European Journal of Inflammation

Original Research Article

Dysregulation of CCL18/CCR8 axis predicts poor prognosis in patients with gastric cancer

European Journal of Inflammation
Volume 16: 1–7

DOI: 10.1177/2058739218796887
journals.sagepub.com/home/eji
SAGE

Jie Yi[1] and Shao-Jie Jiang[2]

Abstract

Increasing data have shown that the dysregulation of C-C motif chemokine ligand 18 (CCL18) and C-C motif chemokine receptor (CCR8) is involved in the development and progression of multiple malignancies. However, the clinical significance of CCL18/CCR8 axis in gastric cancer (GC) was still undocumented. In this study, the expression levels of CCL18 and its receptor CCR8 and their correlation with the clinicopathological characteristics and prognosis in patients with GC were analyzed by TCGA RNA sequencing data. Cox proportional hazard regression model was performed to assess the association between CCL18/CCR8 expression and overall survival (OS) and tumor recurrence in patients with GC. As a consequence, we found that the expression of CCL18 was markedly elevated in GC samples as compared with the adjacent normal tissues and acted as an independent prognostic factor of tumor recurrence in patients with GC. Subsequently, Pearson correlation analysis revealed that CCL18 possessed a positive correlation with CCR8 expression in GC samples. CCR8 expression was upregulated in GC tissues and exhibited the association with poor survival in patients with GC. Taken together, our findings demonstrated that the dysregulation of CCL18/CCR8 axis could predict the poor prognosis in patients with GC and provide a potential antitumor target for the treatment of GC.

Keywords

CCL18, CCR8, gastric cancer, recurrence, survival

Date received: 27 April 2018; accepted: 2 August 2018

Introduction

In spite of the gradual decrease in the incidence and mortality of gastric cancer (GC) worldwide over the few decades, the annual incidence of GC in China is about 680,000 cases, accounting for nearly half of the world's cases[1] and most of the patients with GC have been diagnosed at the advanced stage,[2] owing to the tumor invasiveness and metastasis.[3]With the progress in the application of gastroscopy, early detection and treatment of GC have been greatly improved.[4] But, the poor survival and tumor recurrence of GC patients remain very poor. Therefore, extensive studies are focused on identifying the prognostic predictors or therapeutic targets of GC.

Dysregulation of chemokine CCL18 has been reported implicated in pathologic conditions such as inflammation and cancer.[5] Some studies show that the expression of CCL18 is increased in diffuse large B-cell lymphoma,[6] advanced breast cancer,[7] and low-grade endometrial cancer.[8] In

[1]Department of Gastroenterology, The Jingmen No. 1 People's Hospital, Jingmen, P.R. China
[2]Department of Gastroenterology, Jingzhou Central Hospital, The Second Clinical Medical College, Yangtze University, Jingzhou, P.R. China

Corresponding author:
Shao-Jie Jiang, Department of Gastroenterology, Jingzhou Central Hospital, The Second Clinical Medical College, Yangtze University, No. 1 Renmin Road, Jingzhou 434020, Hubei, P.R. China.
Email: shaojie223@163.com

addition, the serum level of CCL18 is elevated in epithelial ovarian cancer[9] and bladder cancer,[10] acting as a potential biomarker in these patients,[9,10] and associates with poor prognosis as an independent factor in patients with breast cancer[11] and colorectal cancer.[12] Moreover, CCL18 is involved in promoting cell proliferation, epithelial–mesenchymal transition (EMT), invasion and metastasis and repressing cell apoptosis in lymphoma,[6] pancreatic ductal adenocarcinoma,[13] breast cancer,[14] and lung cancer.[15] These studies suggest that CCL18 functions as an oncogenic marker in cancer patients.

