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ORIGINAL RESEARCH

Tumor Response to Irinotecan is Associated with IL-10 Expression Level in Metastatic Colorectal Cancer-Results from mCRC Biomarker Study

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Purpose: Metastatic colorectal cancer (mCRC) is a leading cause of cancer-related death. Resistance to chemotherapy is the main reason for the failure of the treatment of mCRC. IL-10 has been reported to decrease after surgery and increase after mCRC reoccurrence. The role of IL-10 in chemotherapy drug resistance of mCRC is not well elucidated.

Patients and Methods: The retrospective study recruited 264 mCRC patients between January 2012 and December 2016 (NCT03532711). All the enrolled patients received an oxaliplatin-containing or irinotecan-containing regimen. The expression level of IL-10 in 232 patients' plasma and 68 patients' tumor tissue was examined. The relationships between IL-10 and clinicopathological characteristics were analyzed. Kaplan–Meier method and Cox regression were used to evaluate the prognostic impact of IL-10.

Results: The median concentration of IL-10 was 7.60 pg/mL before treatment and 11.08 pg/mL after treatment, which suggested that IL-10 level was significantly increased by treatment with a chemotherapeutic regimen ($p = 0.000$). By utilizing univariate and multivariate Cox proportional hazard analyses, we found that low IL-10 level in plasma was significantly associated with improved overall survival (OS) of mCRC patients treated with irinotecan-containing regimen-with optimal cutoff value of 5.525pg/mL, respectively ($p = 0.002$). In addition, the low IL-10 expression level in tumor tissue was significantly associated with the improved OS for the irinotecan-containing regimen ($p = 0.023$).

Conclusion: Our study demonstrated that IL-10 could act as a prognostic biomarker for mCRC patients undergoing irinotecan-containing chemotherapy.

Keywords: IL-10, metastatic colorectal cancer, chemotherapy, biomarker

Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide. In China, due to the low early diagnosis rate, most CRC patients were diagnosed at advanced stages.[1,2] Despite recent progress in chemotherapeutic approaches, including targeted agents, the prognosis for metastatic CRC is still very poor. Therefore, strategies for improving patients' efficacy with metastatic colorectal cancer (mCRC) are urgently required for diagnosis and treatment in Chinese patients. Currently, chemotherapy remains the standard treatment for first- and second-line management of mCRC. Both oxaliplatin-based and irinotecan-based chemotherapy regimens are two active choices, and either can be alternated in sequential therapy. However, chemoresistance development presents a significant challenge in the treatment and management of mCRC.[3]

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Previously, we conducted a clinical trial named mCRC biomarker study (NCT03532711) to explore reliable biomarkers predicting chemotherapy's drug efficacy for mCRC. By analyzing routine blood counts of mCRC patients, we demonstrated that mean platelet volume (MPV) and its related factor platelet-to-lymphocyte ratio (PLR) may act as a prognostic biomarker for mCRC.[4] Herein, we aimed to identify other potential biomarkers from plasma or tumor tissue.

The association of chronic inflammation with CRC has been fully elucidated. Several inflammatory cytokines promote the development of cancer-associated with chronic inflammation.[5,6] Among them, IL-10 was identified as a key promoter of carcinogenesis. However, the relationship between IL-10 and mCRC chemotherapy drug efficacy has not been reported. In the present study, we aimed to explore the effect of IL-10 expression level on chemotherapy drug effect in mCRC patients.

Patients and Methods

Study Description

A retrospective observational clinical study named mCRC biomarker study was conducted as described before (NCT03532711).[4] Two hundred and sixty-four mCRC patients with histopathologically confirmed mCRC who had at least one measurable lesion were enrolled in this study. According to the investigators' suggestion, the chemotherapy regimens such as FOLFIRI/XELOX/FOLFOX were chosen as first-line or second-line chemotherapy regimen. The irinotecan-containing regimen consisted of the FOLFIRI regimen (infusion and bolus 5-FU with irinotecan), and the oxaliplatin-containing regimen consisted of XELOX/FOLFOX (infusion and bolus 5-FU or oral capecitabine with oxaliplatin). An objective response rate (ORR) to chemotherapy was the primary study endpoint. The overall survival (OS) and progression-free survival (PFS) of patients were the secondary study endpoint. This study was approved by the Ethical Committees of Fudan University Shanghai Cancer Center (Ethics Number: 1203108-10). The consent was obtained from the study participants prior to study commencement. This study was conducted in accordance with the Declaration of Helsinki.

