCL3/miR-425-5p/chr22-38_28785274–29006793.1* network was the hub potential regulator in the pathogenesis of READ.

GSEA on *CXCL3* and *CXCL13* using the TCGA-READ dataset was also presented for downstream functional analysis (Additional file 3: Figure S2). *CXCL3* was positively related to immune-related GO terms such as antimicrobial humoral immune response mediated by antimicrobial peptide and antimicrobial humoral response. *CXCL13* was also positively associated with several immune-related GO terms, such as B cell activation, B cell differentiation, and T cell activation. As for KEGG pathway analysis, most of the pathways predicted in the higher *CXCL3* group are not related to the immune system. On the other hand, terms such as antigen processing and presentation, B cell receptor signaling pathway, and T cell receptor signaling pathway are related to the higher *CXCL13* group.

3. Discussion

Since the imbalance between normal cells and the immune system is considered to induce tumorigenesis, several studies have demonstrated significant roles for CXC chemokines in tumor progression(32–34), tumor microenvironment(35), and prognostic outcomes(36). Previous studies suggested that CXC chemokines, especially *CXCL3* and *CXCL13*, presented as the prognosis markers in rectal adenocarcinoma (READ). For instance, lower expression of *CXCL3* exhibited poor overall survival in READ(13). In the rectal cancer liver metastases, M2 macrophage polarization promoted tumor

colonization by secreting *CXCL13* and activated the *CXCL13*/*CXCR5* axis in rectal cancer cells(37). These findings indicated that both *CXCL3* and *CXCL13* possibly had dual biological functions (tumor promoter/tumor suppressor) in READ, which consisted of the previous researches about chemokines (38). In detail, chemokines might promote metastasis by acting directly on the invasion and migration of tumor cells, while chemokines also recruited immune cells towards tumors (39, 40). In different diseases, each chemokine might have its unique function (38). However, the role of the immune microenvironment in READ remained unclear, and studies with large samples were needed. Therefore, we aimed to investigate whether CXC chemokines played crucial roles in the regulation of the immune microenvironment in READ.

We first characterized the CXC chemokine expression patterns in READ. The aberrant expression of three CXC chemokines (*CXCL3*, *CXCL12*, and *CXCL13*) was consistent in READ tissues compared with normal ones in both TCGA and ONCOMINE databases. Significantly increased *CXCL3* level was observed in READ tissues compared with normal ones, while decreased *CXCL12* and *CXCL13* levels were observed in READ tissues compared with normal ones. Further survival analysis revealed that both lower expression of *CXCL3* and *CXCL13* predicted a poorer prognosis of READ. As CXC chemokines mediate immunocyte migration(4), we also found that the expression of *CXCL3* and *CXCL13* correlated with the infiltration of several immune cells in READ. The expression of *CXCL3* was negatively and significantly correlated with macrophage infiltration, while positively correlated with neutrophil infiltration. On the other hand, *CXCL13* level was negatively and significantly correlated with tumor purity, while positively correlated with the infiltration of B cell, CD8 + T cell, neutrophil, and dendritic cell. Furthermore, there was a significant positive correlation between CNV of *CXCL13* and macrophage infiltration.

CXCL3 is reported as a chemoattractant of neutrophils(41, 42), which is consistent with our TIMER results. Multiple studies indicate that *CXCL3* is an oncogene that promotes the progression of various malignancies, such as prostate cancer, breast cancer, and colorectal cancer(20, 43, 44). Our results show that *CXCL3* is upregulated in READ, indicating that *CXCL3* could also be oncogenic in READ. Surprisingly, upregulated *CXCL3* is associated with a better prognosis. Similarly, *CXCL3* is also upregulated in colon cancer, in which upregulated *CXCL3* indicates better clinical outcomes(45). In esophageal squamous cell carcinoma(46), upregulated *CXCL3* secreted from cancer cells unexpectedly increased the recruitment and the antitumor effect of neutrophils. Collectively, these findings indicate that in READ, *CXCL3* may present dual character, which promotes tumor progression and upregulates neutrophil recruitment simultaneously.

On the other hand, *CXCL13* was originally identified in the stromal cells from B cell follicles which could regulate homing of B cells and follicular T cells (Tfh)(47, 48). It is constitutively secreted by stromal cells in secondary lymphoid tissues, including the spleen and lymph nodes. This is consistent with our results as *CXCL13* expression positively correlated with the infiltration level of B cells in READ. In several solid tumors, *CXCL13* can also promote tumor cell proliferation and metastasis through *CXCL13*/*CXCR5* axis, such as colorectal cancer(49), breast cancer(50, 51), and prostate cancer(52, 53). In colorectal cancer(54), lung cancer(55), and gastric cancer(56), *CXCL13* is upregulated and serves as a prognostic biomarker as well as a therapeutic target, in which lower *CXCL13* expression associates with the better prognostic

outcome. Reversely, our results show that *CXCL13* is downregulated in READ, and lower *CXCL13* expression is linked to a worse prognosis. In terms of mechanism, downregulated *CXCL13* may reduce B cells and Tfh cells infiltration, and thus lead to READ progression and poor prognosis, which is similar to breast cancer[57] and colorectal cancer[58]. These findings indicate the dual character of *CXCL13*, similar to *CXCL3*, in different tumors[59], and *CXCL13* mainly performs anti-tumor effect on READ.

Long non-coding RNAs (lncRNAs) function effect as miRNAs sponges to interfere the functions of miRNAs targeting to mRNAs in various tumors[60, 61]. In our study, 12 miRNAs and 14 lncRNAs were identified as ceRNA networks of *CXCL3*, while eight miRNAs and 22 lncRNAs were identified targeting *CXCL13*. Among all these miRNAs, *miR-425-5p*, which downregulated *CXCL3*[62], was reported as an oncogene[20, 21] and inducing chemo-resistance[63] in colorectal cancer (CRC). It was consistent with our identification that lower expression level of *miR-425-5p* and higher expression level of *CXCL3* were presented in CRC. Additionally, it was reported that overexpression of *miR-425-5p* could induce the M2 polarization of macrophages[31], which enhanced tumor metastasis by secreting vascular endothelial growth factors (VEGF). It suggests that miR-425-5p may regulate M2 polarization of macrophages via *CXCL3*. According to prediction of miRNA-lncRNA from database DIANA, we found that *miR-425-5p* bound to lncRNA *chr22-38_28785274–29006793.1*. Regarding to lncRNA *chr22-38_28785274–29006793.1*, it was considered as the member of mRNA/miRNA/lncRNA ceRNA networks and involved in the infiltration of CD4 + and CD8 + T cell in colon cancer by bioinformatic analysis without biological validation[64]. Thus, we assume that *CXCL3/miR-425-5p/chr22-38_28785274–29006793.1* network may upregulate *CXCL3* in READ and promote immune infiltration to exert the anti-tumor immunological effect.

Several limitations should be addressed. First, the bioinformatics analysis only focused on the transcriptional level of CXC chemokines, which could only reflect specific changes but not global changes of READ immune status. Second, the data were acquired from public resources, so the bias in the study cannot be completely eliminated. Last, ceRNA networks were predicted based on bioinformatics algorithms instead of experiments. To overcome these issues, further experiments in vitro and in vivo are warranted.

