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Characterization of the prognostic values of *CXCR* family in gastric cancer

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ABSTRACT

Introduction: The role of CXC chemokine receptors (CXCRs) in gastric cancer (GC) has been an increasing focus. However, comprehensive prognostic values of CXCR members in GC are yet to be clearly defined.
Methods: Multiple public available datasets, including Kaplan-Meier (KM) plotter, oncomine, the cancer genome atlas (TCGA), SurvExpress platform and the tumor immune estimation resource (TIMER), were used for mRNA expression and prognostic characterization. Nomogram method was used for clinical model prediction.
Results: CXCR3, CXCR4 and CXCR5 displayed significantly up-regulated expression in tumor compared to normal. High mRNA expression of CXCR2 (HR = 0.77, 95%CI: 0.62–0.95, p = 0.014), CXCR3 (HR = 0.74, 95%CI: 0.61–0.90, p = 0.0024), CXCR4 (HR = 0.7, 95%CI: 0.58–0.86, p = 0.00048), CXCR5 (HR = 0.72, 95%CI: 0.59–0.87, p = 0.00093) and CXCR6 (HR = 0.66, 95%CI: 0.54–0.81, p = 4.9e−05) was significantly associated with favorable overall survival (OS). The prognostic values of CXCR members were also explored in subtypes, including HER2 status, Lauren classification, pathological stages. The low risk group of CXCR signature displayed a significantly favorable OS compared to the high risk group (HR = 3.22, 95% CI = 2.21–4.69, p = 1.057e−09). Nomogram clinical models were established for both OS (C-index: 0.692; 95%CI: 0.648–0.736) and recurrence free survival (C-index: 0.731; 95%CI: 0.675–0.786). In addition, CXCR6 and CD8 + T cells featured the highest correlation (partial-cor = 0.781, p = 4.17e−77).
Conclusion: This study identified distinct expression and prognostic values of CXCR members in GC using public databases.

1. Introduction

Gastric cancer (GC) remains one of the leading digestive malignancies in the world, ranking third in cancer-related mortality with more than 819,000 deaths annually [1–5]. Noteworthy, half of the newly occurred cases enrich in Eastern Asia, including China, Japan and South Korea [1–5]. Surgical intervention is the primary therapeutic strategy for advanced GC [2]. Significant survival benefits have been achieved in recent decades due to the improvements both in surgical skills and techniques as well as multi-disciplinary therapeutic regimes and early screen system [2,6,7]. Nonetheless, the overall survival (OS) of GC remains far from satisfactory. In fact, the current therapeutic success has not been fully translated into the individualized treatment. The lack of reliable biomarkers has made it difficult to identify high risk patients and may therefore offset the benefits brought by general multi-agent chemotherapy.

CXC chemokine receptors (CXCRs), comprising of CXCR1-7, are a group of cellular G-protein coupled receptors participating in not only immune system but also tumorigenesis and tumor progression [8,9]. CXCRs facilitate cellular dynamic communications with extracellular microenvironment, cellular trafficking, cell proliferation and invasion [8,9]. In addition, angiogenensis and tumor infiltrating immune cells (TIICs) are also among the increasing focuses associated with CXCRs.

CXCR1 and CXCR2 are cellular membrane receptors for interleukin-8 (IL8) [10,11]. CXCR1 belongs to the largest class of membrane proteins, rhodopsin-like G protein structure (class A), and functions as drug

Abbreviations: GC, gastric cancer; OS, overall survival; CXCRs, CXC chemokine receptors; TIICs, tumor infiltrating immune cells; IL8, interleukin-8; TILs, tumor infiltrating lymphocytes; MMP, matrix metalloproteinase; TGF, transforming growth factor; ERK, extracellular-regulated kinase; NK, natural killer; TCGA, the cancer genome atlas; GEPIA, Gene Expression Profiling Interactive Analysis; KM plotter, Kaplan-Meier plotter; TIMER, Tumor IMmune Estimation Resource; RFS, recurrence-free survival; HR, hazard ratio; CI, confidence interval; C-index, concordance index

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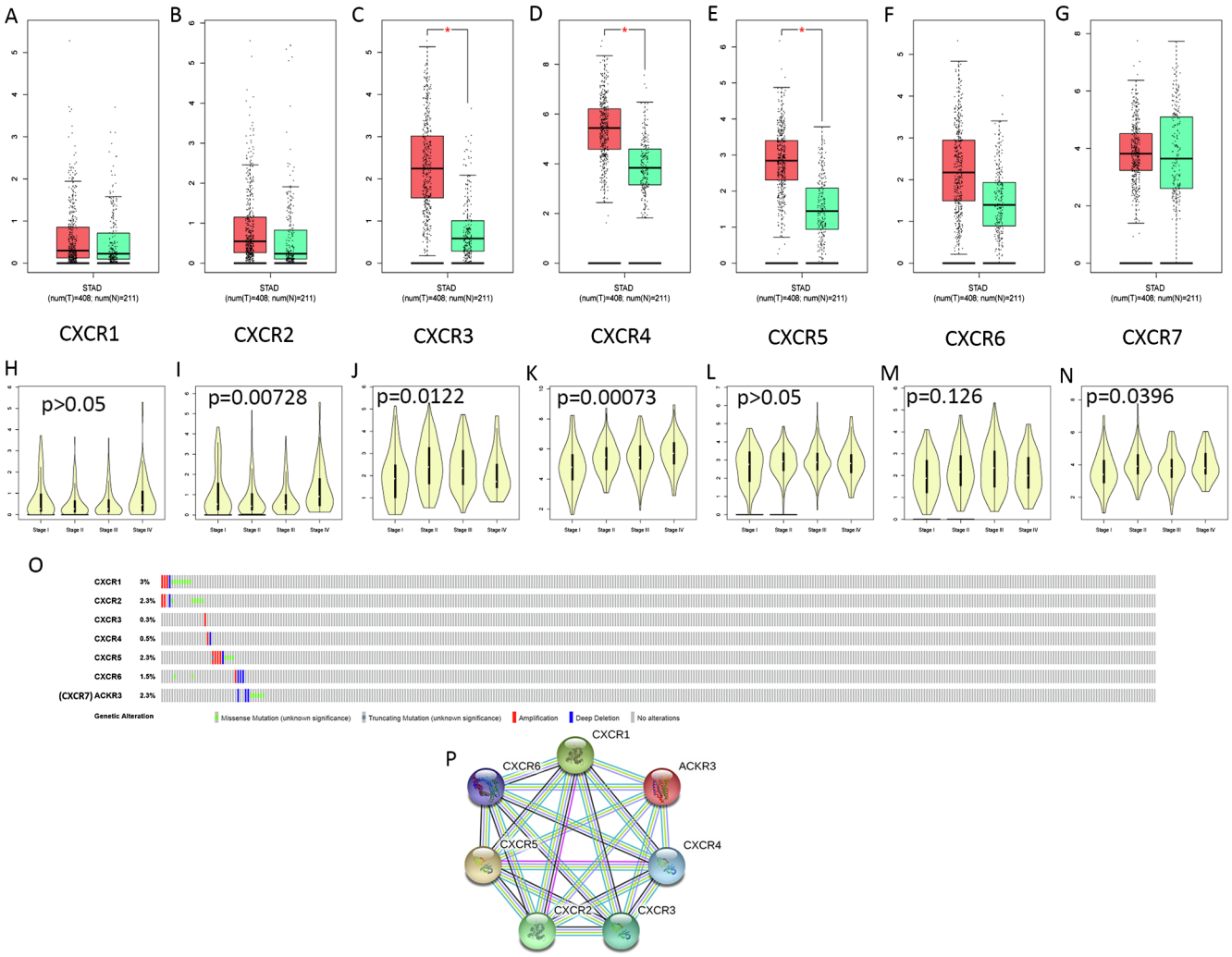

Fig. 1. The mRNA expression and genetic alterations of CXCR family members in the STAD of TCGA. (A–G) The mRNA expression of CXCR members between tumor and normal; red: tumor; green: normal; * $p < 0.05$; (H–N) the stage-specific mRNA expression of CXCR members; (O) the genetic alterations of CXCR members in TCGA; (P) the protein-protein-interaction (PPI) networks of CXCR members. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

receptors and signal transduction [12]. CXCR2 is a G protein-coupled receptor (class B) with close association in angiogenesis and inflammation [13]. Overexpression of CXCR1 and CXCR2 enhances the *in vitro* migration and invasion capability of tumor cells and is associated with metastasis, histological differentiation as well as pathological stages [10].

