Gastric cancer (GC) is a common tumor with a low 5-year survival rate. The chemokine receptor 4 (CXCR4) protein contributes to the progression and prognosis of GC, but the relationship between CXCR4 and immune infiltration, somatic copy number alteration (SCNA), tumor purity, tumor mutation burden (TMB), cytolytic activity (CYT), and drug sensitivity in GC is poorly understood. This study aimed to systematically explore the role of CXCR4 in GC. Microarray and RNA-seq data were collected from the Gene Expression Omnibus and The Cancer Genome Atlas. Our analysis shows that CXCR4 is correlated with various types of immune cells. Patients with high CXCR4 expression had a higher fraction of B cells and CD8+ T cells, and a lower fraction of CD4+ T cells. In addition, high CXCR4 expression was associated with more advanced tumor stage, worse prognosis and higher stromal score, immune score, and cytolytic activity ($P < 0.05$). High CXCR4 expression also correlated with lower tumor purity and TMB. In summary, our analyses suggest that CXCR4 may affect the progression and prognosis of GC by influencing immune infiltration, TMB, CYT, tumor purity, and drug sensitivity.
Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA), and investigated the role of CXCR4 in GC in relation to the clinical characteristics, tumor purity, immune infiltration, TMB, CYT, survival, and other parameters.

Materials and methods

Data collection

Primary observation was performed using the Oncomine database (https://www.oncomine.org/) and TIMER (https://cistrome.shinyapps.io/timer/). We performed a systematic search in the GEO (https://www.ncbi.nlm.nih.gov/geo/) and TCGA (https://cancergenome.nih.gov/) databases. Four data sets on the GEO database with relevant clinical information were considered as cohort 1 (combined n = 726): GSE66229 [21] (n = 400), GSE15459 [22] (n = 200), GSE57303 [23] (n = 70), and GSE34942 [24] (n = 56). In total, 413 GC samples from TCGA were selected as cohort 2. Microarray expression profiles were obtained using Affymetrix Human Genome 133 plus 2.0 Gene Chips. The Batch function was used to consolidate the four data sets. Data normalization was performed, and gene expression levels were computed as mean values of all annotated probe sets [25]. EdgeR was used for the differential gene analysis of RNA sequencing data from TCGA.

Analysis of correlation between CXCR4 expression and basic clinical characteristics

According to the median expression of CXCR4, we divided patients into two groups: a high CXCR4 expression group (CXCR4-H) and a low CXCR4 expression group (CXCR4-L). Differentially expressed genes (P < 0.05, \(|\log(FC)\) ≥ 1) were identified between the two groups and analyzed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) by DAVID (https://david.ncifcrf.gov/) [26]. The following basic clinical characteristics were collected for comparison: patient age, gender, tumor stage, microsatellite instability (MSI), and overall survival (OS).

Analysis of the correlation between CXCR4 expression and immune infiltration, TMB, CYT, SCNA, and drug sensitivity

We calculated the immune infiltration, SCNA, tumor purity, TMB, CYT, and drug sensitivity to identify the correlation between CXCR4 expression. The results with P < 0.05 were considered statistically significant. The TIMER Web site was used for the primary analysis of the correlation between CXCR4 and immune cells, and SCNA of the CXCR4 gene. Then, we performed a Spearman's correlation analysis of the immune cell markers using TIMER, and the immune genes were selected according to previously published methodology [20,27]. The CIBERSORT (https://cibersort.stanford.edu/) method and LM6 gene signature were used for immune infiltration analysis between the CXCR4 expression groups [28]. The CYT of each patient was calculated based on transcript levels of two key cytolytic effectors, granzyme A (GZMA) and perforin (PRF1) [20]. The TMB of TCGA patients was calculated from the Genomic Data Common (GDC) data portal. The ESTIMATE package was used to calculate the tumor purity, stromal score, and immune score [29]. Data from Genomics of Drug Sensitivity in Cancer (GDSC) (https://www.cancerrxgene.org/) were obtained for the analysis of the effect of CXCR4 on drug sensitivity [30]. The Pearson's correlation was calculated between CXCR4 expression and drug half maximal inhibitory concentration (IC50). The results with a threshold of P < 0.01 were considered statistically significant.

