Identification CXCL9 is a Potential Prognostic Biomarker in Ovarian and Gastric Cancer and is Correlated with Immune Infiltrates

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Abstract

Background: CXCL9 also known as an interferon gamma-inducible chemokine that belonging to the CXC chemokine family. It plays a role in promoting chemotaxis, inducing leukocyte differentiation and multiplication, and triggering tissue extravasation.

Methods: The TIMER (Tumor Immune Estimation Resource) and cancer microarray database Oncomine were used to dig at CXCL9 expression. The clinic prognostic level of CXCL9 was evaluated via Kaplan-Meier plotter. Then, Using TIMER and GEPIA, we investigated whether CXCL9 expression impacted cancer immune infiltrates.

Results: CXCL9 expression has been found to be significantly lower in ovarian and gastric cancers relative to normal tissues. In patients with ovarian cancer (OS HR = 0.78, P = 0.0017; PFS HR = 0.85, R = 0.015) and gastric cancer (OS HR = 0.55, P = 1.1e-08; PFS HR = 0.58, R = 7.6e-07), low CXCL9 expression was correlation to PFS (progression-free survival) and OS (poor overall survival). Furthermore, in OV and GC, CXCL9 was shown to have a close interaction with tumor-infiltrating immunity cells (B cells, CD4+ and CD8+ T cells, macrophages, neutrophils, and dendritic cells). CXCL9 expression, on the other hand, was shown to be closely related to several immune markers.

Conclusion: In OV and GC, CXCL9 mRNA level is strongly associated with prognosis and levels of penetration tumor-infiltrating immunity cell. The CXCL9 expression may also play a role in controlling TAMs (tumor-associated macrophages), DCs (Dendritic cells), CTLs (cytotoxic lymphocytes), and NK (natural killer) cells in OV and GC. CXCL9 may be seen as an independent marker that assesses the prognosis in OV and GC patients. Besides, CXCL9 expression level also can assess the immune cell subtypes of tumor microenvironment in OV and GC.

Introduction

Ovarian cancer and Gastric cancer are globally important diseases. Ovarian cancer is the second most common cause of all-year-round, after breast cancer, in women. Annually, there are over 239,000 new cases and 150,000 deaths. Patients in stages III or IV illness have a five-year average survival rate of 29%. Since ovarian cancer is often asymptomatic at first, about 75% of women are already in advanced stages when they are diagnosed. It is estimated that one million new cases of gastric cancer arise per year, and the 5-year survival rate is below 25 percent[1–3]. Fortunately, immunotherapy has been emerging as a promising alternative treatment for some ovarian and gastric cancer patients. Some immunotherapy, for example, CTLA4 (cytotoxic T lymphocyte-associated antigen 4) therapies, PD-L1 (programmed death ligand-1), and PD-1 (programmed cell death protein-1) inhibitors have demonstrated an optimistic antitumor activity in ovarian cancer and gastric cancer. However, like other therapy, only a portion of patients benefit from immunotherapy[4–8]. More and more research found that the immune cell subtype in the tumor microenvironment affects immunotherapy efficacy in cancer treatment[9–13]. Therefore, the understanding of play role of immune cells in immunotherapy is urgently needed,and search new immunity treatment target.

Monokine induced by gamma interferon MIG/CXCL9 belongs to the CXC chemokine family[14]. The function of CXCL9 is to induce chemotaxis, facilitate the multiplication and differentiation of leukocytes, and cause extravasation of tissues. The CXCL9/CXCR3 receptor influences the migration, differentiation, and activation of immune cells [15]. Recently study observed that aberrant abnormal expression of CXCL9 in multiple cancer types. Besides, other research indicated that CXCL9 levels of gene expression associated with clinicopathological features of in multiple cancer types [16–20]. Thus, CXCL9 could have functions in tumorigenesis, growth, and metastases, and may function as the essential prognosis factor for tumors. However, the role of CXCL9 in ovarian and gastric cancers and possible molecular mechanisms has still not been explained so further exploration is necessary.

In this research, we have investigated the relationship between CXCL9 expression and prognosis in cancer patients. We also explored the impact of CXCL9 mRNA expression level on tumor-infiltrating immune cells based on a series of online public

databases. Our result reveals CXCL9 plays a critical role in tumor immunity by regulating tumor-infiltrating immune cells in OV and GC.

Materials And Methods

CXCL9 mRNA expression analysis

In the Oncomine database, the CXCL9 mRNA expression level in different cancer types was identified[21]. The following are the threshold parameters: $p < 0.001$, fold change > 2.

Clinical characteristics analysis

The Kaplan–Meier plotter is an online database, including 54k genes and 14912 cancer samples, which can assess the different genes' effect on survival rates in 21 cancer types[22]. Using KM plotter (http://kmplot.com/analysis/) our team analyzed the association of CXCL9 expression to clinical characteristics in the breast, ovarian, lung, and gastric cancers. There were estimated 95% confidence intervals for hazard ratios (HRs). Log-rank P-values were measured as well.

