Tumor Biol. (2014) 35:9683–9691
DOI 10.1007/s13277-014-2234-0

RESEARCH ARTICLE

CXCL10 mRNA expression predicts response to neoadjuvant chemoradiotherapy in rectal cancer patients

Cong Li · Zhimin Wang · Fangqi Liu · Ji Zhu · Li Yang · Guoxiang Cai · Zhen Zhang · Wei Huang · Sanjun Cai · Ye Xu

Received: 25 March 2014 / Accepted: 12 June 2014 / Published online: 27 June 2014

Abstract Chemoradiotherapy has been commonly used as neoadjuvant therapy for rectal cancer to allow for less aggressive surgical approaches and to improve quality of life. In cancer, it has been reported that CXCL10 has an anti-tumor function. However, the association between CXCL10 and chemoradiosensitivity has not been fully investigated. We performed this study to investigate the relationship between CXCL10 expression and chemoradiosensitivity in rectal cancer patients. Ninety-five patients with rectal cancer who received neoadjuvant chemoradiotherapy (NCRT) were included. Clinical parameters were compared with the outcome of NCRT and *CXCL10* messenger RNA (mRNA) expression between the pathological complete response (pCR) group and non-pathological complete response (npCR) group. *CXCL10* mRNA and protein expressions between groups were analyzed using the Student's *t* test and chi-square test. The mean mRNA level of *CXCL10* in the pCR group was significantly higher than that in the npCR group (p=0.010). In the pCR group, 73.7 % of the patients had high *CXCL10* mRNA expression, and 61.4 % of the patients in the npCR group had low *CXCL10* mRNA expression. Subjects with high *CXCL10* mRNA expression demonstrated a higher sensitivity to NCRT (p=0.011). The receiver operating characteristic curve showed that the diagnostic performance of *CXCL10* mRNA expression had an area under the curve of 0.720 (95 % confidence interval, 0.573–0.867). There were no differences between the pCR and npCR groups in CXCL10 protein expression (p>0.05). High *CXCL10* mRNA expression is associated with a better tumor response to NCRT in rectal cancer patients and may predict the outcome of NCRT in this malignancy.

Keywords *CXCL10* · Neoadjuvant chemoradiotherapy · Rectal cancer · Angiogenesis

Cong Li, Zhimin Wang and Fangqi Liu contributed equally to this work and should be regarded as joint first authors.

C. Li · F. Liu · L. Yang · G. Cai · S. Cai · Y. Xu
Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, Dong'an road, 270, Shanghai 200032, China

C. Li · F. Liu · J. Zhu · L. Yang · G. Cai · Z. Zhang · S. Cai · Y. Xu (✉)
Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China
e-mail: xuye021@163.com

Z. Wang · W. Huang (✉)
Department of Genetics, Shanghai-MOST Key Laboratory of Health and Disease Genomics, Chinese National Human Genome Center and Shanghai Academy of Science & Technology, Bibo road, 250, Shanghai 201203, China
e-mail: weihuangsh@gmail.com

J. Zhu · Z. Zhang
Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Dong'an road, 270, Shanghai 200032, China

Introduction

Colorectal cancer is the third most malignant tumor worldwide, defined by its high morbidity and mortality. Neoadjuvant chemoradiotherapy (NCRT) is a standard strategy recommended by the National Comprehensive Cancer Network guidelines for treatment of stage II/III rectal cancer. This approach has the potential to increase rates of pathologic complete response (pCR) and sphincter preservation. It has previously been shown that NCRT not only can reduce local recurrence rates but also can improve overall survival in patients with locally advanced rectal cancer. Nonetheless, only 5–40 % of the patients achieve a pCR to NCRT [1]. Therefore, it is important to understand this dichotomy in patient response to NCRT and differentiate between patients who will or will not benefit from this treatment. Recently, there have been various studies investigating markers associated with NCRT sensitivity in rectal cancer

patients. However, the markers identified have limitations in clinical application. Therefore, further identification of biomarkers predicting the response to NCRT is required to establish which patients will most likely benefit from this treatment.

Angiogenesis is an essential process in malignant transformation, as the formation of new blood vessels ensures that the growing tumor receives an adequate oxygen supply. The genes associated with angiogenic pathways have been studied in many tumor types and provide a guideline for targeted therapy. Chemokines, a family of small cytokines secreted by cells, participate in pleiotropic functions including angiogenesis. In particular, the chemokine CXCL10 has been reported to play a role in angiostasis and have anti-tumor effects. In addition, CXCL10, as an anti-angiogenesis factor, has been studied in numerous diseases including colorectal cancer [2, 3]. In our previous study, we also demonstrated that high expression of *CXCL10* was related to improved survival in colorectal cancer patients [4].