Plasma IL-10 Determination

The concentration of IL-10 in plasma level was determined in the patients on the day before the first cycle of chemotherapy, the day before the second cycle of chemotherapy, and the day defined as progression disease evaluated by radiologic examination. Plasma levels of IL-10 were established in the sera of all the participants by ELISA, using the Quantikine kit (R&D Systems, UK). The tests were performed following the manufacturer's instructions. Optimal density was measured at 450 nm using a microplate reader (bioMerieux Reader 250). The experiment was triplicated for each patient.

Immunohistochemistry Analysis

Immunohistochemical (IHC) staining was performed as described below. The tumor specimens were fixed with 10% paraformaldehyde and embedded in paraffin. The sections were incubated with IL-10 primary antibody (1:100, Abcam, USA), followed by the appropriate secondary antibody, and visualized with diaminobenzidine (DAB). Finally, the slides were counterstained with hematoxylin. For the evaluation of the IHC results, we defined low expression (immunohistochemistry score 0 or 1) and high expression (immunohistochemistry score 2).

Statistical Analysis

The efficacy of treatment was evaluated according to the RECIST (response evaluation criteria in solid tumors) 1.0 criteria. Progression-free survival (PFS) was defined as the interval between initial treatment and the first documentation of disease progression or death. OS was calculated as the time from initial treatment to death. The differences between-group were assessed using the Student's *t*-test. The optimum cut-off value of IL-10 was selected by using the Receiver-operating characteristics (ROC) curve. A Kaplan-Meier survival analysis was performed a Log rank test to describe the analysis of survival curves. The Cox multivariate proportional hazards regression model was used to determine the independent risk factors that influence OS. P value <0.05 was regarded as significant. Statistical analyses were conducted using SPSS Statistics version 22.0.

Results

A total of 264 patients were enrolled in the mCRC biomarker study. Blood samples from 32 patients were not obtained; therefore, a total of 232 patients were analyzed in the current study. We measured IL-10 level in plasma samples at two-time points: before and after one treatment cycle. The median concentration of IL-10 was 7.60 pg/mL before treatment and 11.08 pg/mL after treatment, which

suggested that IL-10 level was significantly increased by treatment with a chemotherapeutic regimen (p = 0.000).

The optimal cut-off value of baseline IL-10 level was 5.525 pg/mL by using ROC curve analysis (Figure 1,). Our results showed that IL-10 with 5.525 pg/mL contributed the highest prognostic value, with a sensitivity of 0.515 and a specificity of 0.38 (AUC = 0.446, 95% CI: 0.366–0.526, p = 0.192). Therefore, patients were classified into two independent groups: patients with IL-10 ≤5.525 pg/mL (n = 111, 47.8%) and the patients with IL-10 >5.525 pg/mL (n = 121, 52.2%).

The relationship between IL-10 level and clinical characteristics was shown in Table 1. Our result showed the factors including age, gender, primary tumor sites, *RAS* status, metastases organs (liver or lung), treatment regimens (irinotecan-containing regimen or oxaliplatin-containing regimen), and drug efficacy have no difference between the two groups. The ORR (objective response