Statistical analysis

All statistical analyses were conducted using R (The R Foundation, Vienna, Austria) and the SPSS version 23.0 software (IBM, Armonk, NY, USA). Graphical representations were generated using the GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA). Chi-square and Wilcoxon rank-sum tests were used for categorical and continuous variables, respectively. Student's t-test was used for data with normal distribution and homogeneous variance.

Results

Differential gene expression

By using Oncomine and TIMER, we found that CXCR4 expression was higher in GC patients compared with healthy controls. Patients from the GEO were considered as cohort 1, and those from the TCGA were considered as cohort 2. This result was consistent in both databases (Fig. 1A,B).

We divided the samples into two groups based on the CXCR4 expression levels, high (CXCR4-H) and low (CXCR4-L). We found 778 and 1552 differentially expressed genes between the groups in the training and in cohort 2, respectively. We identified the genes by performing a Gene Ontology Biological Process (GO-BP) and KEGG analysis (Fig. 2, Table S1). The GO-BP analysis results showed that the differentially expressed genes were mainly involved in the regulation of the immune response and the inflammatory response. The KEGG results showed that they were mainly involved in migration and adhesion. These biological functions and signaling pathways are closely related to the development of GC.
We then performed a correlation investigation in both cohorts between the CXCR4 expression and various clinical characteristics (Table 1). For the purpose of comparison, we classified clinical stage I and II tumors as early stage and stage III and IV tumors as advanced stage. A similar criterion was used for T and N stage. We found a significant difference between the groups in relation to the clinical stage both in the training and in cohort 2. The CXCR4-H group had more patients with advanced stage tumors compared with the CXCR4-L group in both cohorts ($P < 0.05$), and the expression of CXCR4 gradually increased with the increase in stage, especially in cohort 2 (Fig. 3A). In cohort 1, we also found a statistical difference between the groups regarding age, gender, N stage, and MSI ($P < 0.05$). No significant difference could be found for N stage (Fig. 3B). In cohort 2, we found a significant difference for the T stage ($P < 0.05$). We also found a correlation between earlier T stages and the CXCR4-L group (Fig. 3C). No difference in the M stages was observed in the two cohorts.

**CXCR4 is a predictor of poor OS in patients with GC**

As shown in Fig. 4, we found a strong association between high CXCR4 expression and short OS in GC
Fig. 2. GO-BP (top) and KEGG (bottom) analysis. The BP (left) and KEGG (right) enrichment of the differential genes between the CXCR4-H and CXCR4-L groups in two cohorts. Circle represents cohort 1, and triangle represents cohort 2. The size of the symbol indicates the gene count; the color indicates the −Log10 of the P value evaluating the statistical significance of the relative enrichment.

Table 1. Clinical characteristics. The relationship between CXCR4 expression and clinical characteristics in both training and cohort 2. MSS, microsatellite stability; MSI, microsatellite instability.