CXCR3 has been increasingly involved in tumorigenesis and progression [14,15]. Previous study indicates that the overexpression of CXCR3 is found in tumor compared to normal tissues, with inverse association with tumor invasion depth and metastasis status [14]. Moreover, CXCR3 is also identified as an independent prognostic factor in GC [14,15]. Meanwhile, tumor infiltrating lymphocytes (TILs), including CD8 + T cells and CD4 + T cells, are positively associated with CXCR3 expression [15].

CXCR4, as the most common type of chemokine receptors, has been involved in numerous malignancies, including breast cancer, melanoma, prostate cancer and GC [16,17]. In GC, increased CXCR4 expression is significantly associated with lymph node metastasis, advanced pathological stages and reduced OS (16). Another study highlights the increased expression level of CXCR4 with advanced stages and inferior OS [17]. Moreover, matrix metalloproteinase (MMP)-7, MMP-9, transforming growth factor (TGF)-alpha and extracellular-regulated kinase (ERK) pathway are correlated with CXCR4 expression [17].

CXCR5 is abnormally expressed in numerous tumor and closely associated with tumor progression [18,19]. Nonetheless, no significant correlation is identified between the expression of CXCR5 and metastasis, lymph node status as well as pathological stages [18]. The role of CXCR5 in the progression of GC is yet to be disclosed.

CXCR6 is a chemokine receptor previously known as being selectively expressed in natural killer (NK) cells, T cells and plasma cells [20]. It is responsible for the chemotactic migration of immune cells to inflamed areas. CXCR6 is found inversely correlated with tumor invasion depth and favorable prognosis [20].

Table 1
Comparison of CXCR family members expression between different subtypes of GC and normal tissues via Oncomine platform.

CXCR family members	Types of GC vs normal	Fold change	*t*-test	*p*-value	Ref.
CXCR1	Gastric adenocarcinoma vs normal	1.017	3.172	0.001	[26]
	Gastric cancer vs normal	1.017	2.363	0.011	[26]
	Diffuse type vs normal	1.009	2.059	0.023	[26]
CXCR2	Mixed type vs normal	1.46	2.295	0.016	[27]
	Diffuse type vs normal	2.858	2.209	0.035	[27]
	Gastric adenocarcinoma vs normal	1.017	3.172	0.001	[26]
	Gastric cancer vs normal	1.017	2.363	0.011	[26]
	Diffuse type vs normal	1.009	2.059	0.023	[26]
CXCR3	Diffuse type vs normal	1.924	2.721	0.008	[27]
	Mixed type vs normal	3.016	3.224	0.012	[27]
	Diffuse type vs normal	1.121	2.908	0.002	[26]
CXCR4	Mixed type vs normal	2.033	3.951	8.67E-04	[28]
	Diffuse type vs normal	1.832	3.497	9.96E-04	[28]
	Intestinal type vs normal	1.516	3.497	8.74E-04	[28]
	Gastric cancer vs normal	2.288	3.4	0.001	[29]
	Diffuse type vs normal	2.817	4.107	0.003	[27]
	Mixed type vs normal	4.098	3.524	0.013	[27]
	Gastric cancer vs normal	1.207	2.122	0.018	[30]
	Diffuse type vs normal	1.081	2.671	0.005	[31]
	Mixed type vs normal	1.079	1.951	0.034	[31]
	Gastric adenocarcinoma vs normal	1.017	3.631	2.57E-04	[26]
	Diffuse type vs normal	1.01	2.339	0.012	[26]
	Intestinal type vs normal	1.032	1.796	0.04	TCGA
CXCR5	Diffuse type vs normal	1.291	2.89	0.004	[28]
	Intestinal type vs normal	1.139	2.111	0.02	[28]
	Intestinal type vs normal	1.02	2.045	0.024	[26]
CXCR6	Diffuse type vs normal	1.429	2.004	0.032	[27]
CXCR7	Gastric cancer vs normal	1.566	3.105	0.003	[29]
	Mixed type vs normal	2.281	2.797	0.025	[27]
	Intestinal type vs normal	1.439	1.959	0.028	[27]
	Gastric adenocarcinoma vs normal	1.01	1.825	0.036	[26]
	Diffuse type vs normal	1.01	1.837	0.036	[26]

CXCR7 has been associated with peritoneal dissemination and poor prognosis in GC [21]. Previous gene set enrichment analysis (GSEA) also indicates that CXCR7 is significantly enriched in signatures related to tumor progression [21].

Although a series of studies have elucidated the significant prognostic roles of some CXCR members in GC, the whole picture of the prognostic values of CXCR members remain inequitably characterized in GC. Therefore, this study delineates the prognostic roles of CXCR members via multiple public available datasets and bioinformatics.

2. Materials and methods

2.1. The mRNA expression of CXCR members in the cancer genome atlas (TCGA)

The mRNA expression of CXCR family members in TCGA was analyzed via the Gene Expression Profiling Interactive Analysis (GEPIA) platform (http://gepia.cancer-pku.cn/index.html) [22]. The genomic alterations of CXCR members in TCGA, including missense mutation, truncating mutation, amplification and deep deletion, were identified by the cBioPortal platform (http://www.cbioportal.org/) [23,24]. Moreover, the mRNA expression profile of CXCR members was retrieved from the Xena system for statistical analysis [25].

Table 2
Genetic alterations of CXCR family members in the stomach adenocarcinoma (STAD) of TCGA.

		Cases
CXCR1	Amplification	3
	Deep deletion	1
	Missense mutation	8
	L87I	1
	R144H, R227H	1
	Q310H	1
	T270A	1
	L52R	1
	R203Q	1
	F330V	1
	R144C	1
CXCR2	Amplification	2
	Deep deletion	1
	Missense mutation	6
	D19E, K163N	1
	A98V, R236H	1
	V187I	2
	A106T	1
	S141N	1
CXCR3	Amplification	1
CXCR4	Amplification	1
	Deep deletion	1
CXCR5	Amplification	4
	Deep deletion	1
	Missense mutation	4
	T370S	1
	R201H	1
	A285V	1
	R251C	1
	Truncating mutation	1
	L45Sfs*22	1
CXCR6	Amplification	1
	Deep deletion	3
	Missense mutation	2
	C102Y	1
	S116P	1
CXCR7	Deep deletion	3
	Missense mutation	6
	A229T	1
	V258M	1
	P38L	1
	R197Q	1
	V299I	1
	V175M	1

2.2. Protein-protein interaction (PPI) networks of CXCR members

The PPI networks of CXCR members were established by the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, http://www.string-db.org/) [26].

2.3. Oncomine analysis

The mRNA expression of CXCR members in GC was also explored via the oncomine platform (www.oncomine.org) [27]. The statistical significant cutoff p value was 0.05. The significantly differentially expressed CXCR members between normal and tumor in GC were identified in seven datasets via oncomine [28–33].

2.4. Survival analysis of CXCR members

The prognostic values of OS in CXCR members were investigated via the Kaplan-Meier plotter (KM plotter) (www.kmplot.com) [34].