It has recently been shown that radiation has a considerable effect on chemokine expression, although changes in chemokine expression before radiation have not been well investigated. It seems probable that *CXCL10* expression affects the chemoradiosensitivity of rectal cancer to NCRT. In this study, we measured the messenger RNA (mRNA) levels of *CXCL10* in order to investigate the association between its expression and tumor response to NCRT. We hypothesized that CXCL10 would be a predictive marker for patient outcome after NCRT.

Materials and methods

Patients

This study included 95 patients, selected between 2007 and 2013 in Fudan University Shanghai Cancer Center. *CXCL10* mRNA level analysis was performed in 63 patients; meanwhile, CXCL10 protein expression was analyzed in 53 patients. Among these, 21 patients underwent the two analyses. All patients had histologically confirmed rectal adenocarcinoma within 15 cm from the anal verge. The clinical T and N stages were identified by magnetic resonance imaging. Measurements of carcinoembryonic antigen (CEA) level, complete blood count, serum chemistry tests, colonoscopy, and abdominal and chest computerized tomography were performed before treatment. This study was approved by the institutional ethical committee, and all patients gave written informed consent allowing their tissues to be used in this study.

Treatment

All patients received NCRT. Radiation was given with a total dose of 44, 50, or 55 Gy in 25 fractions. During radiotherapy, patients also received a chemotherapy regimen consisting of oxaliplatin (50 mg/m^2, qw) and capecitabine (625 mg/m^2, bid). Surgery was performed 6–8 weeks following completion of NCRT.

Biopsies and pathological assessment

Pretreatment biopsies of rectal carcinoma were stored in RNAlater (Ambion/Applied Biosystems, Oslo, Norway). Pathological characteristics of post-treatment tumor include T and N stages, vascular and lymphatic invasion, and perineural invasion. Pathologic complete response was defined as the absence of viable tumor cells in the rectal wall and in any of the resected lymph nodes. Tumor downstaging was determined by comparing pretreatment T and N stages with the pathological stage of the surgical specimen [5]. To compare *CXCL10* mRNA expression and protein expression with patient outcome to NCRT, patients were divided into two groups, pCR group and non-pCR (npCR) group, according to tumor response.

RNA extraction and real-time quantitative PCR

Total RNA was extracted from the pretreatment biopsies using an All-Prep RNA/DNA/Protein Mini Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription was performed using a PrimeScript RT reagent kit (TaKaRa, Otsu, Shiga, Japan) with 15-μl volume in each reaction, including 300 ng total RNA. Then, a 1-μl cDNA sample was used to perform a real-time quantitative polymerase chain reaction (RT-qPCR). To determine the amount of RNA in each sample, a standard curve was constructed. Quantification standards were prepared by the cloning of genes of interest. Specific primers and probes for *CXCL10* and an internal control (*ß-actin* [*ACTB*]) were designed according to GenBank sequences (NM_001565.3 and NM_001101.3, respectively) using the Universal Probe Library Assay Design Centre via ProbeFinder software (Roche Diagnostics, Indianapolis, IN, USA). The forward (F) and reverse (R) primers were as follows: *CXCL10*: F, 5′-CAAATCTGCTTTTTAAAGAATG CTC-3′; R, 5′-AAGAATTTGGGCCCCTTG-3′; *ACTB*: F, 5′-ATTGGCAATGAGCGGTTC-3′; R, 5′-TGAAGGTAGTTT CGTGGATGC-3′. Raw expression data for *CXCL10* mRNA levels were adjusted based on *ACTB* levels. RT-qPCR assays were carried out on the sequence detection system (ABI Prism 7900 HT; Applied Biosystems, Foster City, CA, USA) and conducted in triplicate for each sample to ensure experimental accuracy. The mean value was used for calculation. The cycling conditions were as follows: 10 min at 95 °C for 1 cycle; and 15 s at 95 °C, 30 s at 57 °C, and 30 s at 72 °C for 40 cycles.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue sections from preoperative biopsies were deparaffinized in xylene (10 min for

three times) and rehydrated in ethanol series (75, 95, and 100 %, each for 5 min). Slides were treated with 3 % methanol-peroxide for 10 min to block endogenous proxidases and then incubated in a boiling 10-mM sodium citrate buffer for 10 min to retrieve antigen. Subsequently, we applied polyclonal rabbit anti-human CXCL10 antibody (1:300, Abcam, Cambridge, MA, USA) as a primary antibody overnight at 4 °C. Detection was done with a kit (GeneTech, Shanghai, China) for 30 min at room temperature. Finally, antibody binding was visualized with DAB and counter-stained with hematoxylin.

Scoring of slides

The individual tissue cores for each slide (×200 magnification) were viewed using Aperio ImageScope (Aperio Technologies, CA, USA, version 11.2.0.780) and scored by applying the Positive Pixel Count Algorithm (version 9.1). The CXCL10 staining of the tumor tissue was marked with a pen tool for our later analysis. Data was expressed as positivity (number of positive pixel count/total number of positive+ negative pixel count).