4. Conclusions

CXCL3 and *CXCL13* could be valuable prognostic biomarkers in READ. *CXCL3/miR-425-5p/chr22-38_28785274–29006793.1* was identified as the most potential ceRNA network in READ. Our results might provide novel insights in READ immunotherapy.

5. Methods

5.1. The Cancer Genome Atlas (TCGA)

The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/tcga/) is a publicly funded project that aims to catalog and discover major cancer-causing genomic alterations to create a comprehensive

“atlas” of cancer genomic profiles[65]. The transcriptome expression profiles and copy number variation (CNV) of CXC chemokines, along with clinical data from the “TCGA-READ” cohort with 159 READ patients were extracted from the TCGA database. A total of 131 READ patients with full clinicopathological data were applied for further analyses. Co-expression analysis was presented. Prognostic analysis was performed using a Kaplan-Meier method.. The association between expression levels of CXC chemokines and the clinicopathological characteristics of READ patients was also analyzed by the Chi-square test. $P < 0.05$ was considered significant.

5.2. ONCOMINE

ONCOMINE (https://www.oncomine.org/) is an online microarray database for genome-wide expression analysis[66]. We compared the mRNA levels of CXC between READ tissues and corresponding normal tissues. The criteria for data filtration were set as a $P < 0.05$, a |fold change| > 2, and a gene rank in the top 10%. Student’s *t*-test was used to analyze the difference in mRNA levels of CXC chemokines in READ samples.

5.3. GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) is an interactive web application for gene expression analysis based on 9736 tumor samples and 8587 normal samples from the TCGA and the GTEx databases[67]. We used the “Multiple gene comparison” module in the “Expression DIY” to generate a relative expression heatmap of CXC chemokines in READ.

5.4. cBioPortal

The cBio Cancer Genomics Portal (cBioPortal, http://cbioportal.org) is an open-access resource for interactive exploration and visualization of multidimensional cancer genomics data, which provides access to data of more than 5,000 tumor samples from different cancer studies[68]. Genetic alteration analysis of *CXCL3*, *CXCL12*, and *CXCL13* were presented using 155 rectal adenocarcinoma (READ) samples extracted from 594 colorectal adenocarcinoma samples (TCGA, PanCancer Atlas). The criterium of *z*-score for data filtration was set as ± 2.0. The top 50 frequently altered neighbor genes associated with *CXCL3*, *CXCL12*, and *CXCL13* were also identified, in which 41 genes were applied for protein-protein interaction (PPI) and functional analyses as the other nine genes did not functionally relate to others.

5.5. Metascape

Metascape (http://metascape.org/gp/index.html) is an effective and efficient tool to analyze and interpret OMICs-based studies comprehensively[69]. In this study, the “Express Analysis” module was chosen. Metascape subdivided the groups and built a network according to the enriched pathways of *CXCL3*, *CXCL12*, *CXCL13*, and the 41 neighbor genes.

5.6. STRING

The STRING database (https://string-db.org/) aims to collect, score, and integrate all publicly available sources of PPI information, and to complement these with computational prediction[70]. The PPI

networks of upregulated CXC chemokines, as well as frequently altered neighbor genes, were constructed using STRING. The confidence score of ≥ 0.4 was set as the threshold. All the networks were visualized by Cytoscape 3.7.0.

5.7. GeneMANIA

GeneMANIA (http://www.genemania.org) is a flexible, user-friendly web interface for generating hypotheses about gene function, analyzing gene lists, and prioritizing genes for functional assays[71]. We presented the interactive functional association network predicted by GeneMANIA among *CXCL3*, *CXCL12*, *CXCL13*, and the 41 neighbor genes[72].

5.8. Competing endogenous RNA (ceRNA) network construction

The ceRNA network construction of *CXCL3* and *CXCL13* was presented based on the theory of ceRNA[73]. The construction process included two steps. First, miRNA-mRNA interactions of *CXCL3* and *CXCL13* were predicted respectively by DIANA-MicroT-CDS (http://www.microrna.gr/microT-CDS)[74]. The threshold was set as the miTG-score > 0.9. After that, we used the selected miRNA to predict miRNA-lncRNA interactions by DIANA-LncBase Predicted v.2 (www.microrna.gr/LncBase)[75]. The criteria were set as the interaction score = 1.000. For each network, we only selected the long non-coding RNAs(lncRNAs) that interacted with at least two of the predicted miRNAs above for construction. Finally, the two ceRNA networks were visualized by R 3.6.2.

5.9. Gene Set Enrichment Analysis

Gene Set Enrichment Analysis (GSEA) that evaluates microarray data at the level of gene sets can be used to link prior knowledge to newly generated data and thereby help uncover the collective behavior of genes in states of health and disease[76]. We used GSEA for Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of *CXCL3* and *CXCL13* utilizing the TCGA-READ dataset. $P < 0.05$ and FDR $q < 0.25$ were considered significant. The results were presented using R 3.6.2.

Abbreviations

cBioPortal: The cBio Cancer Genomics Portal; ceRNA: Competing endogenous RNA; CNV: copy number variation; CRC: colorectal cancer; GO: Gene Ontology; GSEA: Gene Set Enrichment Analysis; KEGG: Kyoto Encyclopedia of Genes and Genomes; lncRNAs: long non-coding RNAs; PPI: protein-protein interaction; READ: Rectal adenocarcinoma; TCGA: The Cancer Genome Atlas; Tfh: follicular T cells; VEGF: vascular endothelial growth factors.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Wang Xiaoshuai: Methodology, Data curation, Formal analysis, Resources, Writing - Review & Editing **Hu Zhiyang:** Methodology, Data curation, Formal analysis, Software **Du Zefeng:** Methodology, Data curation, Formal analysis, Software **Hong Dongchun:** Investigation, Project administration **Huang Haoyang:** Data curation **Han Yueyin:** Data curation **Hou Yingdong:** Visualization **Feng Haoqian:** Visualization **Chen Tianyu:** Conceptualization, Writing - Review & Editing **Xue Zhicheng:** Conceptualization, Writing - Review & Editing.

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References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-E86.
2. He F, Ju HQ, Ding Y, Jiang Z, Li Z, Huang B, et al. Association between adjuvant chemotherapy and survival in patients with rectal cancer and pathological complete response after neoadjuvant chemoradiotherapy and resection. British journal of cancer. 2020;123(8):1244-52.
3. Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455-65.

4. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annu Rev Immunol. 2004;22:891-928.
5. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity. 2000;12(2):121-7.
6. Zlotnik A, Yoshie O. The chemokine superfamily revisited. Immunity. 2012;36(5):705-16.
7. Nagarsheth N, Peng D, Kryczek I, Wu K, Li W, Zhao E, et al. PRC2 Epigenetically Silences Th1-Type Chemokines to Suppress Effector T-Cell Trafficking in Colon Cancer. Cancer Res. 2016;76(2):275-82.
8. Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature. 2015;527(7577):249-53.
9. Hong YK, Li Y, Pandit H, Li S, Pulliam Z, Zheng Q, et al. Epigenetic modulation enhances immunotherapy for hepatocellular carcinoma. Cell Immunol. 2019;336:66-74.
10. Huang B, Han W, Sheng Z-F, Shen G-L. Identification of immune-related biomarkers associated with tumorigenesis and prognosis in cutaneous melanoma patients. Cancer Cell Int. 2020;20:195.
11. Chen E, Qin X, Peng K, Xu X, Li W, Cheng X, et al. Identification of Potential Therapeutic Targets Among CXC Chemokines in Breast Tumor Microenvironment Using Integrative Bioinformatics Analysis. Cell Physiol Biochem. 2018;45(5):1731-46.
12. Chen J, Chen QL, Wang WH, Chen XL, Hu XQ, Liang ZQ, et al. Prognostic and predictive values of CXCL10 in colorectal cancer. Clin Transl Oncol. 2020;22(9):1548-64.
13. Sun G, Li Y, Peng Y, Lu D, Zhang F, Cui X, et al. Identification of differentially expressed genes and biological characteristics of colorectal cancer by integrated bioinformatics analysis. J Cell Physiol. 2019.
14. Wågsäter D, Hugander A, Dimberg J. Expression of CXCL16 in human rectal cancer. Int J Mol Med. 2004;14(1):65-9.
15. Anisowicz A, Bardwell L, Sager R. Constitutive overexpression of a growth-regulated gene in transformed Chinese hamster and human cells. Proc Natl Acad Sci USA. 1987;84(20):7188-92.
16. Yang C, Yu H, Chen R, Tao K, Jian L, Peng M, et al. CXCL1 stimulates migration and invasion in ER-negative breast cancer cells via activation of the ERK/MMP2/9 signaling axis. Int J Oncol. 2019;55(3):684-96.
17. Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA. 2002;99(26):16899-903.
18. Subat S, Mogushi K, Yasen M, Kohda T, Ishikawa Y, Tanaka H. Identification of genes and pathways, including the CXCL2 axis, altered by DNA methylation in hepatocellular carcinoma. J Cancer Res Clin Oncol. 2019;145(3):675-84.
19. Iida N, Grotendorst GR. Cloning and sequencing of a new gro transcript from activated human monocytes: expression in leukocytes and wound tissue. Mol Cell Biol. 1990;10(10):5596-9.

20. Ruan G-T, Gong Y-Z, Liao X-W, Wang S, Huang W, Wang X-K, et al. Diagnostic and prognostic values of C-X-C motif chemokine ligand 3 in patients with colon cancer. Oncol Rep. 2019;42(5):1996-2008.
21. Li J-Y, Wang Z-J, Deng A-P, Li Y-M. ENA-78 Is a Novel Predictor of Wound Healing in Patients with Diabetic Foot Ulcers. J Diabetes Res. 2019;2019:2695436.
22. Ma J-C, Sun X-W, Su H, Chen Q, Guo T-K, Li Y, et al. Fibroblast-derived CXCL12/SDF-1α promotes CXCL6 secretion and co-operatively enhances metastatic potential through the PI3K/Akt/mTOR pathway in colon cancer. World J Gastroenterol. 2017;23(28):5167-78.
23. Yao W, Sun Y, Sun Y, Chen P, Meng Z, Xiao M, et al. A Preliminary Report of the Relationship Between Gene Polymorphism of and Its Receptors and Systemic Inflammatory Response Syndrome Caused by Wasp Stings. DNA Cell Biol. 2019;38(12):1512-8.
24. Yudate HT, Suwa M, Irie R, Matsui H, Nishikawa T, Nakamura Y, et al. HUNT: launch of a full-length cDNA database from the Helix Research Institute. Nucleic Acids Res. 2001;29(1):185-8.
25. Zhang Y, Zhao W, Li S, Lv M, Yang X, Li M, et al. CXCL11 promotes self-renewal and tumorigenicity of α2δ1 liver tumor-initiating cells through CXCR3/ERK1/2 signaling. Cancer Lett. 2019;449:163-71.
26. Jacobs KA, Collins-Racie LA, Colbert M, Duckett M, Golden-Fleet M, Kelleher K, et al. A genetic selection for isolating cDNAs encoding secreted proteins. Gene. 1997;198(1-2):289-96.
27. Miyata T, Yamashita Y-I, Yoshizumi T, Shiraishi M, Ohta M, Eguchi S, et al. CXCL12 expression in intrahepatic cholangiocarcinoma is associated with metastasis and poor prognosis. Cancer Sci. 2019;110(10):3197-203.
28. Gunn MD, Ngo VN, Ansel KM, Ekland EH, Cyster JG, Williams LT. A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. Nature. 1998;391(6669):799-803.
29. Weinstein EJ, Head R, Griggs DW, Sun D, Evans RJ, Swearingen ML, et al. VCC-1, a novel chemokine, promotes tumor growth. Biochem Biophys Res Commun. 2006;350(1):74-81.
30. Zhu M, Huang Z, Zhu D, Zhou X, Shan X, Qi LW, et al. A panel of microRNA signature in serum for colorectal cancer diagnosis. Oncotarget. 2017;8(10):17081-91.
31. Wang D, Wang X, Si M, Yang J, Sun S, Wu H, et al. Exosome-encapsulated miRNAs contribute to CXCL12/CXCR4-induced liver metastasis of colorectal cancer by enhancing M2 polarization of macrophages. Cancer Lett. 2020;474:36-52.
32. Yin X, Liu Z, Zhu P, Wang Y, Ren Q, Chen H, et al. CXCL12/CXCR4 promotes proliferation, migration, and invasion of adamantinomatous craniopharyngiomas via PI3K/AKT signal pathway. J Cell Biochem. 2019;120(6):9724-36.
33. Guo H, Wang F, Diao Y, Zhang Z, Chen Q, Qian C-N, et al. Knockdown of Notch1 inhibits nasopharyngeal carcinoma cell growth and metastasis via downregulation of CCL2, CXCL16, and uPA. Mol Carcinog. 2019;58(10):1886-96.
34. Wang N, Liu W, Zheng Y, Wang S, Yang B, Li M, et al. CXCL1 derived from tumor-associated macrophages promotes breast cancer metastasis via activating NF-κB/SOX4 signaling. Cell Death Dis. 2018;9(9):880.

