CXCL10 Expression in Tumor Tissues and is An Independent Prognostic Indicator of Pancreatic Cancer

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Research Article

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Abstract

Background: Pancreatic cancer is a malignant disease with difficult diagnosis and high mortality. The most common type is pancreatic duct adenocarcinoma (PDAC). CXCL10 is of great significance in the treatment of immune diseases and cancer. Here, we evaluated the expression of CXCL10 in pancreatic cancer and its clinical significance.

Results: The results of the TCGA database showed that the expression of CXCL10 in pancreatic cancer tissues was higher than that in matched adjacent tissues (P <0.05). Patients with high expression of CXCL10 had a poor prognosis compared with those with low expression of CXCL10 (P=0.0051).We found that CXCL10 was positively and strongly correlated with tumour-infiltrating neutrophils and macrophages in PDAC (P<0.0001). We also performed CXCL10 immunohistochemical staining in a tissue microarray (TMA), which included tumour samples collected from 119 PDAC patients (each sample was matched with an adjacent tissue sample). The expression level of CXCL10 in PDAC tissues was prominently higher than that in the matched adjacent tissues (P = 0.0001). Analysis of clinicopathological factors suggested that the high expression of CXCL10 was correlated with the patient's T stage (P = 0.020). In addition, higher CXCL10 expression was significantly associated with worse overall survival of PDAC patients (P = 0.012). The results of COS multivariate analysis suggested that age (P=0.005), histological grade (P=0.001), TNM stage (P=0.017) and CXCL10 expression (P=0.024) were independent prognostic factors for pancreatic cancer.

Conclusion: Our data indicate that CXCL10 is highly expressed in PDAC and may be used as an independent clinical prognostic indicator for PDAC.

Introduction

Pancreatic cancer is a malignant tumour with difficult early diagnosis, early metastasis, and high mortality. The most common type is pancreatic ductal adenocarcinoma. The prognosis is poor, and mortality is high because late metastases usually occur at the time the diagnosis is confirmed (1). The tumour microenvironment (TME) is composed of several cell types, including endothelial cells, fibroblasts and immune cells, as well as extracellular components that flood around tumour cells and affect the tumour microenvironment, such as chemokines, cytokines, and extracellular matrix(2). Many studies have shown that pancreatic cancer has an unusually complex TME (2, 3). Tang's study reported that Treg cells highly infiltrate tumour tissues and inhibit the immunity of antitumour CD8+ T lymphocytes. Therefore, the level of CD8+ T lymphocyte infiltration in PDAC is lower than that in paracarcinoma tissue. This might be related to the tumour immune escape mechanism (4). There are a variety of abnormally expressed chemokines and their receptor axes in the tumour microenvironment, which play a significant role in tumour migration, invasion, angiogenesis, late metastasis and the growth of various intercellular cells(5). CXCL10 has been reported to induce the polarization of CD4+ and CD8+ T cells and enhance the biological function of migration in tumours. This feature makes CXCL10 a "key chemokine" that has important implications in the therapy of immune diseases and cancer. CXCL10 has two opposite effects

in tumours and can promote and inhibit cancer progression(6). Many researchers have reported that CXCL10 is associated with tumour-infiltrating neutrophils (TINs) and promotes tumour progression in pancreatic, cholangiocarcinoma, breast, gastric, colorectal and bladder cancers (7-12). Most studies have shown that CXCL10 is related to malignant phenotypes such as tumour proliferation and metastasis, suggesting that it is related to poor prognosis in cancer patients.

Therefore, in this research, we used bioinformatics analysis of the Cancer Genome Atlas dataset to explore the expression level of CXCL10 and the correlation between CXCL10 and invasive tumour immune cells. In addition, to confirm whether CXCL10 is a prognostic marker of PDAC, the expression level of CXCL10 in tumour samples from PDAC patients and matched normal adjacent tissues was directly correlated with clinicopathological features by immunohistochemistry and with the prognosis of pancreatic cancer patients.

Results

Differential expression of CXCL10 in public databases (data from the GEO database).

A total of 890 differentially expressed (DE) genes were identified in pancreatic tumour tissues and normal tissues in the GSE56560 dataset. Compared with all chemokines and their receptors discovered to date, 10 DE chemokines or their receptors overlapped in the GSE56560 dataset, of which one chemokine was CXCL10 (Fig. 1).

CXCL10 expression in PDAC samples from the TCGA database.

Studies have reported that CXCL10 is highly expressed in many common tumours and may serve as an oncogene in PAAD, including pancreatic adenocarcinoma (PAAD) and bladder urothelial carcinoma, lung adenocarcinoma, breast invasive carcinoma, prostate adenocarcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, liver hepatocellular carcinoma, colon adenocarcinoma, lymphoid neoplasm diffuse large B-cell lymphoma, thymoma, oesophageal carcinoma, rectum adenocarcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, kidney renal clear cell carcinoma, lung squamous cell carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, stomach adenocarcinoma, testicular germ cell tumours, thyroid carcinoma, and uterine corpus endometrial carcinoma ($P<0.05$) (Fig. 2A). Compared with 171 matched paracancerous tissues, CXCL10 was significantly highly expressed in 179 PAAD tissues (data from the TCGA database) ($P<0.05$) (Fig. 2B).

Patients with PAAD with high CXCL10 expression have a short survival time (data from the TCGA dataset).

In the aggregate, 178 pancreatic cancer patients with CXCL10 expression information from the TCGA dataset were included in the present study. Patients were divided into high- and low-expression groups

according to the median value of the expression. Compared with the low CXCL10 group, the high CXCL10 group had a prominently poorer prognosis (P=0.0051; Fig. 3).

The relationship between CXCL10 and tumour-infiltrating immunocytes.