TIMER analysis

The Tumor IMmune Estimation Resource (TIMER) an online database that comprehensive analysis of tumor-infiltration cells[23]. TIMER analysis reveals the association between the CXCL9 expression and immune cell infiltration (9). TIMER gene modules were used to examine CXCL9 expression in multiple cancers and the association between CXCL9 and the infiltrating level of various immune cells in the tumor microenvironment. Using TIMER correlation modules explored the interaction of CXCL9 expression with immune-related gene markers. Various immune cell molecular markers have been identified in previous research, for example, CCL2, CD68, and IL10 of TAM cell. The gene expression level has been shown by log2 TPM.

GEPIA analysis

Their genes that showed an obvious correlation in the TIMER study were further verified using the online database GEPIA[24]. GEPIA database offers their modules: Gene analysis, Custom data, and Cancer Type Analysis containing tumor and normal samples over18,000 sample from the GTEx projects and TCGA. The correlation of two genes was analyzed by the Spearman method.

Results

CXCL9 Transcriptional Levels in a Variety of Tumors

The Oncomine database analyzed the levels different of CXCL9 mRNA in various tumors and their normal tissues. It found out that CXCL9 expression was elevated in the following cancers: gastric, breast, bladder, cervical, head and neck, colorectal, kidney, leukemia, liver, lymphoma, and prostate (Figure 1A). using the TIMER database, we further assessed the CXCL9 expression difference in various cancer. Results show that CXCL9 expression was elevated in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), cholangiocarcinoma (CHOL), head and neck cancer (HNSC), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), esophageal carcinoma (ESCA), lung squamous (LUSC), lung adenocarcinoma (LUAD), rectum adenocarcinoma (READ), prostate adenocarcinoma (PRAD), uterine corpus endometrial carcinoma (UCEC), and stomach adenocarcinoma (STAD) than their normal tissues. instead, CXCL9 expression was low in thyroid carcinoma (THCA), kidney chromophobe (KICH), and kidney renal papillary (KIRP) than their normal tissues. (Figure 1B).

The Prognostic value of CXCL9 in human cancers

Next, Using KM plotter database to analyze whether the CXCL9 expression level impacts cancer patient's prognosis. The result shows that high CXCL9 level corresponded with better prognosis in OV (OS HR = 0.78, *P* = 0.0017; PFS HR = 0.85, *P* = 0.015), GC patients (OS HR = 0.55, *P* = 1.1e-08; PFS HR = 0.58, 95% CI = 0.46-0.72, *P* = 7.6e-07), Breast patients (OS HR = 0.88, *P* = 0.0011; RFS HR = 0.83, *P* = 0.0039; PPS HR = 0.74, *P* = 0.022, DMFS HR = 0.68, *P* = 0.0011), Lung patients (PFS HR = 0.84, *P* = 0.015) and Liver patient (OS HR = 0.66, *P* = 0.027; PFS HR = 0.65, *P* = 0.0083) (Figure 2(A-H, G-L)). Yet, the CXCL9 expression was no correlation with OS in Lung cancer (Figure 2I). These results indicated that the CXCL9 expression impacts breast, liver, ovarian, and gastric cancer prognosis.

CXCL9 expression impact the clinical characteristic of OV and GC patient

Next, we are again using the Kaplan-Meier Plotter database to investigate whether CXCL9 expression affects the clinical characteristics of OV and GC patients. (Table 1). In stage 3 ovarian cancer patients, low CXCL9 expression show to be associated with a worse OS and PFS (OS HR = 0.73, *P* = 0.0005, PFS HR = 0.71, *P* = 5.1E-05). Overexpression CXCL9 was relationship with better OS and RFS of patients treated with Taxol, platin, and platin + Taxol chemotherapy. In stage 1-3 gastric cancer patients, high CXCL9 expression show to be associated with a better correlation with a better OS and PFS (OS HR = 0.1, *P* = 0.0066, PFS HR = 0.14, *P* = 0.027; OS HR = 0.45, *P* = 0.011, PFS HR = 0.44, *P* = 0.01; OS HR = 0.52, *P* = 2e-04, PFS HR = 0.53, *P* = 0.002). Besides, the gastric patients at the N0-2 stage also showed an obvious correlation with CXCL9 expression. (OS HR = 0.19, *P* = 0.011, PFS HR = 0.2, *P* = 0.014; OS HR = 0.55, *P* = 0.0061, PFS HR = 0.53, *P* = 0.0028; OS HR = 0.36, *P* = 0.0011, PFS HR = 0.42, *P* = 0.0035) (Table 2). These findings show that CXCL9 expression has prognostic signification in OV and GC patients according to their clinical characteristics.

CXCL9 expression impacts the immune cell infiltration from ovarian and gastric cancer.

