Associations of C-X-C motif chemokine ligands 1/2/8/13/14 with clinicopathological features and survival profile in patients with colorectal cancer

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Abstract. The present study aimed to assess the correlation of C-X-C motif chemokine ligand (CXCL)1, CXCL2, CXCL8, CXCL13 and CXCL14 with clinicopathological features and survival profile in patients with colorectal cancer (CRC). Patients with primary CRC (n=232) were retrospectively reviewed, with their tumor tissue specimens acquired from the Department of Pathology (The First Hospital of Jilin University, Changchun, China), their demographic data and preoperative tumor features collected from the hospital database, and their survival data obtained from the follow-up documents. Tumor CXCL expression was detected by immunohistochemistry (IHC). Based on the total IHC score, the expression of CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 was categorized as low expression (IHC score ≤3) and high expression (IHC score >3). CXCL1 (51.3% high and 48.7% low), CXCL2 (59.9% high and 40.1% low), CXCL8 (44.4% high and 55.6% low), CXCL13 (40.9% high and 59.1% low) and CXCL14 (31.0% high and 69.0% low) were expressed in CRC tumor tissues, and their expression levels were correlated with each other, except between CXCL8 and CXCL14, and between CXCL13 and CXCL14. CXCL1 was associated with a larger tumor size, and an advanced T stage, N stage and Tumor-Node-Metastasis (TNM) stage. CXCL2 was associated with an advanced N stage and TNM stage, and CXCL8 was associated with a greater T stage and TNM stage. CXCL13 was associated with a greater T stage, N stage and TNM stage, while CXCL14 was not associated with any clinical characteristics. As for survival, CXCL1, CXCL2, CXCL8 and CXCL13, but not CXCL14, were associated with poor overall survival (OS) rate, and further multivariate Cox's regression model analysis revealed that CXCL1 independently predicted unfavorable OS in patients with CRC. Overall, CXCL1, CXCL2, CXCL8 and CXCL13 have good potential as an indicator for tumor features and survival in patients with CRC.

Introduction

Colorectal cancer (CRC) is ranked the fourth most deadly cancer worldwide, accounting for ~10% of all diagnosed cancer cases and ~10% of cancer-related deaths (1). The treatment options for CRC have been enriched over the decades, with substantial improvements to techniques and a deeper understanding of CRC pathogenesis, which lead to the improvement of overall survival (OS). However, since CRC is not symptomatic until it reaches an advanced stage, and there are high occurrence rates of metastasis, recurrence and drug resistance, the lethality of CRC is yet to be adequately reduced (2). Furthermore, although disease screening using biomarkers has been implemented worldwide to increase the early detection of CRC, there is still a lack of a convincing test that accurately forecasts disease condition or prognosis for patients with CRC. Therefore, constant exploration of novel and reliable biomarkers for CRC monitoring is essential to improve the outcomes of patients with the disease.

C-X-C motif chemokine ligands (CXCLs) are small proteins with a cysteine-containing motif (C represents cysteine and X represents any amino acid) near the N-terminal. The CXCLs are key molecules that attract leukocytes to the inflammation sites, and they bind to the corresponding CXC receptors (CXCRs) to trigger internalization and transduction of downstream signaling pathways (3). A growing body of evidence has shown that CXCLs are involved in the development of a number of malignancies. For example, CXCL1 and CXCL2 facilitate cell survival and metastasis in breast cancer and predict poor OS in gastric cancer (4,5). CXCL8 mediates the initiation and development of prostate cancer, lung cancer and melanoma (6), and CXCL13 is associated with an advanced disease stage, and poor OS and disease-free survival rates of clear cell renal cell carcinoma (7). CXCL14 attenuates tumor progression in squamous cell carcinoma, while predicting poor survival in breast cancer (8,9). As shown by these studies, CXCLs present potential as biomarkers for tumor progression

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Abbreviations: CXCL, C-X-C motif chemokine ligand; CRC, colorectal cancer; IHC, immunohistochemistry; OS, overall survival; SD, standard deviation

Key words: colorectal cancer, CXCL, clinical characteristics, overall survival, biomarker

and prognosis in various cancer types, although the clinical implications of these CXCLs in CRC have not been fully studied yet. According to the existing evidence, we hypothesize that these CXCLs may be of clinical value for the disease management and prognosis of CRC. In the present study, the expression levels of tumor CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 were detected, and their associations with clinicopathological features and survival profile were further assessed in patients with CRC.