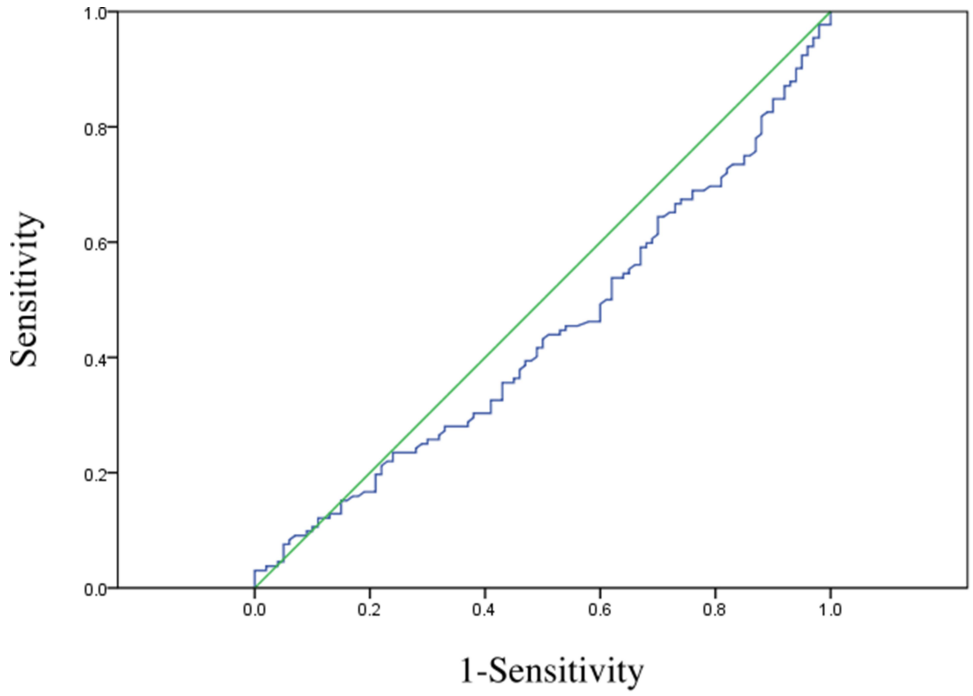

Figure 1 The ROC curve determines the optimal cut-off value of IL-10.

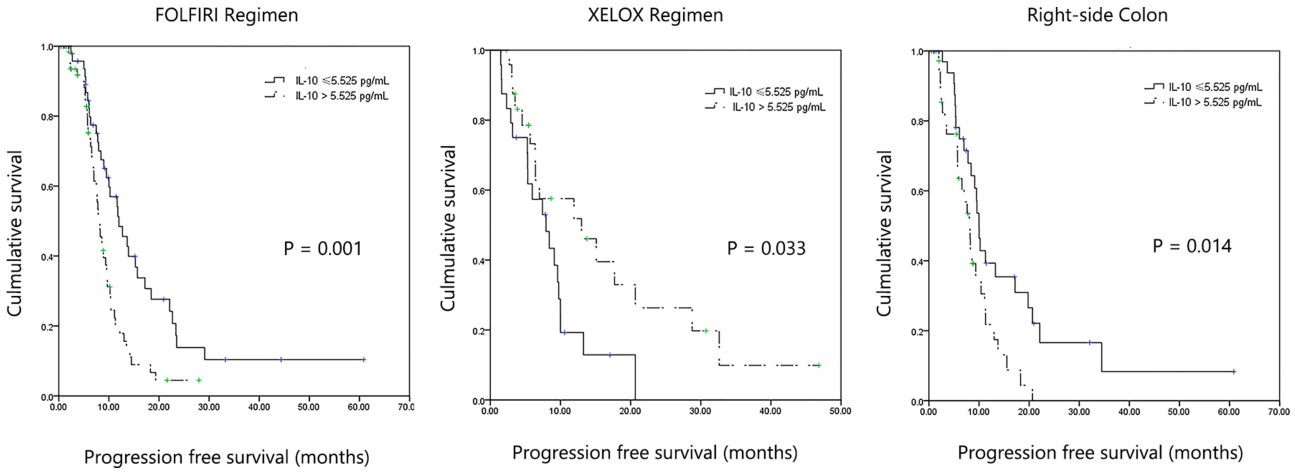

Figure 2 Influence of IL-10 levels in plasma on progression-free survival by Kaplan–Meier analyses. Log-rank analysis was used to determine the statistical significance of the Kaplan–Meier survival curve (P < 0.05).