Furthermore, C-C motif chemokine receptor (CCR8), identified as a chemokine receptor for CCL18 exhibits a key biological role in human chronic diseases.[16] It is mainly expressed in the thymus, and in the periphery, CCR8 is abundantly expressed in activated Th2-polarized cells and in NK1.1+ CD4+ T cells.[17] CCR8 counteracts dexamethasone-induced apoptosis via ERK-dependent pathway[18] and mediates CCL1- and vMIP-I-induced anti-apoptotic activity by RAS/MAPK pathway.[19] CCL1/CCR8 axis as a component of cancer-related inflammation contributes to immune evasion, and inhibition of CCR8 axis may offer a promising strategy for therapeutic intervention in cancers.[20]

Although individual study reveals CCL18 as an independent prognostic factor in GC,[21] the number of patients enrolled is limited and the knowledge about the association between its receptor CCR8 and GC is unknown. In this study, we found that CCL18 expression was markedly elevated in GC samples (n=415) as compared with the adjacent normal tissues (n=32), and its high expression was associated with the tumor recurrence, acting as an independent prognostic factor in patients with GC. CCR8 possessed the positive correlation with CCL18 expression and poor survival, indicating CCL18/CCR8 axis may provide a potential antitumor target for the treatment of GC.

Materials and methods

Clinical data of GC patients

The clinicopathological and prognostic data for 415 cases of GC patients and 32 cases of adjacent normal tissues as well as the relative expression levels of CCL18, PITPNM family member 3 (PITPNM3), and CCR8 in GC samples were downloaded from TCGA 2015 RNA sequencing database (https://genome-cancer.ucsc.edu). All the GC samples were from the biopsies in the TCGA cohort and the TNM stage of GC was diagnosed at stage I + II or stage III + IV. The patients with GC did not receive any chemotherapy. Clinical information on patient's follow-up, overall survival (OS), disease-free survival, and outcome could be downloaded from the TCGA database. The clinicopathologic characteristics and detailed information of 265 GC patients were summarized in Supplementary Table S1. The protocols used in our study were approved by the Ethics Committee of Jingzhou Central Hospital.

Statistical analysis

Statistical analyses were conducted by SPSS 20.0 (IBM, SPSS, Chicago, IL, USA) and GraphPad Prism 7.0. Student's t test or chi-square test was used to evaluate the statistical significance for the comparisons of two groups. Pearson's correlation coefficient analysis was used to analyze the correlations of CCL18 with PITPNM3 and CCR8 expression in GC samples. OS was defined as the interval between the dates of surgery and death, and OS as well as recurrence curves was analyzed with the Kaplan–Meier method and log-rank test. Univariate analysis and multivariate models were performed using a Cox proportional hazards regression model. $P < 0.05$ was considered statistically significant.

Results

The expression of CCL18 was increased in GC samples

In consistence with previous studies indicating the upregulation of CCL18 expression in human malignancies,[6–10] our results demonstrated that the expression level of CCL18 was increased in GC samples (n=415) as compared with the adjacent normal tissues (n=32) using TCGA RNA sequencing data (Figure 1(a)). The similar results were validated in pair-matched GC samples (n=32) as compared with the adjacent normal tissues (n=32) (Figure 1(b)). These findings suggested that elevation of CCL18 expression was a frequent event in patients with GC.

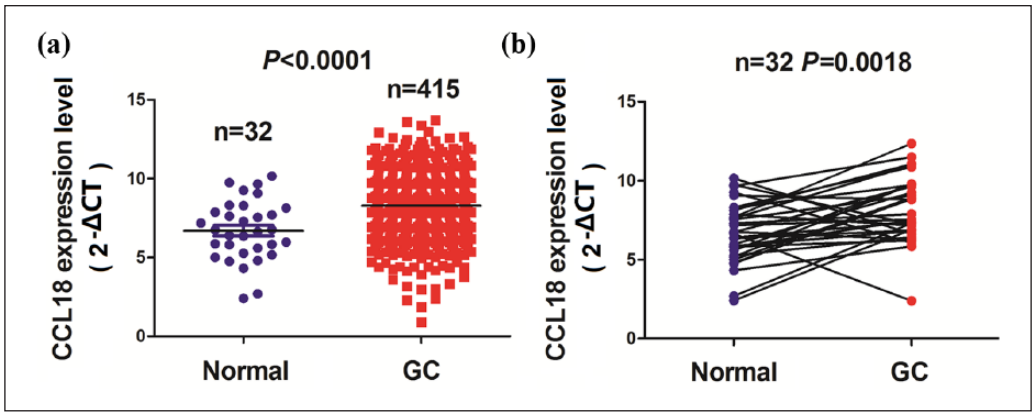

Figure 1. The expression of CCL18 was increased in GC samples. TCGA cohort analysis of the expression level of CCL18 in (a) unpaired and (b) paired GC samples as compared with the adjacent normal tissues.