Materials and methods

Patients. The present study retrospectively reviewed the cases of 232 patients with primary CRC who underwent resection in The First Hospital of Jilin University (Changchun, China) between January 2012 and December 2014. All patients were initially confirmed with primary CRC by histopathology. The age range of the cohort was 18-80 years old. The patients were eligible if they had well-preserved tumor tissue specimens, and complete pre-operation tumor features and survival data, and if they were without distant metastases, did not have recurrent or secondary CRC, had no history of hematological malignancies or other solid tumors and had not received neoadjuvant therapy before resection. Ethical approval for the study was obtained from the Institutional Review Board of The First Hospital of Jilin University (approval no. 2018-413). The First Hospital of Jilin University provided access to the database used in this study. All patients or their family members provided written informed consent.

Data and sample collection. The demographic data (including the age and sex) and preoperative tumor features [including World Health Organization pathological grade (10), tumor size, T stage, N stage and American Joint Committee on Cancer Tumor-Node-Metastasis (TNM) stage (11)] were collected from the database of The First Hospital of Jilin University. The formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens were acquired from the Department of Pathology of The First Hospital of Jilin University. Furthermore, the FFPE normal colon tissues were available for 30 patients of the aforementioned 232 patients with CRC, which were also obtained from the Department of Pathology of The First Hospital of Jilin University.

Immunohistochemistry (IHC) assay. All tumor tissue specimens and normal colon tissues were cut into 4-μm sections, and then the sections were deparaffinized in 65˚C overnight, washed with xylene (Sangon Biotech, Co., Ltd.), rehydrated in a descending ethanol serials and underwent antigen retrieval. After that, 10% goat serum (MilliporeSigma) (30 min, room temperature) and 0.3% H_2O_2 (10 min, room temperature) were added to the sections for the blocking of non-specific binding and peroxidase activity. Subsequently, primary antibodies (CXCL1 rabbit polyclonal antibody; 1:100; cat. no. PA5-86508; CXCL2 recombinant rabbit monoclonal antibody; 1:20; cat. no. 701126; CXCL8 rabbit polyclonal antibody; 1:500; cat. no. PA5-85428; CXCL13 rabbit polyclonal antibody; 1:500; cat. no. PA5-28827; and CXCL14 rabbit polyclonal antibody; 1:500; cat. no. PA5-28820) (all Invitrogen; Thermo Fisher Scientific, Inc.) were added and incubated at 4˚C overnight. The next day, horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L) secondary antibody (1:10,000; cat. no. 31460; Invitrogen; Thermo Fisher Scientific, Inc.) was added and incubated at 37˚C for 60 min. Finally, the tissue sections were stained with diaminobenzidine (MilliporeSigma) and counterstained with hematoxylin (MilliporeSigma) (2 min, room temperature). The IHC staining result was observed on a Nikon ECLIPSE E200 microscope (Nikon Corporation) and assessed by staining intensity and staining density of positive cells, as previously described (12). Based on the total IHC score (staining intensity score x staining density score; score range, 0-12), the expression of CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 was categorized as low expression (IHC score ≤3) and high expression (IHC score >3) (12).

Hematoxylin-eosin staining. The tissues, fixed in 10% formalin (Sangon Biotech, Co., Ltd.) for 24 h at room temperature, were embedded in paraffin and were cut into 4-μm sections. The sections were then deparaffinized with xylene (Sangon Biotech, Co., Ltd.) and rehydrated in a descending ethanol serials. The hematoxylin ((Sangon Biotech, Co., Ltd.) was used to stain the nuclei at room temperature for 5 min. The cytoplasm was stained with eosin (Sangon Biotech, Co., Ltd.) for 2 min at room temperature. The images were taken by a Nikon ECLIPSE E200 microscope (Nikon Corporation).

Follow-up. The survival data were obtained from the patient follow-up documents. According to the survival data, the last follow-up date was December 31, 2018, and the median follow-up duration was 56.0 months (range, 1.0-84.0 months). The OS time was calculated from the date of resection to the date of death.