Table 1 Clinical characteristics compared with tumor response

	Tumor response		*p*
	pCR (*n*=27)	npCR (*n*=68)	
Age (years)			0.897
Mean	52.74	54.03	
<65	24	58	
≥65	3	10	
Gender			0.340
Male	22	49	
Female	5	19	
Tumor distance from anal verge			0.626
<5 cm	13	29	
≥5 cm	14	39	
Pre-T stage			0.261
T2–3	20	56	
T4	6	7	
Missing	1	5	
Pre-N stage			0.164
N0	10	14	
N1–2	16	45	
Missing	1	9	
Vascular invasion			0.157
Present	0	10	
Absent	17	49	
Missing	10	9	
Perineural invasion			0.051
Present	0	15	
Absent	17	45	
Missing	10	8	

pCR pathological complete response, *npCR* non-pathological complete response, *pre* preoperative, *post* postoperative, *T* tumor, *N* node

Statistics

CXCL10 mRNA expression and protein expression were respectively compared between the pCR and npCR groups using the Student's *t* test. Then, the data were ranked based on percentile groups: low gene expression or low positivity for cases below the 50th percentile and high gene expression or high positivity for cases above the 50th percentile. Next, we used the chi-square test to compare differences between the pCR and npCR groups. The diagnostic performance of *CXCL10* mRNA expression was assessed by means of receiver operating characteristic (ROC) curve analysis. All tests were two-tailed, and the significance level was set to 0.05. The analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics compared with tumor response

Clinical parameters, including age, gender, tumor distance from anal verge, pretreatment T and N stages, vascular invasion, and perineural invasion, were compared between tumor response groups. No significant differences were found between the pCR and npCR groups (Table 1).

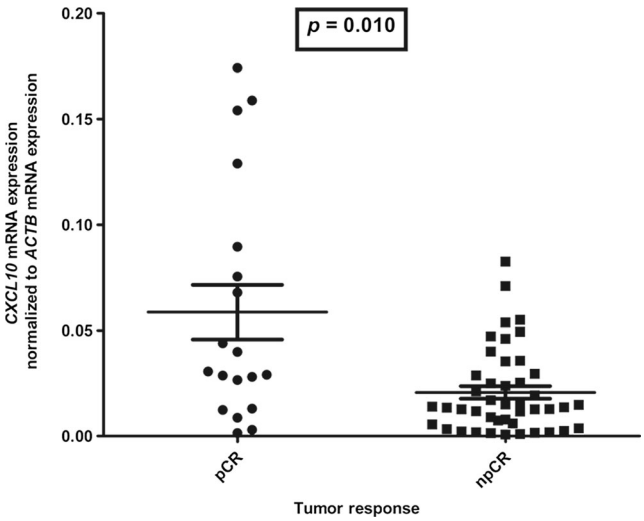

Fig. 1 *CXCL10* mRNA expression in the pCR and npCR groups. The comparison was conducted using the Student's *t* test (p=0.010). *pCR* pathological complete response, *npCR* non-pathological complete response, *ACTB ß-actin* (internal control)

Table 2 Chi-square test for tumor response and *CXCL10* mRNA expression

	CXCL10 mRNA expression, *n* (%)		x^2	*p*	OR	95 % CI
	High	Low				
pCR	14 (73.7 %)	5 (26.3 %)	6.522	0.011*	4.447	1.356–14.586
npCR	17 (38.6 %)	27 (61.4 %)				

pCR pathological complete response, *npCR* non-pathological complete response, *OR* odds ratio, *CI* confidence interval

*$p<0.05$

Tumor response and *CXCL10* mRNA expression

First, we used the normalized data as previously described to directly analyze the difference in *CXCL10* mRNA expression between the pCR (19 patients) and npCR (44 patients) groups using the Student's *t* test (Fig. 1). The mean mRNA expression level of *CXCL10* in the pCR group was significantly higher than that in the npCR group ($p=0.010$). We then performed two-category analysis to compare the distribution of high and low *CXCL10* mRNA expression in the tumor response groups using the chi-square test (Table 2). The results showed that 73.7 % of the patients in the pCR group had high *CXCL10* mRNA expression, and 61.4 % of the patients in the npCR group had low *CXCL10* mRNA expression, and these differences were statistically significant ($p=0.011$). This result indicates that patients with high *CXCL10* mRNA expression are more likely to respond to NCRT (odds ratio 4.447; 95 % confidence interval [CI], 1.356–14.586). The diagnostic performance of *CXCL10* mRNA expression, as assessed by the ROC curve (Fig. 2), showed an area under the curve of 0.720 (95 % CI, 0.573–0.867).