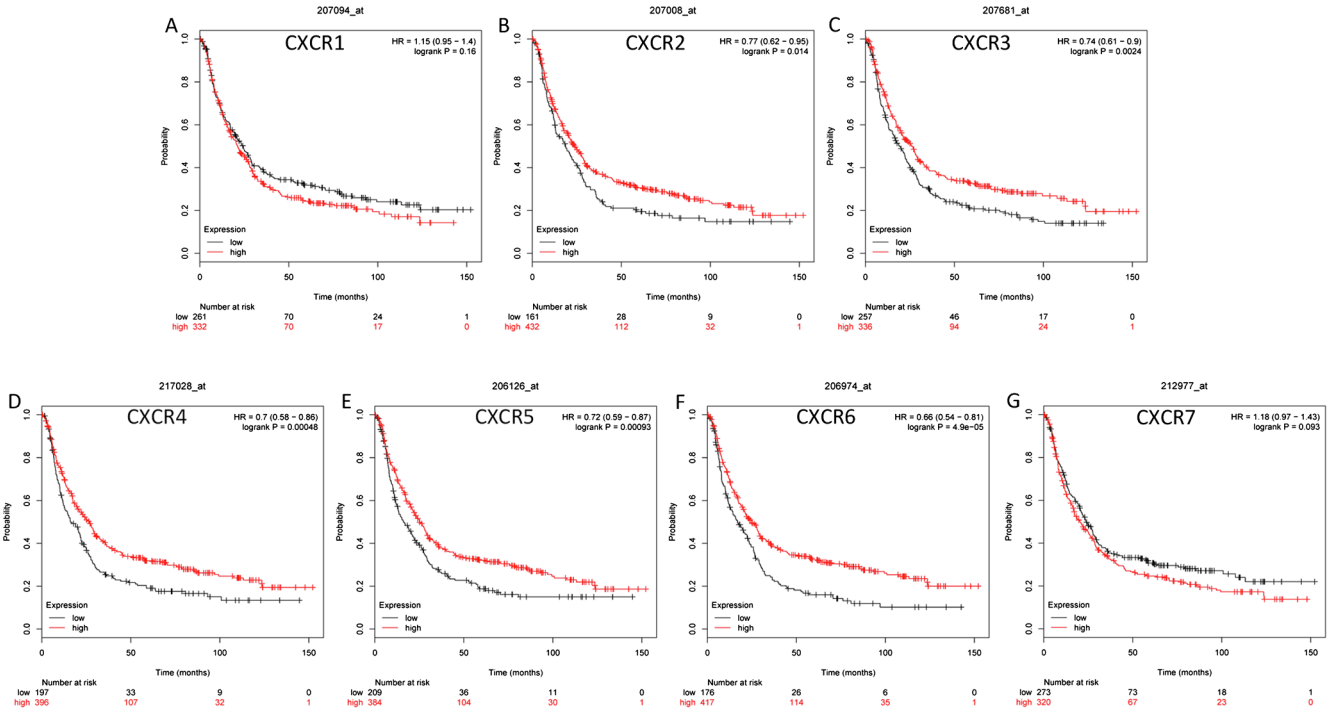

Fig. 2. The prognostic values of CXCR members in all GC by the KM plotter. (A–G) The prognostic values of CXCR members.

Furthermore, given the increasing focus on the prognostic values of genes signature, the prognostic value of CXCR signature was also explored via the SurvExpress platform (http://bioinformatica.mty.itesm.mx:8080/Biomatec/SurvivaX.jsp) [35]. High and low risk groups were divided based on the maximized risk algorithm [35].

2.5. Immune infiltrates correlation via the Tumor Immune Estimation Resource (TIMER)

The correlations between each immune infiltrates (B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages and dendritic cell types) and CXCR members were analyzed via the TIMER web-based platform (https://cistrome.shinyapps.io/timer/) [36]. The correlation was exhibited by the purity-corrected partial Spearman method (partial-cor).

2.6. DNA methylation data of CXCR members

The DNA methylation sites of CXCR members in TCGA were analyzed by MethSurv (https://biit.cs.ut.ee/methsurv/), a comprehensive bioinformatics platform for methylation visualization [37]. Meanwhile, the prognostic values of all the methylation sites associated with CXCR members were also characterized.

2.7. Statistical analysis

Univariate and multivariate cox analysis of CXCR members was performed in both OS and recurrence-free survival (RFS) with hazard ratio (HR) and 95% confidence interval (95%CI). Nomogram models of OS and RFS were established based on the multivariate results using the R software (version 3.3.0) (R foundation for statistical computing, Vienna, Austria, www.r-project.org). $P < 0.05$ was considered as significant. The concordance index (C-index) was used to measure the prediction power.

3. Results

3.1. The mRNA expression levels of CXCR family in GC

CXCR3, CXCR4 and CXCR5 displayed significant up-regulated expression in tumor compared to normal from TCGA (Fig. 1A–F). Next, the expression of CXCR members in different subtypes GC compared was analyzed via Oncomine platform. Noteworthy, all CXCR members were upregulated in diffused type compared to normal (Table 1). Remarkably, the significant upregulation of CXCR4 in tumor was noticed in all datasets (Table 1). Next, CXCR2 ($p = 0.00728$), CXCR3 ($p = 0.0122$), CXCR4 ($p = 0.00073$) and CXCR7 ($p = 0.0396$) displayed significant stage-specifically expression (Fig. 1H–N). The genetic alterations of CXCR members in TCGA were also displayed (Fig. 1O, Table 2). Moreover, all CXCR members displayed close mutual protein-protein interaction (Fig. 1P). In fact, given the comparably low mutation proportion, we further investigated the prognostic roles of CXCR members.

3.2. Prognostic values of CXCR family members in GC

The prognostic values of CXCR members were analyzed by the KM plotter. In fact, the high mRNA expression of CXCR2 (HR = 0.77, 95%CI: 0.62–0.95, $p = 0.014$), CXCR3 (HR = 0.74, 95%CI: 0.61–0.90, $p = 0.0024$), CXCR4 (HR = 0.7, 95%CI: 0.58–0.86, $p = 0.00048$), CXCR5 (HR = 0.72, 95%CI: 0.59–0.87, $p = 0.00093$) and CXCR6 (HR = 0.66, 95%CI: 0.54–0.81, $p = 4.9e-05$) was significantly associated with favorable OS (Fig. 2A–G). Next, the prognostic values of CXCR members in subtypes were analyzed, including HER2 status, Lauren classification, pathological stages.

