Research Article
Plasma CXCL3 Levels Are Associated with Tumor Progression and an Unfavorable Colorectal Cancer Prognosis

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Background. The CXC chemokines belong to a unique family of chemotactic cytokines that influence the initiation, progression, and clinical outcome of many tumor types. Herein, we investigated the association of the CXC-chemokine ligand 3 (CXCL3) with tumor progression and an unfavorable prognosis for colorectal cancer (CRC).

Methods. The quantitative real-time polymerase chain reaction was used to explore the expression of CXCL3 in CRC tissue, adjacent tissue, and plasma. The usefulness of plasma levels of CXCL3 for the diagnosis of CRC was evaluated by receiver operating characteristic curve analysis. Pearson’s correlation analysis assessed relationships among plasma CXCL3, cancer tissue CXCL3, and plasma carcinoembryonic antigen (CEA). Kaplan–Meier analysis was used to assess the survival of CRC patients with high and low expression levels of CXCL3. Survival differences were compared by log-rank test.

Results. Initial analysis of the GSE156720 dataset identified CXCL3 as the most enriched CXCL gene in CRC patients. Higher CXCL3 levels were detected in CRC tissue than in adjacent tissue (P < 0.001). Compared to healthy controls, CRC patient plasma CXCL3 levels were higher (P < 0.001). The area under the curve was 0.81 with a sensitivity of 0.71 and specificity of 0.82, distinguishing CRC from other tumor types. Plasma CXCL3 was positively related to CXCL3 in cancer tissue (r = 0.78, P < 0.01), and also to plasma CEA (r = 0.50, P < 0.01). Plasma CXCL3 was also related to tumor size (P = 0.034), staging (P < 0.001), tumor stage (P = 0.003), differentiation (P = 0.001), and lymph node metastasis (P = 0.007), but not to sex (P = 0.853), age (P = 0.691), tumor site (P = 1.347), or distant metastasis (P = 1.218). Conclusions. CXCL3 levels were increased in CRC patients, with plasma CXCL3 levels associated with tumor progression and an unfavorable CRC prognosis. The results of this study suggest that plasma CXCL3 may be a novel diagnostic and prognostic biomarker for CRC.

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide, with approximately 1 million new cases diagnosed each year [1, 2]. In the past 20 years, the incidence of CRC in China has ranked fourth among all malignant tumors and fifth for mortality [3, 4]. The environment is a significant risk factor for CRC, and while the incidence of CRC can be reduced through lifestyle changes, there is a range of risk factors that cannot be changed, such as age, family, and personal history [5, 6]. Compared with other digestive tract cancers, the prognosis for rectal cancer is relatively good, but because early symptoms are not obvious, most patients are diagnosed during the middle to late disease. Consequently, treatment is often unsatisfactory, with recurrence and metastasis the leading causes of rectal cancer death [7]. As such, the identification of new diagnostic markers is essential for early cancer diagnosis and a reduction in the high CRC mortality rate. CXC’s (CXCL1-17) are a class of soluble, proinflammatory, and highly conserved cytokines. CXC’s are soluble cholinesterases that interact with cognate cellular receptors, stimulating cells, and inducing directed chemotaxis. Chemotaxis, migration, and adhesion play essential roles in developing various tumors [8–10]. CXCL3 encoded by the human GRO gene and located within the chromosomal region, 4q13.3. CXCL3 is a granulocyte chemoattractant (GCP-2) and is an ELR
CXCL3, similar to other ELR+ chemokines that, with alternative splicing, can result in multiple transcript variants. CXCL3 plays a vital role in angiogenesis, tumorigenesis, and cell invasion [11–13]. See et al. demonstrated overexpression of CXCL3 to be closely related to breast cancer metastasis [14]. Multiple studies have found CXCL3 to participate in the development of many inflammatory and autoimmune diseases and the progression and metastasis of many tumor types [15–17]. However, the function of CXCL3 in CRC patients is not well established. This study aimed to explore the expression level and clinical significance of CXCL3 in CRC patients. We found the expression of CXCL3 to be relatively high and stable in cancer tissue and the plasma of CRC patients. CXCL3 levels correlated with carcinoembryonic antigen (CEA) levels, a clinical tumor marker of CRC. Moreover, levels of CXCL3 were found to distinguish CRC from other gastrointestinal tumors.