Table 1 Baseline Characteristics of Patients with mCRC According to IL-10 Levels

Variables	Total n (%)	IL-10 ≤ 5.525 pg/mL	IL-10>5.525 pg/mL	P value
Age (years)				0.762
≤60	109	51 (46.8%)	58 (53.2%)	
>60	123	60 (48.8%)	63 (51.2%)	
Gender				0.884
Male	137	65 (47.4%)	72 (52.6%)	
Female	95	46 (48.4%)	49 (51.6%)	
Location				0.414
Colon-Left	74	31 (41.9%)	43 (58.1%)	
Colon-Right	74	36 (48.6%)	38 (51.4%)	
Rectum	84	44 (52.4%)	40 (47.6%)	
Ras status				0.703
Wild-type	9	5 (55.6%)	4 (44.4%)	
Mutant-type	28	12 (42.9%)	16 (57.1%)	
Liver metastases				0.694
No	91	45 (49.5%)	46 (50.5%)	
Yes	141	66 (46.8%)	75 (53.2%)	
Lung metastases				0.53
No	149	69 (46.3%)	80 (53.7%)	
Yes	83	42 (50.6%)	41 (49.4%)	
Regimen				0.574
XELOX	50	24 (48%)	26 (52%)	
FOLFOX	62	33 (53.2%)	29 (46.8%)	
FOLFIRI	120	54 (45%)	66 (55%)	
Regimen				0.369
Irinotecan-containing	120	54 (45%)	66 (55%)	
Oxaliplatin-containing	112	57 (50.9%)	55 (49.1%)	
Efficacy				0.622
CR+PR	72	36 (50%)	36 (50%)	
SD+PD	140	65 (46.4%)	75 (53.6%)	
NK	20			

Abbreviations: NK, not known; CR, complete response; SD, stable disease; PD, progression disease.

rate) in the two groups was 46.4% and 53.6% (p=0.622), respectively.

Next, we used Kaplan–Meier method to describe the analysis of survival curves. As shown in Table 2, for the whole population, patients with high IL-10 expression level showed no significant difference in PFS or OS compared with patients with low IL-10 expression level (PFS: 9.87± 0.497 months vs 8.27±0.601 months, p = 0.264; OS: 20.97±3.78 months vs 20.2±3.137 months, p = 0.488). The subgroup analysis showed that low IL-10 level was associated with longer PFS for patients treated with the irinotecan-containing regimen than those with high IL-10 level (12±3.75 vs 7.9±1.06 months, p = 0.001). However, for patients treated with oxaliplatin-containing regimen (XELOX), low IL-10 level was associated with shorter PFS than those with high IL-10 level (7.93±3.56 vs 13 ±9.99 months, p = 0.033). Meanwhile, for the patients with primary tumor located on the right-side colon, low IL-10 level was associated with longer PFS than those with high IL-10 level (10±0.93 vs 8.07±1.55 months, p = 0.014). Notably, no marked differences were identified in the OS between the two groups (see Figure 2).

In addition, we performed univariate and multivariate Cox proportional-hazards regression analyses in 120 patients who received an irinotecan-containing regimen. As shown in Table 3, univariate analysis showed that lower IL-10 level was significantly associated with improved PFS (p =0.002). Variables with a p value of less than 0.1 were then included in the multivariate model. As a result, the only gender was proved to be an independent factor for PFS (Table 4).

We next investigated the relationship between the IL-10 expression in tumor tissue and patient prognosis. We collected 68 tissue specimens and performed IHC analysis (Figure 3). As shown in Table 5 and Figure 4, Kaplan-Meier survival analysis showed that patients with high IL-10 expression had significantly poor OS compared with low expression (25.33±18.89 vs 18.37 ±4.66 months, p = 0.035).

Subgroup analysis revealed that patients receiving irinotecan–containing regimen had low expression level of IL-10 and was significantly associated with better OS (34.33±17.84 vs 14.1±11.43 months, p = 0.028), while no significant OS advantage was observed in patients receiving oxaliplatin-containing regimen (17.033± 2.2 vs 21.167±2.054 months, p = 0.483, see Figure 4).