| Group | Cohort 1 | | Cohort 2 | | P |
|-------|----------|--------|----------|--------|--------|
|       | CXCR4-H  | CXCR4-L | P        | CXCR4-H  | CXCR4-L | P        |
| Age (years) | n = 306 | n = 306 | <0.0001* | n = 185 | n = 186 | 0.1642   |
| ≥ 60  | 179      | 236     |          | 123      | 136     |          |
| < 60  | 127      | 70      |          | 62       | 50      |          |
| Gender | n = 306 | n = 306 | 0.0396*  | n = 187  | n = 188 | 0.6387   |
| F     | 114      | 90      |          | 69       | 65      |          |
| M     | 192      | 216     |          | 118      | 123     |          |
| Stage | n = 306 | n = 306 | 0.0176*  | n = 158  | n = 169 | 0.0155*  |
| 1     | 34       | 41      |          | 14       | 35      |          |
| 2     | 58       | 87      |          | 52       | 55      |          |
| 3     | 121      | 106     |          | 71       | 65      |          |
| 4     | 93       | 72      |          | 21       | 14      |          |
| 1 + 2/3 + 4 | 92/214 | 128/178 | 0.0024*  | 66/92    | 90/79   | 0.0378*  |
| T     | n = 191  | n = 175 | 0.4887   | n = 161  | n = 172 | <0.0001* |
| T1    | 0        | 0       |          | 1        | 18      |          |
| T2    | 102      | 92      |          | 32       | 40      |          |
| T3    | 71       | 72      |          | 71       | 86      |          |
| T4    | 18       | 11      |          | 57       | 28      |          |
| T1 + 2/T3 + 4 | 102/89 | 92/83   | 0.8735   | 33/126   | 58/114  | 0.0068*  |
| N     | n = 191  | n = 175 | 0.0267*  | n = 158  | n = 165 | 0.2196   |
| N0    | 36       | 14      |          | 41       | 59      |          |
| N1    | 76       | 80      |          | 49       | 39      |          |
| N2    | 51       | 54      |          | 35       | 36      |          |
| N3    | 28       | 27      |          | 34       | 31      |          |
| N0 + 1/ N2 + 3 | 112/79 | 94/81   | 0.3428   | 90/69    | 98/67   | 0.6109   |
| M     | n = 191  | n = 175 | 0.5298   | n = 158  | n = 166 | 0.2282   |
| M0    | 175      | 157     |          | 144      | 157     |          |
| M1    | 16       | 18      |          | 14       | 9       |          |
| MSS/MSI | n = 150 | n = 150 | 0.0009*  | n = 97   | n = 113 | 0.8915   |
| MSS   | 104      | 128     |          | 80       | 94      |          |
| MSI   | 46       | 22      |          | 17       | 19      |          |

*P < 0.05.
patients in both cohorts. Moreover, we found that CXCR4 expression had a significant effect on both the short-term and long-term survival of GC patients (Wilcoxon and Log-rank test, \( P < 0.05 \)).

**Comparison of immune cell type fractions**

By performing a TIMER analysis, we found a high correlation between CXCR4 expression and CD4\(^+\) T cells, CD8\(^+\) T cells, macrophages, neutrophils, and dendritic cells (Fig. 5A). Thus, we selected several related immune cell markers for further validation (Table S2). We observed a high correlation between CXCR4 expression and T-cell subtypes (T-helper type 1, T-helper type 2, T follicular helper cells, regulatory T cells, and T gamma delta cells), B cells, macrophages subtypes (M1 and M2), neutrophils, natural killer cells, and dendritic cells (Fig. 5B–G). This result is consistent with our previous hypothesis that CXCR4 plays an important role in tumor immune infiltration in GC.

After CIBERSORT calculation, patients with a \( P \) value > 0.05 were removed, and nonparametric testing was performed to evaluate the statistical significance of the different immune cell fractions (Fig. 6). The results showed a significantly higher amount of B cells, CD4\(^+\) T cells, and CD8\(^+\) T cells \(( P < 0.001 \) in the CXCR4-H group than in the CXCR4-L group. In cohort 1, we observed fewer monocytes in the CXCR4-H group \(( P < 0.05 \) ), while in cohort 2 we observed fewer NK cells \(( P < 0.05 \) ).

