CXCL11 Correlates with Anti-tumor Immune Microenvironment and Improved Prognosis in Colon Cancer

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Research

Keywords: CXCL11, colon cancer, prognosis, tumor immune microenvironment

Posted Date: August 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-51986/v1

Abstract

Background: CXCL11 has been considered to be responsible for tumor development, but the specific effect of CXCL11 in colon cancer was still obscure. Therefore, the prognostic value and immunological regulation effect of CXCL11 in colon cancer were evaluated in this study.

Methods: Three independent datasets were used for mRNA-related analysis: one dataset from the Cancer Genome Atlas (TCGA, *n*=451) and two single-cell RNA sequencing (scRNA-seq) datasets from Gene Expression Omnibus (GEO): GSE146771 and GSE132465. In addition, the patient cohort (the Yijishan Hospital cohort, YJSHC, *n*=108) was utilized for cell infiltration-related analysis, accordingly. Both CXCL11 mRNA expression and $CXCL11^+$ (CXCL11-producing) cells were assessed in colon cancer, whose effect on prognosis and immunological regulation was also studied.

Results: High CXCL11 expression were associated with better prognosis in colon cancer, which was still significant even if clinicopathological factors were adjusted. Furthermore, CXCL11 positively correlated with anti-tumor cells infiltration, such as $CD8^+$ T cells and natural killer cells. Meanwhile, CXCL11 correlated positively with several genes associated with DC, NK and T recruitment and a gene set of cytotoxic genes. Notably, CXCL11 correlated positively with several immune checkpoint related genes including of PD-L1.

Conclusions: CXCL11 contributed to anti-tumor immune microenvironment and could improve prognosis in patients with colon cancer. Especially, it's a potential approach that inducible expression of CXCL11 by genetic and pharmacological interventions is able to improve prognosis and response to anti-PD-1 (programmed cell death protein-1) antibody treatment in colon cancer. However, it requires to be verified by further prospective investigations.

Background

Colon cancer is still a major public health problem to be solved around the world [1-3]. It is well known that colon cancer shows significant heterogeneity even within the same stage, which results to the insufficience of predicting prognosis and therapeutic response by means of traditional clinical-pathological staging system [4, 5]. Therefore, the molecular mechanisms of colon cancer development and progression should be further explored and the novel biomarkers for prognosis evaluation in colon cancer should be identified.

Carcinogenesis represents sustained proliferation and resistance to apoptosis due to the reorganization of the tumor immune microenvironment and genomic variation. Notably, lots of studies have presented a consensus that robust anti-tumor immune microenvironment improved prognosis in patients with colon cancer [6-10]. Immunoscore had strong correlation with clinicopathological parameters containing Union for International Cancer Control TNM classification system and the AJCC (American Joint Committee on Cancer) [11, 12]. The distinct roles of tumor immunity are dependent on cytokine-cytokine interaction, which constitutes the cytokine networks to maintain intestinal homeostasis normally [13]. So far, various

cytokines and cytokine receptors participating in inflammatory diseases and tumor development have been illustrated, but less is known about the specific effect of CXCL11 in colon cancer.

CXCL11 (or I-TAC, IFN-inducible T cell a-chemoattractant) belongs to the chemokines, which are a series of low-molecular-weight cytokines and can be categorized into 4 families (i.e. CXC, CC, C, and CX3C chemokines) according to the number and spacing of the amino-terminal cysteines [14, 15]. CXC chemokines including of CXCL11 play a role in intercellular communication by mediating the activation and migration of specific populations of leukocytes [16]. CXCL11 can be stimulated by interferon in leukocytes. However, CXCL11 is not only expressed in leukocytes, but also in fibroblast, endothelial cells and tumor cells [17-19]. Therefore, CXCL11 could make complex and unique effects by binding to CXCR3 and CXCR7 of all kinds of cells. On the one hand, CXCL11 may promote tumor development and progression in some situations [20-22]. On the other hand, it may inhibit tumor growth by enhancing antitumor immunity and inhibiting angiogenesis [23]. Although several gene expression signatures including of CXC chemokines have been set as the potential prognostic biomarkers in some studies [24-26], the role of CXCL11 in the tumor immune microenvironment and response to therapeutic treatment in colon cancer was still obscure. In order to provide additional information to help clinicians select more appropriate drugs and improve outcome in patients with colon cancer, more attention should be attracted to the prognostic value and immunological regulation effect of CXCL11 in colon cancer.

In this study, the prognostic value of CXCL11 mRNA expression and $CXCL11^+$ cells infiltration in TCGA and YJSHC was investigated. Furthermore, the effect of CXCL11 on tumor immune microenvironment was evaluated. Currently, our work is the first research to analyze the clinical value of CXCL11 mRNA and $CXCL11^+$ cells, as well as the correlation between CXCL11 and tumor immune microenvironment comprehensively in colon cancer.

Methods

Patient samples

Three independent datasets were adopted in this study, which consisted of one dataset from TCGA (the Cancer Genome Atlas, *n*=451) and two scRNA-seq (single-cell RNA sequencing) datasets from GEO (Gene Expression Omnibus): GSE146771 and GSE132465. In addition, the patient cohort: YJSHC (the Yijishan Hospital cohort, *n*=108) was also involved. The characteristics of patients in TCGA were downloaded from http://www.cbioportal.org at 18 April 2020. The patients' statistical description of TCGA and YJSHC in this study was provided in supplementary Table S2. The YJSHC consisted of patients with colon cancer from Yijishan Hospital affiliated to Wannan Medical College (Wuhu, China), who received surgery during August 2011 to December 2014. All of the research was carried out in accordance with the Declaration of Helsinki and was approved by the ethics committee of Wannan Medical College, Yijishan Hospital. At the same time, informed consent was obtained from every patient.