35. Liao W, Overman MJ, Boutin AT, Shang X, Zhao D, Dey P, et al. KRAS-IRF2 Axis Drives Immune Suppression and Immune Therapy Resistance in Colorectal Cancer. Cancer Cell. 2019;35(4).
36. Cabrero-de Las Heras S, Martínez-Balibrea E. CXC family of chemokines as prognostic or predictive biomarkers and possible drug targets in colorectal cancer. World J Gastroenterol. 2018;24(42):4738-49.
37. Zhao S, Mi Y, Guan B, Zheng B, Wei P, Gu Y, et al. Tumor-derived exosomal miR-934 induces macrophage M2 polarization to promote liver metastasis of colorectal cancer. J Hematol Oncol. 2020;13(1):156.
38. Wang JM, Deng X, Gong W, Su S. Chemokines and their role in tumor growth and metastasis. Journal of immunological methods. 1998;220(1-2):1-17.
39. Dangaj D, Bruand M, Grimm AJ, Ronet C, Barras D, Duttagupta PA, et al. Cooperation between Constitutive and Inducible Chemokines Enables T Cell Engraftment and Immune Attack in Solid Tumors. Cancer Cell. 2019;35(6):885-900.e10.
40. Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001;410(6824):50-6.
41. Ahuja SK, Murphy PM. The CXC chemokines growth-regulated oncogene (GRO) alpha, GRObeta, GROgamma, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. J Biol Chem. 1996;271(34):20545-50.
42. Smith DF, Galkina E, Ley K, Huo Y. GRO family chemokines are specialized for monocyte arrest from flow. Am J Physiol Heart Circ Physiol. 2005;289(5):H1976-H84.
43. Gui S-L, Teng L-C, Wang S-Q, Liu S, Lin Y-L, Zhao X-L, et al. Overexpression of CXCL3 can enhance the oncogenic potential of prostate cancer. Int Urol Nephrol. 2016;48(5):701-9.
44. See ALP, Chong PK, Lu S-Y, Lim YP. CXCL3 is a potential target for breast cancer metastasis. Curr Cancer Drug Targets. 2014;14(3):294-309.
45. Zhao Q-Q, Jiang C, Gao Q, Zhang Y-Y, Wang G, Chen X-P, et al. Gene expression and methylation profiles identified CXCL3 and CXCL8 as key genes for diagnosis and prognosis of colon adenocarcinoma. J Cell Physiol. 2020;235(5):4902-12.
46. Chen C-L, Wang Y, Huang C-Y, Zhou Z-Q, Zhao J-J, Zhang X-F, et al. IL-17 induces antitumor immunity by promoting beneficial neutrophil recruitment and activation in esophageal squamous cell carcinoma. Oncoimmunology. 2017;7(1):e1373234.
47. Ansel KM, Ngo VN, Hyman PL, Luther SA, Förster R, Sedgwick JD, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature. 2000;406(6793):309-14.
48. Förster R, Mattis AE, Kremmer E, Wolf E, Brem G, Lipp M. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. Cell. 1996;87(6):1037-47.
49. Meijer J, Zeelenberg IS, Sipos B, Roos E. The CXCR5 chemokine receptor is expressed by carcinoma cells and promotes growth of colon carcinoma in the liver. Cancer research. 2006;66(19):9576-82.

50. Biswas S, Sengupta S, Roy Chowdhury S, Jana S, Mandal G, Mandal PK, et al. CXCL13-CXCR5 co-expression regulates epithelial to mesenchymal transition of breast cancer cells during lymph node metastasis. Breast Cancer Res Treat. 2014;143(2):265-76.
51. Panse J, Friedrichs K, Marx A, Hildebrandt Y, Luetkens T, Barrels K, et al. Chemokine CXCL13 is overexpressed in the tumour tissue and in the peripheral blood of breast cancer patients. British journal of cancer. 2008;99(6):930-8.
52. El-Haibi CP, Singh R, Sharma PK, Singh S, Lillard JW. CXCL13 mediates prostate cancer cell proliferation through JNK signalling and invasion through ERK activation. Cell Prolif. 2011;44(4):311-9.
53. El Haibi CP, Sharma PK, Singh R, Johnson PR, Suttles J, Singh S, et al. PI3Kp110-, Src-, FAK-dependent and DOCK2-independent migration and invasion of CXCL13-stimulated prostate cancer cells. Mol Cancer. 2010;9:85.
54. Yan Q, Yuan Y, Yankui L, Jingjie F, Linfang J, Yong P, et al. The Expression and Significance of CXCR5 and MMP-13 in Colorectal Cancer. Cell Biochem Biophys. 2015;73(1):253-9.
55. Wang G-Z, Cheng X, Zhou B, Wen Z-S, Huang Y-C, Chen H-B, et al. The chemokine CXCL13 in lung cancers associated with environmental polycyclic aromatic hydrocarbons pollution. Elife. 2015;4.
56. Wei Y, Lin C, Li H, Xu Z, Wang J, Li R, et al. CXCL13 expression is prognostic and predictive for postoperative adjuvant chemotherapy benefit in patients with gastric cancer. Cancer Immunol Immunother. 2018;67(2):261-9.
57. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, et al. CD4 follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest. 2013;123(7):2873-92.
58. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity. 2013;39(4):782-95.
59. Rubio AJ, Porter T, Zhong X. Duality of B Cell-CXCL13 Axis in Tumor Immunology. Front Immunol. 2020;11:521110.
60. Xu J, Li Y, Lu J, Pan T, Ding N, Wang Z, et al. The mRNA related ceRNA-ceRNA landscape and significance across 20 major cancer types. Nucleic Acids Res. 2015;43(17):8169-82.
61. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell. 2011;146(3):353-8.
62. Tan Y, Lin JJ, Yang X, Gou DM, Fu L, Li FR, et al. A panel of three plasma microRNAs for colorectal cancer diagnosis. Cancer epidemiology. 2019;60:67-76.
63. Cristóbal I, Madoz-Gúrpide J, Rojo F, García-Foncillas J. Potential therapeutic value of miR-425-5p in metastatic colorectal cancer. Journal of cellular and molecular medicine. 2016;20(11):2213-4.
64. Yang X, Wu W, Pan Y, Zhou Q, Xu J, Han S. Immune-related genes in tumor-specific CD4(+) and CD8(+) T cells in colon cancer. BMC cancer. 2020;20(1):585.

65. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;19(1A):A68-A77.
66. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004;6(1):1-6.
67. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1).
68. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401-4.
69. Bader GD, Hogue CWV. An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics. 2003;4:2.
70. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019;47(D1):D607-D13.
71. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res. 2010;38(Web Server issue):W214-W20.
72. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504.
73. Sen R, Ghosal S, Das S, Balti S, Chakrabarti J. Competing endogenous RNA: the key to posttranscriptional regulation. ScientificWorldJournal. 2014;2014:896206.
74. Paraskevopoulou MD, Georgakilas G, Kostoulas N, Vlachos IS, Vergoulis T, Reczko M, et al. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. Nucleic Acids Res. 2013;41(Web Server issue):W169-W73.
75. Paraskevopoulou MD, Vlachos IS, Karagkouni D, Georgakilas G, Kanellos I, Vergoulis T, et al. DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts. Nucleic Acids Res. 2016;44(D1):D231-D8.
76. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005;102(43):15545-50.

Tables

Tables 1 and 2 are not available with this version.