Human Protein Atlas Database validation. The expression of CXCLs and CXCRs were re-assessed using the Human Protein Atlas Database (www.proteinatlas.org), derived from The Cancer Genome Atlas (TCGA) database. In detail, the IHC score for 597 patients for CXCL1, CXCL2, CXCL8, CXCL13, CXCL14 are shown in Fig. S1. Besides, the survival data were downloaded from TCGA database for subsequent analysis of the correlation between CXCL1, CXCL2, CXCL8, CXCL13, CXCL14, CXCR1, CXCR2, CXCR3 and CXCR5 and survival, which are shown in Figs. S1 and S2 (available from www.proteinatlas.org).

Statistical analysis. The descriptive analysis of continuous variables is expressed as mean ± standard deviation (SD), and the descriptive analysis of categorical variables is displayed as count (percentage). The correlations among CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 were determined using Spearman's correlation analysis. The comparison of quantitative data with a normal distribution (including age and tumor size) was determined using an unpaired Student's t-test. The comparison of an unordered categorical variable (including sex) was assessed by χ^2 test. The comparison of ordered categorical variables (including pathological grade, T stage, N stage and TNM stage) was performed using Wilcoxon's rank sum test. Comparisons between the tumor tissue and normal colon tissue with regard to CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 expression were achieved by the paired t-test. OS

was displayed using Kaplan-Meier curves, and comparisons of OS between two groups were determined by log-rank test. Multivariate logistic regression analysis was performed to combine CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 as CXCLs, and the calculation formula is shown in Table SI. Receiver operative curves were used for analyzing the ability of CXCLs to distinguish between tumor tissue and normal colon tissue. Factors affecting OS were analyzed by univariate and backward stepwise multivariate Cox's proportional hazard regression model. Statistical analyses were performed using SPSS (version 22.0; IBM Corp.), and figures were plotted using GraphPad Prism (version 7.00; GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics of patients with CRC. The mean age ± SD for the cohort was 65.2±10.7 years, and the median age (range) was 67.5 years (39.0-80.0 years). The sex composition was 106 (45.7%) females and 126 (54.3%) males. A total of 34 (14.7%), 166 (71.6%) and 32 (13.8%) patients were in pathological grades G1, G2 and G3, respectively. The mean tumor size was 4.4±1.2 cm, and for the tumor stage, the number of patients at TNM stage I, II and III was 30 (12.9%), 109 (47.0%) and 93 (40.1%), respectively. Other detailed clinical characteristics are shown in Table I.

Expression of CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 in CRC tumor tissues and normal colon tissues. Representative staining of CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 in tumor tissues and normal colon tissues is shown in Fig. 1. In addition, the tumor tissues and normal colon tissues stained using hematoxylin-eosin staining are shown in Fig. S3. Compared with the normal colon tissue, the tumor tissue exhibited elevated levels of CXCL2 and CXCL8 expression (both $P<0.01$), but similar levels of CXCL1, CXCL13 and CXCL14 expression (all $P>0.05$) (Table SII). The combination of CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 (referred to as CXCLs) had a certain ability for distinguishing the CRC tumor tissues from the normal colon tissues (Fig. S4). The correlations among CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 are shown in Table SIII. All CXCLs were associated with each other (all $P<0.05$), with the exception of CXCL14 and CXCL8, and CXCL14 and CXCL13 (both $P>0.05$).

Comparison of CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 between CRC patients with different clinicopathological features. In patients with CRC, those with high CXCL1 expression exhibited a larger tumor size (P=0.009), and advanced T stage (P=0.004), N stage (P=0.015) and TNM stage (P=0.006). Patients with high CXCL2 expression presented with advanced N stage (P=0.016) and TNM stage (P=0.015). Patients with high CXCL8 expression presented with an advanced T stage (P=0.027) and TNM stage (P=0.041), and those with high CXCL13 expression presented with a higher T stage (P=0.003), N stage (P=0.001) and TNM stage (P=0.001) (Table II). However, there were no differences with regard to clinical characteristics among patients with CRC with different levels of CXCL14 expression (all $P>0.05$).

Table I. Clinical characteristics of patients with colorectal cancer (n=232).

Characteristic	Value
Mean age ± SD, years	65.2±10.7
Sex, n (%)	
Female	106 (45.7)
Male	126 (54.3)
Pathological grade, n (%)	
G1	34 (14.7)
G2	166 (71.6)
G3	32 (13.8)
Mean tumor size ± SD, cm	4.4±1.2
T stage, n (%)	
T1	5 (2.2)
T2	25 (10.8)
T3	199 (85.8)
T4	3 (1.3)
N stage, n (%)	
N0	139 (59.9)
N1	61 (26.3)
N2	32 (13.8)
TNM stage, n (%)	
I	30 (12.9)
II	109 (47.0)
III	93 (40.1)

SD, standard deviation.