Clinical characteristics and *CXCL10* mRNA expression

The *t* test used to analyze clinical parameters and *CXCL10* mRNA expression (Table 3) showed an association between tumor distance from the anal verge and *CXCL10* expression level ($p=0.028$). In addition, there was a statistically significant association between post-operative T stage and *CXCL10* mRNA expression ($p=0.024$).We then analyzed the difference in clinical parameters between the two *CXCL10* mRNA expression groups using the chi-square test. As shown in Table 4, the *CXCL10* mRNA expression level was not statistically related to any of the clinical parameters ($p>0.05$), with the exception of post-operative T stage ($p=0.016$).

Tumor response and CXCL10 protein expression

Immunohistochemistry results of 53 pretreatment biopsies of rectal cancer patients, with 13 in the pCR group and 40 in the npCR group, were chosen to be analyzed. CXCL10 protein expression was mostly detected in the cytoplasm of rectal cancer cells, and partly in the tumor stroma (Fig. 3). Positivity of CXCL10 protein expression ranged from 0.01 to 62.88 %.

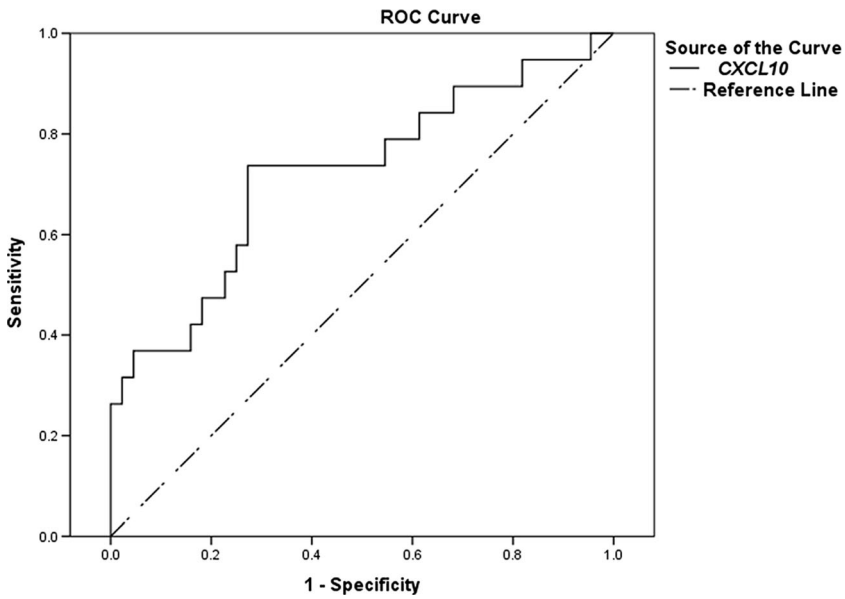

Fig. 2 Diagnostic performance of *CXCL10* mRNA expression. The receiver operating characteristic (ROC) curve shows the combined sensitivity and specificity across different values of the predictive index, with an area under the ROC curve (AUC) of 0.720 (95 % confidence interval, 0.573–0.867)

Table 3 *T* test for significant associations between clinical characteristics and *CXCL10* mRNA expression

	Patients (*n*)	*p*
Age (years)		0.304
Mean	52.08	
<65	57	
≥65	6	
Gender		0.433
Male	53	
Female	10	
Tumor distance from anal verge		0.028*
<5 cm	27	
≥5 cm	36	
Pre-T stage		0.223
T2–3	50	
T4	9	
Missing	4	
Pre-N stage		0.768
N0	23	
N1–2	35	
Missing	5	
Post-T stage		0.024*
T0–1	25	
T2–4	38	
Post-N stage		0.150
N0	40	
N1–2	23	
T downstage		0.066
No downstage	25	
Downstage	34	
Missing	4	
N downstage		0.944
No downstage	32	
Downstage	26	
Missing	5	
Vascular invasion		0.303
Present	5	
Absent	41	
Missing	17	
Perineural invasion		0.699
Present	9	
Absent	37	
Missing	17	

pre preoperative, *post* postoperative, *T* tumor, *N* node

*$p<0.05$

Table 4 Chi-square test for significant associations between clinical characteristics and *CXCL10* mRNA expression

	CXCL10 mRNA expression		*p*
	High	Low	
Age (years)			1.000
Mean	53.16	51.56	
<65	28	29	
≥65	3	3	
Gender, *n*			1.000
Male	26	27	
Female	5	5	
Tumor distance from anal verge			0.181
<5 cm	16	11	
≥5 cm	15	21	
Pre-T stage, *n*			0.164
T2–3	27	23	
T4	2	7	
Missing	2	2	
Pre-N stage, *n*			0.788
N0	11	12	
N1–2	18	17	
Missing	2	3	
Post-T stage, *n*			0.016*
T0–1	17	8	
T2–4	14	24	
Post-N stage, *n*			0.490
N0	21	19	
N1–2	10	13	
T downstage, *n*			0.497
No downstage	11	14	
Downstage	18	16	
Missing	2	2	
N downstage, *n*			1.000
No downstage	16	16	
Downstage	13	13	
Missing	2	3	
Vascular invasion, *n*			0.918
Present	3	2	
Absent	19	22	
Missing	9	8	
Perineural invasion, *n*			1.000
Present	4	5	
Absent	18	19	
Missing	9	8	