Figures

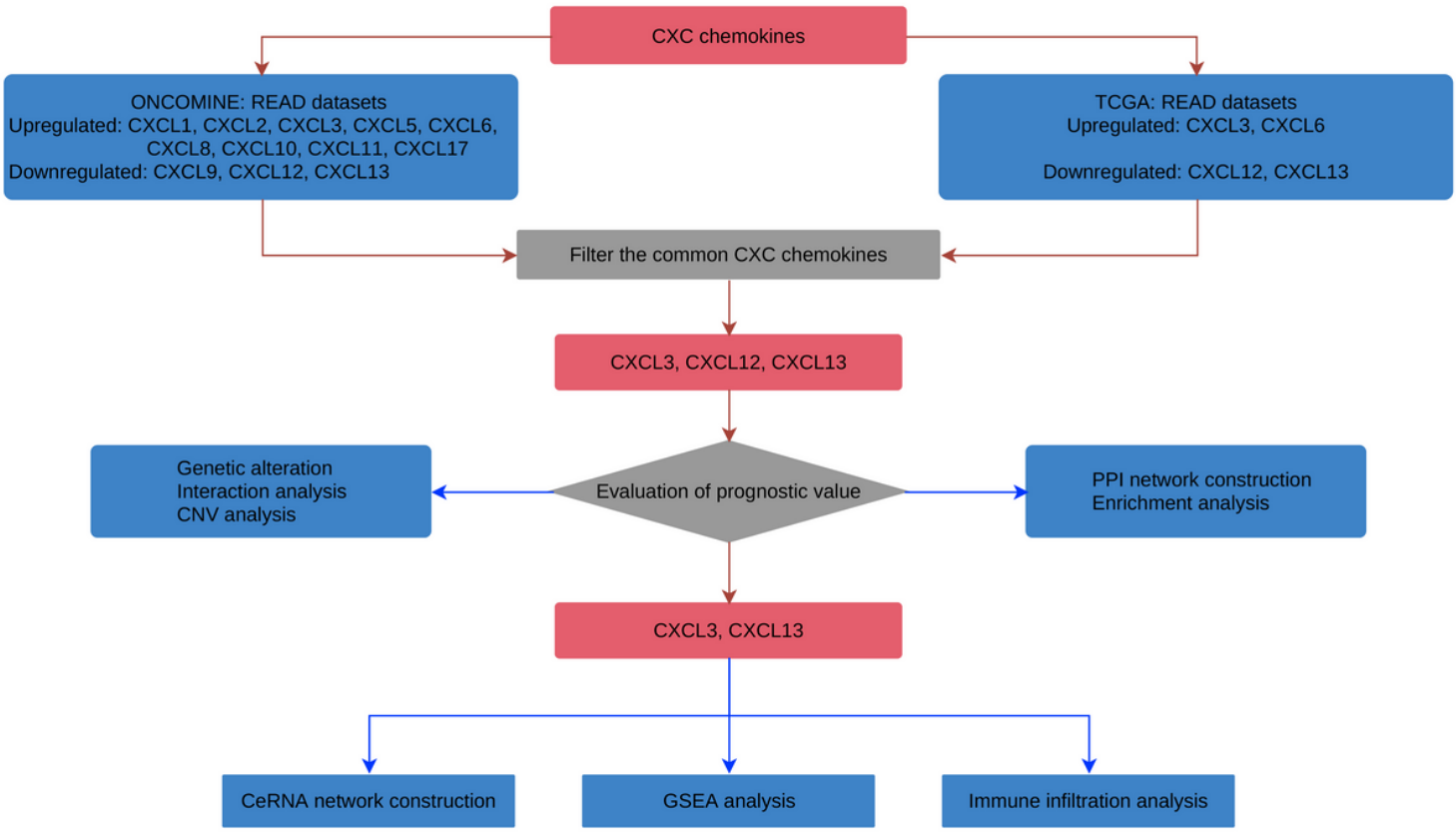

Figure 1

Flow chart of the analyses. READ: rectal adenocarcinoma; CeRNA: competing endogenous RNA; GSEA: Gene set enrichment analysis.

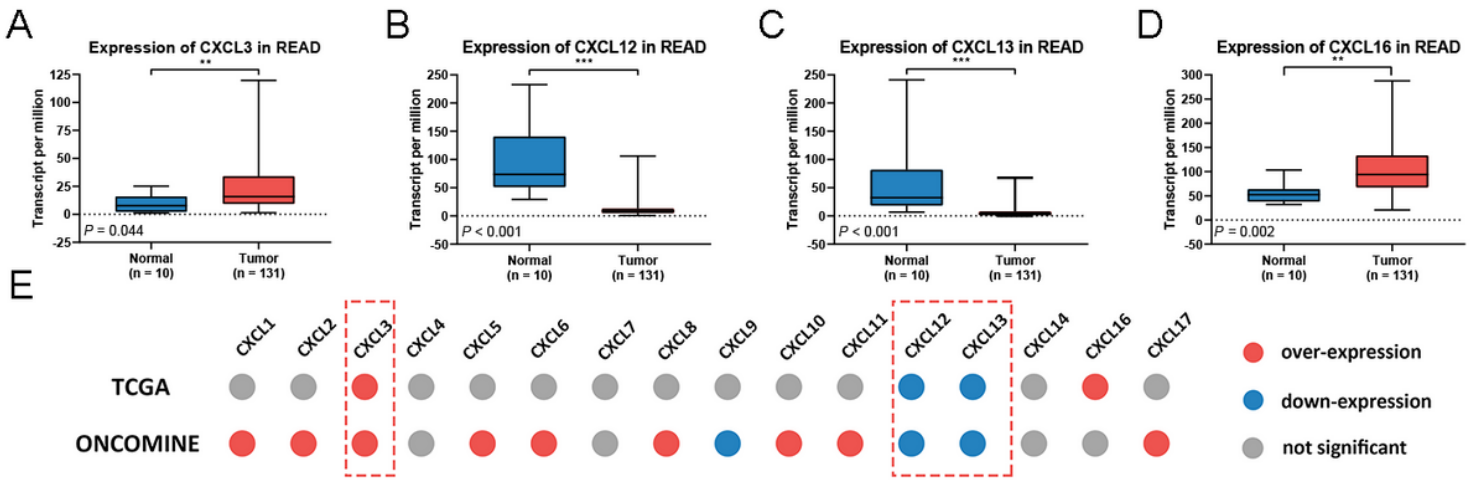

Figure 2

The transcription levels of CXC chemokines in READ. The expression levels of (A) CXCL3, (B) CXCL12, (C) CXCL13, and (D) CXCL16 in READ tissues and normal rectum tissues generated from the TCGA database were shown. Data were analyzed by Student's t-test and P < 0.05 was considered significant. * P < 0.05, **

P < 0.01, *** P < 0.001. (E) Expression patterns of CXC chemokines generated from TCGA database and ONCOMINE database, respectively. READ: Rectal adenocarcinoma.

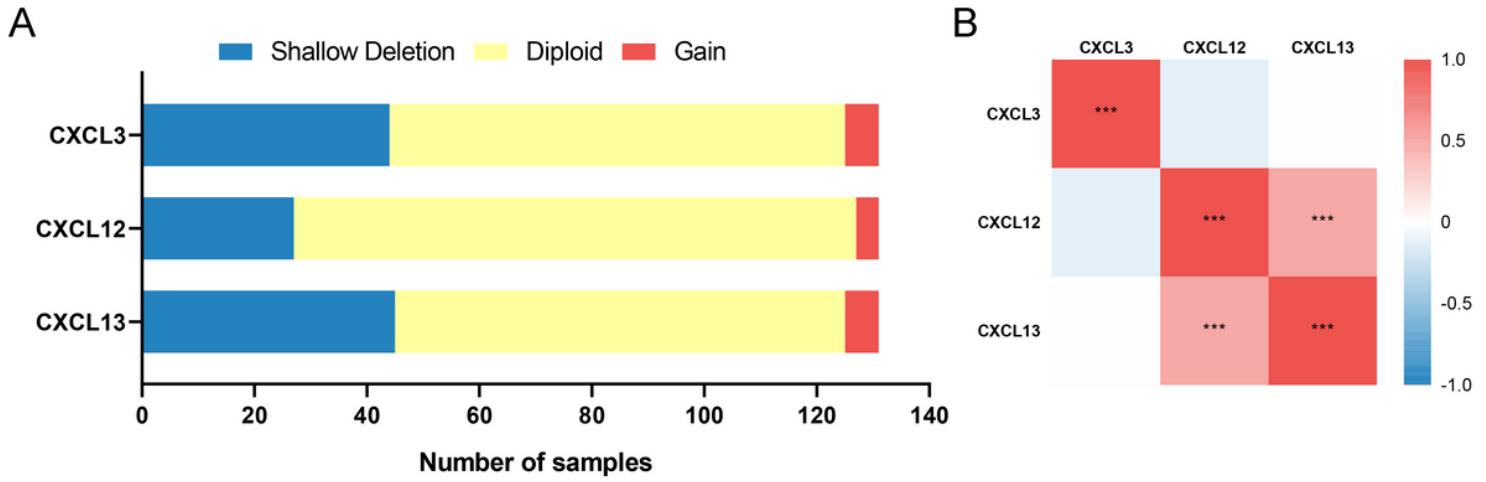

Figure 3

Copy number variation and interaction analyses of CXCL3, CXCL12, and CXCL13 in READ. (A) Summary of copy number variation in CXCL3, CXCL12, and CXCL13. (B) Correlation heatmap of CXCL3, CXCL12, and CXCL13 in READ. READ: Rectal adenocarcinoma. * P < 0.05, ** P < 0.01, *** P < 0.001.