We estimated the relationship between CXCL10 and tumour-infiltrating immune cells (B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils and dendritic cells). The analysis showed that neutrophils (PAAD: R=0.39, P<0.001) and dendritic cells (PAAD: R=0.442, P<0.001) showed the most significant correlation (Fig. 4).

Expression of CXCL10 in PAAD patients (data from the tissue microarray dataset).

For each individual point, the final H-SCORE for CXCL10 expression was represented by the average H-SCORE of duplicates in TMA. The mean H-SCORE in PAAD tissues was 116.7 (95% CI, 110.5-122.8). The mean H-SCORE in matched paracancerous tissues was 71.32 (95% CI, 66.44-76.20). The discrepancy in the CXCL10 expression level between PAAD tumour tissues and matched paracancerous tissues was significant (P<0.0001; Fig. 5A). Positive staining for CXCL10 in PAAD tissues (Fig. 5B) and negative staining for CXCL10 in matched paracancerous tissues (Fig. 5C).

Correlation between CXCL10 and clinicopathological features (data from tissue microarray dataset).

Statistical results revealed that for patients with PAAD, an increased level of CXCL10 expression was associated with T stage (P=0.020). No significant correlations were observed between CXCL10 expression and sex, tumour site, histological grade, tumour site, age, vascular invasion, N stage, or M stage (P >0.05; Table II).

High expression of CXCL10 predicted a shorter survival time for patients with PAAD (data from the tissue microarray dataset).

Prognostic analysis was also conducted based on the tissue microarray dataset. Kaplan-Meier survival analysis and the log-rank test suggested that patients with high CXCL10 expression suffered a prominently poorer prognosis than those with low expression (log-rank test: P = 0.012, Fig. 6). This also shows that CXCL10 may be used as an independent target for the treatment of PAAD.

Multivariate Cox regression analysis suggested that CXCL10 was an independent prognostic factor for PAAD patients (data from the tissue microarray dataset).

First, univariate analysis was used to screen for prognostic factors. Clinicopathological evaluation suggested that CXCL10 expression (P=0.015), age (P=0.037), histological grade (P=0.010), N stage (P=0.003) and TNM stage (P=0.003) were prominently related to poor overall survival. Due to associations of N stage, M stage and TNM stage, only the TNM stage variable was included in the Cox regression analysis. The results suggested that histological grade (P=0.001), age (P=0.005), CXCL10 (P=0.024) and TNM stage (P=0.017) were independent prognostic factors for pancreatic cancer patients.

CXCL10 [adjusted hazards ratio 1.697 (95% CI 1.073 to 2.684)] in Cox multivariate regression analysis suggested that in PAAD patients, patients with high CXCL10 expression had an approximately 1.7 times higher risk of death than patients with low CXCL10 expression. Therefore, this also indicates that CXCL10 is an independent prognostic factor for PAAD patients (Fig. 7).

Discussion

Chemokines are a very small but highly conserved family of cytokines that mediate a variety of biological processes, including chemotaxis, haematopoiesis, and angiogenesis, and act by interacting with G-protein coupled receptors (GPCRs) on the cell surface. Because chemokines and chemokine receptors play an important role in various biological and pathological functions, they have been the focus of clinical intervention discovery(16). Chemokines and their receptors bind to each other in multiple nonspecific ways, forming various ligand and receptor axes(17). They can make cells move in a directional way and regulate the functional and physiological processes of cells (16). CXCL10/IP-10 (also known as IFN-gamma-inducible protein 10) is secreted by multiple cells, such as keratinocytes, macrophages, fibroblasts, endothelial cells, and activated T cells(18). CXCR3 is the chemokine receptor of CXCL10, which is abundant in T cells (both CD4+ and CD8+) and NK cells(19). To date, the main research on the two main chemokines is the chemokine receptor axis: the CXCR4-CXCL12 axis and the CCR2-CCL2 axis. The CXCR4-CXCL12 axis attracts tumour cells to the tissue via CXCL12, which is a growth factor for a variety of malignancies, inducing TAMS and CD4+ T cells to produce IL-10 at tumour sites to suppress antitumour immunity (20, 21). Accumulation of the CCR2-CCl2 axis via TAMS is a key factor in tumour progression, and this chemokine-chemokine receptor interaction is important for growth-promoting malignancies in an autocrine manner(22-24). Medoff. BD reports that CXCL10 facilitates the migration of recruiting effector T cells to inflammatory tissues. In a variety of diseases, CXCL10-deficient or CXCL10-overexpressing mouse models have proven that CXCL10 promotes CD4+ and CD8+ effector T cell aggregation in inflammatory or tumour tissues(25).

Various cells present in the tumour microenvironment have indisputable contributions to tumour growth(26, 27). Studies have found that tumour-infiltrating immune cells have a variety of types, including neutrophils, B cells, DCs, NK cells, T cells and macrophages, which can infiltrate the tumour and co-constitute and take part in the regulation of the tumour microenvironment together with other nonimmune stromal cells (28). Tumour-infiltrating neutrophils are present in many different types of tumours, and many studies have shown that they have a tumour-promoting effect (29, 30). Histological studies on a variety of tumour types have shown that high tumour-infiltrating neutrophil expression is related to poor prognosis in cancer patients(31). Chen reported that CXCL10 expression was related to tumour-infiltrating immune cells in colorectal cancer and was related to the progression of colorectal cancer(32). In a public database, we verified whether there is a connection between CXCL10 and tumour-infiltrating immune cells, and the results suggested that CXCL10 was significantly positively correlated with tumour-infiltrating neutrophils (TINs) and tumour-infiltrating dendritic cells (TIDCs). The poor prognosis of PAAD patients may be due to the association of CXCL10 with various tumour-infiltrating immune cells that are abundant in the tumour microenvironment.