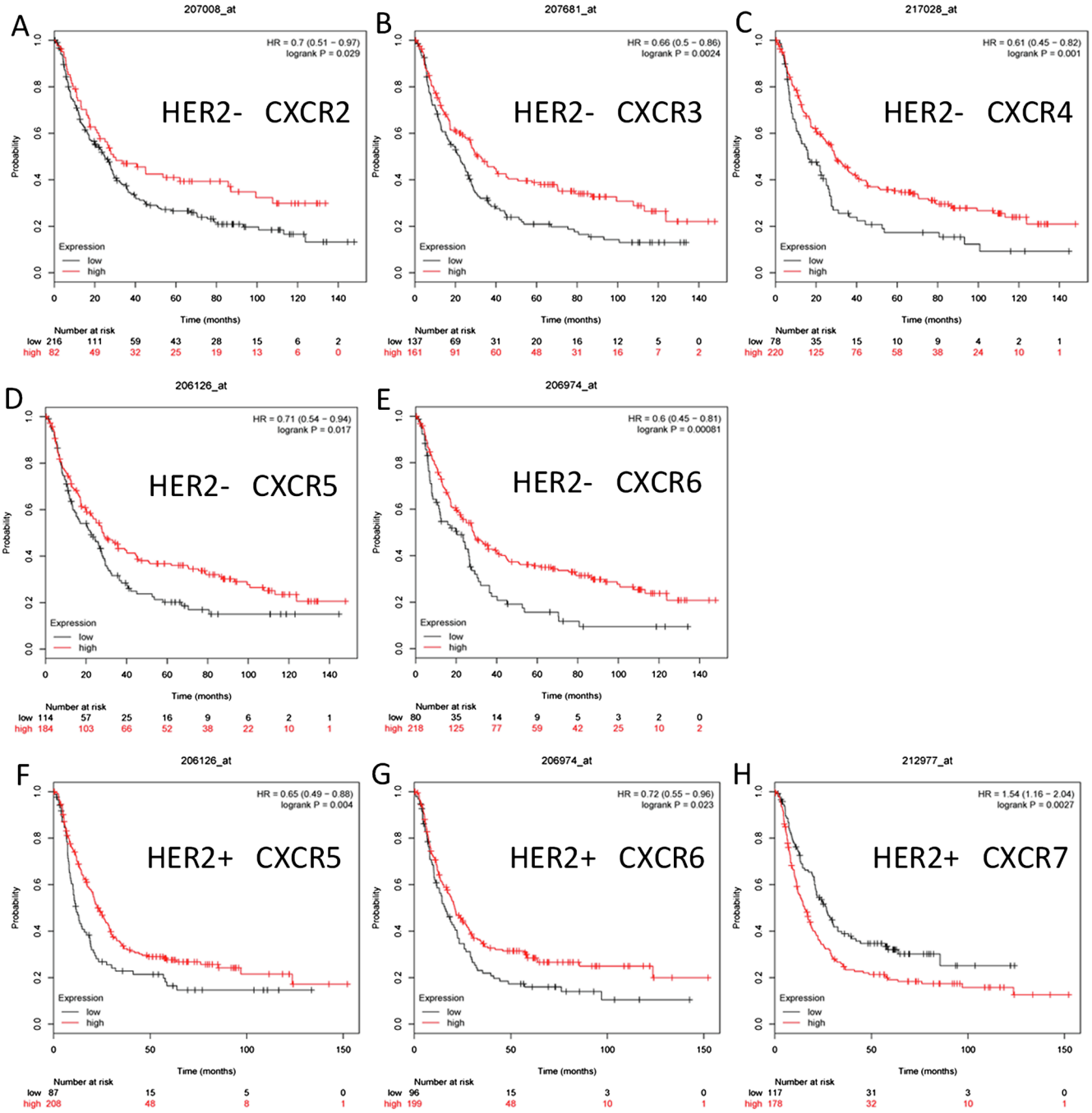

Fig. 3. The prognostic values of CXCR members in HER2 subtypes of GC. (A–E) The prognostic values of CXCR2-6 in HER2 negative GC; (F–H) the prognostic values of CXCR5-7 in HER2 positive GC.

For HER2-group, high expression of CXCR2 (HR = 0.7, 95%CI: 0.51–0.97, p = 0.029), CXCR3 (HR = 0.66, 95%CI: 0.5–0.86, p = 0.0024), CXCR4 (HR = 0.61, 95%CI: 0.45–0.82, p = 0.001), CXCR5 (HR = 0.71, 95%CI: 0.54–0.94, p = 0.017) and CXCR6 (HR = 0.6, 95%CI: 0.45–0.81, p = 0.00081) showed significant association with favorable OS (Fig. 3 A–E). For HER2 + group, only high expression of CXCR5 (HR = 0.65, 95%CI: 0.49–0.88, p = 0.004) and CXCR6 (HR = 0.72, 95%CI: 0.55–0.96, p = 0.023) showed significant association with favorable OS whereas high expression of CXCR7 (HR = 1.54, 95%CI: 1.16–2.04, p = 0.0027) showed significant association with poor OS (Fig. 3 F–H). CXCR members with insignificant prognosis were not displayed.

For Lauren classification, four members, including CXCR1, CXCR2, CXCR6 and CXCR7, displayed significant association with OS in intestinal type of GC (Fig. 4 A–D). CXCR1, CXCR3, CXCR5 and CXCR6 showed significant association with OS in diffuse type (Fig. 4E–H). Only

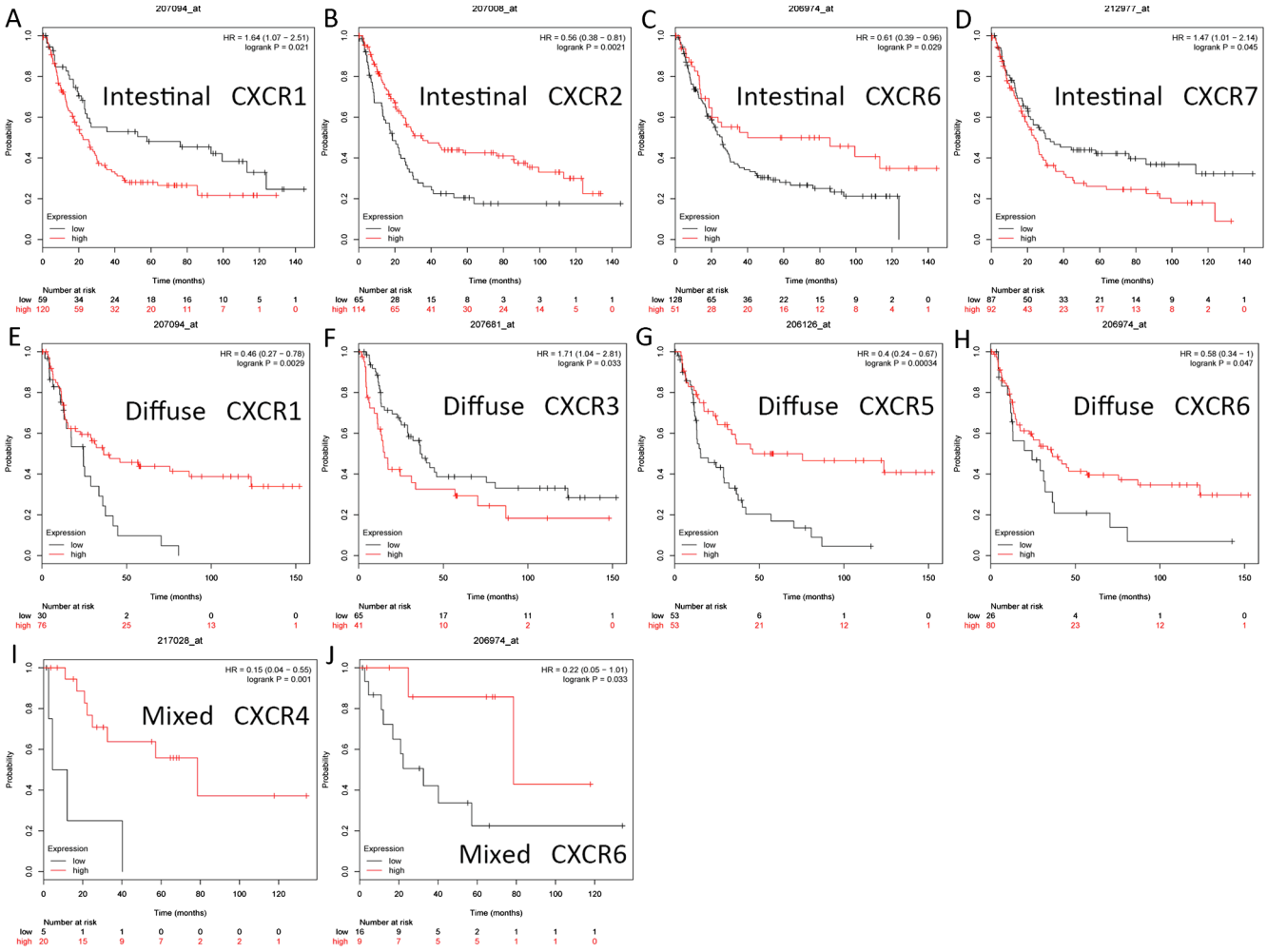

Fig. 4. The prognostic values of CXCR members in Lauren classification subtypes. (A–D) The prognostic values of CXCR1,2,6 and 7 in intestinal type; (E–H) the prognostic values of CXCR1,3,5 and 6 in diffuse type; (I, J) the prognostic values of CXCR4 and CXCR6 in mixed type.

CXCR4 and CXCR6 showed significant association with OS in mixed type (Fig. 4I, J). Intriguingly, CXCR1 displayed inverse prognostic values between intestinal type (HR = 1.64, 95%CI: 1.07–2.51, p = 0.021) and diffuse type (HR = 0.46, 95%CI: 0.27–0.78, p = 0.0029) (Fig. 4A, E). Furthermore, the prognostic values of CXCR members were explored in pathological stages (Table 3). Of note, CXCR5 and CXCR7 displayed significant association with stage I GC, indicating as potential predictors.