Discussion

IL-10 is known as an important immunoregulatory cytokine, plays multiple biological roles. It has been well documented in inflammatory diseases due to its anti-inflammatory and immunoregulation properties.[7,8] However, the role of IL-10 in tumorigenesis remains not well elucidated. Previous studies indicated that IL-10 could promote tumorigenesis and systemic tumor immune suppression.[9,10] Conversely, IL-10 itself has been reported to have potent immune-dependent anti-tumor effects.[11,12] To the best of our knowledge, IL-10 and its impact on chemotherapy efficacy in mCRC has not been investigated yet. In the current study, we aimed to

Table 2 Association of IL-10 with the Survival Data According to the Chemotherapeutic Regimen and Primary Tumor Location

		IL-10 ≤ 5.525	IL-10>5.525	P value
PFS (mons)		9.87 (8.895–10.845) (n=111)	8.27 (7.092–9.448) (n=121)	0.264
	Regimen			
	FOLFIRI (n=120)	12 (8.25–15.75) (n=54)	7.9 (6.837–8.963) (n=66)	0.001
	FOLFOX (n=62)	9.03 (6.474–11.586) (n=33)	8.7 (4.458–12.942) (n=29)	0.998
	XELOX (n=50)	7.93 (4.371–11.489) (n=24)	13 (3.011–22.989) (n=26)	0.033
	Primary tumor location			
	Colon-Left (n=74)	9.87 (5.562–14.178) (n=31)	8.27 (7.150–9.390) (n=43)	0.94
	Colon-Right (n=74)	10 (9.074–10.926) (n=36)	8.07 (6.518–9.622) (n=38)	0.014
	Rectum (n=84)	9.03 (6.820–11.240) (n=44)	10.23 (5.050–15.405) (n=40)	0.683
OS (mons)		20.97 (19.080–22.860) (n=111)	20.2 (17.063–23.337) (n=121)	0.488
	Regimen			
	FOLFIRI (n=120)	22.37 (12.879–31.861) (n=54)	20.5 (15.420–25.580) (n=66)	0.122
	FOLFOX (n=62)	21.7 (14.974–28.426) (n=33)	21.47 (13.264–29.676) (n=29)	0.662
	XELOX (n=50)	14.63 (13.099–16.161) (n=24)	19.1 (14.697–23.503) (n=26)	0.112
	Primary tumor location			
	Colon-Left (n=74)	19.9 (15.684–24.116) (n=31)	20.2 (15.076–25.324) (n=43)	0.828
	Colon-Right (n=74)	20.67 (15.185–26.155) (n=36)	16.4 (12.589–20.211) (n=38)	0.208
	Rectum (n=84)	25.47 (17.738–33.302) (n=44)	26.93 (7.952–45.908) (n=40)	0.688

Table 3 Results of Univariate Analysis of Progression-Free Survival in Patients with mCRC Receiving FOLFIRI Regimen

Variables	Hazard Ratio	95% CI	P-value
Age (years) (≥60 versus <60)	1.256	0.79–1.996	0.335
Gender	1.567	0.993–2.475	0.054
IL-10 (≤ 5.525 versus >5.525)	2.112	1.329–3.355	0.002
Primary tumor location	1.156	0.875–1.527`	0.307
Liver metastasis	0.914	0.588–1.421	0.689
Lung metastasis	0.896	0.57–1.408	0.634
Efficacy	1.079	0.63–1.849	0.782

Table 4 Results of Multivariate Analysis of Progression-Free Survival in Patients with mCRC Receiving FOLFIRI Regimen

Variables	Hazard Ratio	95% CI	P-value
Gender	2.037	1.277–3.248	0.003
IL-10 (≤5.525 versus >5.525)	1.453	0.916–2.304	0.112

identify the role of IL-10 in predicting first-line chemotherapy drug sensitivity in mCRC patients.

Previously, a prospective clinical study was conducted to identify the promising biomarkers from chemotherapy in mCRC. The expression of IL-10 in plasma samples from 234 mCRC patients was examined. Our results showed that compared with the baseline, IL-10 was significantly increased in patient plasma after chemotherapy, both with oxaliplatin-containing and irinotecan-containing regimen. Interestingly, IL-10 was significantly decreased after surgery and increased when the tumor recurred in operable CRC.[13,14] It may be a different situation in early-stage CRC. Moreover, our results showed that the group of mCRC patients with low IL-10 level had better PFS than those with high IL-10 level ($p = 0.001$). Notably, these patients refer to patients treated with irinotecan-containing regimen but not oxaliplatin-containing regimen. More importantly, IL-10 expression in tumor tissue also demonstrated that low expression level of IL-10 was significantly associated with better OS ($p = 0.035$), again in patients treated with irinotecan-containing regimen ($p = 0.023$); however, not in the oxaliplatin-containing regimen ($p = 0.483$).