Upregulation of CCL18 expression is correlated with tumor recurrence in patients with GC

CCL18 increased expression inspired us to analyze its correlation with the clinicopathological features and prognosis in patients with GC. According to the OS time, OS status, and CCL18 expression level, we gained its cut-off value (6.694) (Figure 2(a)) in GC samples (n=265) and divided the patients into two groups: CCL18 high expression (n=212) and CCL18 low expression (n=53) (Figure 2(b)). Receiver operating characteristic (ROC) curve and area under curve (AUC) were used to assess whether CCL18 was a potential marker in patients with GC, which showed that AUC of CCL18 was 0.59 (Figure 2(a)). This result indicated that CCL18 might be a potential marker in patients with GC. As shown in Table S1, we further analyzed the correlation of CCL18 expression with the clinicopathological characteristics and prognosis in patients with GC and found that CCL18 high expression had no correlation with age, gender, pathological stage, and TNM stage (each $P > 0.05$). Kaplan–Meier analysis indicated that the patients with CCL18 high expression harbored no difference in OS (Figure 2(c)), but had a higher recurrence rate (Figure 2(d)) as compared with those with CCL18 low expression. In addition, we further analyzed the correlation of CCL18 high expression with the tumor recurrence in patients of early stage or late stage. We found that the patients of early stage (stage I + II) with CCL18 high expression displayed no difference in tumor recurrence as compared with those with CCL18 low expression (Figure 2(e)), but the patients of late stage (stage III + IV) with CCL18 high expression had a higher recurrence rate as compared with those with CCL18 low expression (Figure 2(f)). Univariate and multivariate Cox regression analysis revealed that CCL18 expression as well as gender was an independent prognostic factor of tumor recurrence in patients with GC (Table 1).

CCL18 displayed a positive correlation with CCR8 expression in GC samples

According to the previous report,[16] PITPNM3 and CCR8 act as the receptors of CCL18. First, we examined their expression levels in GC samples using the TCGA RNA sequencing data, which indicated that the expression of CCR8 was markedly increased in GC samples (n=32) as compared with the pair-matched adjacent normal tissues (n=32), but that of PITPNM3 was significantly decreased in GC samples (n=25) as compared with the pair-matched adjacent normal tissues (n=25) (Figure 3(a)). The consistent results for PITPNM3 and CCR8 expression were further verified in total GC samples (Figure 3(b)). The spearman correlation analysis showed that CCL18 exhibited a negative correlation with PITPNM3 expression (Figure 3(c)) but a positive association with CCR8 expression (Figure 3(d)) in GC samples. Taken account into the promoting role of CCL18 in cancer,[6,13–15] its receptor was selected for further analysis.

Increased expression of CCR8 was associated with poor survival in patients with GC

Having identified an increased expression of CCR8 (Figure 3(a) and (b)) in GC samples, we