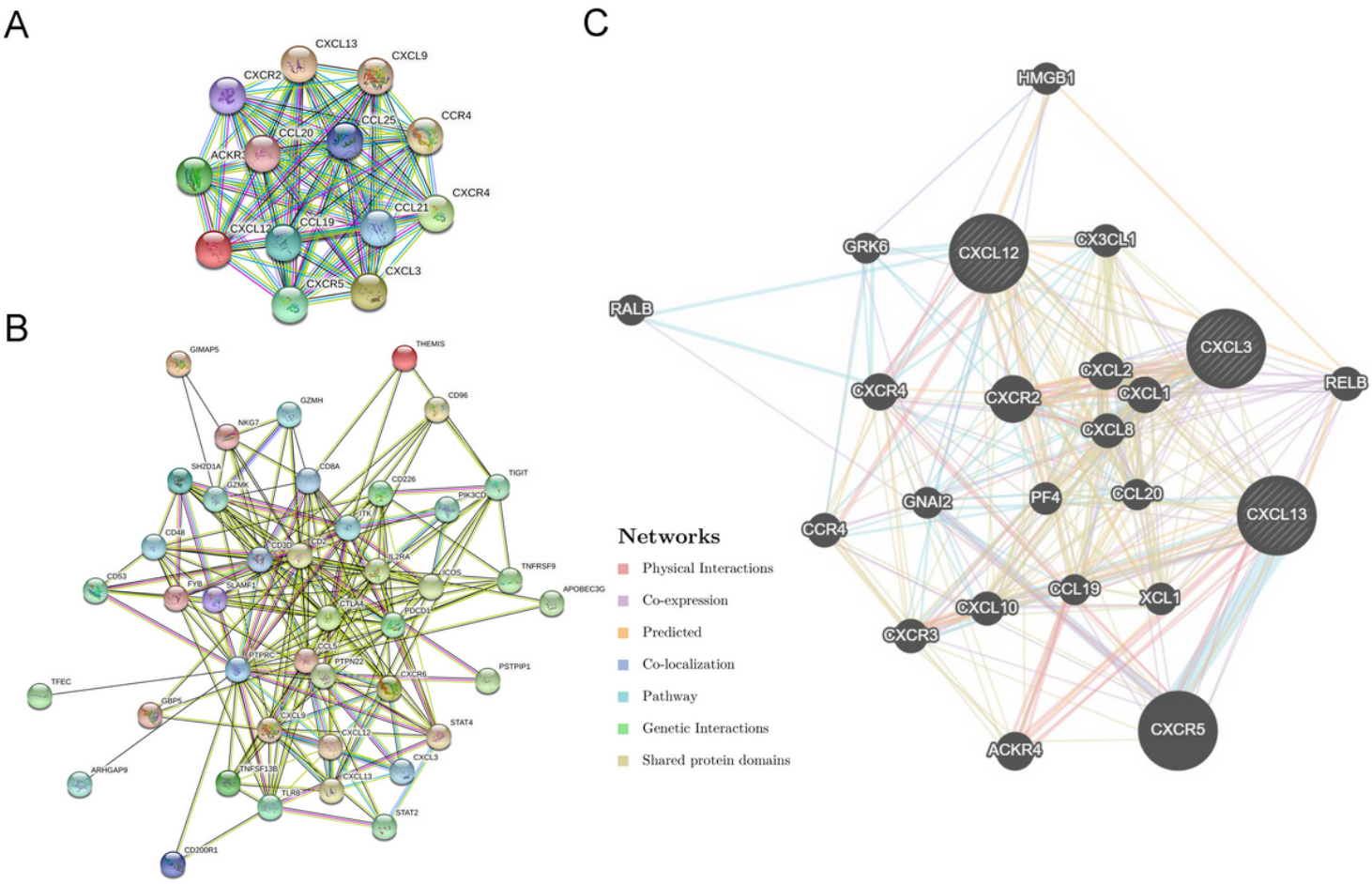

Figure 4

Protein-protein interaction and gene network analyses of CXCL3, CXCL12, and CXCL13. (A) Extended protein-protein interaction (PPI) network of CXCL3, CXCL12, and CXCL13. (B) PPI network of CXCL3, CXCL12, CXCL13, and 41 correlated genes generated by STRING. (C) Interaction network of CXCL3, CXCL12, and CXCL13 and related genes in READ determined by GeneMANIA.