3.3. Prognostic values of CXCR signature in GC

Given the increasing focus on the prognostic value of genes signature, hereby, the CXCR signature was also explored via the SurvExpress platform. High/low risk groups were divided by prognostic risk algorithms. Remarkably, the low risk group displayed a significant favorable OS outcome compared to the high risk group (HR = 3.22, 95% CI = 2.21–4.69, p = 1.057e−09) (Fig. 5A). The expression of each CXCR member was also displayed between high and low risk groups (Fig. 5B, C). In fact, the expression of CXCR4 and CXCR7 in low risk group was significantly lower than in high risk group whereas CXCR6 showed the opposite feature (Fig. 5C). Furthermore, the receiver operating characteristics (ROC) curves were also present in various survival time point (Fig. 5D, E). The highest area under the curve (AUC) were 0.762 by KM method while 0.748 by NNE method (Fig. 5D, E).

3.4. Univariate and multivariate analysis of CXCR members in GC

Furthermore, whether the CXCR members could be used as independent prognostic predictors (OS + RFS) were under investigation by univariate and multivariate cox regression. In fact, only CXCR4 (HR = 1.72, 95%CI = 1.19–2.48, p = 0.004), CXCR6 (HR = 0.51, 95%CI = 0.34–0.75, p = 0.001) and CXCR7 (HR = 1.61, 95%CI = 1.09–2.38, p = 0.016) were identified as independent prognostic predictor in OS whereas CXCR1 (HR = 2.62, 95%CI = 1.01–6.75, p = 0.047), CXCR2 (HR = 0.36, 95%CI = 0.17–0.79, p = 0.011), CXCR3 (HR = 3.49, 95%CI = 1.01–12.07, p = 0.049), CXCR4 (HR = 3.20, 95%CI = 1.72–5.96, p < 0.001), CXCR6 (HR = 0.22, 95%CI = 0.12–0.42, p < 0.001) were identified as independent prognostic predictor in RFS (Tables 4 and 5).

Table 3
Correlation of CXCR members mRNA expression for GC patients with stages in KM plotter.

Gene	Stages	Cases	HR(95%CI)	*P*-value
CXCR1	I	19	2.9(0.92–9.13)	0.058
	II	27	1.39(0.6–3.22)	0.44
	III	93	0.85(0.61–1.18)	0.32
	IV	41	0.68(0.38–1.22)	0.2
CXCR2	I	19	2.3(0.71–7.48)	0.15
	II	27	0.63(0.27–1.5)	0.29
	III	93	0.57(0.4–0.8)	0.0012*
	IV	41	1.32(0.76–2.31)	0.33
CXCR3	I	19	1.61(0.53–4.96)	0.4
	II	27	0.64(0.26–1.58)	0.33
	III	93	0.75(0.54–1.03)	0.077
	IV	41	1.59(0.88–2.85)	0.12
CXCR4	I	19	0.34(0.11–1.05)	0.05
	II	27	2.14(0.9–5.11)	0.078
	III	93	0.75(0.53–1.06)	0.099
	IV	41	0.77(0.41–1.44)	0.41
CXCR5	I	19	4.98(1.1–22.51)	0.021*
	II	27	0.56(0.2–1.51)	0.24
	III	93	1.37(0.99–1.88)	0.056
	IV	41	0.67(0.39–1.16)	0.15
CXCR6	I	19	0.44(0.12–1.69)	0.22
	II	27	0.57(0.23–1.44)	0.23
	III	93	0.62(0.43–0.89)	0.0082*
	IV	41	1.34(0.77–2.34)	0.3
CXCR7	I	19	0.15(0.02–1.17)	0.037*
	II	27	3.18(1.16–8.73)	0.018*
	III	93	1.17(0.84–1.65)	0.35
	IV	41	2.33(1.15–4.7)	0.015*

* $P < 0.05$.

3.5. CXCR-based prognostic nomogram models for GC

Next, nomogram clinical models were established for both OS and RFS (Figs. 6 and 7). In fact, the C-index for nomogram model in OS was 0.692 (95%CI: 0.648–0.736) whereas the C-index for nomogram model in RFS was 0.731 (95%CI: 0.675–0.786) (Figs. 6 and 7), indicating potential clinical model values of CXCR members.

3.6. TIICs associated with CXCR members

Given the distinct prognostic values of CXCR members, the potential immunological correlation of CXCR members and TIICs were investigated. The expression of CXCR members against tumor purity showed negative association indicating highly expression in the microenvironment. Intriguingly, the highest correlation was identified between CXCR6 and CD8+T cells (partial-cor = 0.781, p = 4.17e−77). Moreover, the correlation between CXCR6 and neutrophils (partial-cor = 0.617, p = 3.19e−40), CXCR6 and dendritic cells (partial-cor = 0.652, p = 2.64e−46), CXCR3 and CD8+T cells (partial-cor = 0.657, p = 4.05e−47), CXCR3 and dendritic cells (partial-cor = 0.606, p = 1.57e−38), CXCR4 and dendritic cells (partial-cor = 0.618, p = 2.02e−40) and CXCR5 and CD4+T cells (partial-cor = 0.617, p = 8.62e−40) were comparably high (Fig. 8).

3.7. Prognostic values of the DNA methylation sites of CXCR members

The DNA methylation levels of each CpG in CXCR members were displayed, respectively (Figure Supplementary Fig. 1, Supplementary Fig. 2, Supplementary Fig. 3, Supplementary Fig. 4, Supplementary Fig. 5, Supplementary Fig. 6 and Supplementary Fig. 7). Next, the