Associations between CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 expression levels and OS in patients with CRC. High CXCL1 expression (P=0.005) (Fig. 2A), high CXCL2 expression (P=0.003) (Fig. 2B), high CXCL8 expression (P=0.024) (Fig. 2C) and high CXCL13 expression (P=0.018) (Fig. 2D) were associated with poor OS in patients with CRC, whereas no association was observed between CXCL14 expression level and OS (P=0.408) (Fig. 2E).

Factors affecting OS in patients with CRC. In total, 101 patients died during the follow-up period. Of these, 92 patients died of cancer or its related causes and 9 patients died from other causes, including 8 patient deaths due to complications of their disease and 1 patient death from an accident (a fall causing a head injury). High CXCL1 (P=0.006, HR=1.756), high CXCL2 (P=0.004, HR=1.883), high CXCL8 (P=0.025, HR=1.561) and high CXCL13 (P=0.019, HR=1.593) expression levels, as well as higher pathological grade ($P<0.001$, HR=2.166), greater tumor size (P=0.023, HR=1.657) and advanced TNM stage ($P<0.001$, HR=1.826) were associated with a lower OS rate in the patients with CRC (Table III). Backward stepwise multivariate Cox's regression further illustrated that CXCL1 high expression (P=0.043, HR=1.563), higher pathological grade ($P<0.001$, HR=2.191), greater tumor size (P=0.003, HR=1.975) and advanced TNM stage (P=0.001, HR=1.662) were independent predictive factors for poor OS rate in patients with