pre preoperative, *post* postoperative, *T* tumor, *N* node

*$p<0.05$

Student's *t* test showed no significant difference between the pCR and npCR groups in CXCL10 protein expression (Fig. 4). Still, we performed two-category analysis to compare the distribution of high and low positivity of CXCL10 protein expression in the tumor response groups using the chi-square test. As shown in Table 5, 53.8 % of the patients had high positivity of CXCL10 protein expression in the pCR group,

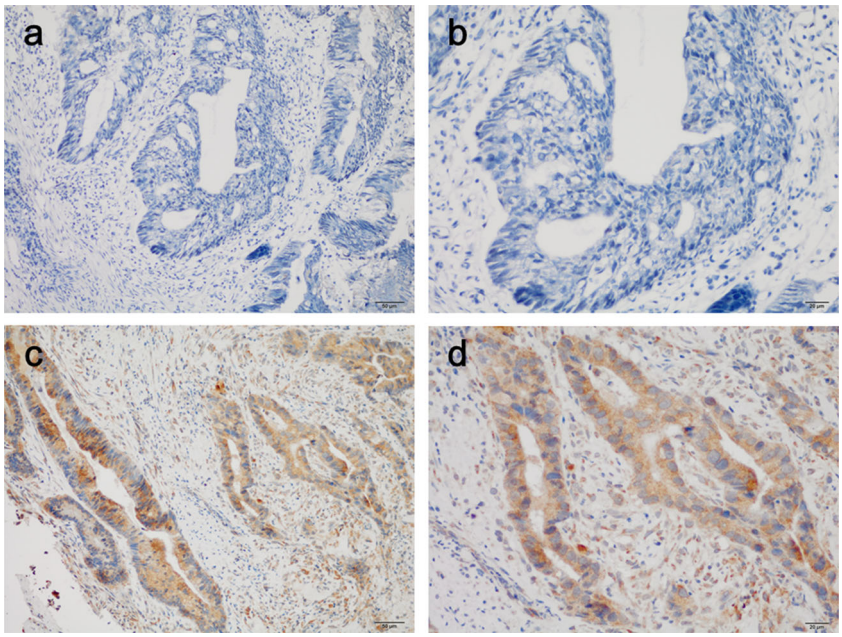

Fig. 3 Immunohistochemistry analysis of CXCL10. **a** (×200 magnification) and **b** (×400 magnification): low positivity of CXCL10 protein expression in pretreatment biopsies. **c** (×200 magnification) and **d** (×400 magnification): high positivity of CXCL10 protein expression in pretreatment biopsies. CXCL10 expression was seen both in the cytoplasm of tumor cells and tumor stroma

and 50.0 % had low positivity in the npCR group, although there were no statistically significant differences ($p>0.05$) (Table 5).

Correlation between CXCL10 mRNA levels and protein expression

The CXCL10 mRNA levels and protein expression of 21 patients were examined in our research. There was no significantly statistical correlation found between the mRNA levels and protein expression ($r=-0.070$, $p>0.05$) (Fig. 5).

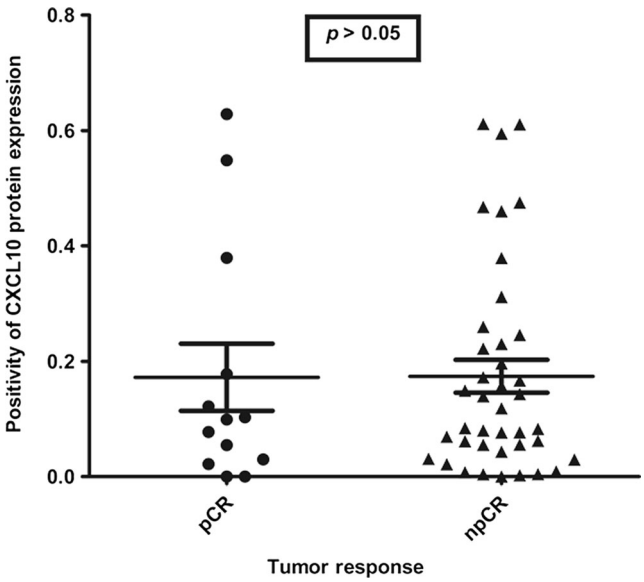

Fig. 4 Positivity of CXCL10 protein expression in the pCR and npCR groups. The comparison was conducted using the Student's *t* test ($p>0.05$). *pCR* pathological complete response, *npCR* non-pathological complete response