Immunohistochemistry (IHC) staining and evaluation

We collected Formalin-fixed paraffin-embedded surgical specimens, which were used for tissue microarray construction and subsequent immunohistochemistry studies. Firstly, tissue microarray sections were rehydrated, which were treated with hydrogen peroxide and heat-mediated antigen retrieval by microwave with sodium citrate. Next, the slides were incubated with indicated antibodies which were obtained from Abcam (Cambridgeshire, UK) in a humidified box at 4℃ overnight. The instructions of four antibodies in this study were provided in supplementary Table S1. Following, color-reaction was realized by DAB substrate kit and nucleus counterstaining was carried out by hematoxylin. The level of protein expression was assessed based on the intensity of staining and the extent of staining at 200X under the microscope. For the extent of staining, the quantity score (0-4) denoted 0%, 1-10%, 11-50%, 51-80% and 81-100% of positive cells, respectively. The degree of staining intensity was divided into three grades, which were weak, moderate and strong staining. What should be noted was that the corresponding intensity scores were 1-3. The final IHC score was calculated by multiplying the quantity and intensity scores.

Immune cells infiltration analysis

The relative abundance of the tumor infiltrated lymphocytes (TILs) in different CXCL11 mRNA expression status of colon cancer was calculated by CIBERSORT (https://cibersort.stanford.edu/) [27] and TISIDB (http://cis.hku.hk/TISIDB/index.php) [28]. For each cancer type, GSVA (gene set variation analysis) was adopted to infer the relative proportion of various TILs based on the immune-related gene expression profile of 28 TIL types from Charoentong's study [29]. The correlation between CXCL11 and TILs was measured by Spearman’s test. In this study, we also investigated the differential expression of CXCL11 between tumor and adjacent normal tissues across all TCGA tumors in TIMER (https://cistrome.shinyapps.io/timer/) [30].

Differential expression analysis

Differential expression analysis was conducted by R package “limma”. Differentially expressed genes were up-regulated or down-regulated genes with fold change ≥2 and false discovery rate (FDR) p value <0.05 between CXCL11-high and CXCL11-low tumor samples.

Gene set enrichment analysis

The significantly enriched pathways in CXCL11-high tumor samples were identified by gene set enrichment analysis (GSEA) performed by “MSigDB” (Molecular Signatures Database, version 6.0). Meanwhile, the enrichment score was assessed by the expression of a gene set and the “enriched” gene set represented the majority of a gene set had higher expression.

Single-cell transcriptome analysis

External single-cell transcriptome data of colon cancer patients were retrieved from Gene Expression Omnibus (GSE146771 and GSE132465). Single-cell data analysis was performed using scanpy v.1.4 (https://scanpy.readthedocs.io/en/stable/). Since single-cell technologies currently capture only a portion of the transcripts in any cell, we generated the “pseudobulks” as technical replicates by summing the raw UMI counts for each gene from the same patient.

Statistical analysis

R and corresponding R packages were utilized for the statistical analysis and the Cutoff Finder platform (http://molpath.charite.de/cutoff) was utilized to automatically determine the cutoff points in this study [31]. Results were all displayed by mean ± SD. The two-tailed *p* value <0.05 was considered statistically significant. Student’s t-test was adopted for the analysis of continuous variables. Spearman's correlation was made for the analysis of the correlation. Kaplan-Meier analysis was used for survival analysis and the log-rank test were used for the comparison of survival among multiple groups, respectively.

Results

Aberrant expression and prognostic value of CXCL11 in colon cancer

As shown in Figure 1a, the Kaplan–Meier survival curve revealed that patients with high CXCL11 mRNA expression had better prognosis (p=0.0053) in TCGA. Next, TISIDB was utilized to examine the association between CXCL11 and clinical outcome across all TCGA tumors and we found that patients with high expression of CXCL11 mRNA had good prognosis especially in COAD (colon cancer) (Figure 1b). Furthermore, we explored the differential expression of CXCL11 between tumor and adjacent normal tissues across all TCGA tumors in TIMER and found that CXCL11 was significantly up-regulated in tumor than adjacent normal tissues across several tumors (Figure 1c). In addition, we also validated the up-regulated expression of CXCL11 in tumor compared to adjacent normal tissues (Figure 2a and 2b) by immunochemistry staining in the Yijishan Hospital cohort (YJSHC).

Prognostic value of $CXCL11^+$ cells and predictive value of $CXCL11^+$ cells for response to ACT

Patients with high abundance of intratumoral $CXCL11^+$ cells had better OS (overall survival, p=0.001) within the YJSHC, as shown in Figure 2c. Meanwhile, in Figure 2d, it could be observed that the effect of intratumoral $CXCL11^+$cells infiltration kept significant after confounders were adjusted (HR= 0.35; 95% CI 0.17-0.72; p= 0.004).

Association of CXCL11 with tumor immune microenvironment

Firstly, the differential tumor immune microenvironment in different groups of CXCL11 mRNA expression in TCGA was investigated by CIBERSORT. High CXCL11 mRNA expression group had higher proportion of anti-tumor immune cells, such as: $CD8^+$ T cells (CD8T, $p<0.001$), activated natural killer cells (NKa, $p<0.05$), as shown in Figure 3a. On the contrary, high CXCL11 mRNA expression group had lower proportion of pro-tumor immune cells, such as: M0 macrophages (M0, $p<0.05$), resting natural killer cells (NKr, $p<0.001$), monocytes (Mono, $p<0.05$).