**Influence of CXCR4 expression on somatic copy number alterations in immune cells**

We compared the immune infiltration levels among tumors with the presence of different somatic copy number alterations for the CXCR4 gene, by performing a TIMER analysis. We observed that when the CXCR4 gene had an arm-level deletion, the expression of CXCR4 was reduced \(( P < 0.05 \) ) and the proportion of all types of immune cells was significantly...
decreased. When an arm-level gain appeared instead, the expression of CXCR4 was reduced without significance, and the proportion of macrophages and dendritic cells decreased slightly, while the other types showed no significant changes (Fig. 7).

**Tumor purity, CTY, and TMB comparison**

The tumor purity, stromal score, and immune score were calculated by the ESTIMATE package, which inferred the level of infiltrating stromal and immune cells in tumor tissues and tumor purity using gene expression data [29]. We calculated the tumor purity value in the two cohorts. We found significant differences in tumor purity, immune score, and stromal score between the CXCR4-H group and the CXCR4-L group. The CXCR4-H group had a higher stromal score and immune score, but a lower tumor purity (Fig. 8A–C). Both cohorts had similar results. Regarding the CYT activity, patients with high CXCR4 expression showed a stronger CYT activity in both cohorts (Fig. 8D). Regarding TMB, we converted the TMB count of the TCGA database into log2TMB, and the result showed that the TMB amount was lower in the CXCR4-H group, indicating that the CXCR4-H group may be less recognized and therefore less likely to be attacked by immune cells (Fig. 8E).

**Influence of CXCR4 on drug sensitivity**

To analyze the effect of CXCR4 on drug sensitivity, we used data from the Genomics of Drug Sensitivity in Cancer database (GDSC). We found that three drugs, 17-allylamino-17-demethoxygeldanamycin (17-agg), trametinib, and docetaxel, which are used for GC treatment, met the screening criteria ($P < 0.01$) (Fig. 9, Table S3); their Pearson’s correlation coefficients were 0.391, 0.338, and 0.293, respectively. The results showed that with the increase in CXCR4 expression, the drug IC50 is also increased.

**Discussion**

Gastric cancer is a common malignant tumor characterized by a low 5-year survival rate. In recent years, because of advances in chip and next-generation sequencing technologies, more pathological parameters have been measured, such as TMB, CYT, immune infiltration, and tumor purity. These new parameters could potentially be used as therapeutic markers. Thus, numerous studies have focused on investigating their role in the development and prognosis of GC.

CXCR4 is a member of the C-X-C chemokine receptor family, which has been widely investigated in many cancers. It can regulate the biological behavior of GC cells through multiple pathways, such as the classical AKT pathway, or the alternative MAPK and Wnt pathways. Most studies have shown that high expression of CXCR4 is closely related to a poor prognosis. Therefore, CXCR4 plays an important role in the occurrence and development of GC. However, whether the expression of CXCR4 is correlated with the TMB, CYT, immune infiltration, and tumor purity of GC is still unknown.

In this present study, we showed that CXCR4 is overexpressed in GC tumor tissue, with a significant correlation between the CXCR4 levels and the survival rate, consistent with previous studies. Moreover, we found that a high CXCR4 expression in patients was correlated with a more advanced clinical stage, which
**Fig. 5.** Correlation between CXCR4 expression and immune cells. (A) The correlation between CXCR4 expression and six types of immune cells. (B–G) The validation of the correlation between CXCR4 and gene markers of B-cell and T-cell subtypes (Th1, Th2, Thf, Tregs, and T gamma), macrophages subtypes (M1 and M2), neutrophils, natural killer cells, and dendritic cells (Spearman’s correlation).
is an extremely important prognostic factor for tumor progression [31,32]. For the T stage, we found a statistical difference only in cohort 2 and not in cohort 1. However, in cohort 1, the total number of patients with a T3 and T4 stage tumor in the CXCR4-H group was higher than that in the CXCR4-L group. These results are consistent with previous studies reporting that in patients with high CXCR4 expression, both the depth of tumor invasion and the amount of distant metastasis are higher than those in patients with a low expression [33,34]. The difference between the cohorts may be explained by the absence of patients with a T1 stage tumor in cohort 1, which may limit the power of the statistical analysis. We also found no significant difference between the two groups regarding the M stage, probably because of the small number of M1 patients available. In terms of N stage, we found a significant difference in cohort 1, but no significant progressiveness. Collectively, our results indicate that CXCR4 may have an effect on local and distant invasion in GC. More patient data should be collected to validate our analysis.