Discussion

In recent years, NCRT has improved the treatment outcomes for rectal cancer patients. However, some patients still fail to respond to this therapy. Numerous studies have been carried out to discover the association between tumor response to NCRT and pretreatment clinical characteristics, such as age, gender, CEA level, tumor position, tumor differentiation, and clinical staging. However, few factors have been verified as significant predictors. Likewise, in this study, no clinical parameters were found to be significantly different between the pCR and npCR groups. Therefore, it is important to identify molecular biomarkers, rather than disease characteristics, that may predict treatment response. Several genes have been proposed as predictive biomarkers associated with chemoradiosensitivity in rectal cancer, including *EGFR*,

Table 5 Chi-square test for tumor response and positivity of CXCL10 protein expression

	Positivity of CXCL10 protein expression, *n* (%)		x^2	*p*
	High	Low		
pCR	7 (53.8 %)	6 (46.2 %)	0.058	0.810
npCR	20 (50.0 %)	20 (50.0 %)		

pCR pathological complete response, *npCR* non-pathological complete response

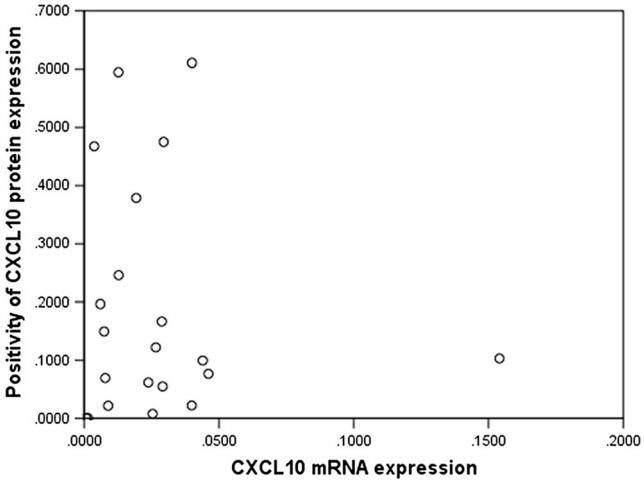

Fig. 5 Correlation between CXCL10 mRNA levels and protein expression. Pearson correlation value ($r=-0.07$) was calculated when performing the correlation analysis ($p>0.05$)

CD133, and thymidylate synthase, in addition to circulating free DNA and RNA [6–10]. A number of microarray studies have also identified genes with a predictive value [11–13]. However, most of these results remain controversial.

To our knowledge, the mRNA expression of *CXCL10* has never been reported as a predictive biomarker for tumor response to NCRT in rectal cancer patients. As an angiostatic chemokine, CXCL10 plays a key role in the course of tumor growth, especially in the process of new vessel formation. Yates-Binder et al. demonstrated that a CXCL10-derived peptide can inhibit vascular endothelial growth factor (VEGF)-induced endothelial motility and tube formation. They also showed that this peptide could both prevent vessel formation and induce involution of newly formed vessels [14]. Further, Sato et al. demonstrated that CXCL10 levels showed a significant inverse correlation with VEGF levels in uterine cervical cancers. They concluded that CXCL10 might act through suppression of angiogenesis associated with VEGF [15]. The angiostatic effects of CXCL10 may be mediated by the p38 mitogen-activated protein kinase signaling pathway [16]. In the present study, we measured the mRNA levels of *CXCL10* in rectal cancer patients by RT-qPCR and found high *CXCL10* expression in patients who achieved pCR. A number of studies have reported that treatment with anti-angiogenic agents may increase the benefit of chemotherapy and radiotherapy [17, 18]. We hypothesized that high expression of *CXCL10* in rectal cancer patients before treatment was indicative of angiostasis. Therefore, fewer abnormal vessels would form in the developing tumor, resulting in reduced vessel density. Similarly, a study by Yang et al. concluded that CXCL10 overexpression could lead to fewer abnormal vessels and reduce tumor growth in an ovarian cancer xenograft model [19]. One explanation for this result is that reduced vessel formation may lead to decreased oxygen perfusion among the tumor cells, resulting in hypoxia and consequently radiation resistance. However, it is also believed that the amount of newly formed vessels in the tumor stroma does not necessarily lead to increased blood flow [20]. Furthermore, not only the tumor cells but also endothelial cells require oxygen. The newly formed vessels are inefficient and may compromise oxygen perfusion and consumption [21]. According to this hypothesis, normal vessels will reorganize to increase the oxygen perfusion of tumor cells and decrease the oxygen supply to the endothelial cells of inefficient vessels. Under such circumstances, more oxygenic tumor cells will be sensitive to the radiation and killed. However, this proposed mechanism needs to be confirmed by additional studies.