Irinotecan, a water-soluble and semisynthetic derivative of camptothecin, is one of the widely used first- and second-line chemotherapeutics drugs for treating patients with mCRC. Several factors may reduce the sensitivity of irinotecan drug efficacy, including downregulation of topoisomerase-I, decreased activity of the metabolic enzyme and increased drug transporters to the membrane.[15–17] Previously, we reported that mCRC patients bearing single nucleotide polymorphisms in MTHFR and ABCG2 might benefit from irinotecan-containing regimen.[18] Herein, we first demonstrated that IL-10 expression level was significantly associated with irinotecan therapeutic efficacy. Our results suggested that IL-10 could serve as a promising biomarker for evaluating the chemotherapeutic regimen choose for patients with mCRC.

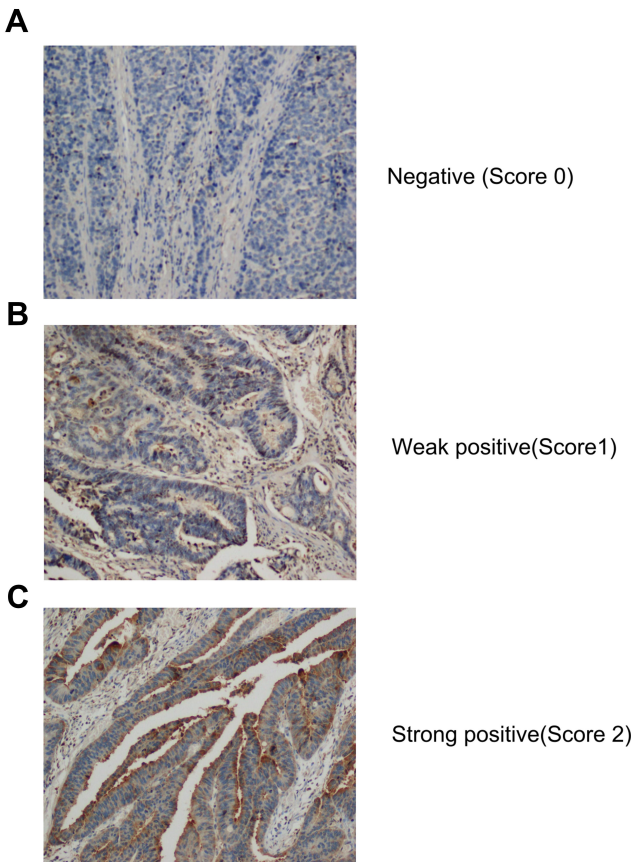

Figure 3 Immunohistochemical staining patterns of IL-10 in mCRC. (**A**) A CRC tumor negative for IL-10 expression; (**B**) a CRC tumor with part of cancer cells expressing IL-10 in the cytoplasm; (**C**) a CRC tumor with most carcinoma cells positive for IL-10 (original magnification: ×400).

IL-10 has been established as a novel therapeutic target for malignancies. A recent study reported that IL-10 recombination could specifically target tumor-infiltrating memory $CD8^+$ T cells and activate anti-tumor immune response.[19] CpG plus anti-IL-10R treatment also showed robust antitumor therapeutic activity in CRC and breast cancer xenograft tumor.[20] In this study, we demonstrated that a high IL-10 plasma level could abrogate the drug efficacy of an irinotecan-containing regimen, thus providing the possibility to combine anti-IL-10 and irinotecan for the treatment of the patient with mCRC. As known, oxaliplatin and irinotecan are alternative regimens for metastatic colorectal cancer in the

Table 5 Association of IL-10 Expression Level with the Survival Data in mCRC Primary Tumor Tissue

Variables		Low Expression (n=34)	High Expression (n=34)	P value
IL-10	PFS	7.967 (7.384–8.550)	7.933 (7.278–8.589)	0.973
	OS	25.33 (6.445–44.222)	18.367 (13.708–23.025)	0.035