2. Materials and Methods

2.1. Patients and Samples. This study was conducted in Xuzhou Central Hospital and was approved by the Research Ethics Committee of Xuzhou Central Hospital. A total of 59 CRC patients (42 males and 17 females, mean age 56 ± 19 years) underwent surgery at Xuzhou Central Hospital from January 2018 to June 2020. CRC cancer and adjacent tissue (5 cm removed from the cancer tissue) were excised and digested with collagenase for 15 minutes to prepare cell suspensions that were stored in liquid nitrogen. 169 patients (119 males, 50 females, mean age 55 ± 17 years) with nonoperative CRC and 219 healthy subjects (153 males, 66 females, mean age 57 ± 18 years) were also evaluated. Plasma samples were collected from all patients and control subjects. Patients had no other underlying medical history and did not receive radiotherapy, chemotherapy, or immunotherapy. All patients signed informed consent before sampling. All
tissue and plasma samples were kept at -80°C before assessment. Plasma levels of the classical tumor marker CEA (carcinoembryonic antigen) were assessed using the CMIA (chemiluminescent microparticle immunoassay) method on ARCHITECT 8200 ci (Abbott Laboratories, Abbott Park, IL, USA).

2.2. RNA Extraction. Total cells from tissue samples or plasma were collected and resuspended in lysis buffer (10 mM NaCl, 20 mM MgCl, ten mM Tris-HCl, pH 7.8, 5 mM DTT, 0.5% NP-40) kept in ice for 5 minutes. And then, the fraction was subjected to protease treatment for 20 minutes at 37°C by adding an equal volume of proteinase K solution (300 mM NaCl, 0.2 M Tris-HCl, pH 7.5, 25 mM EDTA, 2% SDS, and 0.1 mg/ml proteinase K), and RNA was purified using the QIAzol Lysis Reagent (Qiagen, Germany). The RNA was subjected to DNase treatment; cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen, Germany) according to the manufacturer’s protocol.

2.3. qRT-PCR Analysis. qRT-PCR was performed using a Bio-Rad My cycle (Bio-Rad, CA, USA) according to the manufacturer’s instructions. β-actin was used as a reference gene. The PCR primer for CXCL3 was F: 5’-CGCCAAACCGAAGTCATAG-3’ and β-actin F: 5’-AAATGTGTGTGCACGG GATG-3’, R: 5’-CTCCTTAATGCACGCAACAG-3’. The reaction conditions were: 95°C, 15 s; 60°C, 30 s; 74°C, 30 s; and 72°C, 20 s, with 40 cycles of amplification. All experiments were repeated at least three times. The analysis was performed using the 2^-ΔΔCt method, with β-actin as the endogenous control.

2.4. Statistical Analysis. Statistical analysis was conducted using GraphPad prism 8.0. The data conforming to normal distribution were expressed by mean ± standard deviation. The baseline mean between the two groups was compared by independent sample t-test, the rate was compared by χ² test, and the data not conforming to normal distribution were compared by Wilcoxon sign rank-sum test. Pearson’s correlation analysis was used to assess the relative expression levels of CXCL3 and CEA in plasma and CRC tissues. The CRC diagnostic value of plasma CXCL3 was estimated by receiver operating characteristic curve analysis (ROC). P < 0.05 was considered to be statistically significant.

3. Results

3.1. CXCL3 Was Highly Expressed in CRC Tissues. The relative mRNA expression of CXCLs in CRC was analyzed with the public GEO dataset, GSE156720. CXCL1, CXCL2, CXCL3, CXCL5, CXCL8, and CXCL12 were highly expressed in CRC tissue compared to adjacent nontumor tissue (AT) (mean log2 (CC/AT) ≥ 1), while CXCL12, CXCL13, and CXCL14 were poorly expressed in CRC tissue (mean log2 (CC/AT) ≤ 1). CXCL3 was the most enriched

| Characteristics | AUC | SE  | P     | 95% CI    | Sen | Spe | Youden index |
|-----------------|-----|-----|-------|-----------|-----|-----|--------------|
| CXCL3 + CEA     | 0.85| 0.04| <0.001| 0.81–0.89 | 0.74| 0.96| 0.70         |
| CXCL3           | 0.81| 0.03| <0.001| 0.77–0.86 | 0.71| 0.82| 0.53         |
| CEA             | 0.72| 0.05| <0.001| 0.67–0.77 | 0.61| 0.80| 0.41         |

CXCL3: CXC-chemokine ligand 3; CEA: carcinoembryonic antigen; CRC: colorectal cancer.
were measured for CRC patients and HC. Compared to HC subjects (HC)) were enrolled in this study. Plasma CXCL3 levels in 59 pairs of CRC and adjacent tissues were greater in CRC tissues compared to matched adjoining tissue (AT) were examined by qRT-PCR. mRNA levels of CXCL3 in plasma of CRC patients was positively related to plasma CXCL3 level (HR: 2.017, 95% CI: 0.916-3.274, P < 0.01) and tumor stage (HR: 1.809 95% CI: 0.726-4.223, P < 0.01). Further, the level of CXCL3 in plasma of CRC patients was positively related to plasma CEA (50.85 ± 46.93; r = 0.50, P < 0.01) (Figure 2(b)).