There may also be other mechanisms that underlie CXCL10-mediated chemoradiosensitivity. One laboratory in China elucidated the molecular mechanism behind the antitumor activity of CXCL10 using HeLa cells. They demonstrated that CXCL10 upregulation after irradiation may prolong the G1 phase and delay the S phase of the cell cycle, as evidenced by upregulated p27 protein and downregulated cyclin E. Therefore, more cells stayed in the G1 phase, which resulted in higher sensitivity to radiation treatment [22].

Recently, Rentoftet al. has found that high *CXCL10* mRNA expression was associated with a poor response to radiotherapy in patients with squamous cell carcinoma of the tongue. Besides, patients with low *CXCL10* mRNA expression had a better survival [23]. Their conclusions are opposite to ours, which means that the effect of CXCL10 to radiation may be various in different cancers.

Through the comparison of clinical parameters and *CXCL10* expression, we found that there was a significant difference between post-operative T stage and *CXCL10* expression level. In the high *CXCL10* expression group, there were more patients with a low post-operative T stage. This finding is similar to the previous result demonstrating an association between *CXCL10* and tumor response. In addition, we found an association between low-sited rectal cancer and higher *CXCL10* expression, although the basis for this relationship is currently unclear.

It has long been a question of what the correlation between mRNA and protein expression is. In the present study, we have not found any correlation between CXCL10 mRNA and protein expressions. Likewise, some other researchers have discovered a lack of mRNA–protein correlation when comparing the results of RT-PCR and immunohistochemistry [24, 25]. Different regulation mechanisms may play a role in influencing mRNA–protein correlation, such as synthesis and degradation rates, which will affect the amount of the two molecules differently. Specifically, transcription, mRNA degradation, post-transcription, translation, and protein degradation may all contribute to the variation in mRNA and protein concentrations [26]. These possible mechanisms in

the regulation process of mRNA and protein expression inspired us to have a further investigation in the future.

There were certain limitations to our study, such as the small sample size. Therefore, additional studies with larger sample sizes and more paired samples may be necessary in order to further validate *CXCL10* as a predictive biomarker. Moreover, because it has not been clarified through which mechanism CXCL10 may increase the sensitivity to NCRT in rectal cancer patients, further studies are necessary to further elucidate this association.

In conclusion, our results indicated that *CXCL10* mRNA expression, rather than CXCL10 protein expression, has the potential to be a biomarker for predicting chemoradiosensitivity in rectal cancer patients who receive NCRT. We found that high expression of *CXCL10* before treatment was associated with sensitivity to NCRT. However, further studies investigating the mechanism linking chemoradiosensitivity with CXCL10 are warranted.

Acknowledgments We thank all the people who have so willingly participated in this study. This work was partially supported by the Chinese National Key Program (2012CB722308), National Natural Science Foundation of China (81272307, 30900814), Shanghai Science and Technology Commission of Shanghai Municipality (10DJ1400500, 10DJ1400502), and Pudong New Area Science and Technology Development Fund (Pkj2013-z02).

Conflicts of interest None.

Authors' contributions The work presented here was collaboratively performed by all authors. YX, SJC, and WH defined the research theme. CL and ZMW co-designed the methods and experiments. JZ, LY, FQL, GXC, and ZZ collected samples and helped document clinical parameters of patients. JZ and ZZ provided knowledge on radiology. CL performed the laboratory experiments, analyzed the data, interpreted the results, and wrote the paper. YX and ZMW discussed the analyses, interpretation, and presentation. All authors read and approved the final manuscript.