Cancer patients' survival times are affected by tumor-infiltrating lymphocytes. So we analyzed the correlation between the 39 cancer in TIMER and the CXCL9 expression level. The analyzed result indicated that CXCL9 expression associates with tumor purity in 27 types of cancer. besides, CXCL9 expression obviously association with the infiltration levels of immune cell. Such as $CD4^+$ (26 types cancer), B cell (23 types cancer), $CD8^+$T cells (32 types cancer), Macrophage cells (12 types cancer), Neutrophil cell (32 types cancer) and Dendritic cell (34 types cancer) (supplementary Figure 1). In OV and GC, high CXCL9 transcription was association to better prognosis and elevated immune cell infiltration levels. High CXCL9 expression was found to be obviously positive associated with the infiltration level of immune cell including $CD4^+$ (r = 0.268, P = 2.37e-09), B cells (r = 0.199, P = 1.14e-05), macrophages (r = 0.025, p = 5.79e-01), $CD8^+$ T cells (r = 0.401, P = 5.15e-20), neutrophils (r = 0.338, P = 2.72e-14), and DCs (r = 0.39, P = 7.42e-14), in OV tissues (Figure 3A). Similar, in Gastric cancer has been found to be obviously correlation with better prognosis and elevated immune cell infiltration levels. Immune cell including $CD4^+$ T cells (r = 0.131, P = 1. 20E-02) $CD8^+$ T cells (r = 0.595, P = 8.33e-37), neutrophils (r = 0.521, P = 3.60e-27), macrophages (r = 0.179, P = 5.34e-04), and DCs (r = 0.551, P = 8.53e-31). Interestingly, Only B cells in GC had a negative association (r = -0.209, P = 5.44e-05) (Figure 3B). The results clearly indicate that CXCL9 can recruit immune cells in the OV and GC microenvironment.

CXCL9 Expression and Immune Marker Association Analyze

The immune cell that infiltration level obviously correlation with the CXCL9 expression in OV and GC by KM plotter were further analyzed in TIMER and GEPIA database (Figure 4, Table3 and Table4). we found markers of immune cells in OV was to be strongly correlated with CXCL9 expression, including CD8+ T cell marker, CD8A (r = 0.739; P =2.78e-44), CD8B (r = 0.585; P = 2.77e-24), T cell marker, CD3D (R = 0.83; P = 1.56e-64), CD3E (R = 0.842; P = 4.47e-68), CD2 (r = 0.852; P = 1.73e-71), B cell marker, CD79A (r = 0.584; P = 3.49e-24), Monocyte marker, CD86 (r = 0.512, P = 4.56e-18), TAM marker, CD68 (r = 0.488; P = 2.73e-16), M2 macrophage marker, MS4A4A (r = 0.446, P = 1.37e-13) Neutrophils marker,CCR7 (r = 0.602, P = 6.66e-26), Nature killer cell marker, KIR2DL4 (r = 0.482; P = 7.41e-16), Dendritic cell marker, HLA-DPB1 (r = 0.507; P = 1.12e-17), HLA-DRA (r = 0.447; P = 1.49e-15), HLA-DPA1 (r = 0.512, P = 4.74e-18) and CD11C (r = 0.421; P = 4.09e-12). Besides, we also found markers of immune cells in GC was to be strongly correlated with CXCL9 expression, including CD8+ T cell

marker, CD8A (r = 0.737; P = 4.56e-66), CD8B (r = 0.604; P = 5.27e-39), T cell marker, CD3D (r = 0.707; P = 9.99e-59), CD3E (r = 0.706; P = 1.82e-58), CD2 (r = 0.745; P = 2.97e-68), Monocyte marker, CD86 (r = 0.643, P = 1.27e-45), CD115 (r = 0.499; P = 2.83e-25), TAM marker, IL10 (r = 0.404; P = 2.57e-16), M2 macrophage marker, CD163 (r = 0.521; P = 1.02e-27),VSIG4 (r = 0.465; P = 1.10e-21), MS4A4A (r = 0.523; P = 6.11e-28), Neutrophils marker, CD11b (r = 0.411; P = 7.33e-17), CCR7 (r = 0.412, P = 5.96e-17), Nature killer cell marker, KIR2DL1 (r = 0.412; P = 5.96e-17), KIR2DL4 (r = 0.549; P = 3.34e-31), KIR3DL1 (r = 0.443; P = 1.08e-19), KIR3DL2 (r = 0.437; P = 4.10e-19),Dendritic cell marker, HLA-DPB1 (r = 0.644; P = 1.02e-45), HLA-DQB1 (0.508; P = 3.06e-26), HLA-DRA (r = 0.637; p = 1.90e-44), HLA-DPA1 (r = 0.623; P = 1.90e-44) and CD11C (r = 0.497; P = 5.24e-25) (Figure 4 and Table3). GEPIA analysis resulted have consistent with TIMER (Table 4). These results indicated that CXCL9 expression correlation with infiltration of immune cells in OV and GC.