In the present study, we evaluated the significance of CXCL10 expression in PAAD. Through a literature review, many studies have reported that chemokines are connected with the prognosis of pancreatic cancer patients. Therefore, we searched the GEO and TCGA databases and found that CXCL10 was overexpressed in PAAD tumour tissues and was related to poor prognosis in PAAD patients. In addition, we further explored CXCL10 expression in tumour tissues and matched paracancerous tissues and selected normal tumour tissues of 119 pancreatic cancer patients for tissue microarray chip immunohistochemistry analysis. Student's t-test results showed that CXCL10 expression in tumour tissue was higher than that in matched paracancerous tissues (P<0.0001). Next, the chi-square test showed that CXCL10 was significantly related to T stage in clinicopathological factors (P=0.020). Univariate analysis suggested that the expression of CXCL10 (P= 0.015), age (P=0.037), histological grade (P=0.010), stage N (P=0.003) and TNM stage (P=0.003) were prominently related to the difference in overall survival, which may be related to the tumour suppressant pathway of CXCL10. Then, parameters (clinicopathological factors) with P <0.05 were set for multivariate analysis. Since N stage, M stage and TNM interval were not independent, only TNM stage was included in the COS multivariate analysis. The results suggested that histological grade (P=0.001), age (P=0.005), CXCL10 (P=0.024) and TNM stage (P=0.017) were independent prognostic factors for PAAD patients. Kaplan-Meier survival analysis showed that patients with CXCL10 overexpression had significantly shorter survival times than patients with low expression of CXCL10 (P=0.012), which may be used as a potential biomarker and target for therapeutic intervention of PAAD.

Conclusion

Our data indicate that CXCL10 is highly expressed in PDAC and may be used as an independent clinical prognostic indicator for PDAC. Although we confirmed the expression and prognosis of CXCL10 in pancreatic cancer, a study using a large sample size is needed to verify our conclusions, and the mechanism of action needs to be further investigated.

Materials And Methods

Overlapping gene Venn diagram (data from the GEO database).

Overlapping gene Venn diagrams of clinical data (numbers for GSE56560) are from the GEO database (https://www.ncbi.nlm.nih.gov/geo/)(13, 14). First, through "limma" (http://www.bioconductor.org/packages/release/bioc/html/limma.html) in the PDAC in the R group, the difference between the corresponding tissue adjacent to the carcinoma gene was analysed, and the selection criteria were adjusted for “P≤0.10 and |logfold-change|≥1.5”(15). Differential genes include DE chemokines and DE chemokine receptors, which are identified by overlapping all genes in the dataset and all chemokines and their receptors discovered to date. Diagram by "Calculate and draw the custom Venn diagrams" online tools (http://bioinformatics.psb.ugent.be/webtools/Venn/).

Expression and survival analysis (data from the TCGA database).

The survival curves of CXCL10 in PAAD (the name of pancreatic cancer in the TCGA database) patients were plotted on the gene expression profile interactive analysis website (http://gepia.cancer-pku.cn/). The clinical data of a total of 178 patients with pancreatic cancer were obtained from the TCGA database (https://cancergenome.nih.gov/).

Correlation analysis between CXCL10 and tumour-infiltrating immunocytes.

A web tool called Tumour Immune Estimation Resource (TIMER), a comprehensive resource for tumour-infiltrating immune cells (http://cistrome.dfci.harvard.edu/TIMER/), was used to investigate the relationships between CXCL10 and tumour-infiltrating immunocytes.

Tissue microarray chips.

Tissue microarray (TMA) preparation equipment was used to prepare tissue chips, for a total of 238 points, including 119 pairs of tumour tissues and corresponding adjacent tissues (National Human Genetic Resources Sharing Service Platform, Shanghai, China). The patients underwent surgery for the removal of pancreatic tumours, including those on the head or tail of the pancreas. Clinicopathological feature data included sex, age, tumour site, vascular invasion, grade, T stage, N stage and M stage. Overall survival (OS), calculated as the time from surgery to death or censoring, is an indicator of patient prognosis. Permission was obtained from the Ethics Committee of Taizhou Hospital, and all pancreatic cancer tissue samples were collected in Taizhou Hospital. All patients signed informed consent forms, received routine chemotherapy or other adjuvant treatment after surgery, and received regular outpatient clinics or follow-up telephone calls to obtain information.

Immunohistochemical staining of tissue microarray chips.

For immunohistochemical staining, according to the product instructions, the UltraSensitive SP IHC Kit (Maxim Biotech Inc., CA, USA) and IHC Biotin Block Kit (Maxim Biotech Inc., CA, USA) dewaxed and rehydrated tissue slices via 100% xylene and 80% ethanol. The IHC Biotin Block kit contains two reagents, A and B. After the antigen was retrieved, it was washed with PBS, and 50 μL of reagent A, the anti-biotin protein solution, was added to the tissue microarray and incubated for 10 min at room temperature. The tissue microarray was rinsed 3 times for 5 min each time with PBS. Then, 50 μL of reagent B, d-biotin solution, and anti-biotin protein solution were added to the tissue microarray, incubated for 10 min at room temperature, and then rinsed 3 times for 5 min each time with PBS. Antibody incubation was performed using CXCL10 (rabbit monoclonal antibody, dilution 1:500, cat. no. ab9807, Abcam). The stained tissue microarray chips were scanned using an Aperio Digital Pathology Slide Scanner (Aperio CS; Leica Microsystems, Inc., IL, USA) to obtain images. Visualization of tissues was performed using a high-capacity digital slide scanner and Pannoramic Viewer Software (3DHISTECH, Ltd.). After preliminary screening, the unqualified samples in tissue microarray sections and staining images were rejected. The images were independently assessed by two clinical pathologists who were blind to all information. The histo-score (H-score) method, a semiquantitative scoring method that responds to results and reflects, was used in which both staining intensity and positive areas were recorded to evaluate CXCL10 staining.