We also focused our attention on another important characteristic, tumor immune infiltration. The effect of immune infiltration cells on the prognosis of GC is still controversial [35]. In the present study, genes involved in the immune and the inflammation response were enriched in the fraction of genes showing a differential expression between the CXCR4-H and CXCR4-L groups. CXCR4 expression was highly correlated with T cells, macrophages, dendritic cells, and other immune cells. Moreover, we verified this correlation by observing a similar correlation between CXCR4 expression and immune gene markers. Then, we calculated the immune infiltration levels of each patient by CIBERSORT, and we found that, in both cohorts, in
the CXCR4-H group, the fraction of B cells and CD8+ T cells was higher, while the fraction of CD4+ T cells was lower compared to the CXCR4-L group. A recent study showed that a high number of CD8+ T cells positively correlate with poor OS [36]. Another study reported that an increased intratumor CD8+ lymphocyte ratio is correlated with a decreased OS [37]. Moreover, an increased CD4+ T-cell number represents a favorable prognostic factor [38]. We also found that in cohort 1, the number of monocytes in the CXCR4-H group was lower than that in the CXCR4-L group, while in cohort 2, the number of NK cells was lower. These results are consistent with previous studies showing an antitumor role for monocytes and NK cells in the tumor microenvironment. These two cell types also have a positive effect on GC prognosis [39,40].

We then investigated whether there was a difference in the extent of immune cell infiltration and SCNA of the CXCR4 gene. We observed that the majority of changes occurred in concomitance with arm-level deletion and arm-level gain. When CXCR4 had arm-level deletion or arm-level gain, the mRNA expression level of both groups decreased, but only the decrease in the arm-level deletion group was significant compared with that of the normal group. There was no significant difference in the gain group. As shown in Fig. 5, the expression of CXCR4 was positively correlated with B cells, CD4, CD8, macrophages, neutrophils, and dendritic cells, indicating that when the expression of CXCR4 was greatly reduced, the infiltration degree of these immune cells would also be reduced, so the immune cells in the arm-level deletion group all decreased compared with the normal group. The decrease in the arm-level gain group was not statistically significant. Thus, the decrease in the number of immune cells was only different between macrophages and dendritic cells, but the difference was not obvious. Therefore, it can be observed that when copy number variation of CXCR4 occurs, especially when arm-level deletion occurs, the expression of CXCR4 will be decreased, thus affecting the infiltration of immune cells.

Tumor purity is another aspect that has been thoroughly investigated in recent years. Many studies showed that tumor purity could become an important parameter in tumor research [18,41]. By calculating the tumor purity in our cohorts, we observed that in the
CXCR4-H group, the tumor stromal score and the immune score were higher than in the CXCR4-L group, while the tumor purity was lower than that in the CXCR4-L group. Previous studies showed that patients with a high tumor purity have a better prognosis. A high stromal and immune score in tumor tissues are correlated with a lack of infiltration and function of chemotherapy drugs [42,43]. Consistently, our results showed that patients with high CXCR4 expression have a high IC50 response to three antitumor drugs.

The TMB value is generally expressed in terms of the total number of nonsynonymous mutations or the number of mutations per Mb (1 Mb base). Our immune system eliminates abnormal cells; the idea behind tumor immunotherapy is to boost and strengthen the body’s immune system with various methods to kill the tumor cells. In this context, the higher the TMB is, the greater the difference between tumor cells and normal cells. Thus, high TMB values reflect a high predisposition to be the target of antitumor immunity. Consistent with our survival and drug sensitivity results, numerous studies confirmed that immunotherapy is more efficient in patients with high TMB than in patients with low TMB [43,44]. In our study, patients with a high expression of CXCR4 had a lower TMB than patients with a low CXCR4 expression, suggesting that patients with high expression of CXCR4 may be resistant to immunotherapy, thus explaining the worse prognosis.