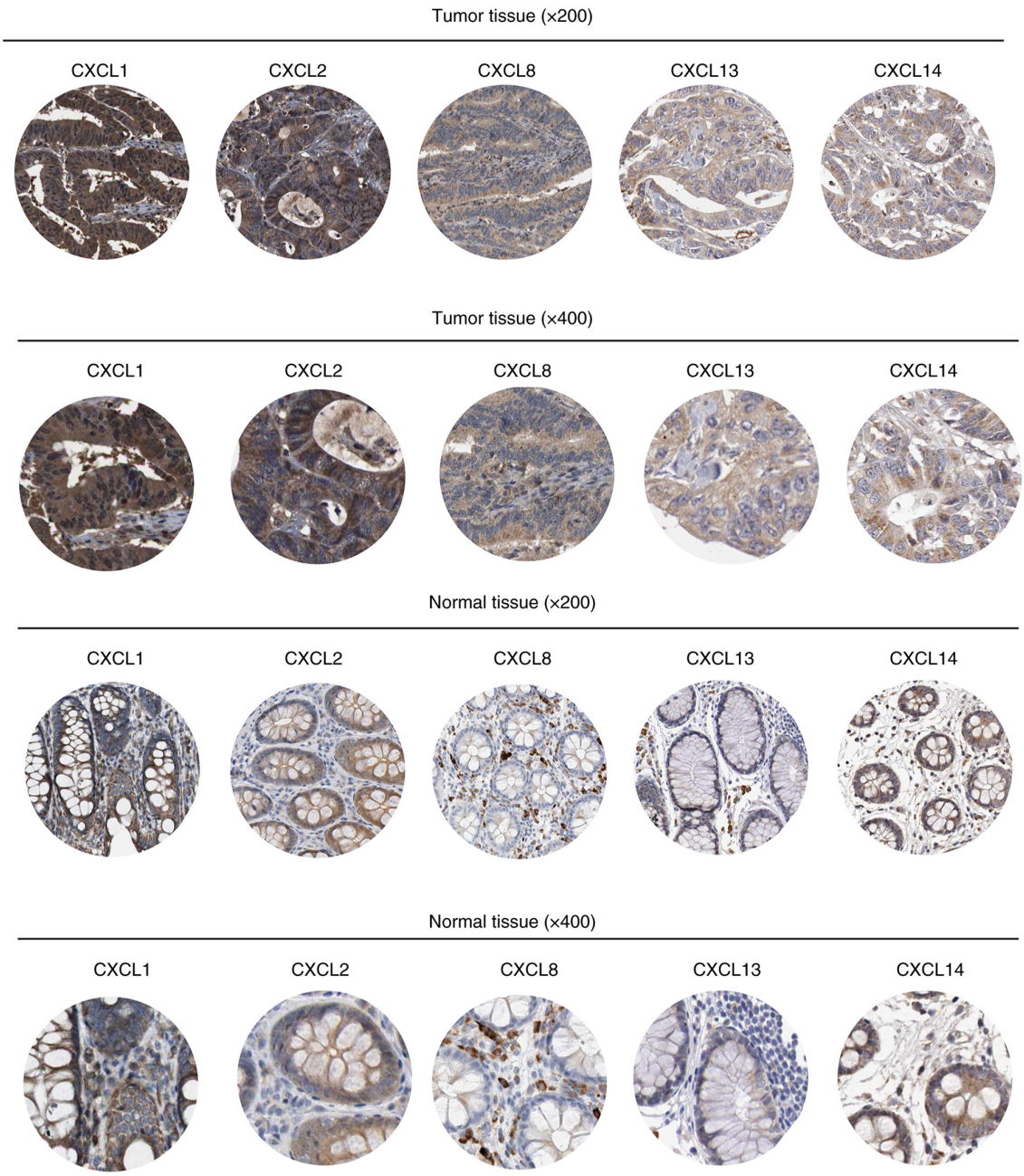

Figure 1. Representative expression of CXCLs in colorectal cancer tissues and normal colon tissues. CXCL, C-X-C motif chemokine ligand.

CRC. All the enrolled patients in the study received a surgical resection with curative intent. After the surgical resection, 220 patients achieved an R0 resection. An analysis was performed for R0 resection status and OS. The results showed that patients with an R0 resection exhibited a prolonged accumulating OS time compared with those patients who did not achieve an R0 resection (P=0.001; Fig. S5).

Validation of CXCL expression and correlation with survival in CRC patients. The expression of CXCLs in CRC was re-assessed using the Human Protein Atlas Database and divided into high and low expression according to the median expression value (fragments per kilobase per million) (Fig. S1A, C, E, G and I). High CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 expression was associated with poor 5-year survival in patients with CRC (all P<0.05) (Fig. S1B, D, F, H and J). Furthermore, the associations of the CXCRs with survival were also determined, which showed that only high CXCR1 expression was associated with prolonged OS time (P=0.035), while CXCR2, CXCR3 and CXCR5 were not associated with OS (all P>0.05) (Fig. S2A-D).

Discussion

The present study found that CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 were sufficiently expressed in CRC tissues and they were closely correlated with each other, with the exception of CXCL8 and CXCL14, and CXCL14 and CXCL13. Most importantly, CXCL1, CXCL2, CXCL8, CXCL13, but not CXCL14, were associated with advanced tumor features and poor OS in patients with CRC.