Total H-SCORE(0 to 300) using the following formula =$\sum$ (PI x I)= where ‘PI’ represents The proportion of cells with the same staining intensity in the population(0% to 100%)and ‘I’ represents the score of staining intensity (0 to 3). The patients were divided into low and high groups according to the optimal cut-off value of the CXCL10 expression level. The optimal cut-off value was determined using X-tile software version 3.6.1 (Yale University School of Medicine, New Haven, CT, USA).

Statistics of patient clinicopathological features (data from the tissue microarray dataset).

A total of 119 pancreatic cancer patients were included in this analysis after the removal of samples from TMA that had been lost as a result of unsuccessful biopsy and staining. For each patient, tumour samples and matched paracancerous tissues were collected. Statistics of the clinicopathological features of pancreatic cancer and the corresponding number of patients (Table I).

Statistical analysis.

In this study, quantitative data were tested by Student's t-test, and qualitative data were tested by the chi-square test, Fisher's exact test or likelihood ratio test. Survival was estimated with the Kaplan-Meier method. The log-rank test was used to compare the survival curves. Multivariate analysis was conducted with the Cox regression mode for analysis. Clinicopathological features with significant correlations (defined as $P < 0.05$) in univariate survival analysis were selected for Cox multivariate analysis. SPSS statistical software, version 25 (SPSS Inc., IL), was used for statistics and analysis. GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA), Adobe Photoshop CS5 and Adobe Illustrator CC2019 (Adobe Systems Inc., San Jose, CA) were used to prepare composite figures. A statistically significant difference was considered at $P < 0.05$.

Declarations

Ethics approval and consent to participate

Permission was obtained from the Ethics Committee of Taizhou Hospital, and all pancreatic cancer tissue samples were collected in Taizhou Hospital.

Consent for publication

Not applicable.

Conflicts of interest

All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

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Authors' contributions

BW, and ZX conceived the idea; LY and HK performed the experiments; LY, HK and BW analyzed the data; LY wrote the manuscript. All authors have read and approved the final version of the manuscript.

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Tables

Table I. Clinicopathological features and statistics of the patients.

Clinicopathological features	Group	No. of patients
Sex		
	Male	71
	Female	48
Age, years		
	<70	81
	≥70	38
Tumor site		
	Head	72
	Other	47
VI		
	Yes	64
	No	55
Grade		
	1	15
	2	59
	3	45
T		
	T1+T2	76
	T3+T4	43
N		
	N0	63
	N1+N2	56
M		
	M0	87
	M1	32
TNM stage[a]		
	I+II	102
	III+IV	17

[a]TNM stage of patients with pancreatic adenocarcinoma according to the American Joint Commission on Cancer guidelines (8th edition)(33). T, tumor; N, node; M, metastasis□VI, vascular invasion

Table II. Correlation between CXCL10 expression and clinicopathological features.

Features	No. of patients	Expression level of CXCL10		P-value
		Low (%)	High (%)	
Sex				0.565
Male	71	48(68)	23 (32)	
Female	48	30 (62)	18 (38)	
Age, years				0.969
<70	81	53 (65)	28 (35)	
≥70	38	25 (66)	13 (34)	
Tumor site				0.098
Head	72	43 (60)	29 (40)	
Other	47	35 (72)	12 (28)	
VI				0.684
Yes	64	43(67)	21 (33)	
No	55	35(64)	20 (36)	
Grade				0.597
1	15	9 (60)	6 (40)	
2	59	37 (63)	22 (37)	
3	45	32(71)	13(29)	
T				0.020
T1+T2	76	44 (58)	32 (42)	
T3+T4	43	34(79)	9 (21)	
N				0.785
N0	63	42 (67)	21(33)	
N1+N2	56	36 (64)	20 (36)	
M				0.188
M0	87	54(62)	33 (38)	
M1	32	24(75)	8 (25)	
TNM stage				0.083
I+II	102	70 (69)	32 (31)	

III+IV	17	8 (47)	9(53)

VI, vascular invasion; TNM, Tumor-Node-Metastasis.