Discussion

The role of CXCL9 in the tumorigenesis and metastasis of different tumors has always been controversial. For instance, Addison et al[15]. Found CXCL9 expression at high levels can inhibit tumor-derived angiogenesis, which inhibits NSCLC tumor development. Liber et al[25]. Found OV patients with elevated levels of CXCL9 had improved RFS. Chang et al[16]. Found a significant correlation between high cxcl9 expression and worse OS in OSCC (oral squamous cell carcinoma) patients. CXCL9 expression was associated with the prognosis of OV and GC in our research. In OV and GC, low CXCL9 expression was associated with a poor survival outcome. Besides, we discovered that the infiltration level of immune cells and different immunological markers are related to CXCL9 expression in OV and GS. Thus, our study suggested that CXCL9 potential plays a vital part in recruiting immune cells into tumor microenvironment progress, and it also can use as a prognosis biomarker. In the present research, analyzed CXCL9 mRNA expression level in multiple kinds of cancer by ONCOMINE and TIMER online database. levels of CXCL9 expression have been identified in multiple cancers and their normal tissues. Due to differences in data collection and processing methods between databases, these two databases' analyzed results differ slightly. Furthermore, KM plotter analysis shows increased CXCL9 expression level was shown to be associated with favorable prognosis in breast, ovarian, gastric, liver, and cancers (Fig. 3). In gastric patients, low CXCL9 expression with poor survival outcomes in stage 1–3, T2-3, and N0-2 disease. In ovarian patients, low CXCL9 expression was associated with worse survival outcomes in patients who underwent suboptimal debulking surgery and stage 3 disease. This finding strongly supports the notion that CXCL9 is a prognosis biomarker for ovarian and gastric cancers.

This study also discovered that the CXCL9 expression impacted the immune cell's infiltration level in OV and GC. There was a strong correlation between infiltration of CD8 + T cell, CD4 + T cell, Neutrophil, Dendritic cell, and B cell and CXCL9 expression in OV and GC. Moreover, the immune cells marker gene was positively correlated with CXCL9 expressions, such as TAM marker CD68, monocyte marker, CD86, and M2 macrophage marker, MS4A4A. This indicates that CXCL9 regulates the infiltration and activity of tumor-associated macrophages (TAMs) and activates cytotoxic lymphocytes (CTLs) and natural killer cells (NKs). These findings suggest that CXCL9 is essential for the recruitment and modulation of immune infiltrating cells in OV and GC.

Conclusion

In conclusion, our results suggest increasing CXCL9 expression associated with better prognosis and evaluating immune cell infiltration in OV and GC patients. Therefore,CXCL9 is a potential new independent biomarker evaluate for OV and GS patient prognosis and play a critical role regulation immune cell into the tumor microenvironment.

Abbreviations

CXCL9/MIG: Monokine Induced by Gamma Interferon (MIG); GC: Gastric cancer; OV: Ovarian Cancer; PD-1: programmed death-1; CTLA-4: Cytotoxic T - Lymphocyte Antigen 4; TIMER: Tumor Immune Estimation Resource; GEPIA: Gene Expression Profiling Interactive Analysis; OS: overall survival; DSS: disease-specific survival; PFS: progression-free survival; RFS, relapse-

free survival; RFS: relapse-free survival; DMFS: distant metastasis-free survival; PPS: post progression survival; FP: first progression; TAMs: tumor-associated macrophages; NK cell: natural killer cells; DCs: Dendritic cells, CTLs: cytotoxic lymphocytes.

Declarations

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Author Contributions

SL, TW and YL conceived the project and wrote the manuscript. HZ, RS, DW, KZ, SX, and JZ participated in data analysis.

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Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the article.

Ethics approval and consent to participate Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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Tables

Table1. Correlation of CXCL9 mRNA expression and clinical prognosis in ovarian cancer

Variables of ovarian cancer	Overall survival (N=1656)			Progression-free survival (N=1436)		
N	Hazard ratio	*P*-value		*N*	Hazard ratio	*P*-value
Histology						
Endometroid	37	2.76 (0.46-16.52)	0.25	51	2.94 (1.16-7.46)	0.018
Serous	1207	0.76 (0.63-0.91)	0.0035	1104	0.77(0.66-0.89)	0.0005
Stage						
1	74	2.81 (0.88-8.94)	0.068	96	2.77 (0.96-8)	0.05
2	61	0.44 (0.15-1.32)	0.13	67	1.64 (0.83-3.24)	0.15
3	1044	0.73 (0.61-0.87)	0.0005	919	0.71 (0.61-0.84)	5.10E-05
4	176	0.81 (0.56-1.19)	0.28	162	1.47(0.96-2.26)	0.075
Grade						
1	56	0.43 (0.16-1.17)	0.09	37	4.05 (1.31-12.58)	0.009
2	324	0.82 (0.59-1.13)	0.22	256	1.33 (0.99-1.8)	0.059
3	1015	0.66 (0.55-0.81)	0.000031	837	0.71 (0.6-0.85)	0.00011
TP53 mutation						
Mutated	506	0.62 (0.47-0.81)	0.0006	483	0.52 (0.4-0.69)	1.60E-06
Wild type	94	1.22 (0.69-2.19)	0.49	84	1.43 (0.79-2.58)	0.23
Debulk						
Optimal	801	0.81 (0.63-1.05)	0.11	696	1.26 (1.02-1.57)	0.034
Suboptimal	536	0.7 (0.56-0.87)	0.0016	459	0.62 (0.49-0.8)	0.00013
Chemotherapy						
contains platin	1409	0.71 (0.6-0.84)	8.5e-05	1259	0.77 (0.66-0.89)	0.0005
contains Taxol	793	0.77 (0.62-0.94)	0.011	715	0.8 (0.66-0.98)	0.03
contains Taxol+platin	776	0.77 (0.62-0.95)	0.015	698	0.8 (0.66-0.99)	0.035