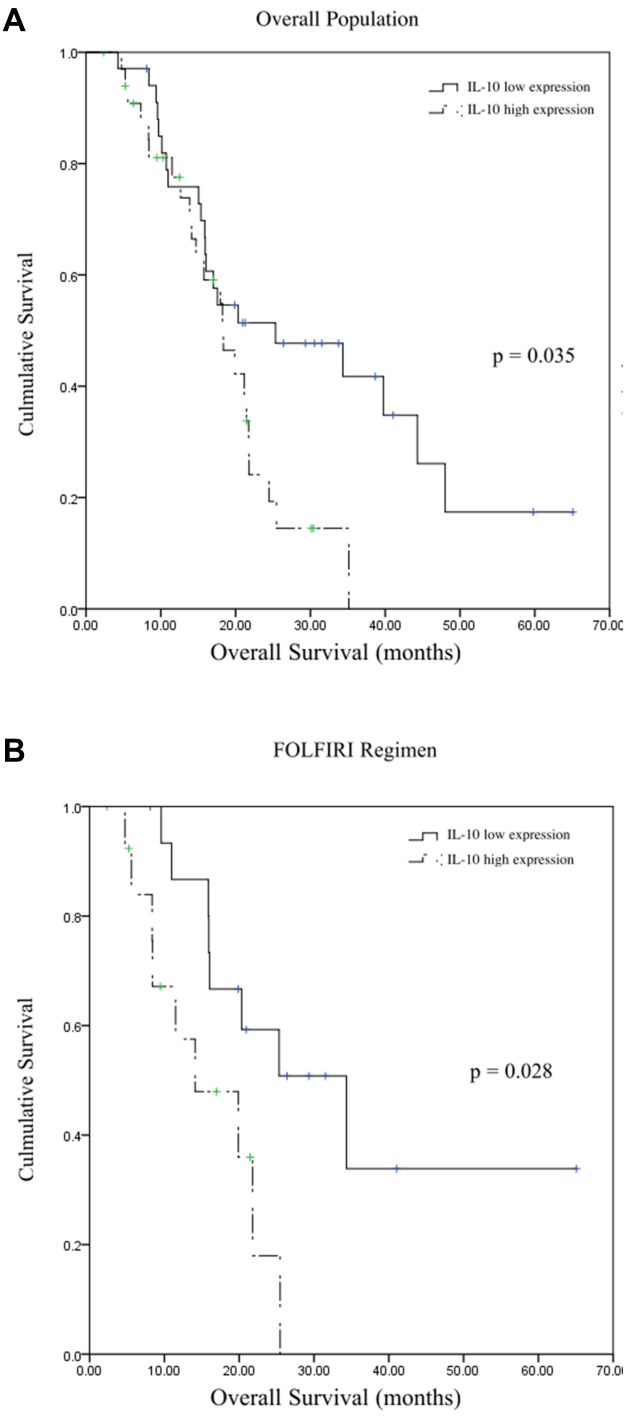

Figure 4 Influence of IL-10 levels in tumor tissue on the progression of overall survival by Kaplan-Meier analyses. Log-rank analysis was used to determine the statistical significance of the Kaplan–Meier survival curve (P < 0.05). (**A**) Patients with high IL-10 tissue expression level had significant shorter OS than those with low IL-10 expression level (p = 0.035). (**B**) Subgroup analysis showed that in irinotecan containing regimen treated patients, high IL-10 tissue expression level had significant shorter OS than those with low IL-10 expression level (p = 0.028).

first-line setting, with similar overall efficacy. In order to distinguish which patients could benefit more from irinotecan-based regimen, our research has provided a simple and convenient choice, which is helpful to solve practical clinical problems.

The current study has some limitations. We did not discuss the underlying mechanism of IL-10 mediated irinotecan drug resistance. Besides, the findings in the plasma-based sample should be verified in a larger size of tumor tissue. Further studies are needed to discover the possibility of combined treatment of IL-10 and irinotecan in mCRC.

Conclusion

In conclusion, IL-10 was proved to act as a prognostic biomarker for mCRC patients undergoing irinotecan-containing chemotherapy. We believed that the current study's findings provided a valuable reference for the first-line chemotherapy for mCRC patients.

Ethics Approval and Informed Consent

This study was approved by the Ethical Committees of the Fudan University Shanghai Cancer Center (Ethics Number: 1203108–10).

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Disclosure

The authors report no conflicts of interest in this work.

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