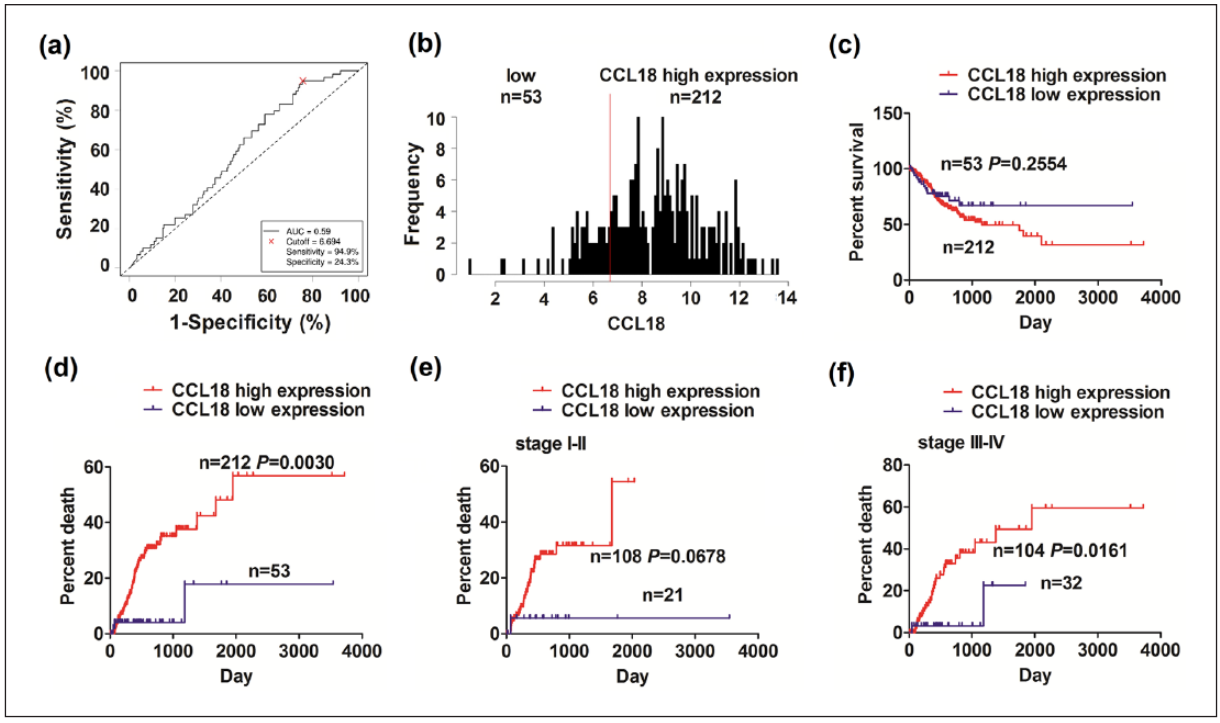

Figure 2. Upregulation of CCL18 expression was associated with tumor recurrence in patients with GC. (a) The cut-off value and AUC of CCL18 were assessed in GC samples (n = 265). (b) The patients with GC were divided into CCL18 high expression group and low expression group. Kaplan–Meier analysis of the correlation of CCL18 high or low expression with the (c) OS and (d) tumor recurrence in patients with GC. Kaplan–Meier plotter analysis of the correlation of CCL18 high or low expression with the tumor recurrence in GC patients of (e) early stage (stage I + II) or (f) late stage (stage III + IV).

Table 1. Cox regression analysis of CCL18 expression as recurrence predictor in patients with GC.

Variables	Univariate Cox regression analysis		Multivariate Cox regression analysis	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Age (years)				
⩾60 versus <60	0.882 (0.520–1.495)	0.641	NA	NA
Gender				
Male versus female	2.146 (1.159–3.975)	0.015	2.133 (1.152–3.950)	0.016
Pathological stage				
III/IV versus I/II	1.065 (0.638–1.778)	0.811	NA	NA
T stage				
T3 + T4 versus T1 + T2	0.764 (0.444–1.314)	0.330	NA	NA
N staging				
Positive versus negative	1.138 (0.654–1.982)	0.648	NA	NA
M stage				
Positive versus negative	1.016 (0.406–2.543)	0.973	NA	NA
CCL18 expression				
High versus low	4.890 (1.530–15.630)	0.007	4.864 (1.521–15.551)	0.008

GC: gastric cancer; NA: not analyzed.

further analyzed the relationship between its expression with clinicopathological features and prognosis in patients with GC. According to the OS time, OS status, and CCR8 expression level, a cut-off value (4.899) of CCR8 (Figure 4(a)) in GC samples (n = 293) was determined, by which the patients were divided into two groups: CCR8 high expression and CCR8 low expression (Figure 4(b)).