Next, TISIDB[28] was employed to validate which kinds of intratumoral lymphocytes might be regulated by CXCL11 across all TCGA tumors (supplementary Figure S1a). Surprisingly, we found that CXCL11 positively correlates with activated $CD8^+$ T cells (Act CD8, r=0.55, $p<0.001$; Figure 3b), natural killer T cells (NKT, r=0.438, $p<0.001$; Figure 3c), activated dendritic cells (Act DC, r=0.488, $p<0.001$; Figure 3d) in colon cancer.

Genetic alteration and enriched biological process in the high and low CXCL11 metagene expression groups

Firstly, we performed GSEA and identified that biological pathways enriched in high-CXCL11 tumor samples were immune-activated process, which consisted of chemokine signaling pathway, cytokine-cytokine receptor interaction, antigen processing and presentation, T cell receptor signaling pathway natural killer cell mediated cytotoxicity, intestinal immune network for IgA production, interferon alpha response interferon gamma response , as shown in Figure 3e-3l.

We then analyzed the genetic alteration and found that differentially expressed genes in tumors revealed several immune-activated genes in high CXCL11 mRNA expression group. The volcano plot was shown in Figure 4a. As shown in Figure 4b and 4c, CXCL11 positively correlated with a gene set associated with DC, NK and T recruiting genes: CCL4 (r=0.543, $p<0.001$), CCL5 (r=0.59, $p<0.001$), CXCL9 (r=0.717, $p<0.001$), CXCL10 (r=0.821, $p<0.001$).

Cytotoxic genes [32-34] including IFNG, GZMA, GZMB, GZMK, GZMM and PRF1 were positively correlated with CXCL11 mRNA expression (Spearman's q=0.49, 0.55, 0.22, 0.37, 0.21 and 0.37; $p<0.001$, $p<0.001$, $p<0.001$, $p=0.045$, and $p=0.004$; respectively), as shown in Figure 4d. Interestingly, CXCL11 positively correlated with several immunosuppressive molecules [34] including of PDCD1,PD-L1, CTLA4 (Spearman's q=0.35, 0.56, and 0.44; $p<0.001$, $p<0.001$ and $p<0.001$; respectively), as shown in Figure 4e. Surprisingly, we also found the up-regulated expression of CXCL11 in tumor compared to adjacent normal tissue (Figure 4f and 4h), and the positive correlation between the expression of CXCL11 and PD-L1 (r=0.66, r=0.78; $p<0.001$, $p<0.001$; respectively; Figure 4g and 4i) in colon cancer single-cell RNA-seq dataset GSE146771 and GSE132465.

Validating the association of $CXCL11^+$ cells with TILs and PD-L1

Further analysis in YJSHC was conducted to validate the findings in TCGA. As was expected, tumor with high abundance of $CXCL11^+$ cells infiltration tended to have high abundance of intratumoral $CD8^+$ T cells and $CD56^+$ NK cells infiltration. In addition, high abundance of $CXCL11^+$ cells infiltration was associated with high abundance of intratumoral PD-$L1^+$ cells. Accordingly, the expression of PD-L1, CD8A, CD56 was higher in CXCL11-high group than CXCL11-low group (Figure 5b-5d) and the positive correlation between the expression of CXCL11 and PD-L1, CD8A, CD56 (r=0.62, p<0.001; r=0.34, p<0.001; r=0.32, p<0.001; respectively; Figure 5e-5g) was significant within YJSHC.

Discussion

In this study, we found that patients with high abundance of intratumoral $CXCL11^+$ cells infiltration had better survival and CXCL11 was found to have independent prognostic value in colon cancer. Considering that CXCL11-producing cells were a variety of heterogeneous cells [17] and the intercellular communication mediated by CXCL11 was complicated, the discoveries make sense [26, 35].

Furthermore, we found that CXCL11 positively correlated with CCL4, CCL5, CXCL9, CXCL10 in colon cancer, which were associated with DC, NK and T recruitment and may played an important role in inhibiting tumor growth and improving prognosis [36, 37]. Consequently, we found that tumor with high CXCL11 mRNA expression and $CXCL11^+$ cells had high abundance of anti-tumor $CD8^+$ T cells, $CD56^+$ NK cells infiltration [38, 39], which was validated in TCGA and YJSHC. These data indicated that CXCL11 in colon cancer could function by mediating the infiltration of other immunocytes. Accordingly, the positive correlation between CXCL11 and cytotoxic genes (IFNG, GZMA, GZMB, GZMK and GZMM) [32-34] was discovered, which indicated that CXCL11 could enhance the cytotoxic function and contribute to the immunostimulatory microenvironment. However, it requires further investigation to discover the molecular mechanism underlining the correlation between CXCL11 and tumor immune microenvironment.

It is well known that ICIs (immune checkpoint inhibitors) therapy has been widely adopted in a variety of cancers [40, 41]. Several studies have showed that tumor immune signatures could provide more information for clinicians to make appropriate choice of immunotherapy treatment [42-44]. In this study, we found that CXCL11 positively correlated with PD-L1 in TCGA and YJSHC, which was also verified in colon cancer single-cell RNA-seq dataset GSE146771 and GSE132465. The discovery was consistent with the initial study that PD-L1 blockade combined with an oncolytic vaccinia virus expressing CXCL11 in murine tumor models significantly shrinked tumor burden and improved prognosis [45]. Several studies have showed that massive intratumoral $CD8^+$ T cells infiltration was able to alleviate the resistance to ICB (immune checkpoint blockade) in some way and intratumoral $CD8^+$ T cells infiltration had the predictive value of therapeutic response to ICB treatment [41]. Nevertheless, prospective investigations need to be conducted to validate whether CXCL11 can predict the response to ICIs treatment in colon cancer. It's

promising that increased expression of CXCL11 contributes to the anti-tumor immune microenvironment and sensitize patients who are resistant to anti-PD-1blockade treatment.