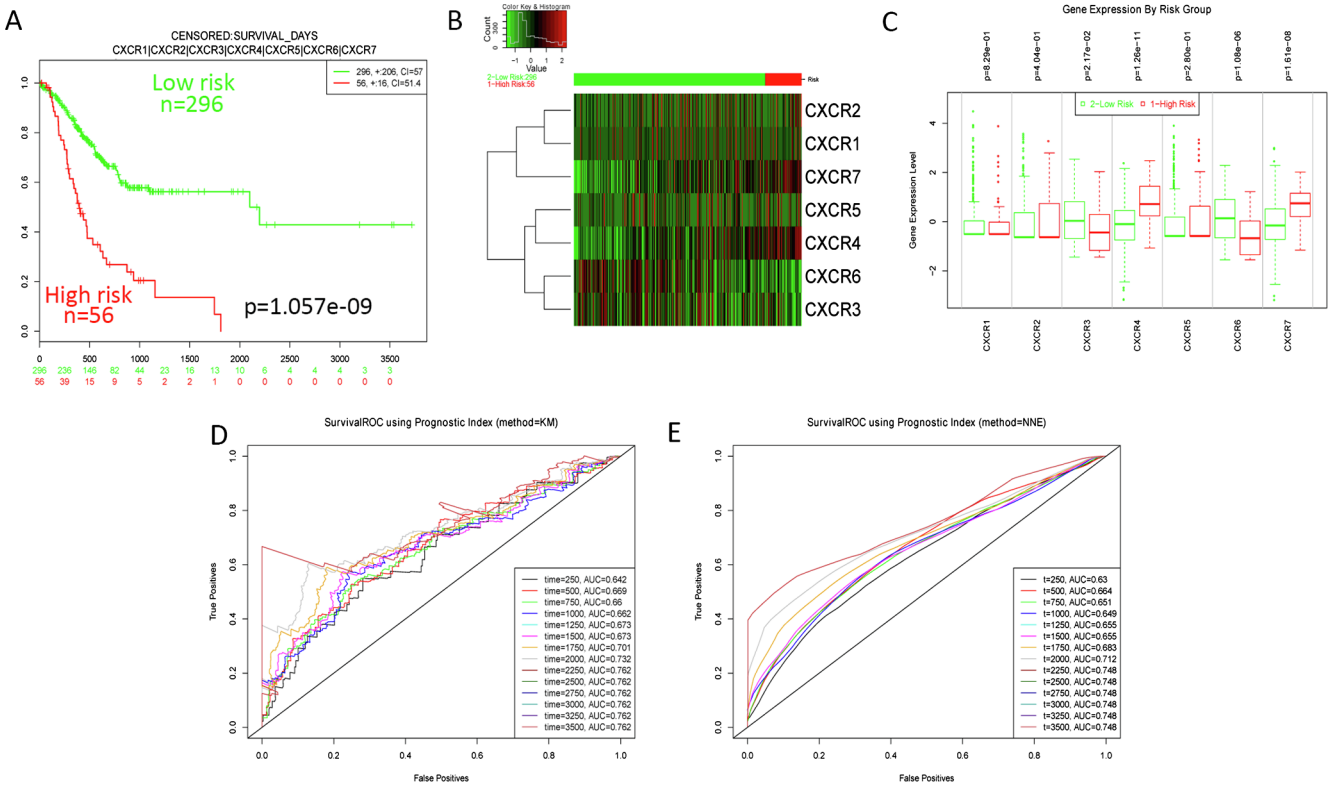

Fig. 5. The prognostic values of CXCR signature via the SurvExpress. (A) The survival analysis between low risk (green) and high risk (red) groups; (B) the expression of CXCR members in a heat map; (C) the expression comparison of CXCR members between low and high risk groups; (D, E) the receiver operating characteristics (ROC) curves of CXCR signature by Kaplan-Meier (KM) method and Nearest Neighbor Estimation (NNE) method. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Univariate and multivariate overall survival (OS) analysis of CXCR members in GC patients.

Variables	Univariate analysis*	Multivariate analysis†	
	P-value	HR (95%CI)	P-value
Age	0.020		
< 60		Reference	
60–75		1.85(1.23–2.78)	0.003
> 75		2.31(1.3–4.08)	0.004
Gender	0.120		
Male		Reference	
Female		0.88(0.6–1.29)	0.525
Pathologic_T	0.009		
T1		Reference	
T2		3.76(0.49–28.62)	0.202
T3		4.57(0.61–34.09)	0.139
T4		4.84(0.64–36.62)	0.126
Pathologic_N	< 0.001		
N1		Reference	
N2		1.38(0.83–2.28)	0.213
N3		1.55(0.90–2.67)	0.117
N4		2.34(1.40–3.92)	0.001
Pathologic_M	0.031		
M0		Reference	
M1		1.67(0.88–3.19)	0.117
Pathologic_stage	< 0.001		
stage I		Reference	
stage II		–	–
stage III		–	–
stage IV		–	–
Neoplasm_histologic_grade	0.042		
G1		Reference	
G2		1.76(0.41–7.61)	0.449
G3		2.57(0.6–10.97)	0.203
CXCR1	0.061		
Low		Reference	
High		1.36(0.92–2.01)	0.118
CXCR2	0.077		
Low		Reference	
High		0.67(0.4–1.12)	0.127
CXCR3	0.262		
Low			
High			
CXCR4	0.003		
Low		Reference	
High		1.72(1.19–2.48)	0.004
CXCR5	0.223		
Low			
High			
CXCR6	0.015		
Low		Reference	
High		0.51(0.34–0.75)	0.001
CXCR7	0.001		
Low		Reference	
High		1.61(1.09–2.38)	0.016

* Univariable Cox regression analysis.
† Multivariate analysis adjusted by age, gender, pathologic_T, pathologic_N, pathologic_M, neoplasm_histologic_grade, CXCR1, CXCR2, CXCR4, CXCR6, CXCR7.

Table 5
Univariate and multivariate recurrent-free survival (RFS) analysis of CXCR members in GC patients.

Variables	Univariate analysis*	Multivariate analysis†	
	P-value	HR (95%CI)	P-value
Age	0.681		
< 60			
60–75			
> 75			
Gender	0.004		
Male		Reference	
Female		0.54(0.3–0.98)	0.043
Pathologic_T	0.291		
T1			
T2			
T3			
T4			
Pathologic_N	0.147		
N1		Reference	
N2		0.81(0.42–1.57)	0.534
N3		0.93(0.45–1.93)	0.839
N4		0.87(0.42–1.8)	0.702
Pathologic_M	0.678		
M0			
M1			
Pathologic_stage	0.410		
Stage I			
Stage II			
Stage III			
Stage IV			
Neoplasm_histologic_grade	0.107		
G1		Reference	
G2		1.12(0.14–9)	0.918
G3		1.70(0.21–13.51)	0.615
CXCR1	0.112		
Low		Reference	
High		2.62(1.01–6.75)	0.047
CXCR2	0.099		
Low		Reference	
High		0.36(0.17–0.79)	0.011
CXCR3	0.063		
Low		Reference	
High		3.49(1.01–12.07)	0.049
CXCR4	0.003		
Low		Reference	
High		3.20(1.72–5.96)	< 0.001
CXCR5	0.053		
Low		Reference	
High		3.29(0.72–15.12)	0.125
CXCR6	0.009		
Low		Reference	
High		0.22(0.12–0.42)	< 0.001
CXCR7	0.131		
Low		Reference	
High		0.94(0.47–1.86)	0.854

* Univariable Cox regression analysis.
† Multivariate analysis adjusted by gender, pathologic_N, CXCR1, CXCR2, CXCR3, CXCR4, CXCR6.

prognostic value of each CpG was characterized. Only the CpGs with significant prognostic values were displayed in KM curves (Figure Supplementary Fig. 8). CXCR1 and CXCR6 only had one prognostic-related CpG, respectively. CXCR4 had 6 prognostic-related CpGs, the most compared to other members (Figure Supplementary Fig. 8).

4. Discussion

Given tumor immunotherapy has been emerged as a hotpot in both basic and clinical senses, the values of CXCR members, key components involved in the immune system, have been increasingly realized. In fact, an increasingly sophisticated knowledge of the biological and clinical values of CXCR members has been obtained during the past decade. However, their prognostic values in GC are yet to be equally characterized. Of note, describing the prognostic values of CXCR members