Figures

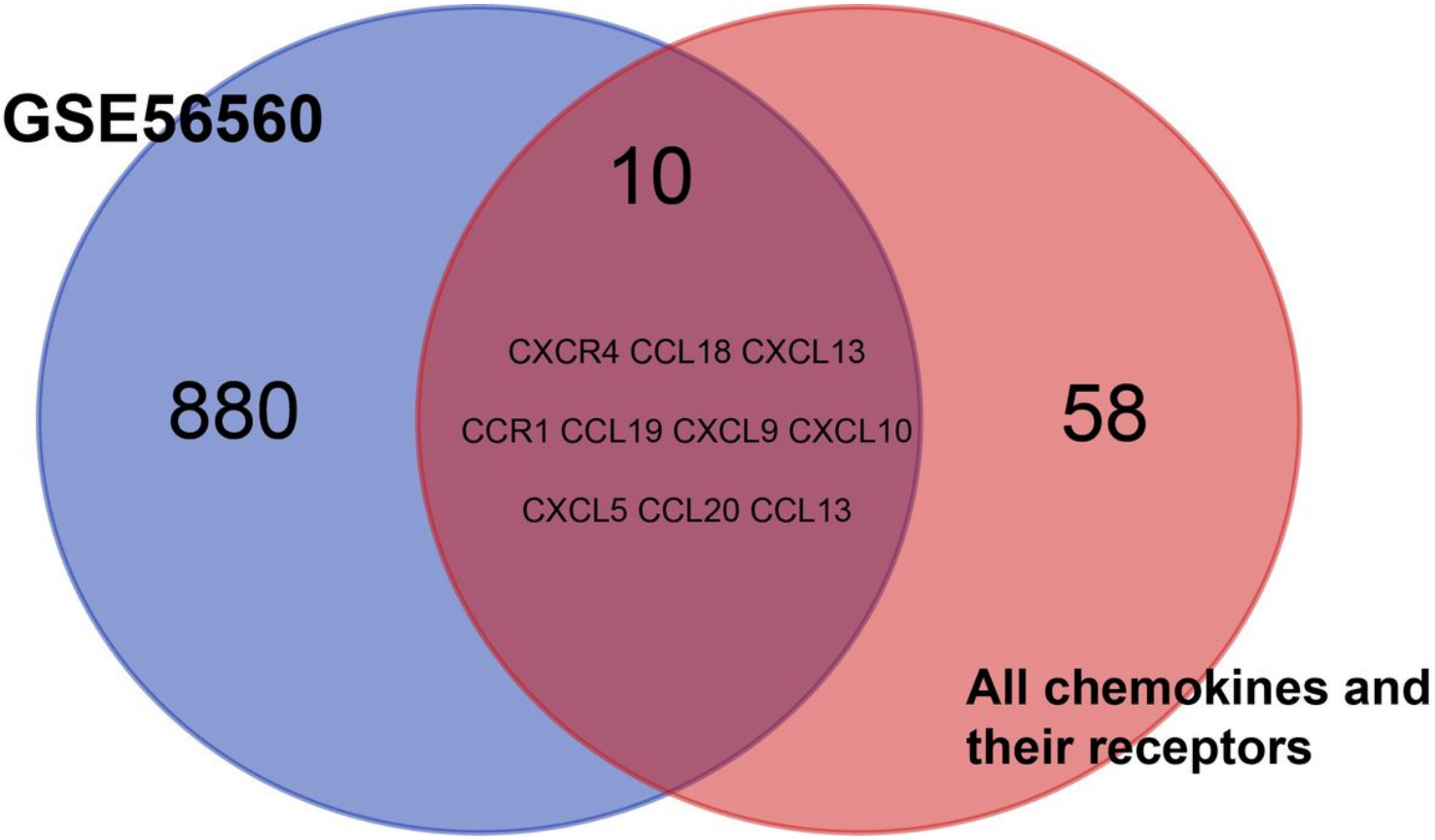

Figure 1

Venn diagram showing that CXCL10 is one of the DE chemokines and their receptors. DE, differentially expressed.

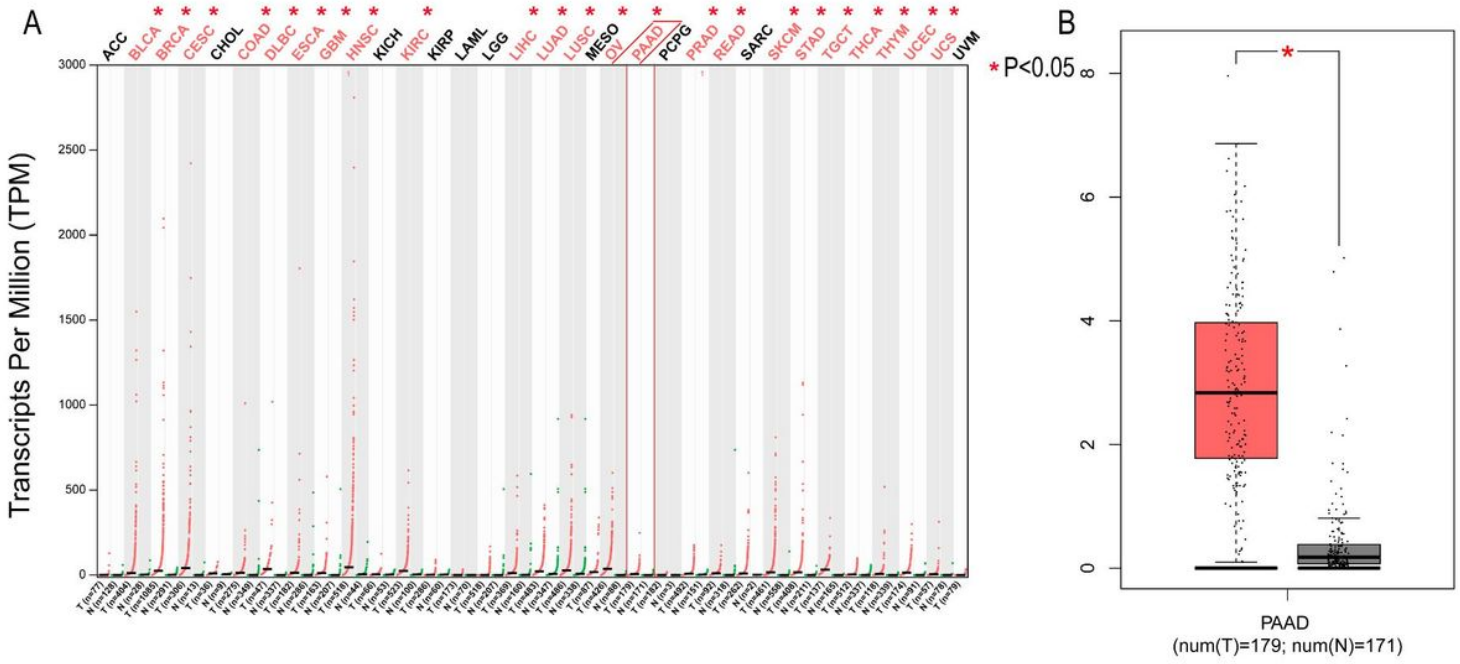

Figure 2

(A). The red font indicates that CXCL10 is highly expressed in the tumour, and the black font indicates that there is no prominent difference in CXCL10 expression level between the tumour and matched paracancerous tissues. (B). Box-plot of CXCL10 expression in PAAD. Red represents PAAD tumour tissue, grey represents PAAD matched paracancerous tissues;*P<0.05