Somehow, our study also has some limitations. On the one hand, our analysis can only reflect certain aspects of tumor status and need to be verified in additional patient cohorts. On the other hand, in vitro and in vivo studies should be performed to discover the latent molecular mechanism.

Conclusions

In conclusion, CXCL11 was identified as a prognostic biomarker in patients with colon cancer in this study. This was the very first study which revealed the clinical value and the correlation between CXCL11 and anti-tumor immune microenvironment in colon cancer. In addition, inducible expression of CXCL11 in colon cancer may improve the therapeutic response of patients receiving anti-PD-1 antibody treatment. No matter how, our findings can provide some novel insights to assist clinicians in choosing appropriate drugs for their patients and improve the long-term outcome of patients with colon cancer.

Abbreviations

TCGA: Cancer Genome Atlas; scRNA-seq: single-cell RNA sequencing; GEO: Gene Expression Omnibus; YJSHC: the Yijishan Hospital cohort; ACT: adjuvant chemotherapy; AJCC: American Joint Committee on Cancer; TILs: tumor infiltrated lymphocytes; GSVA: gene set variation analysis; FDR: false discovery rate; GSEA: gene set enrichment analysis; MSigDB: Molecular Signatures Database; OS: overall survival; ACC: adrenocortical carcinoma; BLCA: bladder urothelial carcinoma; BRCA: breast carcinoma; CESC: cervical squamous carcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; HNSC: head and neck squamous cell carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LGG: lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; MESO: mesothelioma; OV: ovarian serous adenocarcinoma; PAAD: pancreatic ductal adenocarcinoma; PCPG: paraganglioma&pheochromocytoma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; SARC: sarcoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell cancer; THCA: thyroid papillary carcinoma; UCEC: uterine corpus endometrioid carcinoma; UCS: uterine corpus squamous carcinoma; UVM: uveal melanoma; Bn: naive B cells; Bm: memory B cells; Plasma: plasma cells; CD8T: $CD8^+$ T cells; CD4Tn: naive $CD4^+$T cells; CD4Tmr: resting memory $CD4^+$T cells; CD4Tma: activated memory $CD4^+$T cells; Tfh: follicular helper T cells; Tregs: regulatory T cells; Tgd: γδT cells; NKr: resting natural killer cells; NKa: activated natural killer cells; Mono: monocytes; M0: M0 macrophages; M1: M1 macrophages; M2: M2 macrophages; DCr: resting dendritic cells; DCa: activated dendritic cells; Mastr: resting mast cells; Masta: activated mast cells; Eos: eosinophils; Neut: neutrophils.

Declarations

Acknowledgements

The authors thank the members of their laboratory for their research work.

Authors’ contributions

Y.Y.Cao, T.T.Sun and Y.R.Ma performed the experiments. Y.W.Zhang, N.L.Jiao and X.Y.Zhang collected patient samples. Y.Y.Cao and H.Y.Chen performed the data analysis work and wrote the manuscript. H.Y.Chen and J.Hong designed the study and assisted in writing the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by grants from the National Natural Science Foundation of China (31701250).

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Patient samples were obtained after informed consent according to the Declaration of Helsinki. This study was approved by local ethic committee institutional review board of Wannan Medical College, Yijishan Hospital (Wuhu, China).

Consent for publication

Not applicable.

Competing interests

All other authors declare no competing financial interests.