Table2. Correlation of CXCL9 mRNA expression and clinical prognosis in gastric cancer

Variables of gastric cancer	Overall survival (N=875			Progression-free survival (N=640)		
	N	Hazard ratio	*P*-value	*N*	Hazard ratio	*P*-value
Gender						
Female	236	0.46 (0.3-0.72)	0.0005	201	0.53 (0.33-0.86)	0.009
Male	544	0.59 (0.47-0.74)	4.00E-06	437	0.53 (0.4-0.68)	1.20E-06
Stage						
1	67	0.1 (0.01-0.77)	0.0066	60	0.14 (0.02-1.08)	0.027
2	140	0.45 (0.24-0.84)	0.011	131	0.44 (0.23-0.84)	0.01
3	305	0.52 (0.36-0.74)	2.00E-04	186	0.53 (0.35-0.8)	0.002
4	148	0.52 (0.32-0.83)	0.0054	141	0.65 (0.41-1.04)	0.069
Stage T						
2	241	0.56 (0.33-0.95)	0.029	239	0.42 (0.24-0.76)	0.0032
3	204	0.54 (0.36-0.79)	0.0016	204	0.61 (0.42-0.89)	0.0089
4	38	0.67 (0.26-1.71)	0.4	39	1.67 (0.63-4.42)	0.3
Stage N						
0	74	0.19 (0.04-0.8)	0.011	72	0.2 (0.05-0.83)	0.014
1	225	0.55 (0.36-0.85)	0.0061	222	0.53 (0.35-0.81)	0.0028
2	121	0.36 (0.19-0.68)	0.0011	125	0.42 (0.23-0.77)	0.0035
3	76	0.61 (0.32-1.16)	0.13	76	0.65 (0.34-1.24)	0.19
Stage M						
0	444	0.53 (0.39-0.71)	2.40E-05	443	0.56 (0.42-0.75)	6.60E-05
1	56	0.58 (0.32-1.04)	0.065	56	0.73 (0.37-1.44)	0.36
HER2 status						
negative	532	0.43 (0.32-0.59)	3.90E-08	408	0.49 (0.36-0.66)	2.80E-06
positive	343	0.74 (0.54-1.01)	0.058	232	1.36 (0.98-1.89)	0.063
Lauren classification						
Intestinal	320	0.6 (0.42-0.84)	0.0026	263	0.5 (0.33-0.76)	0.0008
Diffuse	241	0.61 (0.41-0.91)	0.015	231	0.6 (0.39-0.92)	1.80E-02
Differentiation						
poorly	165	0.54 (0.35-0.84)	0.0051	121	1.78 (0.99-3.2)	0.05
moderately	67	1.88 (0.98-3.6)	0.053	67	1.83 (0.98-3.42)	0.054

Table3. Correlation analysis between CXCL9 and relate genes and markers of immune cells in TIMER.

		OV				STAD			
		Purity		None		Purity		None	
Description	Gene markers	Cor	*P*	Cor	*P*	Cor	*P*	Cor	*P*
CD8+ T cell	CD8A	0.739	***	0.811	***	0.737	***	0.754	***
	CD8B	0.585	***	0.676	***	0.604	***	0.621	***
T cell (general)	CD3D	0.83	***	0.875	***	0.707	***	0.72	***
	CD3E	0.842	***	0.874	***	0.706	***	0.71	***
	CD2	0.852	***	0.885	***	0.745	***	0.757	***
B cell	CD19	0.267	***	0.293	***	0.328	***	0.346	***
	CD79A	0.584	***	0.679	***	0.375	***	0.405	***
Monocyte	CD86	0.512	***	0.677	***	0.643	***	0.658	***
	CD115 (CSF1R)	0.319	***	0.518	***	0.499	***	0.505	***
TAM	CCL2	0.292	***	0.461	***	0.229	***	0.267	***
	CD68	0.488	***	0.632	***	0.35	***	0.364	***
	IL10	0.161	0.011	0.366	***	0.404	***	0.416	***
M1 macrophage	INOS (NOS2)	-0.13	0.040	-0.014	0.803	0.065	0.209	0.075	0.126
	IRF5	0.11	0.083	0.219	**	0.215	***	0.229	***
	COX2 (PTGS2)	0.003	0.963	0.157	**	-0.089	0.084	-0.061	0.216
M2 macrophage	CD163	0.358	***	0.536	***	0.521	***	0.532	***
	VSIG4	0.278	***	0.491	***	0.465	***	0.481	***
	MS4A4A	0.446	***	0.6	***	0.523	***	0.537	***
Neutrophils	CD11b (ITGAM)	0.35	***	0.532	***	0.411	***	0.419	***
	CD66b (CEACAM8)	-0.093	0.145	-0.104	0.065	-0.014	0.791	-0.002	0.791
	CCR7	0.602	***	0.671	***	0.412	***	0.44	***
Natural killer cell	KIR2DL1	0.124	0.051	0.228	***	0.367	***	0.39	***
	KIR2DL3	0.139	0.028	0.203	**	0.373	***	0.395	***
	KIR2DL4	0.482	***	0.544	***	0.549	***	0.566	***
	KIR3DL1	0.259	***	0.361	***	0.443	***	0.458	***
	KIR3DL2	0.188	*	0.361	***	0.437	***	0.468	***
	KIR3DL3	0.121	0.057	0.159	**	0.177	**	0.165	***