The CYT is calculated based on the GZMA and PRF1 levels. CYT is dramatically upregulated upon CD8\(^+\) T-cell activation and during productive clinical responses to anti-CTLA-4 and anti-PD-L1 immunotherapies [20]. Previous studies [45,46] showed that in glioma and colorectal cancers, an increased CYT is correlated with an improved prognosis [47]. However, the relevance of CYT in GC is unknown. In our study, CYT and the CD8\(^+\) T-cell fraction were higher in the CXCR4-H group. Thus, it seems that in GC, CYT is higher in the CXCR4 groups characterized by a worse survival rate, in contrast with previous studies. We think that the CYT with worse survival could be high in this group for several reasons. Patients with a high CXCR4 expression have a lower tumor purity, which can lead to poor survival. An increase in CD8\(^+\) T-cell infiltration could also affect the prognosis. Moreover, whether a high CYT can lead to an improved survival also in GC patients is still unknown. More studies on the prognostic role of CYT in GC patients are needed. Our results showed that despite a high CYT in the CXCR4-H group, the prognosis of these patients is lower than that of patients in the CXCR4-L group for a variety of reasons.

Drug sensitivity is an important prognostic factor for GC patients. Previous studies indicated that CXCR4 overexpression could protect tumor cells from chemotherapy drugs [48,49]. The 17-agg drug is an HSP90 inhibitor. Recent studies showed that in lung adenocarcinoma and breast cancer, 17-aag can inhibit the growth and induce the apoptosis of tumor cells [50-52]. Other studies showed that 17-agg combined with platinum can inhibit the growth of GC cells [53]. Trametinib, a specific MEK inhibitor, is mainly used for the treatment of adult patients with unresectable melanoma or metastatic melanoma who carry either the BRAF V600E or the V600K mutations [54]. Its use for the treatment of advanced non-small-cell lung cancer with the BRAF V600E mutation has also been reported [55]. Some studies also showed that trametinib can be used to induce apoptosis of GC cells in combination with other drugs. However, 17-agg and trametinib are rarely used in the conventional clinical treatment of GC. Docetaxel has been shown to be more effective for GC treatment; various studies suggested that a combination of docetaxel and other drugs can be used to prolong the survival in patients with advanced GC [56-58]. In the present study, we discovered that the CXCR4 levels can affect the resistance of cancer cells to three drugs. When CXCR4 expression is increased, the resistance to the three drugs also increases. These results could guide physicians in clinical treatment selection, especially for docetaxel. Patients with a low expression of CXCR4 may benefit from a docetaxel treatment, although further verification with more samples is needed.

In summary, CXCR4 is an important independent factor to evaluate the prognosis of GC patients.
found a statistically significant correlation between CXCR4 and TMB, CYT, tumor purity, tumor immune infiltration, drug sensitivity, and other clinical parameters playing an important role in GC insurgence and development.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

YL and LL conceived and designed the project. H-CW and BS acquired the data. YL and H-CW analyzed and interpreted the data. J-SW completed the statistical calculations. YL complete the original draft and interpreted the data. J-SW completed the statistic and BS acquired the data. YL and H-CW analyzed the data sets used and/or analyzed in the current study are available from the TIMER, GEO, and TCGA database. We thank all the authors for agreeing to share their data. Our study was supported by the National Natural Science Foundation of China (General Program, Grant No. 81572355).

Role of CXCR4 in gastric cancer

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**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** GO-BP and KEGG analysis of the two cohorts.

**Table S2.** Immune gene markers.

**Table S3.** GDSC analysis.