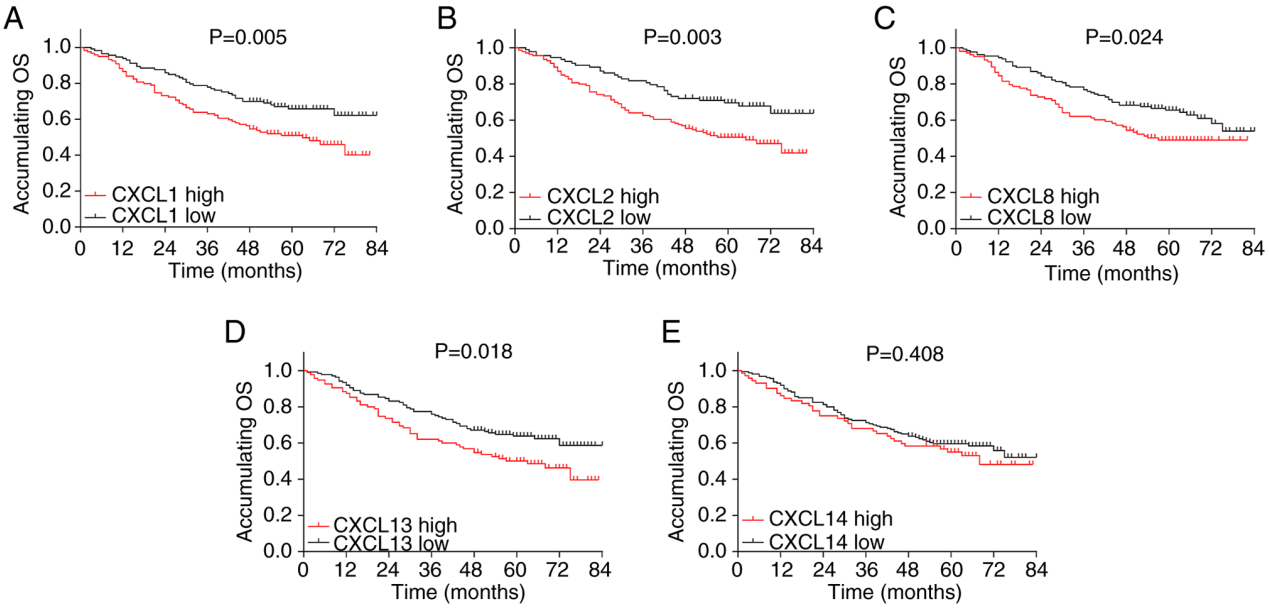

Figure 2. Associations between CXCLs and OS in patients with colorectal cancer. Comparison of OS between patients with high and low (A) CXCL1, (B) CXCL2, (C) CXCL8, (D) CXCL13 and (E) CXCL14 expression. OS, overall survival; CXCL, C-X-C motif chemokine ligand.

CXCLs are known to be associated with tumor formation and metastasis (4,5,7-9,13-15). In the pathology of CRC, CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 have been shown to modulate tumor progression, such as the tumor-specific immune response, angiogenesis and metastasis (5,7-9,13-18), whereas their associations with clinicopathological features in patients with CRC are obscure (19). The high level of CXCLs may activate the carcinoma-associated fibroblasts that promote cancer cell growth, migration and invasion, leading to lymph node metastasis and a higher TNM stage of CRC (8,18). From another prospective, CXCL13 activates the Wnt/β-catenin pathway and the production of IL-12, IL-17 and IgG4, and CXCL1, CXCL2 and CXCL8 activate the NF-κB pathway, both of which are contributors for the tumorigenesis and metastasis of CRC (17,20). With regard to CXCL14, its role in cancer is controversial, being tumor suppressive in squamous cell carcinoma, but tumor promotive in breast cancer (8,9). Therefore, no association between CXCL14 and any clinical characteristics was observed in the patients with CRC in the present study. Considering these results, the detection of CXCL levels may assist pathological assessment in clinical settings.

Overall, the upregulation of CXCLs is associated with a poor prognosis in cancer. Specifically, CXCL1 and CXCL2 are independent predictive factors for poor OS in patients with gastric cancer (5), CXCL8 is closely associated with unfavorable survival in papillary thyroid carcinoma (21), CXCL13 predicts poor OS and disease-free survival in clear cell renal cell carcinoma, as well as the recurrence of hepatocellular carcinoma after hepatectomy (16), and CXCL14 accelerates cell growth in breast cancer, and induces drug resistance and metastasis, which leads to a poor prognosis for patients with breast cancer (7,16). Regarding CRC, although previous studies have reported the influence of these CXCLs on tumor initiation and development, the prognostic value of these CXCLs towards CRC has not been fully investigated and needs further validation (2,3,19). The present study observed that CXCL1, CXCL2, CXCL8 and CXCL13, but not CXCL14, predicted poor OS, and that CXCL1 was an independent predictive factor for unfavorable OS in patients with CRC. Moreover, the negative association of high CXCL expression with poor 5-year survival was observed via analysis using the Human Protein Atlas Database. These results regarding prognosis were in accordance with an existing study indicating that CXCL1, CXCL2, CXCL8 and CXCL13 were predictive factors for poor survival in patients with colorectal cancer (3). There are several explanations for these CXCLs being able to predict poor OS: i) CXCL1, CXCL2 and CXCL8 are ligands binding to receptor CXCR2, and are proangiogenic and facilitate chemoresistance under chemotherapeutic drugs in various cancer types. Therefore, they are associated with poor survival in patients with CRC. ii) CXCL13 binds to CXCR5 and regulates lymphocyte migration, promotes inflammation, and promotes CRC cell growth, invasion and metastasis via the PI3K/AKT pathway, which leads to poor survival (22). CXCL14 was not associated with clinical characteristics of the patients with CRC in the present study, and considering that the role of CXCL14 in cancer is controversial, it is predictable that CXCL14 was not associated with the survival of these patients. Due to the accessibility of CXCL expression levels, the evaluation of CXCLs might be of clinical value for identifying patients at risk of a poor prognosis in order for appropriate treatment approaches. One unneglectable limitation in the present study needed to be clarified: The study was retrospective, and when it was performed, 101 patients had already died; therefore the consent was signed by the family members on behalf of the patients. .