	KIR2DS4	0.111	0.080	0.203	**	0.362	***	0.376	***
Dendritic cell	HLA-DPB1	0.507	***	0.641	***	0.644	***	0.659	***
	HLA-DQB1	0.333	***	0.452	***	0.508	***	0.535	***
	HLA-DRA	0.447	***	0.604	***	0.637	***	0.691	***
	HLA-DPA1	0.512	***	0.638	***	0.623	***	0.639	***
	BDCA-1 (CD1C)	0.219	**	0.397	***	0.18	***	0.212	***
	BDCA-4 (NRP1)	0.105	0.098	0.303	***	0.274	***	0.288	***
	CD11c (ITGAX)	0.421	***	0.57	***	0.497	***	0.506	***

Table4. Correlation analysis between CXCL9 and relate genes and markers of monocyte and macrophages in GEPIA

Description	Gene markers	OV		STAD	
		Tumor		Tumor	
		R	P	R	P
Monocyte	CD86	0.7	***	0.64	***
	CD115 (CSF1R)	0.55	***	0.48	***
TAM	CD68	0.63	***	0.33	***
	CCL2	0.47	***	0.2	***
	IL10	0.45	***	0.4	***
M1 Macrophage	INOS (NOS2)	0.11	0.08	0.077	0.12
	IRF5	0.27	***	0.2	***
	COX2 (PTGS2)	0.22	***	-0.04	0.42
M2 Macrophage	CD163	0.57	***	0.53	***
	VSIG4	0.53	***	0.47	***
	MS4A4A	0.63	***	0.52	***

Figures

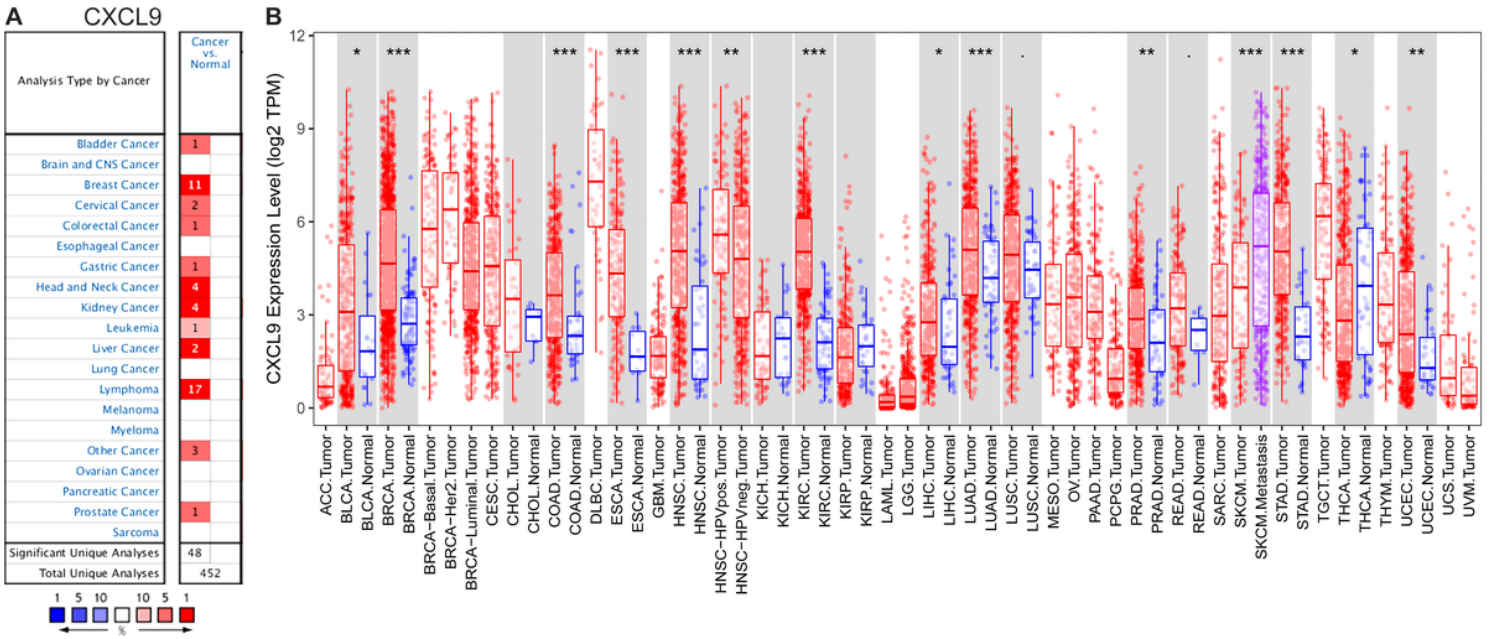

Figure 1

CXCL9 expression levels in different types of cancer. (A) CXCL9 expression level in data sets of different cancer types compared with normal tissues from Oncomine database. (B) CXCL9 expression levels in different types of cancer from the TCGA database in TIMER. Note: *P < 0.05, **P < 0.01, ***P < 0.001.