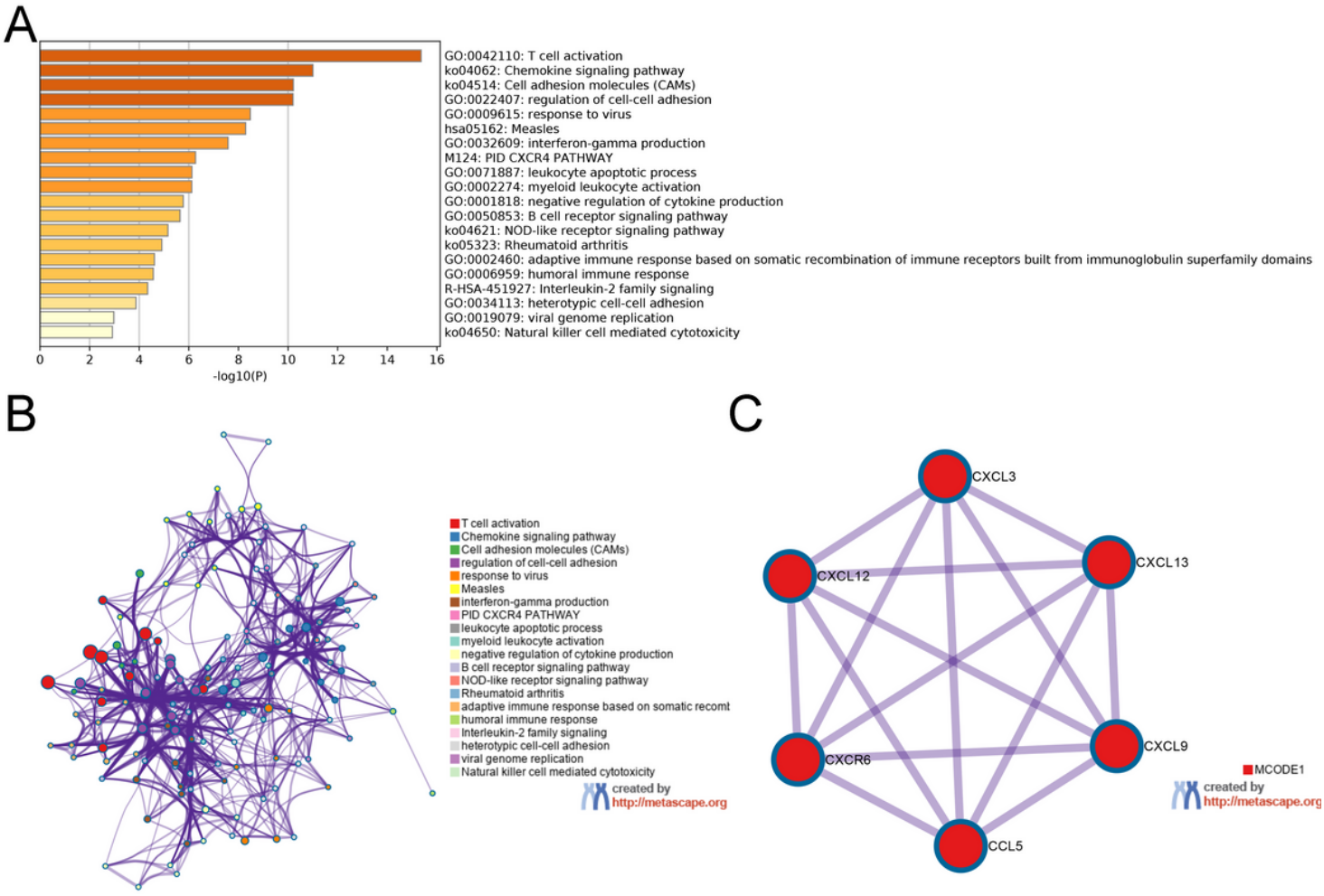

Figure 5

Enrichment analysis of CXCL3, CXCL12, CXCL13 and 41 correlated genes in READ carried out by Metascape database. (A) Bar graph demonstrated biological processes enrichment analysis of CXCL3, CXCL12, CXCL13 and 41 correlated genes. (B) Biological processes enrichment of CXCL3, CXCL12, CXCL13 and 41 correlated genes. Each node represented an enriched term, and the nodes were colored by their cluster IDs. (C) Significant module construction by Metascape. $P < 0.05$ was considered significant.

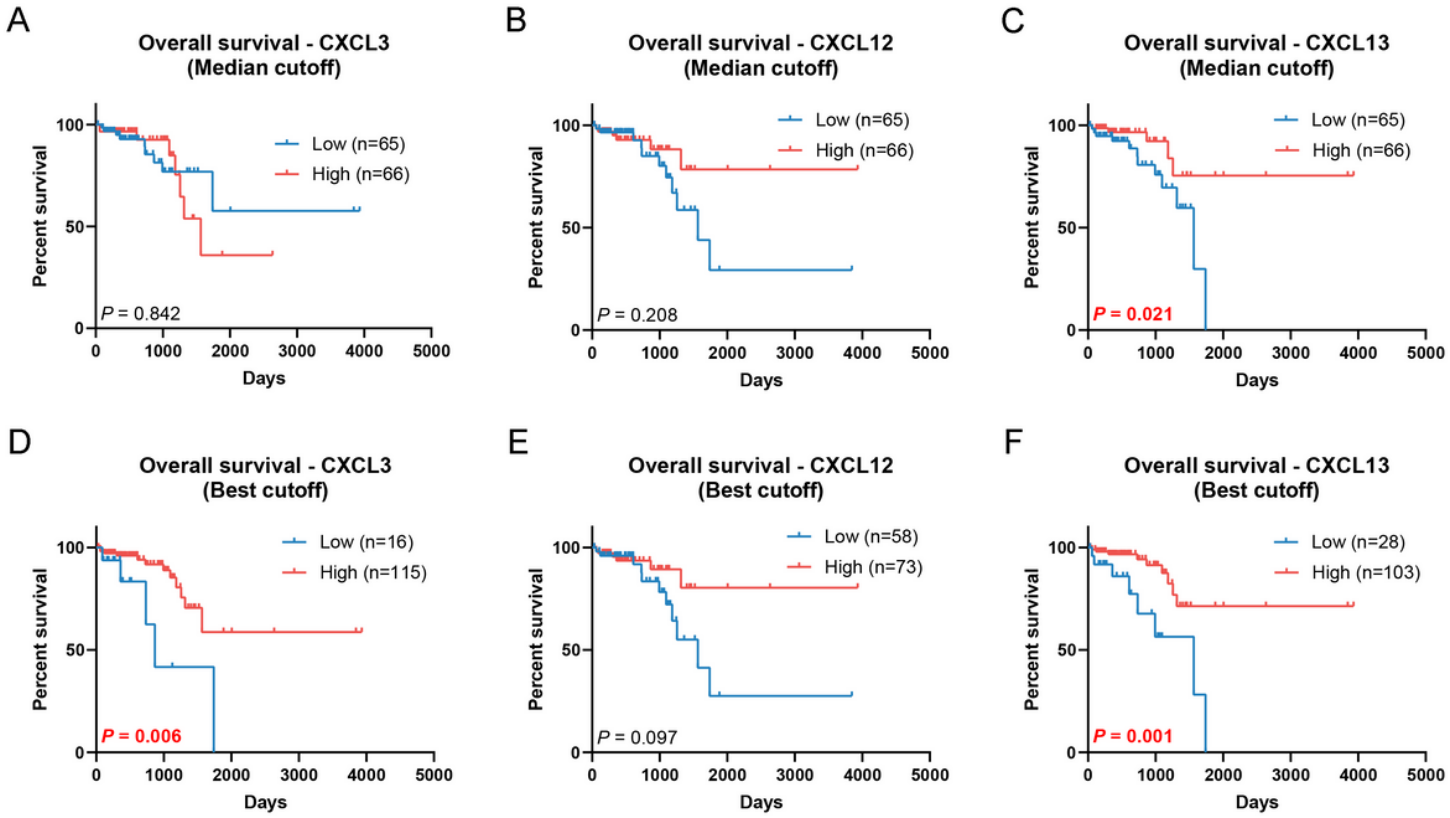

Figure 6

The prognostic value of CXCL3, CXCL12, and CXCL13 in READ cohort. Kaplan-Meier analysis revealed the correlation between overall survival rate and the expression levels of CXCL3, CXCL12, and CXCL13 with (A-C) median cutoff and (D-F) best cutoff. P < 0.05 was considered significant.