In this study, the associations of key CXCLs with clinical characteristics and survival in patients with CRC were explored; however, there were still several restrictions. Above all, since this was a small-scale study with limited samples recruited from a single geographic area, the results might be subjected to selection bias. Further large-scale and multi-center investigation is necessary to validate the findings.

Table II. Comparison between CXCL1, CXCL2, CXCL8, CXCL13 and CXCL14 expression levels in patients with colorectal cancer with regard to different clinical characteristics.

	CXCL1 expression			CXCL2 expression			CXCL8 expression			CXCL13 expression			CXCL14 expression		
Items	High (n=119)	Low (n=119)	P-value	High (n=139)	Low (n=93)	P-value	High (n=103)	Low (n=129)	P-value	High (n=95)	Low (n=137)	P-value	High (n=72)	Low (n=160)	P-value
Mean age ± SD, years	64.4±10.2	66.0±11.2	0.241	64.9±10.6	65.6±11.0	0.648	65.0±10.7	65.4±10.8	0.786	63.8±10.4	66.2±10.9	0.097	64.1±9.7	65.7±11.2	0.289
Sex, n (%)			0.052			0.345			0.179			0.147			0.549
Female	47 (39.5)	59 (52.2)		60 (43.2)	46 (49.5)		42 (40.8)	64 (49.6)		38 (40.0)	68 (49.6)		35 (48.6)	71 (44.4)	
Male	72 (60.5)	54 (47.8)		79 (56.8)	47 (50.5)		61 (59.2)	65 (50.4)		57 (60.0)	69 (50.4)		37 (51.4)	89 (55.6)	
Pathological grade, n (%)			0.218			0.072			0.338			0.090			0.483
G1	16 (13.5)	18 (15.9)		18 (12.9)	16 (17.2)		14 (13.6)	20 (15.5)		13 (13.7)	21 (15.3)		7 (9.7)	27 (16.9)	
G2	83 (69.7)	83 (73.5)		97 (69.8)	69 (74.2)		72 (69.9)	94 (72.9)		63 (66.3)	103 (75.2)		56 (77.8)	110 (68.7)	
G3	20 (16.8)	12 (10.6)		24 (17.3)	8 (8.6)		17 (16.5)	15 (11.6)		19 (20.0)	13 (9.5)		9 (12.5)	23 (14.4)	
Mean tumor size ± SD, cm	4.6±1.3	4.2±1.1	0.009	4.5±1.3	4.3±1.2	0.241	4.6±1.3	4.3±1.2	0.064	4.6±1.4	4.3±1.1	0.056	4.4±1.3	4.4±1.2	0.900
T stage, n (%)			0.004			0.056			0.027			0.003			0.862
T1	1 (0.8)	4 (3.5)		2 (1.4)	3 (3.2)		1 (1.0)	4 (3,1)		0 (0.0)	5 (3.6)		1 (1.4)	4 (2.5)	
T2	8 (6.8)	17 (15.1)		12 (8.6)	13 (14.0)		7 (6.8)	18 (13.9)		6 (6.3)	19 (13.9)		8 (11.1)	17 (10.6)	
T3	107 (89.9)	92 (81.4)		122 (87.8)	77 (82.8)		93 (90.3)	106 (82.2)		86 (90.5)	113 (82.5)		62 (86.1)	137 (85.6)	
T4	3 (2.5)	0 (0.0)		3 (2.2)	0 (0.0)		2 (1.9)	1 (0.8)		3 (3.2)	0 (0.0)		1 (1.4)	2 (1.3)	
N stage, n (%)			0.015			0.016			0.140			0.001			0.579
N0	63 (52.9)	76 (67.3)		75 (54.0)	64 (68.8)		56 (54.4)	83 (64.3)		46 (48.4)	93 (67.9)		45 (62.5)	94 (58.8)	
N1	34 (28.6)	27 (23.9)		40 (28.8)	21 (22.6)		31 (30.1)	30 (23.3)		28 (29.5)	33 (24.1)		18 (25.0)	43 (26.8)	
N2	22 (18.5)	10 (8.8)		24 (17.2)	8 (8.6)		16 (15.5)	16 (12.4)		21 (22.1)	11 (8.0)		9 (12.5)	23 (14.4)	
TNM stage, n (%)			0.006			0.015			0.041			0.001			0.699
I	9 (7.6)	21 (18.6)		14 (10.1)	16 (17.2)		8 (7.8)	22 (17.0)		6 (6.3)	24 (17.5)		9 (12.5)	21 (13.1)	
II	54 (45.4)	55 (48.7)		61 (43,9)	48 (51.6)		48 (46.6)	61 (47.3)		40 (42.1)	69 (50.4)		36 (50.0)	73 (45.6)	
III	56 (47.0)	37 (32.7)		64 (46.0)	29 (31.2)		47 (45.6)	46 (35,7)		49 (51.6)	44 (32.1)		27 (37.5)	66 (41.3)	