3.2. Preoperative Plasma CXCL3 Levels Are Elevated in CRC Patients. 228 patients diagnosed with CRC (included 59 surgical cases and 169 non-surgical cases and 219 healthy control subjects (HC)) were enrolled in this study. Plasma CXCL3 levels were measured for CRC patients and HC. Compared to HC (8.82 ± 6.79 pg/ml), preoperative plasma CXCL3 levels were significantly elevated (P < 0.001) for patients with CRC (71.15 ± 67.84 pg/ml, Figure 2(a)). To explore the diagnostic value of CXCL3, a ROC curve was constructed. The outcome showed an AUC of 0.81 (95% confidence interval (CI): 0.77~0.86) with a sensitivity of 0.71 and a specificity of 0.82, and the cutoff value of 15.5. When plasma CXCL3 and CEA values were combined for the diagnosis of CRC, the AUC increased to 0.85 (95% CI: 0.81~0.89), and the sensitivity and specificity were 0.74 and 0.96, respectively, indicating that CXCL3 added a significant advantage for a diagnosis of CRC (Figure 2(b) and Table 1).

3.3. Relationships among CXCL3 in CRC Plasma, CXCL3 in Cancer Tissue, and Plasma CEA. Pearson’s correlation analysis showed that the level of CXCL3 in plasma of CRC patients was positively related to the expression of CXCL3 in cancer tissue (r = 0.78, P < 0.01), Figure 3(a). Further, the level of CXCL3 in plasma of CRC patients was positively related to plasma CEA (50.85 ± 46.93; r = 0.50, P < 0.01) (Figure 3(b)).

3.4. Relationships among Plasma CXCL3, CRC Clinicopathologic Features, and CRC Patient Survival Time. The patients with CRC were divided into high- and low-expression groups based on the cutoff value derived from the ROC curve. There were 94 cases in the increased expression group and 75 in the low expression group. The plasma expression of CXCL3 in CRC was related to many clinicopathological Characteristics (Table 2) such as tumor size (P = 0.034, Figure 4(a)), staging (P < 0.001, Figure 4(b)), tumor stage (P = 0.003, Figure 4(g)), tumor differentiation (P = 0.001, Figure 4(c)), lymph node metastasis (P = 0.007, Figure 4(e)), and distant metastasis (P = 0.128, Figure 4(i)). To further assess the independently correlated clinical parameters of plasma CXCL3 levels, multivariate stepwise regression analysis was performed. When plasma CXCL3 levels were set as the dependent variable, and sex, age, tumor site, tumor size, staging, tumor stage, differentiation, lymph node metastasis, and distant metastasis were set as the independent variables, staging (standard $\beta = 0.773$, P = 0.042), tumor stage (standard $\beta = 1.038$, P = 0.038), and differentiation (standard $\beta = 1.229$, P = 0.024) were found to be independent factors of plasma CXCL3 levels (Table 3).

Kaplan–Meier analysis demonstrated an overall survival of patients in the high CXCL3 expression group to be shorter than in the low expression group (HR: 2.556, 95% CI: 1.306-4.223, P < 0.01, Figure 4(j)). Next, we used COX regression analysis to assess the correlation between plasma CXCL3 level and overall survival. The death events and follow-up time were the dependent variables; independently correlated clinical parameters such as sex, age, tumor site, tumor size, staging, tumor stage, differentiation, lymph node metastasis, distant metastasis, and plasma CXCL3 level were set as the independent variables; plasma CXCL3 level (HR: 2.017, 95% CI: 0.916-3.274, P < 0.01, Figure 4(j)) and tumor stage (HR: 1.809 95% CI: 0.726-2.612, P < 0.01) were found to be independent factors of overall survival.