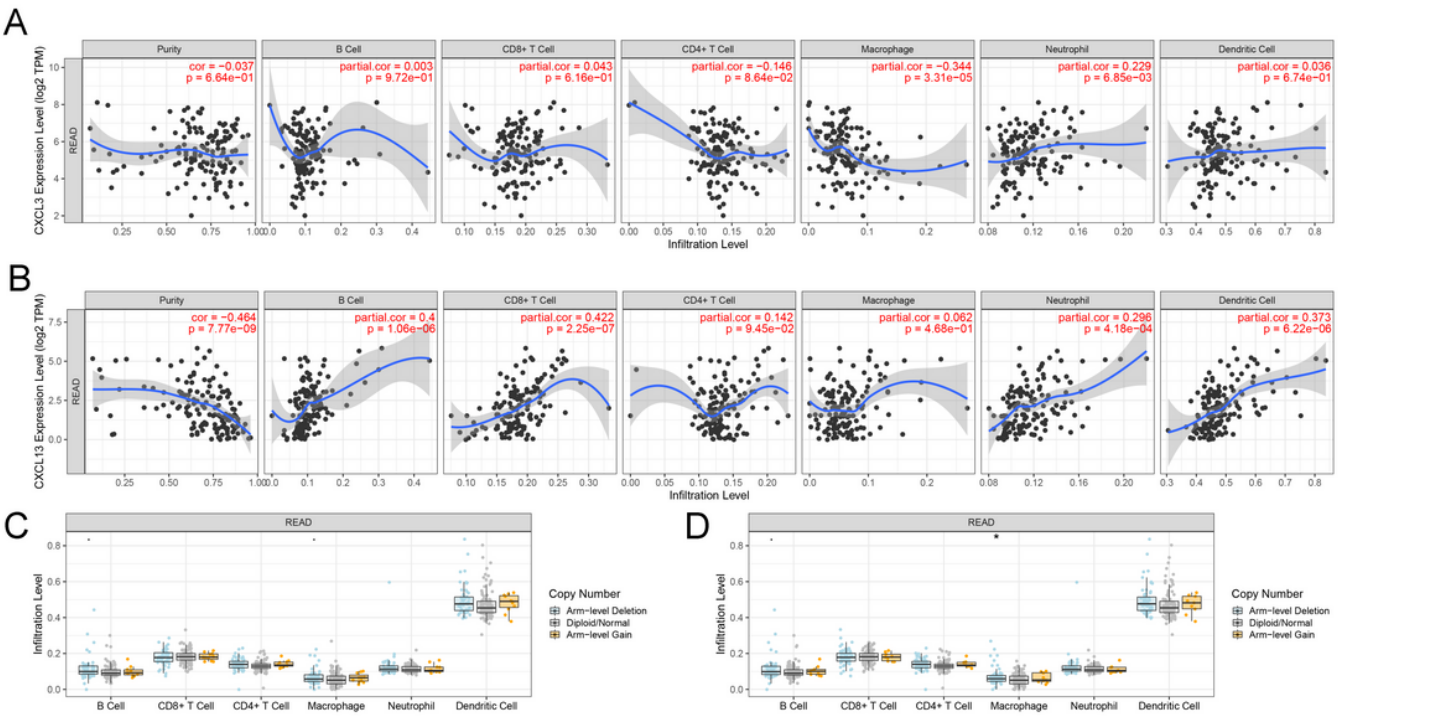

Figure 7

Correlation of CXCL3 and CXCL13 with immune infiltration level in READ. (A) CXCL3 expression is negatively correlated with the infiltration level of macrophages and positively correlated with the infiltration level of neutrophils. (B) CXCL13 expression is significantly related to tumor purity and has positive correlations with the infiltration levels of B cells, CD8+ T cells, neutrophils and dendritic cells. (C) CXCL3 CNV has no significant effect on immune infiltrate of READ. (D) CXCL13 CNV affects the infiltration level of macrophages. READ: rectal adenocarcinoma; CNV: copy number variation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

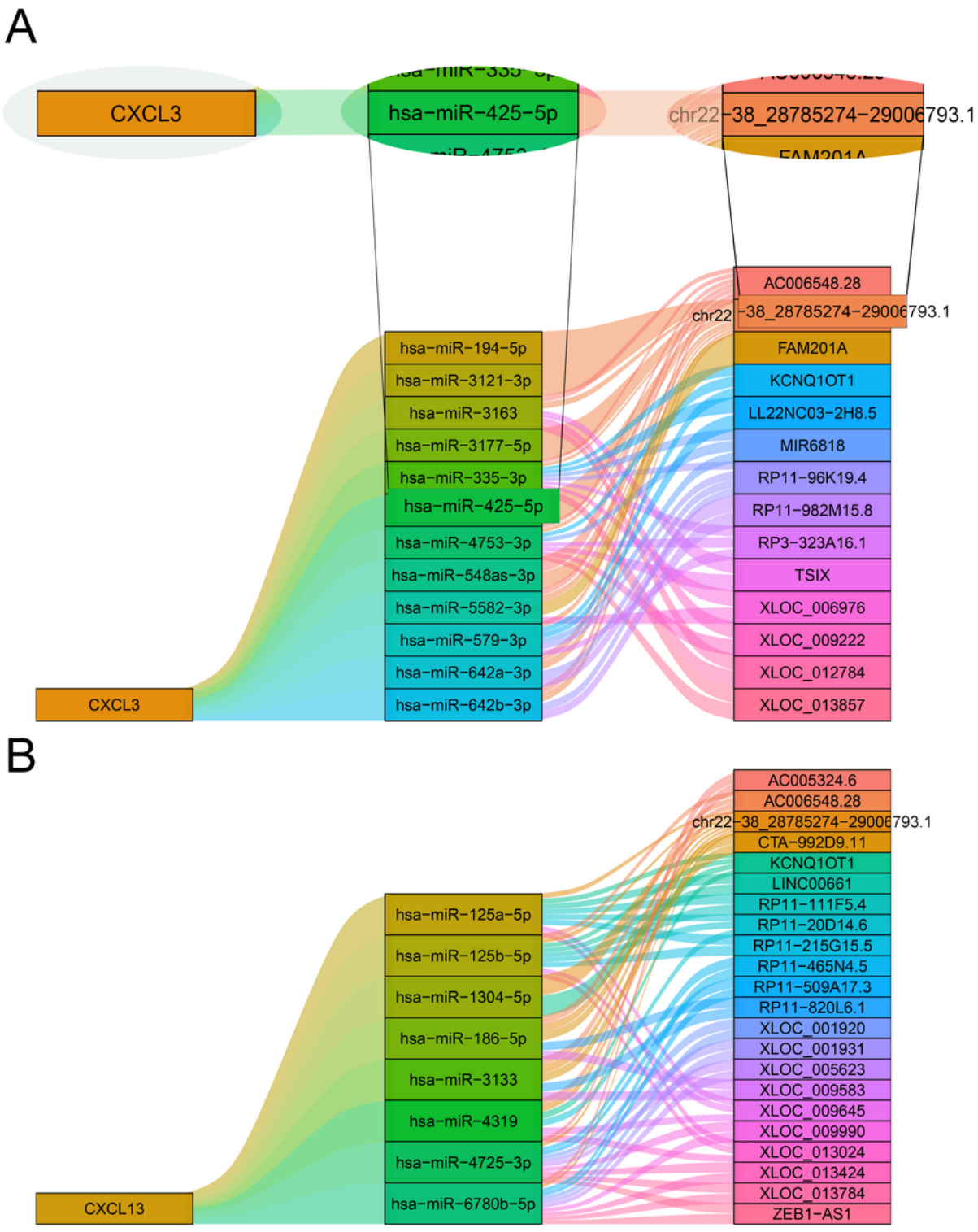

Figure 8

The ceRNA network construction of (A) CXCL3 and (B) CXCL13.

Supplementary Files

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- Additionalfile1TableS1.xlsx
- Supplementaryfigure1.png
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