References

1. Dedemadi G, Wexner SD. Complete response after neoadjuvant therapy in rectal cancer: to operate or not to operate? Dig Dis. 2012;30 Suppl 2:109–17.
2. Toiyama Y, Fujikawa H, Kawamura M, Matsushita K, Saigusa S, Tanaka K, et al. Evaluation of CXCL10 as a novel serum marker for predicting liver metastasis and prognosis in colorectal cancer. Int J Oncol. 2012;40:560–6.
3. Zipin-Roitman A, Meshel T, Sagi-Assif O, Shalmon B, Avivi C, Pfeffer RM, et al. CXCL10 promotes invasion-related properties in human colorectal carcinoma cells. Cancer Res. 2007;67:3396–405.
4. Jiang Z, Xu Y, Cai S. CXCL10 expression and prognostic significance in stage II and III colorectal cancer. Mol Biol Rep. 2010;37:3029–36.
5. Janjan NA, Khoo VS, Abbruzzese J, Pazdur R, Dubrow R, Cleary KR, et al. Tumor downstaging and sphincter preservation with preoperative chemoradiation in locally advanced rectal cancer: the M. D. Anderson Cancer Center experience. Int J Radiat Oncol Biol Phys. 1999;44:1027–38.
6. Agostini M, Pucciarelli S, Enzo MV, Del Bianco P, Briarava M, Bedin C, et al. Circulating cell-free DNA: a promising marker of pathologic tumor response in rectal cancer patients receiving preoperative chemoradiotherapy. Ann Surg Oncol. 2011;18:2461–8.
7. Jao SW, Chen SF, Lin YS, Chang YC, Lee TY, Wu CC, et al. Cytoplasmic CD133 expression is a reliable prognostic indicator of tumor regression after neoadjuvant concurrent chemoradiotherapy in patients with rectal cancer. Ann Surg Oncol. 2012;19:3432–40.
8. Pucciarelli S, Rampazzo E, Briarava M, Maretto I, Agostini M, Digito M, et al. Telomere-specific reverse transcriptase (hTERT) and cell-free RNA in plasma as predictors of pathologic tumor response in rectal cancer patients receiving neoadjuvantchemoradiotherapy. Ann Surg Oncol. 2012;19:3089–96.
9. Stoehlmacher J, Goekkurt E, Mogck U, Aust DE, Kramer M, Baretton GB, et al. Thymidylate synthase genotypes and tumour regression in stage II/III rectal cancer patients after neoadjuvant fluorouracil-based chemoradiation. Cancer Lett. 2008;272:221–5.
10. Kim J, Kim J, Li S, Yoon W, Song K, Kim K, et al. Epidermal growth factor receptor as a predictor of tumor downstaging in locally advanced rectal cancer patients treated with preoperative chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2006;66:195–200.
11. Nishioka M, Shimada M, Kurita N, Iwata T, Morimoto S, Yoshikawa K, et al. Gene expression profile can predict pathological response to preoperative chemoradiotherapy in rectal cancer. Cancer Genomics Proteomics. 2011;8:87–92.
12. Rimkus C, Friederichs J, Boulesteix AL, Theisen J, Mages J, Becker K, et al. Microarray-based prediction of tumor response to neoadjuvantradiochemotherapy of patients with locally advanced rectal cancer. Clin Gastroenterol Hepatol. 2008;6:53–61.
13. Casado E, Moreno Garcia V, Javier Sanchez J, Blanco M, Maurel J, Feliu J, et al. A combined strategy of SAGE and quantitative PCR provides a 13-Gene signature that predicts preoperative chemoradiotherapy response and outcome in rectal cancer. Clin Cancer Res. 2011;17:4145–54.
14. Yates-Binder CC, Rodgers M, Jaynes J, Wells A, Bodnar RJ, Turner T. An IP-10 (CXCL10)-derived peptide inhibits angiogenesis. PLoS One. 2012;7:e40812.
15. Sato E, Fujimoto J, Toyoki H, Sakaguchi H, Alam SM, Jahan I, et al. Expression of IP-10 related to angiogenesis in uterine cervical cancers. Br J Cancer. 2007;96:1735–9.
16. Petrai I, Rombouts K, Lasagni L, Annunziato F, Cosmi L, Romanelli RG, et al. Activation of p38MAPK mediates the angiostatic effect of the chemokine receptor CXCR3-B. Int J Biochem Cell Biol. 2008;40:1764–74.
17. Lee CG, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, et al. Anti-vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res. 2000;60:5565–70.
18. Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res. 2004;64:3731–6.
19. Yang F, Gou M, Deng H, Yi T, Zhong Q, Wei Y, et al. Efficient inhibition of ovarian cancer by recombinant CXC chemokine ligand 10 delivered by novel biodegradable cationic heparin-polyethyleneiminenanogels. Oncol Rep. 2012;28:668–76.
20. Koukourakis MI. Tumour angiogenesis and response to radiotherapy. Anticancer Res. 2001;21:4285–300.
21. Wachsberger P, Burd R, Dicker AP. Tumor response to ionizing radiation combined with antiangiogenesis or vascular targeting agents: exploring mechanisms of interaction. Clin Cancer Res. 2003;9:1957–71.

22. Yang LL, Wang BQ, Chen LL, Luo HQ, Wu JB. CXCL10 enhances radiotherapy effects in HeLa cells through cell cycle redistribution. Oncol Lett. 2012;3:383–6.
23. Rentoft M, Coates PJ, Loljung L, Wilms T, Laurell G, Nylander K. Expression of CXCL10 is associated with response to radiotherapy and overall survival in squamous cell carcinoma of the tongue. Tumour Biol. 2014;35:4191–8.
24. Stark AM, Pfannenschmidt S, Tscheslog H, Maass N, Rosel F, Mehdorn HM, et al. Reduced mRNA and protein expression of BCL-2 versus decreased mRNA and increased protein expression of BAX in breast cancer brain metastases: a real-time PCR and immunohistochemical evaluation. Neurol Res. 2006;28:787–93.
25. Chong YM, Williams SL, Elkak A, Sharma AK, Mokbel K. Insulin-like growth factor 1 (IGF-1) and its receptor mRNA levels in breast cancer and adjacent non-neoplastic tissue. Anticancer Res. 2006;26:167–73.
26. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet. 2012;13:227–32.