### Table 2: Associations among preoperative plasma CXCL3 levels and clinical parameters of CRC patients.

| Clinicopathological characteristics | Total (n = 228) | Expression of CXCL3 High (n = 129) | Low (n = 99) | P value |
|-----------------------------------|----------------|-----------------------------------|-------------|---------|
| Sex                               |                |                                   |             | 0.853   |
| Female                            | 71             | 39                                | 32          |         |
| Male                              | 157            | 91                                | 66          |         |
| Age (years)                       |                |                                   |             | 0.691   |
| ≥ 60                              | 155            | 88                                | 67          |         |
| < 60                              | 73             | 39                                | 34          |         |
| Tumor site                        |                |                                   |             | 1.347   |
| Left colon                        | 49             | 38                                | 11          |         |
| Right colon                       | 78             | 32                                | 46          |         |
| Rectum                            | 101            | 57                                | 44          |         |
| Tumor size (cm)                   |                |                                   |             | 0.034   |
| ≥ 5                               | 142            | 74                                | 68          |         |
| < 5                               | 86             | 52                                | 34          |         |
| Staging                           |                |                                   |             | <0.001  |
| I + II                            | 76             | 42                                | 34          |         |
| III                               | 132            | 75                                | 57          |         |
| IV                                | 20             | 11                                | 9           |         |
| Tumor stage                       |                |                                   |             | 0.003   |
| T1 + T2                           | 9              | 6                                 | 3           |         |
| T3                                | 179            | 108                               | 71          |         |
| T4                                | 40             | 29                                | 16          |         |
| Differentiation                   |                |                                   |             | 0.001   |
| CRC, WD                           | 9              | 6                                 | 3           |         |
| CRC, MD                           | 96             | 53                                | 43          |         |
| CRC, PD                           | 123            | 81                                | 42          |         |
| Lymph node metastasis             |                |                                   |             | 0.007   |
| N0                                | 81             | 43                                | 38          |         |
| N1                                | 58             | 45                                | 13          |         |
| N2                                | 89             | 70                                | 19          |         |
| Distant metastasis                |                |                                   |             | 1.218   |
| M0                                | 199            | 110                               | 89          |         |
| M1                                | 29             | 18                                | 11          |         |

CXCL3: CXC-chemokine ligand 3; TNM, tumor (T), nodes (N), and metastases (M). CRC: colorectal cancer; CRC WD: well-differentiated cancer; CRC MD: moderately differentiated cancer; CRC PD: poorly-differentiated cancer.

(mean log2 (CC/AT) = 3.96) (Figure 1(a)). The mRNA levels of CXCL3 in 59 pairs of CRC and adjacent tissues (AT) were examined by qRT-PCR. mRNA levels of CXCL3 were greater in CRC tissues compared to matched adjoining tissue (P < 0.001) Figure 1(b).
4. Discussion

CRC is the third most malignant tumor worldwide. Diagnosis is typically at a middle or advanced stage, which is challenging to treat, resulting in a high degree of mortality. Studies have shown the incidence of CRC to be closely related to the composition of the diet. Unhealthy high fat or high carbohydrate western diets significantly increase the incidence of CRC [18]. Despite the wide range of screening programs available for clinical use, poor early detection of CRC remains a global

Figure 4: Associations among preoperative plasma CXCL3 levels and clinical parameters of CXC patients. (a) tumor size, (b) staging, (c) tumor stage, (d) differentiation, (e) lymph node metastasis, (f) sex, (g) age, (h) tumor site, (i) distant metastasis, and (j) Kaplan–Meier estimated disease-free survival in patients with high or low CXCL3 expression. Higher CXCL3 expression was associated with poorer disease-free survival ($P < 0.01$).
Table 3: Multivariate stepwise regression analysis of plasma CXCL3 levels.

| Independent variable | β   | S.E. | P value | OR   | 95% CI          |
|----------------------|-----|------|---------|------|-----------------|
| Staging              | 0.773 | 0.347 | 0.042  | 2.163 | 1.016–4.522    |
| Tumor stage          | 1.038 | 0.441 | 0.038  | 0.306 | 0.139–0.995    |
| Differentiation      | 1.229 | 0.572 | 0.024  | 3.227 | 1.185–7.952    |

Variables of the original model included: sex, age, tumor site, tumor size, staging, tumor stage, differentiation, lymph node metastasis, and distant metastasis.

There are limitations to this study. First, results may be confounded by variations in clinical case screening and differences in treatment. Second, cases were selected from only one hospital and may not represent other local or national sites. Third, the study was cross-sectional and requires confirmation of the role of CXCL3 in the pathogenesis of CRC cell differentiation, invasion, metastasis, clinical treatment, and prognosis. Such confirmation will be the purpose of our subsequent studies.

In summary, this study analyzed the expression of CXCL3 in cancer tissue and plasma of CRC patients and found relationships among CXCL3 and patient clinic pathological characteristics. Also, a potential clinical diagnostic value for the measurement of plasma CXCL3 in CRC patients was found. The area under the curve analysis allowed for a better understanding of the CRC diagnostic value of CXCL3. It is important to note that the onset of CRC is insidious and that patients are often referred to a doctor at an advanced disease stage, limiting treatment options. Therefore, there is an urgent need for an early diagnostic marker with high sensitivity and specificity as a means by which to improve clinical diagnosis. That need will be the focus of our future work.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of these results.

Authors’ Contributions

CC and RZ contributed equally to this work. CC, RZ, GPN, and JL designed and conceived this project. RZ, YFP, and JL analyzed the data. CC, YYH, and LS revised the manuscript and provided important intellectual content. CC, FG, LS, and RZ collated the data. YFP, LS, JL, YYH, and FG performed immunohistochemistry. CC and RZ contributed equally to this work and are co-first authors for this article. GPN and JL contributed equally to this work and are corresponding authors for this manuscript.

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