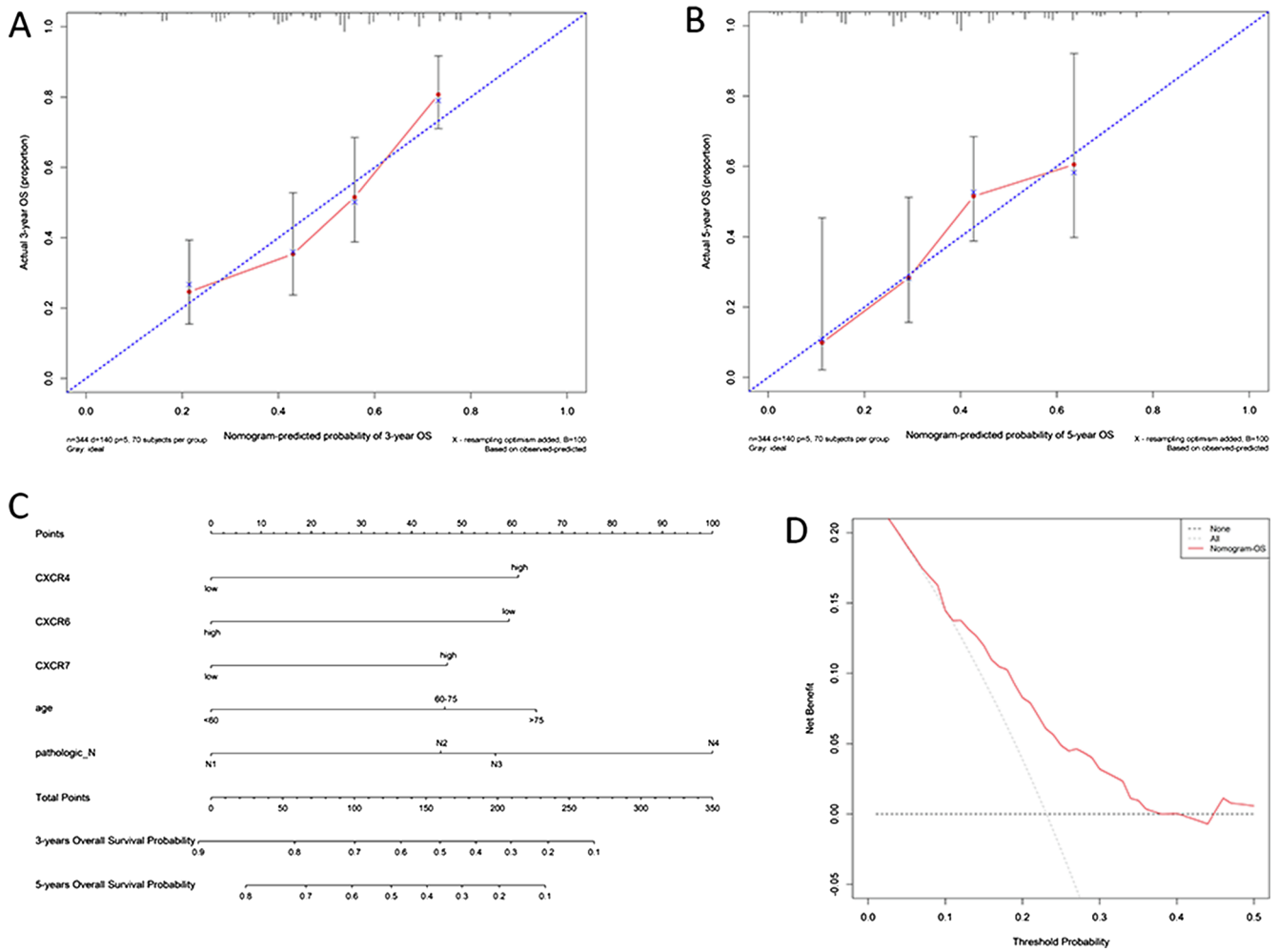

Fig. 6. Nomogram model for the OS of CXCR members. (A, B) The calibration plots for the 3- and 5- year OS; (C) nomogram model for 3- and 5-year associated OS; (D) the decision curve analysis.

in GC is important for identifying and stratification of patients with diverse survival risks.

This study found out that high expression of CXCR2-6 were significantly associated with favorable OS by KM plotter, while apparently contradictory, CXCR3-5 were significantly increased in tumor compared to normal. It is partially because of the different datasets. Specifically, the expression dataset used was retrieved from TCGA while the survival datasets were from GEO platform, processed by KM plotter web tool. The KM plotter contained six independent profiles, including GSE14210, GSE15459, GSE22377, GSE29272, GSE51105 and GSE62254 (GSE62254 was excluded for heterogeneity).

CXCR1/2 have been found as key mediators for tumorigenesis and tumor progression in multiple cancers and responsible for poor outcome and early recurrence [38,39]. Mechanistically, CXCR1/2 modulates the migration and invasion capability of GC cells [39]. In addition, metalloproteinase-9 (MMP9) is closely correlated to the expression of CXCR1/2 [39]. Of note, CXCR1/2 are also responsible for the regulation of phosphorylated ERK1/2, JNK and c-Jun [39]. In our study, CXCR1 showed significantly inverse prognostic values between intestinal and diffuse GC subsets, indicating a possible sophisticated mechanism in association with histological classification. CXCR2 showed a significantly favorable prognostic value in general group and numerous subsets, including HER2- and intestinal. Moreover, CXCR1/2 were identified as independent prognostic factors associated with RFS, instead of OS. Nonetheless, the expression of CXCR1/2 between high/low risk groups in the prognostic signature of CXCR remains insignificant. In fact, our results complemented the prognostic values of CXCR1/2 in GC with multi-dimensional clues. The expression of CXCR4 displayed a stage-specific pattern, indicating the potential association between tumor progression and CXCR4 expression. Previously, Masuda et al. reported that the immunoreactivity of nuclear CXCR4 was an independent prognostic factor and associated with reduced 5-year OS [40]. Moreover, Yasumoto et al. reported that positive CXCR4 in GC significantly associated with the peritoneal metastases, indicating the metastatic role of CXCR4 in GC [41]. Consistently, the signature prognosis analysis also highlighted the distinct upregulated expression pattern of CXCR4 in high risk group compared to the low risk group. The low risk group, with lower expression of CXCR4, showed a significant favorable OS than high risk group (HR = 3.22, 95% CI = 2.21–4.69, p = 1.057e−09). It may seem contradictory to the favorable survival outcome of CXCR4 evidenced by KM plotter using optimal cutoff (HR = 0.7, 95%CI: 0.58–0.86, p = 0.00048). However, using multivariate analysis, CXCR4 was finally identified as an independent risk factor in both OS (HR = 1.72, 95% CI = 1.19–2.48,

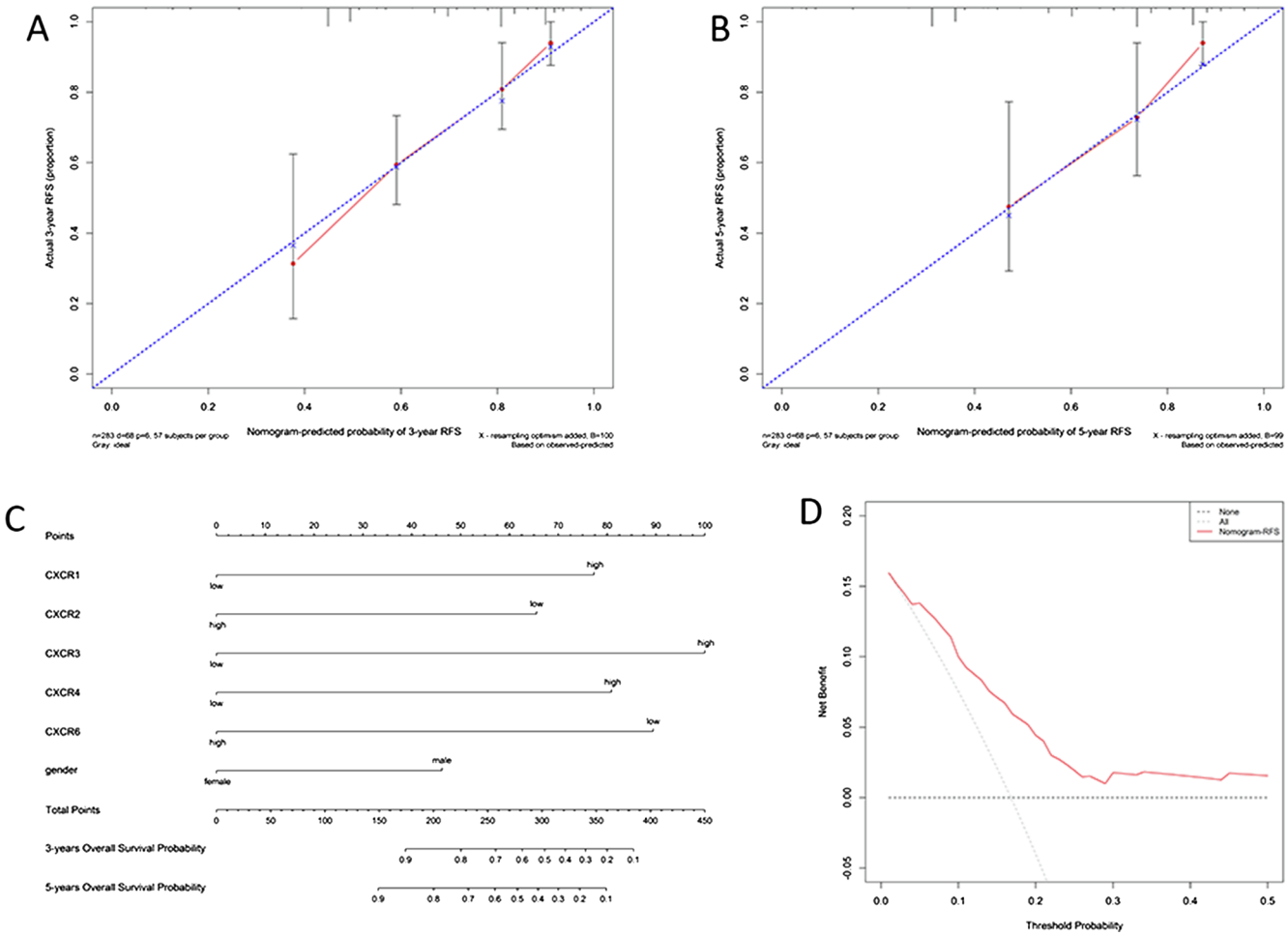

Fig. 7. Nomogram model for the RFS of CXCR members. (A, B) The calibration plots for the 3- and 5- year RFS; (C) nomogram model for 3- and 5-year associated RFS; (D) the decision curve analysis.