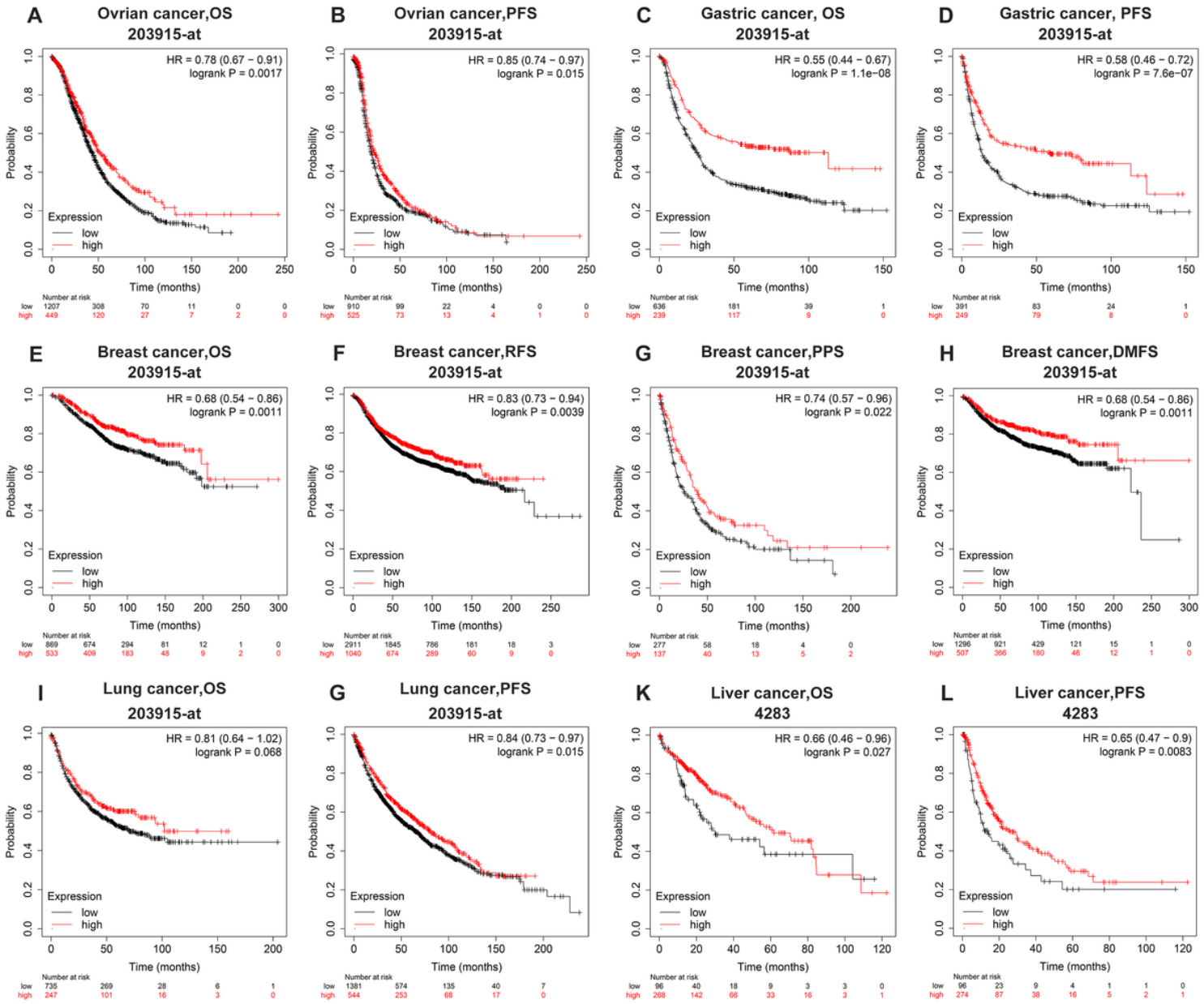

Figure 2

Kaplan-Meier survival curve analysis of the prognostic significance of high and low expression of CXCL9 in different types of human cancers using the Kaplan-Meier plotter database. (A-B) Kaplan-Meier survival analysis of CXCL9 in OS and PFS in ovarian cancer (n = 1656, n = 1435). (C-D) Kaplan-Meier survival analysis of CXCL9 in OS and PFS in gastric cancer (n = 875, n = 636). (E-H) Kaplan-Meier survival analysis of CXCL9 in OS, RFS, PPS and DMFS in breast cancer (n = 1402, n = 3951, N = 414, N = 1803). (I-G) Kaplan-Meier survival analysis of CXCL9 in OS and PFS in lung cancer (n = 982, n = 1925). (K-L) Kaplan-Meier survival analysis of CXCL9 in OS and PFS in liver cancer (n = 364, n = 370).