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Figures

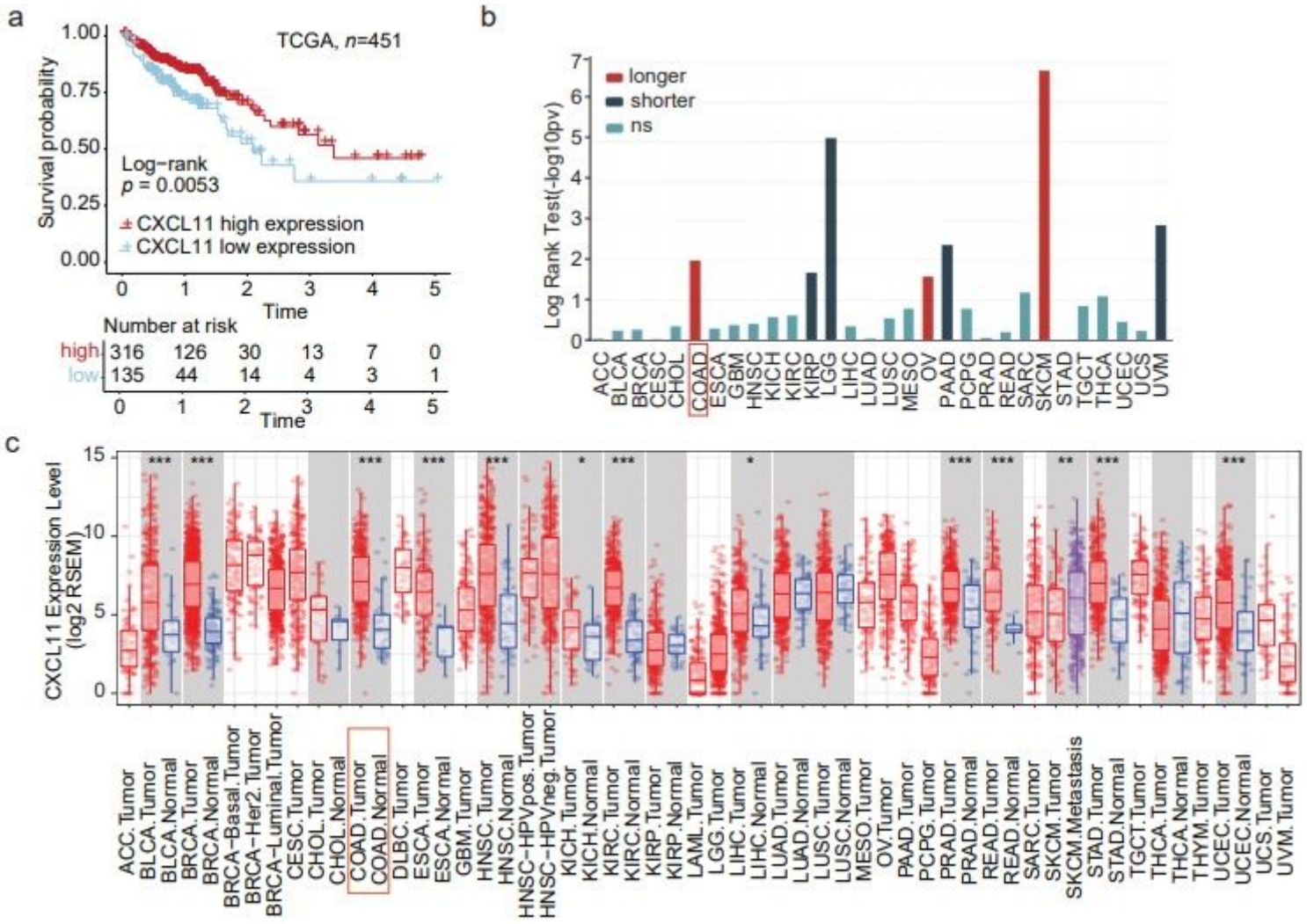

Figure 1

Aberrant Expression and prognostic value of CXC11 in TCGA (the Cancer Genome Atlas). Survival curves of CXCL11 mRNA expression for TCGA (a). Correlation between CXCL11 and clinical outcome in all TCGA tumors, *p<0.05, ** p<0.01 and *** p<0.001 (b). The relative level of CXCL11 mRNA expression in all TCGA tumors (c). ACC = adrenocortical carcinoma; BLCA = bladder urothelial carcinoma; BRCA = breast carcinoma; CESC = cervical squamous carcinoma; CHOL = cholangiocarcinoma; COAD = colon adenocarcinoma; ESCA = esophageal carcinoma; GBM = glioblastoma multiforme; HNSC = head and neck squamous cell carcinoma; KICH= Kidney Chromophobe; KIRC = Kidney renal clear cell carcinoma; KIRP = Kidney renal papillary cell carcinoma; LGG = lower grade glioma; LIHC = liver hepatocellular carcinoma; LUAD = lung adenocarcinoma; LUSC = lung squamous cell carcinoma; MESO = mesothelioma; OV = ovarian serous adenocarcinoma; PAAD = pancreatic ductal adenocarcinoma; PCPG = paraganglioma&pheochromocytoma; PRAD = prostate adenocarcinoma; READ = rectum adenocarcinoma; SARC = sarcoma; SKCM = skin cutaneous melanoma; STAD; TGCT = testicular germ cell cancer; THCA = thyroid papillary carcinoma; UCEC = uterine corpus endometrioid carcinoma; UCS = uterine corpus squamous carcinoma; UVM = uveal melanoma.