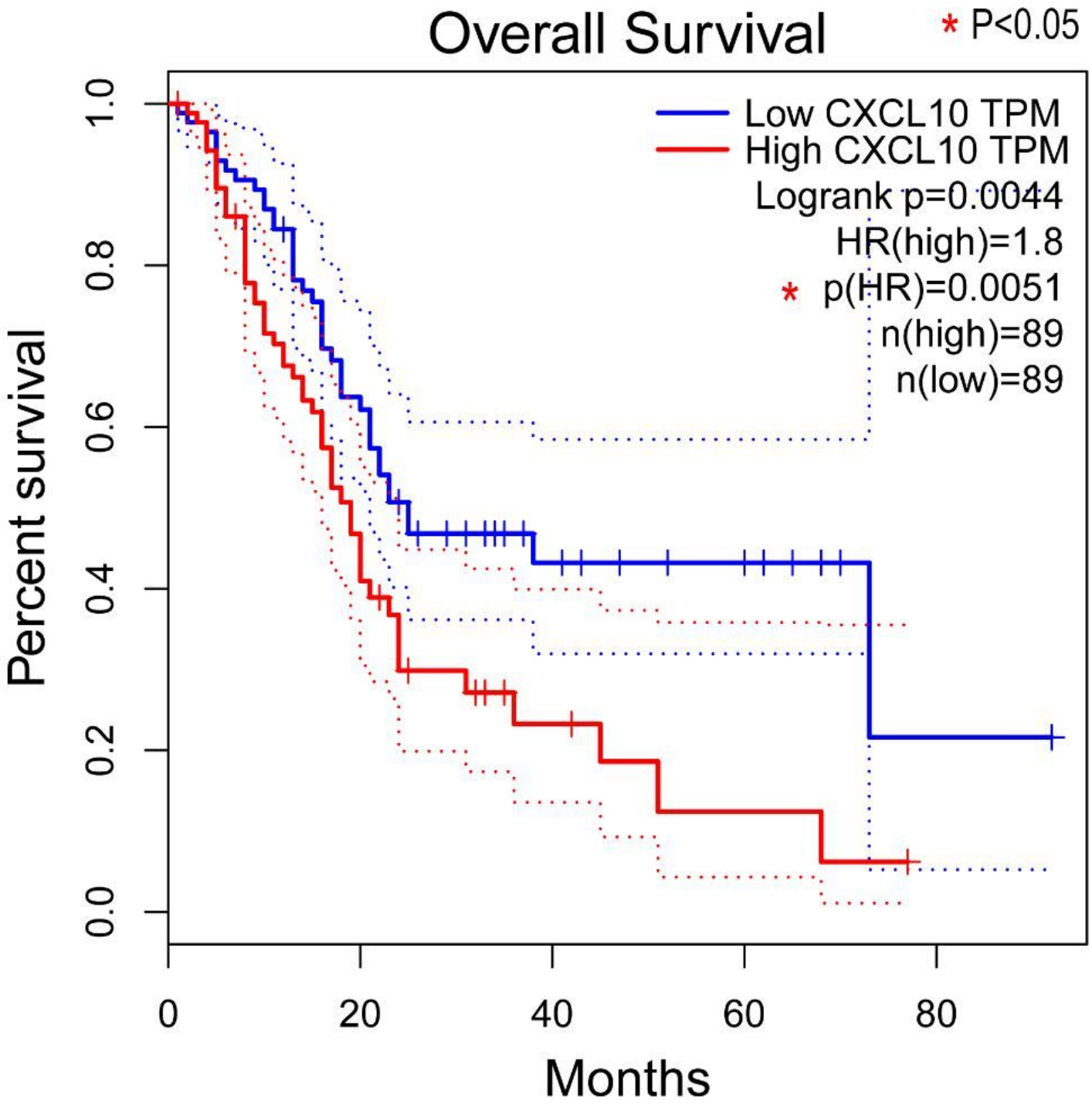

Figure 3

The survival curves for the expression of CXCL10 in PAAD (data from the TCGA dataset); HR, hazard ratio; *P<0.05