p = 0.004) and RFS (HR = 3.20, 95% CI = 1.72–5.96, p < 0.001). Several issues needed to be addressed. Firstly, the different datasets in KM plotter and SurvExpress (TCGA) may account for the contradictory facts. Secondly, different statistic strategies were involved. Log rank analysis was used for KM plotter with optimal cutoff value while the entire CXCR member were included for prognostic risk algorithm analysis prior to log rank analysis in SurvExpress. Thirdly, this study used univariate and multivariate analysis to confirm the independent prognostic values of CXCR4. Noteworthy, the prognostic values of CXCR signature were not further analyzed either by multivariate method or external validation.

CXCR7 is the latest chemokine receptor implicated in oncological field [42]. Previous study indicated that high CXCR7 mRNA expression was a poor prognostic indicator for gastric cancer in both Singapore and Japanese cohorts [21]. Consistently, in our study, high expression of CXCR7 showed significantly poor prognosis in different pathological stages, HER2+ subset and intestinal subset, respectively. High level of five methylation sites of CXCR7 showed significant favorable OS. Moreover, CXCR7 was also an independent prognostic factor in the multivariate OS analysis. Reasonably presume, the prognostic values of CXCR7 could be further characterized and validated in several subsets, including HER2+ and intestinal type. In terms of HER2, the association between CXCR7 and HER2 is yet to be clarified. Previous studies indicated that CXCR7 expression was significantly reduced in HER2 type compared to luminal type in breast cancer [43]. Mechanistically, CXCR7/ERK1/2 signaling pathway could be involved with the regulation of BRCA1 and OTUB1 and further impact upon the stabilization of estrogen receptor α (ERα) [43]. Moreover, CXCR7 depletion could reduce the phosphorylation site of ERK1/2 and epidermal growth factor receptor (EGFR) (Tyrosine 1110) [42]. In fact, the correlation between CXCR7 and ErbB family (EGFR, HER2) in GC remains further investigation.

Commonly, the prognostic values of each gene were based on high/low expression group via the expression optimal cutoff. In fact, this commonly used method may neither fully reflect the intricacy of potential biomarkers, nor maximally distinguish the survival benefits in two given groups. Therefore, one of the highlights in this study was the CXCR signature analysis.

Moreover, clinical nomogram models were also established based on the multivariate results. Nomogram-based statistical method has been increasingly used for prognosis-associated clinical practice. By receiving individual clinical variables, nomogram model could provide précised indications based on survival scores and was featured by reproducibility and feasibility. Collectively, this study provided a multilevel prognostic characterization of CXCR members.

The limitation of this study was the lack of external and

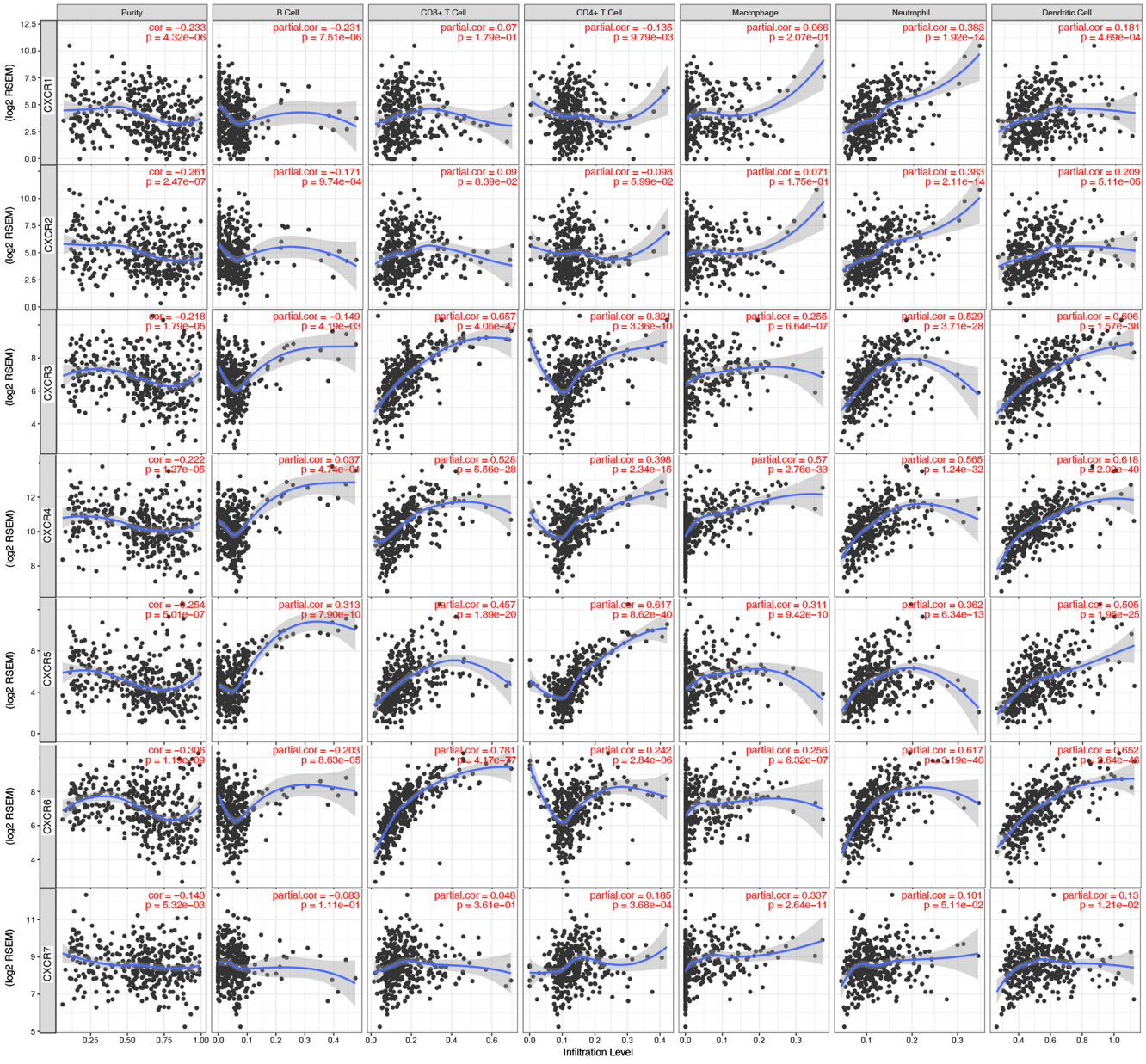

Fig. 8. Correlation of tumor infiltrating immune cells (TIICs) and CXCR members. Tumor purity was showed at the left panel. The correlation of CXCR memers and TIICs (B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages and dendritic cell types) was displayed, respectively, in the stomach adenocarcinoma (STAD).

experimental validation of the results. Moreover, larger samples are warranted for more solid prognostic evaluation.

5. Conclusion

This study identified distinct expression and prognostic values of CXCR members in GC using public databases.

6. Contributorship statement

CY and YZ carried out experiments and data analysis; CY and YZ drafted the manuscript; CY and YZ participated in study design and data collection. All authors read and approved the final manuscript.

Declaration of Competing Interest

All authors declare no conflict of interest in this study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.154785.

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