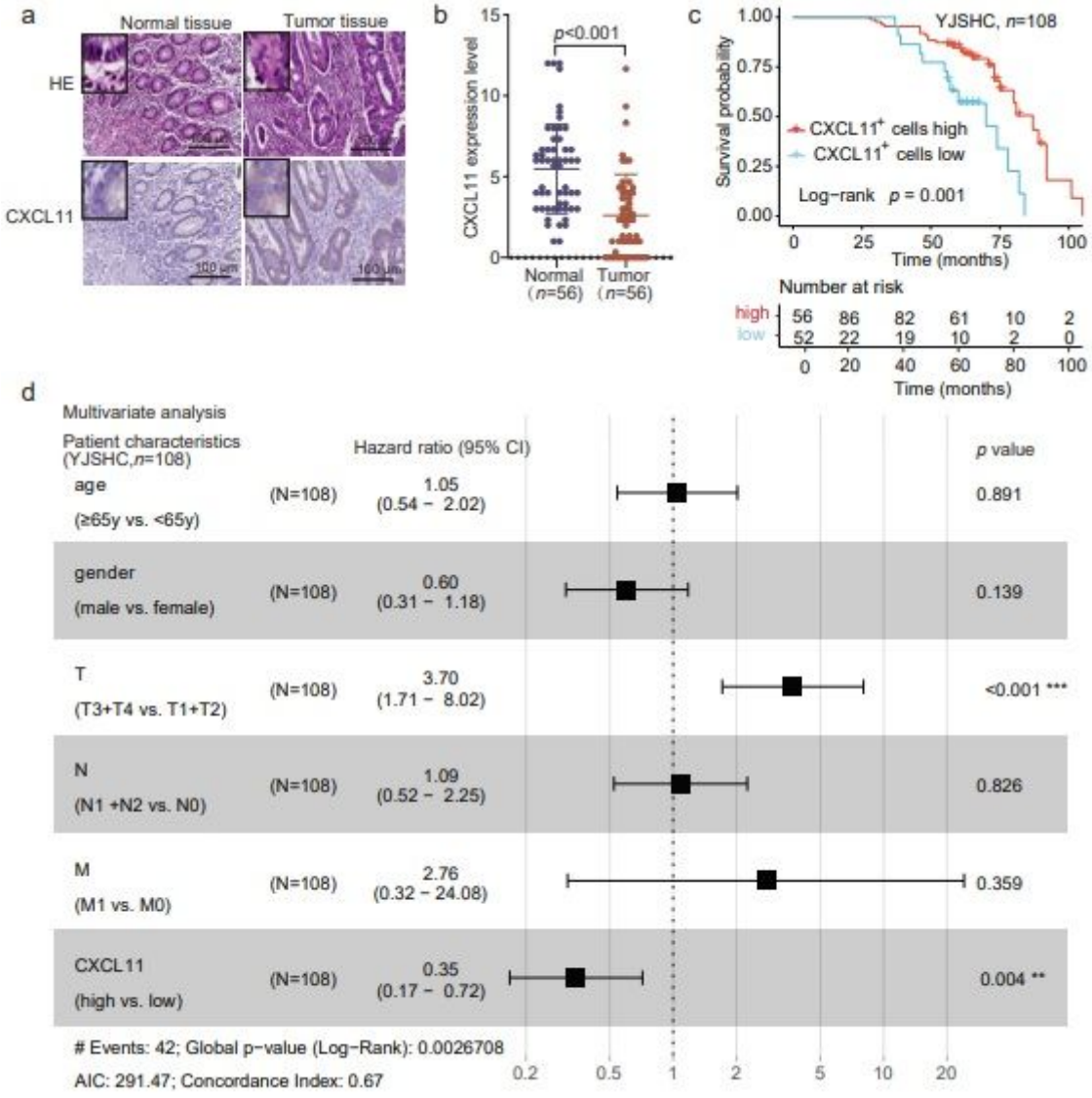

Figure 2

Expression of CXCL11 and Effect of CXCL11+ cells on patient survival in Yijishan Hospital cohort (YJSHC). Comparison of CXCL11+ cells between tumor and adjacent normal tissues (a and b) in YJSHC. Survival curves of intratumoral CXCL11+ cells for overall survival in YJSHC (c). multivariate cox analysis for CXCL11+ cells infiltration and clinicopathological variables (d). *p<0.05, ** p<0.01 and *** p<0.001.