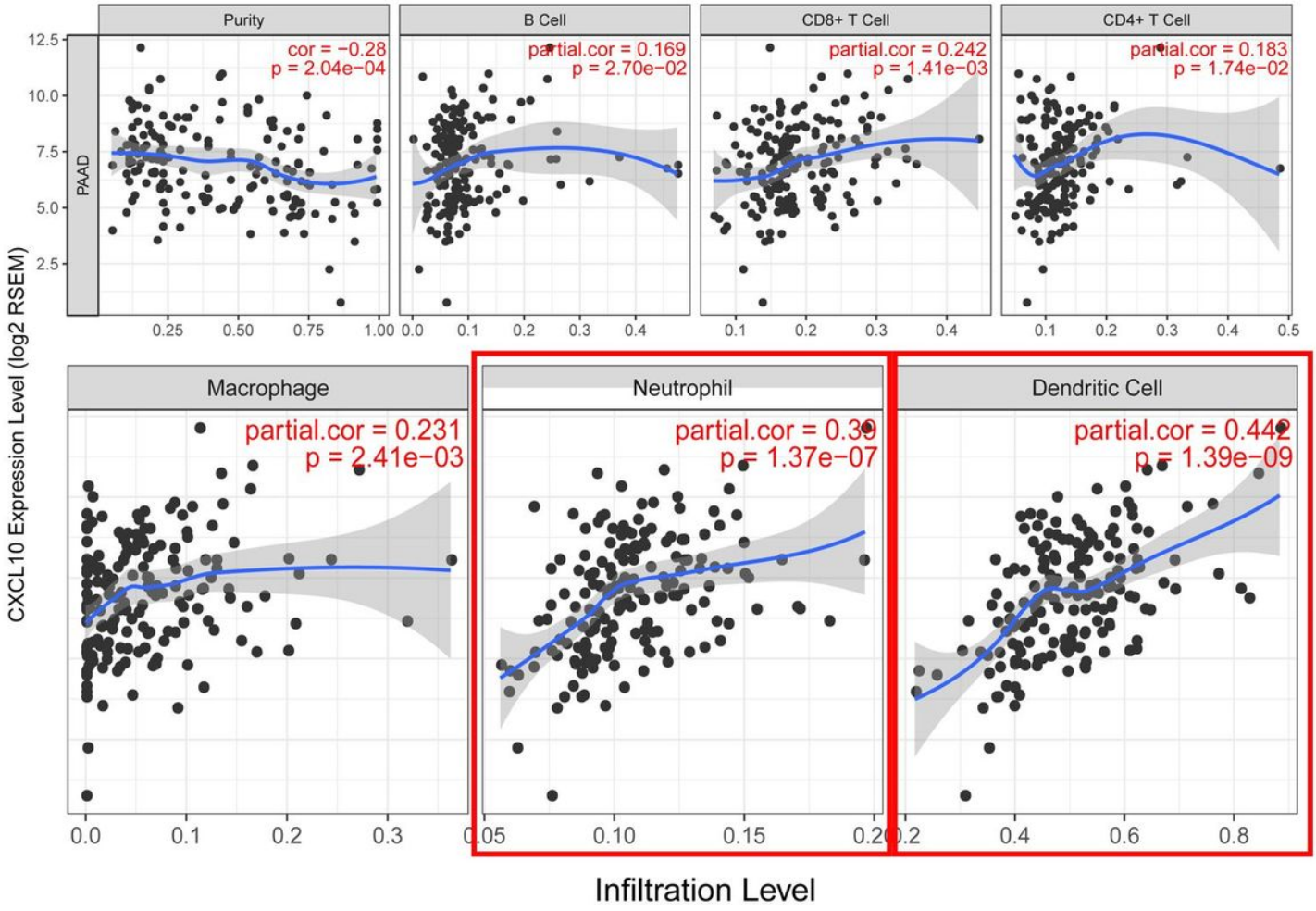

Figure 4

A correlation between CXCL10 expression and tumour-infiltrating immunocytes. The red box represents a significant correlation with a P value <0.001; partial.cor =partial correlation coefficient=R; P, P value.

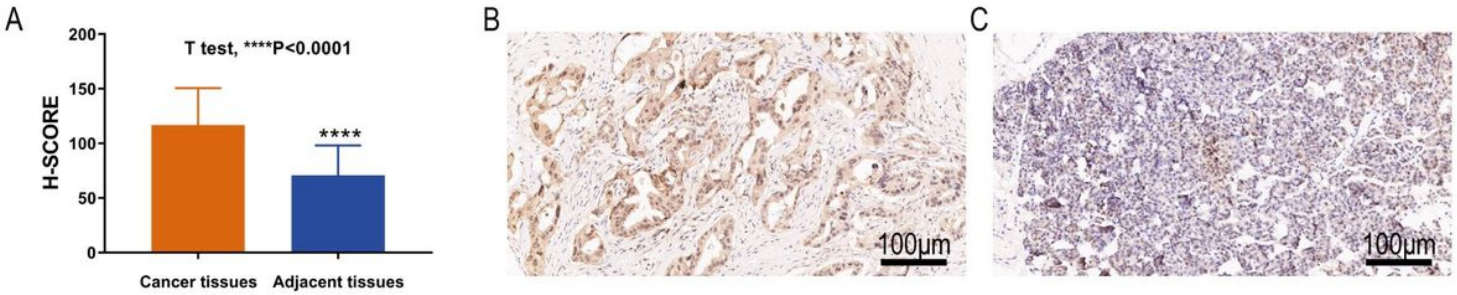

Figure 5

The expression level of CXCL10 in PAAD. (A) T-test results of the CXCL10 H-score in PAAD tumour tissues and matched paracancerous tissues. (B) Positive staining for CXCL10 in PAAD tissues (magnification, ×20). (C) Negative staining for CXCL10 in matched paracancerous tissues (magnification, ×20).