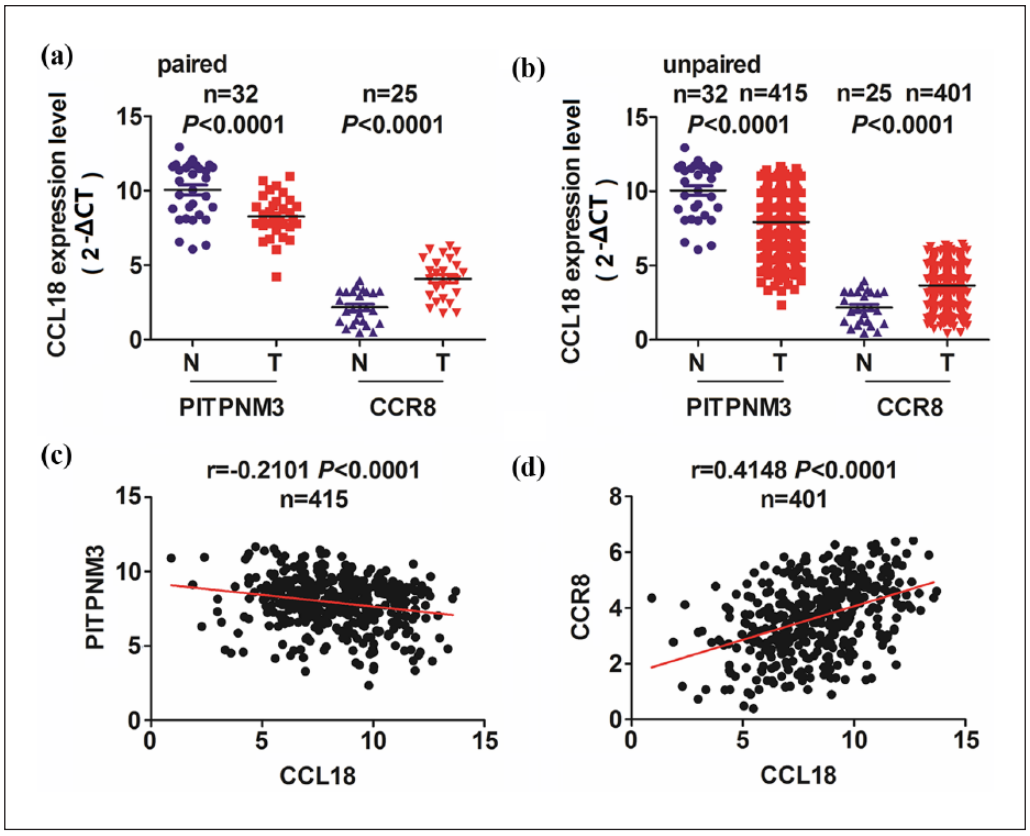

Figure 3. The correlation of CCL18 with the expression of its receptors PITPNM3 and CCR8 in GC. TCGA cohort analysis of the expression levels of PITPNM3 and CCR8 in (a) paired and (b) unpaired GC samples as compared with the adjacent normal tissues. Spearman correlation analysis of the association between CCL18 expression and its receptors (c) PITPNM3 and (d) CCR8 in GC.

ROC curve was used to assess the sensitivity and specificity of CCR8 in GC, which indicated that the AUC, sensitivity, and specificity of CCR8 were, respectively, 0.5, 26.5%, and 83.8% (Figure 4(a)), suggesting that CCR8 might be a potential marker for GC patients. Furthermore, we found that CCR8 high expression had no correlation with the age, gender, pathological stage, and TNM stage (each $P > 0.05$, Table S2). Kaplan–Meier analysis demonstrated that the patients with CCR8 high expression presented a shorter survival (Figure 4(c)) but had no difference in tumor recurrence rate (Figure 4(d)) as compared with those with CCR8 low expression. The patients of early stage (stage I + II) (Figure 4(e)) rather than late stage (stage III + IV) (Figure 4(f)) with CCR8 high expression displayed a poor survival as compared with those with CCR8 low expression. Univariate and multivariate Cox regression analysis illuminated that CCR8 expression as well as age was an independent prognostic factor of poor survival in GC patients (Table S3).