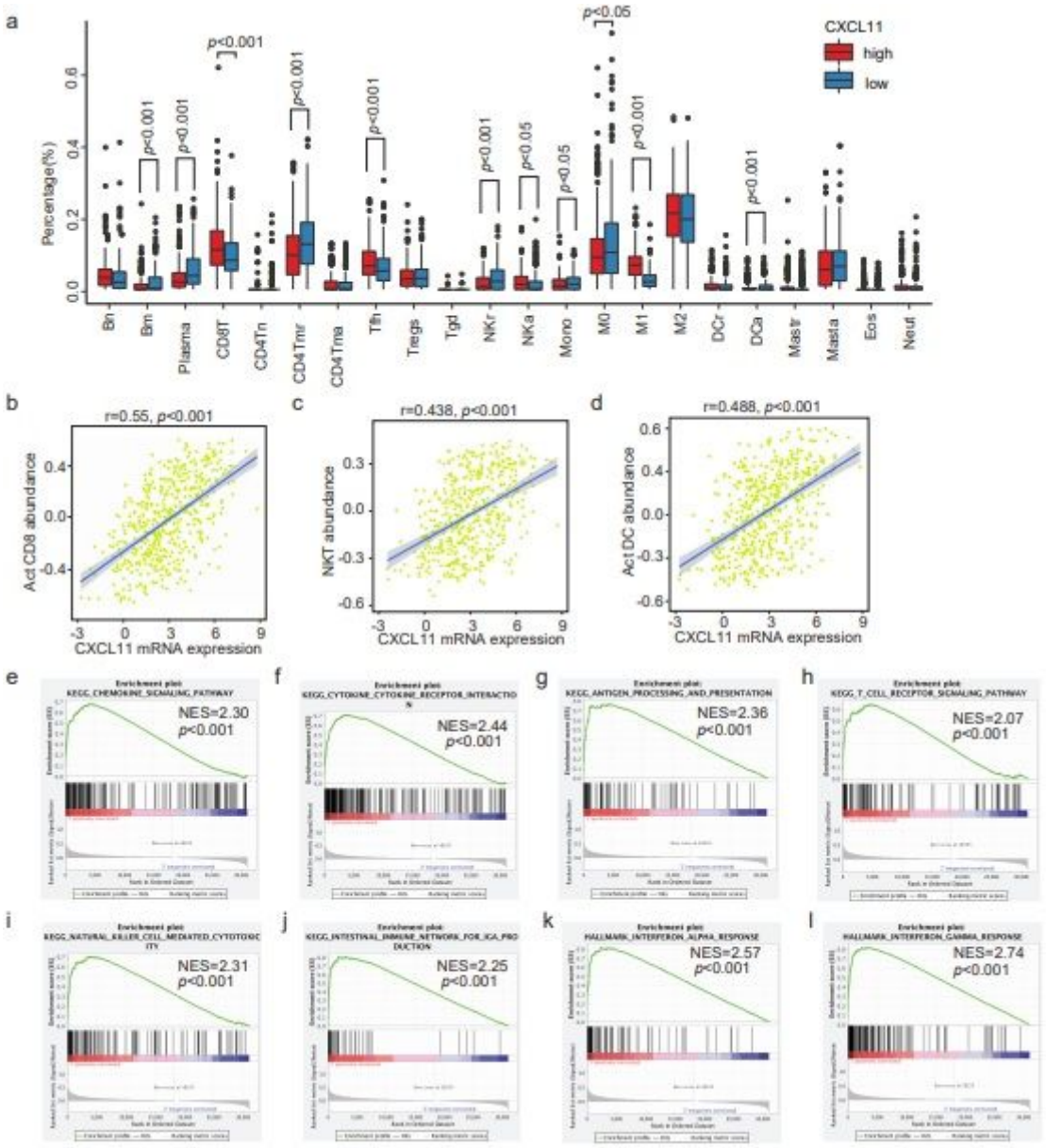

Figure 3

The infiltration of various TILs and enriched biological pathways between high and low CXCL11 groups. The infiltration of various TILs identified by CIBERSORT between high and low CXCL11 groups in TCGA (a). Bn = naive B cells; Bm = memory B cells; Plasma = plasma cells; CD8T = CD8+ T cells; CD4Tn = naive CD4+T cells; CD4Tmr = resting memory CD4+T cells; CD4Tma = activated memory CD4+T cells; Tfh = follicular helper T cells; Tregs = regulatory T cells; Tgd = γδT cells; NKr = resting natural killer cells; NKa = activated natural killer cells; Mono = monocytes; M0 = M0 macrophages; M1 = M1 macrophages; M2 = M2 macrophages; DCr = resting dendritic cells;DCa = activated dendritic cells; Mastr = resting mast cells; Masta = activated mast cells; Eos = eosinophils; Neut = neutrophils. The correlation between CXCL11 and the abundance of Act CD8 (activated CD8+ T cells), NKT (natural killer T cells), Act_DC (activated dendritic cells) in TISIDB (b-d). Gene set enrichment analysis (GSEA) revealed the enriched biological pathways in high CXCL11 group (e-l). NES, normalized enrichment score.