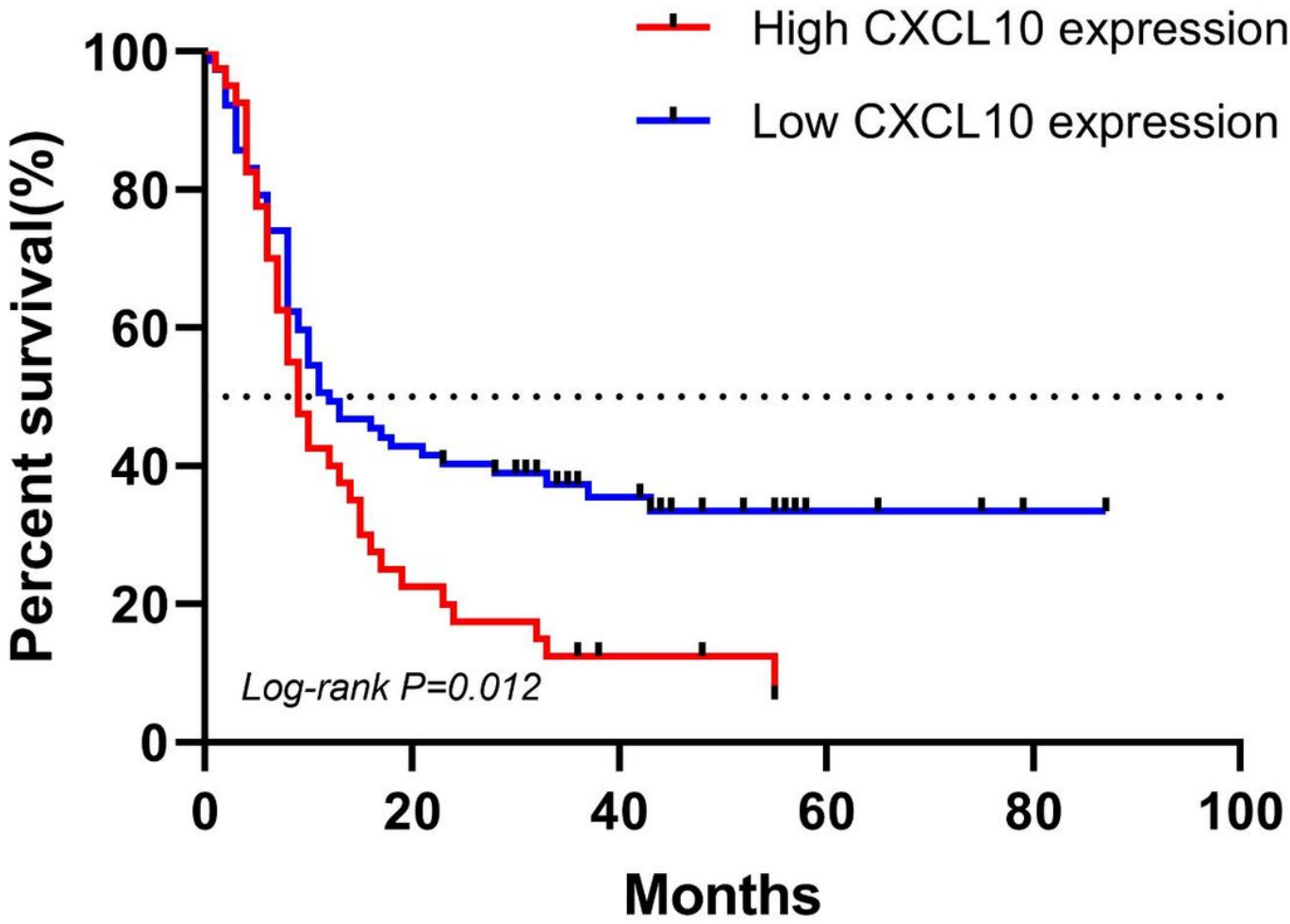

Figure 6

Overall survival displayed graphically as a Kaplan–Meier curve according to CXCL10 expression. (Data from the tissue microarray dataset).

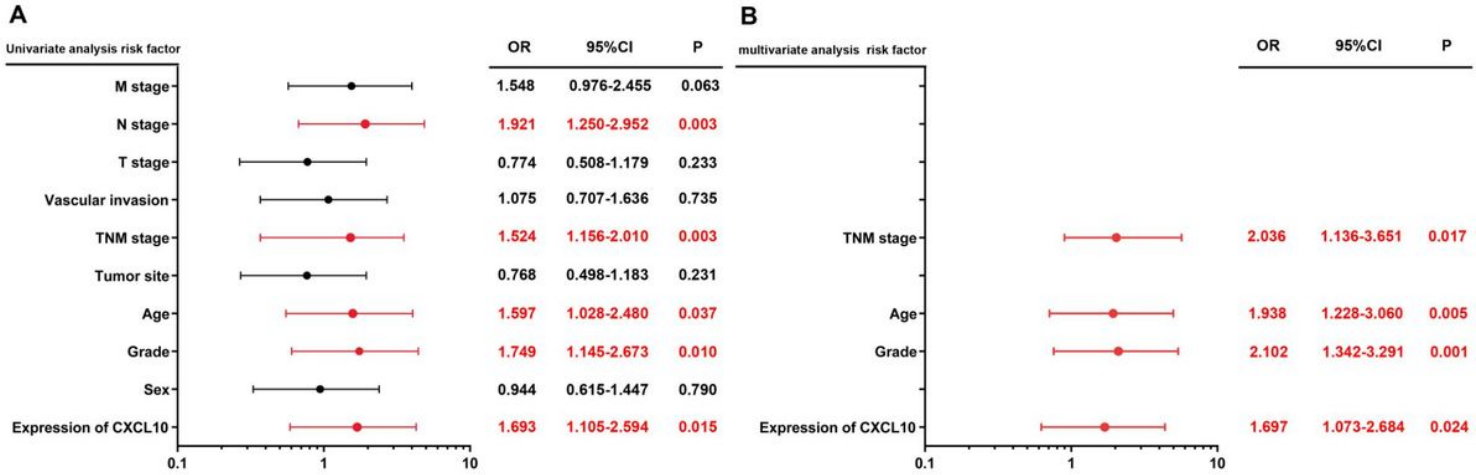

Figure 7

(A). Univariate analysis of clinicopathological factors. CXCL10 expression (P=0.015), age (P=0.037), histological grade (P=0.010), N stage (P=0.003) and TNM stage (P=0.003) were chosen in the COS multivariate analysis; (B). The clinicopathological factors were screened by univariate analysis for COS multivariate analysis. The results suggested that age (P=0.005), histological grade (P=0.001), TNM stage (P=0.017) and CXCL10 (P=0.024) were independent prognostic factors for pancreatic cancer patients; OR, odds ratio; CI, confidence interval; red represents statistical significance (P<0.05);