Discussion

CCL18 exhibits crucial functions in the immunological regulation and promoting tumor progression. Its expression is substantially increased in lymph node-positive diseases and associates with tumor size, invasiveness, and distant metastasis in non-small cell lung cancer (NSCLC).[22,23] The expression of CCL18 is increased in cancer samples[6–10,22–24] and facilitates EMT, migration, and metastasis by proline-rich tyrosine kinase 2 or mTORC2 signaling in ovarian cancer,[24,25] by Nir1-ELMO1/DOC180 signaling in NSCLC,[23] and by NF-κB/VCAM-1 pathway in pancreatic ductal adenocarcinoma.[26] In this study, in accordance with previous studies,[6–10,21–24] CCL18 expression was found significantly elevated in GC samples, but inconsistent with these results,[21–23] our study showed that CCL8 high expression had no correlation with the clinical features in patients with GC, which might be attributed to the tissue source diversity and the difference in sample size. These findings indicated that the increased expression of CCL18 is a frequent event in cancer.

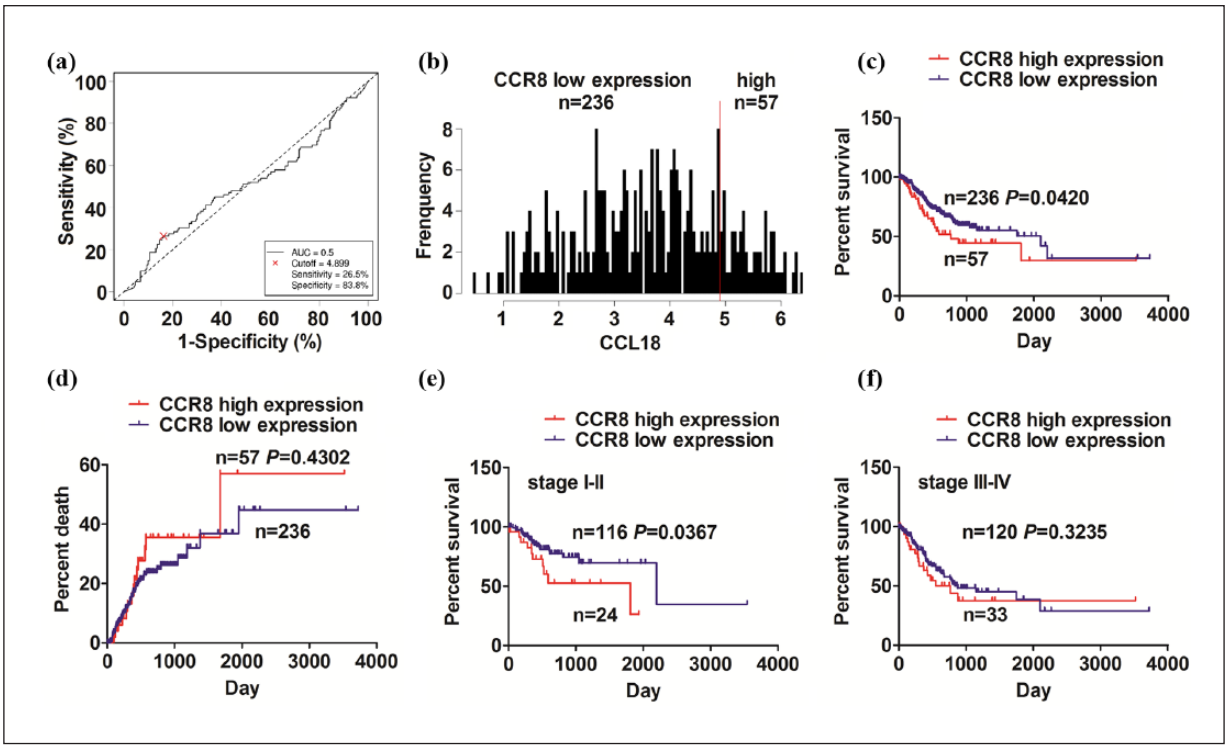

Figure 4. Upregulation of CCR8 expression was associated with poor survival in patients with GC. (a) The cut-off value and AUC of CCR8 were determined in GC samples (n = 293). (b) The patients with GC were divided into CCR8 high expression group and low expression group. Kaplan–Meier analysis of the correlation of CCR8 high or low expression with the (c) OS and (d) tumor recurrence in patients with GC. Kaplan–Meier plotter analysis of the correlation of CCR8 high or low expression with the poor survival in GC patients of (e) early stage (stage I + II) or (f) late stage (stage III + IV).