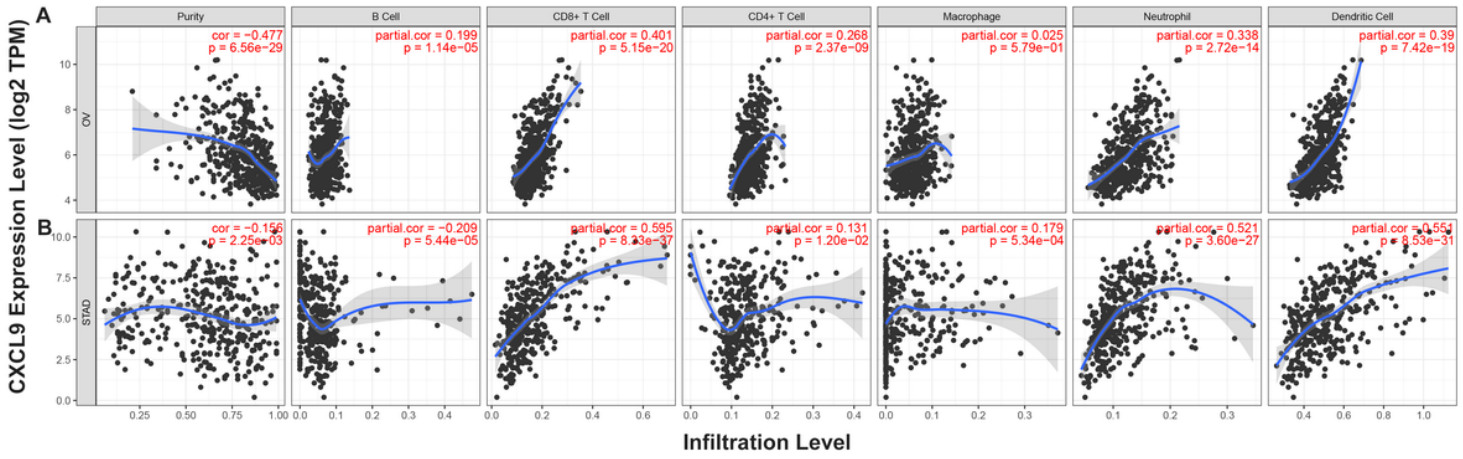

Figure 3

Correlation of CXCL9 expression with immune infiltration level in OV (ovarian serous cystadenocarcinoma) and STAD (stomach adenocarcinoma). (A) CXCL9 expression was significantly negatively related to tumor purity and had significant positive correlations with the levels of infiltrating B cells, CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells in OV. CXCL9 expression showed no correlation with macrophage infiltration in OV. (B) CXCL9 expression was significantly negatively related to tumor purity and had significant positive correlations with the levels of infiltrating CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells but had significant negative correlations with the levels of infiltrating B cells in STAD.

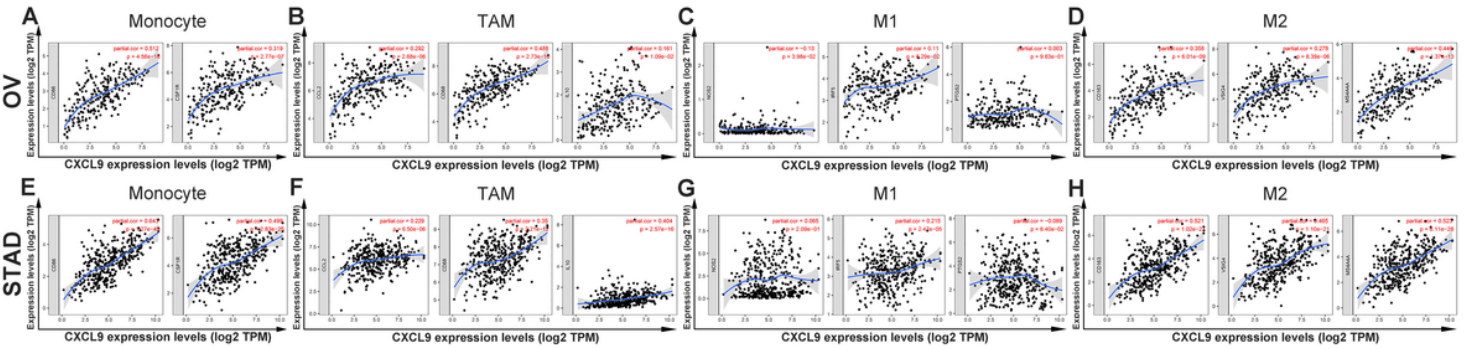

Figure 4

The correlation between the expression level of CXCL9 and immune marker genes in STAD and OV. Markers include CD86 and CSF1R of monocytes; CCL2, CD68, and IL10 of TAMs; NOS2, IRF5, and PTGS2 of M1 macrophages; and CD163, VSIG4, and MS4A4A of M2 macrophages. (A–D) Scatterplots of correlations between CXCL9 expression and gene markers of monocytes (A), TAMs (B), and M1 (C) and M2 macrophages (D) in OV. (E–H) Scatterplots of correlation between CXCL9 expression and the expression of gene markers of monocytes (E), TAMs (F), and M1 (G) and M2 macrophages (H) in STAD.

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