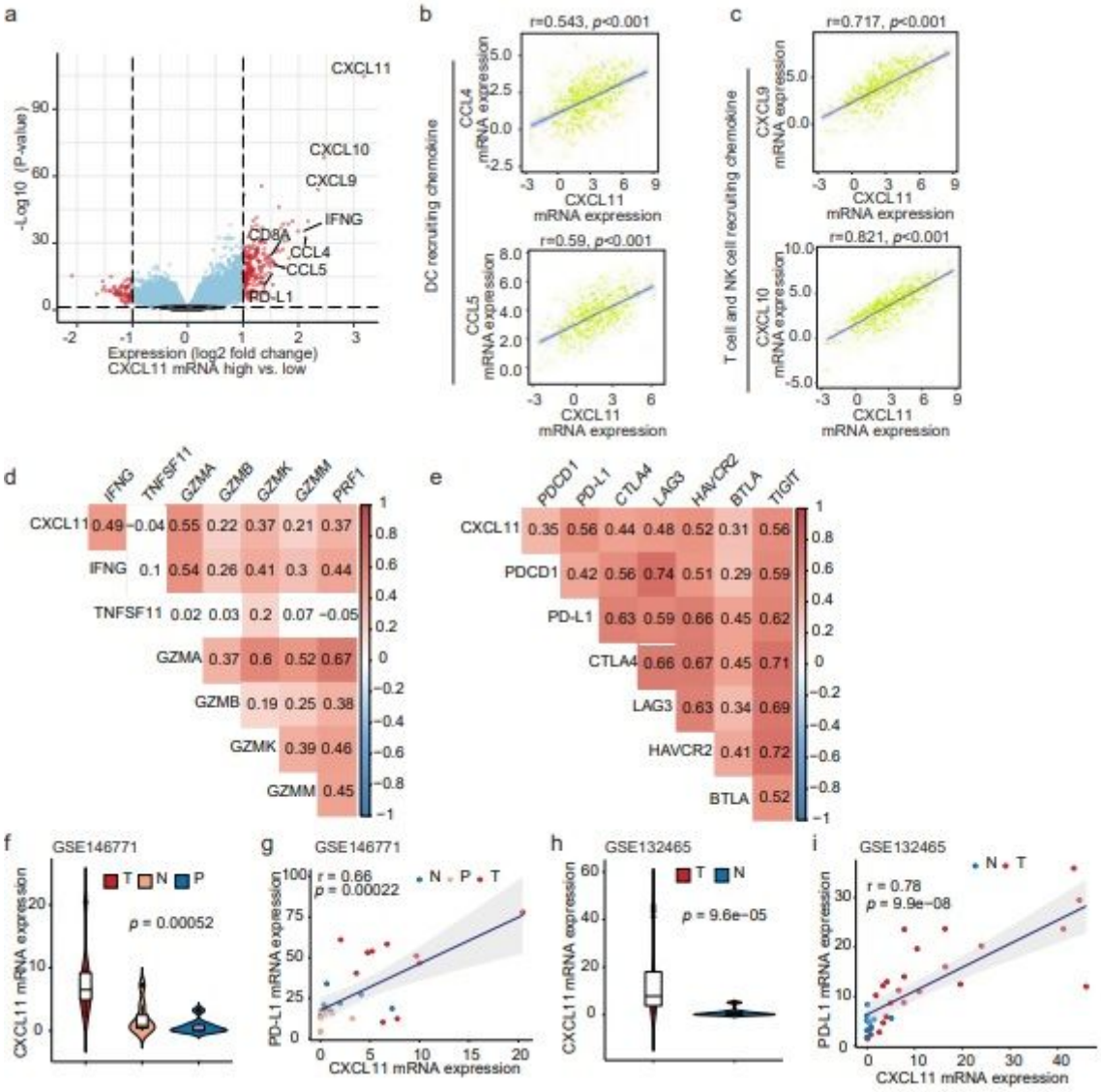

Figure 4

Association of CXCL11 with immune-related genes. The volcano plot showed the differentially expressed genes between high and low CXCL11 groups (a). Correlation between CXCL11 and indicated transcripts of DC and T cell-recruiting chemokines (b and c). Correlation between CXCL11 and cytotoxic molecules (d), immunosuppressive genes (e). The differential expression of CXCL11 in N (normal tissue), P (para-carcinoma tissue) and C (cancer tissue) of single-cell RNA-seq dataset GSE146771 (g) and GSE132465 (i). Spearman's correlation between CXCL11 and PD-L1 in single-cell RNA-seq dataset GSE146771 (f) and GSE132465 (h). Each dot represents one patient in GSE146771 and GSE132465.

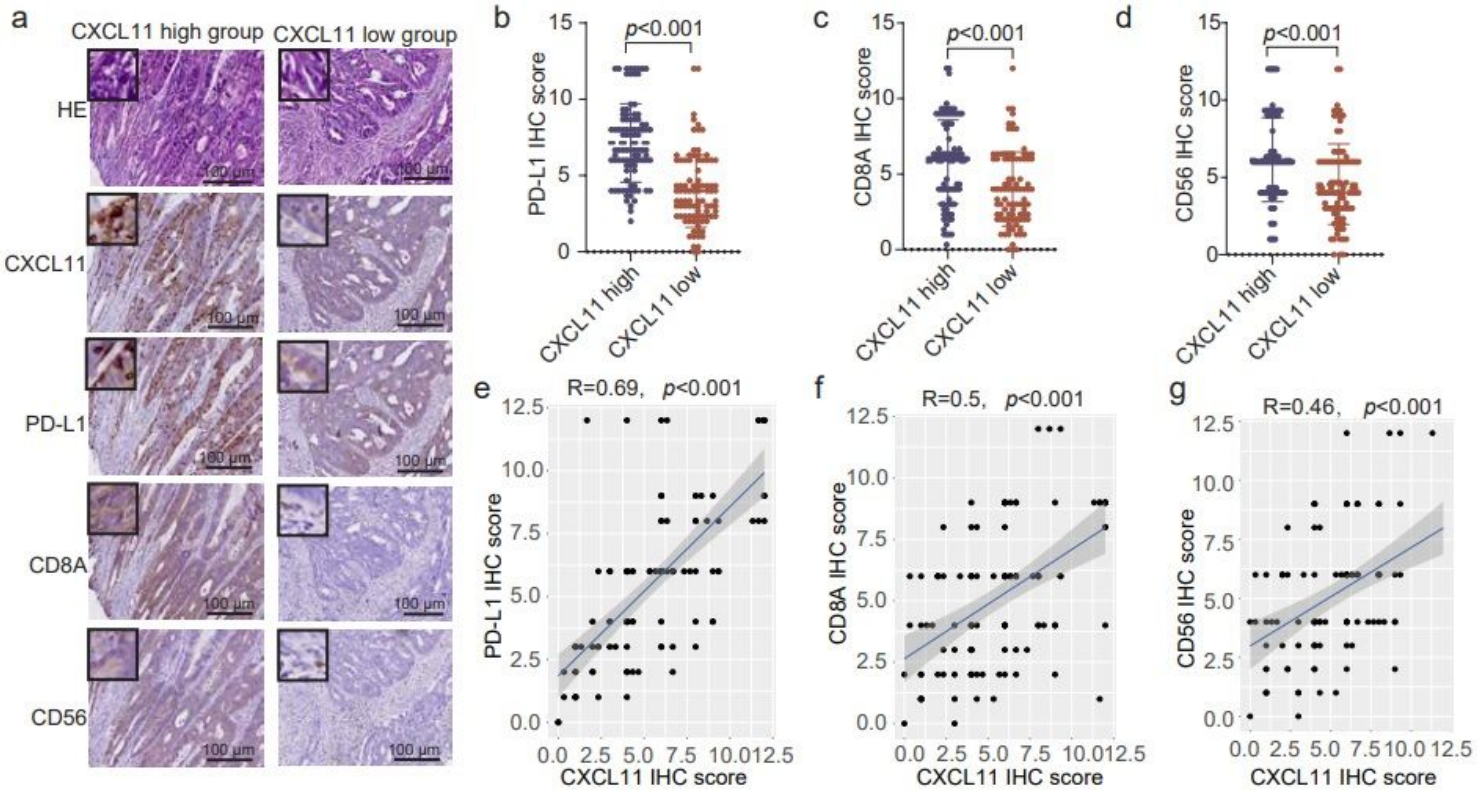

Figure 5

Verification of the TILs between different groups of CXCL11+ cell infiltration by immunohistochemistry (IHC). Comparison of the infiltation of PD-L1+, CD8A+, CD56+ cells between high and low intratumoral CXCL11+ cells groups in YJSHC (a-d). Correlation of CXCL11 with PD-L1, CD8, CD56 in YJSHC (e-g).

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