It is known to us that the patients with advanced GC have poor prognosis.[2] Identification of the prognostic predictors or therapeutic targets is essential for the diagnosis and treatment of GC. Some studies showed that CCL18 expression has a link with disease severity and poor prognosis in patients with lymphoma.[27] CCL18 acts as a biomarker,[9,10] associated with poor prognosis as an independent factor in cancer patients.[11,12] In this study, in consistence with previous results,[21] we found that CCL18 high expression had an association with tumor recurrence, but had no correlation with OS in patients with GC. Multivariate analysis revealed CCL18 as an independent prognostic factor of tumor recurrence in patients with GC. These findings suggested that CCL18 might be used to predict the tumor recurrence in patients with GC.

CCR8 as a receptor of CCL18[16] is also implicated in tumorigenesis. It not only induces the anti-apoptosis effects,[18,19] but also leads to the immune evasion.[20] However, the correlation of CCR8 with CCL18 expression and the prognosis in patients with GC is unclear. In this study, we found that CCR8 exhibited a positive correlation with CCL18 expression in GC samples, but showed no correlation with the clinical factors in patients with GC. CCR8 high expression was associated with poor survival rather than tumor recurrence in patients with GC. Multivariate analysis showed that CCR8 expression as well as age was an independent prognostic factor of poor survival in patients with GC. These findings indicated that CCR8 could predict the poor survival in patients with GC.

In conclusion, our findings demonstrated that the dysregulation of CCL18/CCR8 axis predicts the poor prognosis in patients with GC and may provide a potential antitumor target for the treatment of GC.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This work was supported by grants from the Jingzhou Municipal Science and Technology Bureau (No. 2016086).