Comparison of quantitative data with a normal distribution (including age and tumor size) was determined by unpaired Student's t-test. Comparison of unordered categorical variables (including sex) was checked by χ^2 test. Comparison of the ordered categorical variables (including pathological grade, T stage, N stage and TNM stage) was performed by the Wilcoxon rank sum test. CXCL, C-X-C motif chemokine ligand; SD, standard deviation; TNM, Tumor-Nide-Metastasis.

Table III. Factors affecting OS.

	Cox's proportional hazard regression			
			95% CI	
Factor	P-value	HR	Lower	Higher
Univariate Cox's regression				
CXCL1 high	0.006	1.756	1.176	2.623
CXCL2 high	0.004	1.883	1.228	2.888
CXCL8 high	0.025	1.561	1.056	2.308
CXCL13 high	0.019	1.593	1.078	2.354
CXCL14 high	0.410	1.189	0.787	1.797
Age (>60 years)	0.991	0.998	0.665	1.496
Male	0.409	1.180	0.797	1.747
Pathological grade	<0.001	2.166	1.495	3.139
Tumor size (>5 cm)	0.023	1.657	1.071	2.562
TNM stage	<0.001	1.826	1.337	2.492
Backward stepwise multivariate Cox's regression				
CXCL1 high	0.043	1.563	1.013	2.411
Pathological grade	<0.001	2.191	1.505	3.190
Tumor size (>5 cm)	0.003	1.975	1.263	3.090
TNM stage	0.001	1.662	1.226	2.255

Factors affecting OS were analyzed by univariate and backward stepwise multivariate Cox's proportional hazard regression model. HR, hazard ratio; CI, confidence interval; CXCL, C-X-C motif chemokine ligand; SD, standard deviation; OS, overall survival; TNM, Timor-Node-Metastasis.

Secondly, although CXCLs have been studied for their potential as CRC prognostic markers in this study, the molecular mechanisms of CXCLs in CRC were not investigated. In this study, the expression of CXCLs was detected by IHC, and the cutoff value for CXCL high and low expression was an IHC score of 3. However, the optimal cutoff for CXCLs should be determined by Youden index or the Maxstat method, or the χ^2 test, which was not performed since control data was lacking. Furthermore, the association of CXCLs with prognosis in patients with CRC could be validated with more thorough analysis, such as use of nomograms, or using an additional cohort from a second hospital assessed with IHC. This study was a retrospective study; therefore, its inherent limitation should not be neglected such as patient selection bias and insufficient data. Even though the multivariate Cox's regression analysis was performed to eliminate the potential cofounding factors such as age, the range of the enrolled patients was large (mean age, 65.2±10.7 years; range, 39-80 years), which may affect the generalization of the results. Fresh frozen tissues were not stored for use in this study; therefore, the RNA level of the CXCLs was not determined in the present study. Finally, the CXCR levels in patients with CRC and their associations with survival should be determined in further studies.

In conclusion, the present study found that CXCL1, CXCL2, CXCL8 and CXCL13, but not CXCL14, were associated with worse tumor features and unfavorable OS in patients with CRC. This may be of potential in assisting predictive and individualized CRC treatments.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XL and LW made substantial contributions to conception and design. XL, JT, YZ, PZ, DS and LW collected and analyzed the data. XL, JT, YZ, PZ and DS were involved in drafting the manuscript and revising it critically for important intellectual content. XL and LW confirm the authenticity of all the raw data. All authors given final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have read and approved the manuscript.

Ethics approval and consent to participate

Ethical approval for the study was obtained from the Institutional Review Board of The First Hospital of Jilin University (approval no. 2018-413). All patients or their family members provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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