ORCID iD

Shao-Jie Jiang https://orcid.org/0000-0001-7403-5554

References

1. Chen W, Zheng R, Zhang S et al. (2017) Cancer incidence and mortality in China, 2013. *Cancer Letters* 401: 63–71.
2. Ajani JA, Lee J, Sano T et al. (2017) Gastric adenocarcinoma. *Nature Reviews Disease Primers* 3: 17036.
3. Huang Y, Zhang J, Hou L et al. (2017) LncRNA AK023391 promotes tumorigenesis and invasion of gastric cancer through activation of the PI3K/Akt signaling pathway. *Journal of Experimental & Clinical Cancer Research* 36: 194.
4. Ngamruengphong S, Abe S and Oda I (2017) Endoscopic management of early gastric adenocarcinoma and preinvasive gastric lesions. *Surgical Clinics of North America* 97: 371–385.
5. Chenivesse C and Tsicopoulos A (2018) CCL18—Beyond chemotaxis. *Cytokine* 109: 52–56.
6. Zhou Q, Huang L, Gu Y et al. (2018) The expression of CCL18 in diffuse large B cell lymphoma and its mechanism research. *Cancer Biomarkers* 21: 925–934.
7. Narita D, Seclaman E, Anghel A et al. (2016) Altered levels of plasma chemokines in breast cancer and their association with clinical and pathological characteristics. *Neoplasma* 63: 141–149.
8. Sakane R, Tsubamoto H, Sakata K et al. (2014) Expression of chemokine ligand 18 in stage IA low-grade endometrial cancer. *Anticancer Research* 34: 5331–5336.
9. Yuan L, Wan J, Huang C et al. (2017) Evaluation of serum CCL18 as a potential biomarker for ovarian cancer. *Cancer Biomarkers* 21: 97–104.
10. Miyake M, Ross S, Lawton A et al. (2013) Investigation of CCL18 and A1AT as potential urinary biomarkers for bladder cancer detection. *BMC Urology* 13: 42.
11. Sun JH, Fan N and Zhang Y (2016) Correlation between serum level of chemokine (C-C motif) ligand 18 and poor prognosis in breast cancer. *Genetics and Molecular Research* 15(3). doi: 10.4238/gmr.15038632.
12. Yuan R, Chen Y, He X et al. (2013) CCL18 as an independent favorable prognostic biomarker in patients with colorectal cancer. *Journal of Surgical Research* 183: 163–169.
13. Meng F, Li W, Li C et al. (2015) CCL18 promotes epithelial-mesenchymal transition, invasion and migration of pancreatic cancer cells in pancreatic ductal adenocarcinoma. *International Journal of Oncology* 46: 1109–1120.
14. Chen J1, Yao Y, Gong C et al. (2011) CCL18 from tumor-associated macrophages promotes breast cancer metastasis via PITPNM3. *Cancer Cell* 19: 541–555.
15. Ploenes T, Scholtes B, Krohn A et al. (2013) CC-chemokine ligand 18 induces epithelial to mesenchymal transition in lung cancer A549 cells and elevates the invasive potential. *PLoS ONE* 8: e53068.
16. Islam SA, Ling MF, Leung J et al. (2013) Identification of human CCR8 as a CCL18 receptor. *Journal of Experimental Medicine* 210: 1889–1898.
17. Zingoni A, Soto H, Hedrick JA et al. (1998) The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. *Journal of Immunology* 161: 547–551.
18. Spinetti G, Bernardini G, Camarda G et al. (2003) The chemokine receptor CCR8 mediates rescue from dexamethasone-induced apoptosis via an ERK-dependent pathway. *Journal of Leukocyte Biology* 73: 201–207.
19. Louahed J1, Struyf S, Demoulin JB et al. (2003) CCR8-dependent activation of the RAS/MAPK pathway mediates anti-apoptotic activity of I-309/ CCL1 and vMIP-I. *European Journal of Immunology* 33: 494–501.
20. Eruslanov E, Stoffs T, Kim WJ et al. (2013) Expansion of CCR8(+) inflammatory myeloid cells in cancer patients with urothelial and renal carcinomas. *Clinical Cancer Research* 19: 1670–1680.
21. Leung SY, Yuen ST, Chu KM et al. (2004) Expression profiling identifies chemokine (C-C motif) ligand 18 as an independent prognostic indicator in gastric cancer. *Gastroenterology* 127: 457–469.
22. Schmid S, Le UT, Haager B et al. (2016) Local concentrations of CC-chemokine-ligand 18 correlate with tumor size in non-small cell lung cancer and are elevated in lymph node-positive disease. *Anticancer Research* 36: 4667–4671.
23. Shi L, Zhang B, Sun X et al. (2016) CC chemokine ligand 18(CCL18) promotes migration and invasion of lung cancer cells by binding to Nir1 through Nir1-ELMO1/DOC180 signaling pathway. *Molecular Carcinogenesis* 55: 2051–2062.
24. Lane D, Matte I, Laplante C et al. (2016) CCL18 from ascites promotes ovarian cancer cell migration through proline-rich tyrosine kinase 2 signaling. *Molecular Cancer* 15: 58.
25. Wang Q, Tang Y, Yu HY et al. (2016) CCL18 from tumor-cells promotes epithelial ovarian cancer metastasis via mTOR signaling pathway. *Molecular Carcinogenesis* 55: 1688–1699.
26. Ye H, Zhou Q, Zheng S et al. (2018) Tumor-associated macrophages promote progression and the Warburg effect via CCL18/NF-kB/VCAM-1 pathway in pancreatic ductal adenocarcinoma. *Cell Death & Disease* 9: 453.
27. Miyagaki T, Sugaya M, Suga H et al. (2013) Increased CCL18 expression in patients with cutaneous T-cell lymphoma: Association with disease severity and prognosis. *Journal of the European Academy of Dermatology and Venereology* 27: e60–e67.