Early low blood MALT1 expression levels forecast better efficacy of PD-1 inhibitor-based treatment in patients with metastatic colorectal cancer

CHUANMING LI[1*], FAN YU[1*] and WANLI XU[2]

Departments of [1]Anorectal Surgery and [2]Gastroenterology, Wuhan No. 8 Hospital (Wuhan Anorectal Hospital), Wuhan, Hubei 430000, P.R. China

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Abstract. Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) modulates colorectal cancer (CRC) malignant behaviors and tumor immune escape. The present study aimed to explore the association of MALT1 with treatment response and survival time among patients with metastatic CRC (mCRC) after programmed cell death protein-1 (PD-1) inhibitor-based treatment. MALT1 from the blood samples of 75 patients with unresectable mCRC receiving PD-1 inhibitor-based treatment at baseline and after 2-cycle treatment, as well as 20 healthy controls (HCs), was detected by reverse transcription-quantitative PCR. In the patients with mCRC, the objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS) and overall survival (OS) were calculated. MALT1 expression was elevated in patients with mCRC compared with that in HCs (P<0.001). In patients with mCRC, MALT1 expression was positively correlated with multiple (vs. single) metastasis (P=0.032) and peritoneum metastasis (P=0.029). MALT1 levels before treatment were decreased in ORR patients vs. non-ORR patients (P=0.043) and in DCR patients vs. non-DCR patients (P=0.007). Additionally, MALT1 expression was reduced after treatment compared with that before treatment (P<0.001). Meanwhile, MALT1 expression after treatment was notably decreased in ORR patients vs. non-ORR patients (P<0.001) and in DCR patients vs. non-DCR patients (P<0.001). Furthermore, a low MALT1 level before treatment was associated with longer PFS (P=0.030) and OS (P=0.025) times. Decreased MALT1 expression after treatment and a decline in MALT1 expression of >30% after treatment (ratio to MALT1 before treatment) (both P≤0.001) presented more significant associations with prolonged PFS and OS times. In conclusion, early low levels of blood MALT1 during therapy may predict an improved response to PD-1 inhibitor-based treatment and survival time in patients with mCRC.

Introduction

Colorectal cancer (CRC) is the third most frequent cancer and it causes a huge medical burden globally (1,2). According to the recent Global Cancer Statistics, the number of CRC-associated deaths reached ~1 million worldwide in 2020 (2). Meanwhile, there exists ~25% of CRC patients diagnosed with metastatic CRC (mCRC), which is the major cause for the CRC high mortality observed (3). At present, the survival time of mCRC is still poor and the management choices for mCRC are limited (4,5).

Programmed cell death in CRC can be regulated by several factors, including radiation and chemotherapeutic drug treatments. Immune surveillance also modulates the induction of programmed cell death of CRC cells; for example, $CD8^+$ T cells modulate the programmed cell death of CRC cells via perforin or the Fas ligand pathway (6-8). Meanwhile, programmed cell death protein 1 (PD-1) can induce immune escape and decrease antitumor immunity in CRC (9). Over the decades, PD-1 inhibitor-based treatment has been widely applied for cancer treatment, where it plays an antitumor role by inhibiting immune escape through accelerating the antitumor immune response and promoting sensitivity to radio-chemotherapy (10-12). Certain studies have also reported that PD-1 inhibitor-based treatment provides survival benefits in patients with mCRC (13-15). However, >50% of patients with mCRC fail to respond to PD-1 inhibitor-based treatment, which consequently impairs their prognosis (16,17). Thus, the exploration

Correspondence to: Dr Wanli Xu, Department of Gastroenterology, Wuhan No. 8 Hospital (Wuhan Anorectal Hospital), 1288 Jianshe Avenue, Wuhan, Hubei 430000, P.R. China
E-mail: xinci7160204@163.com

*Contributed equally

Abbreviations: CRC, colorectal cancer; mCRC, metastatic CRC; PD-1, programmed cell death protein-1; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; PBMC, peripheral blood mononuclear cells; RT-qPCR, reverse transcription-quantitative PCR; ROC, receiver operating characteristic; IQR, interquartile range; ORR, objective response rate; DCR, disease control rate

Key words: mCRC, MALT1, PD-1 inhibitor, treatment response, survival

of potential biomarkers to reflect the treatment response and survival of patients receiving PD-1 inhibitor-based treatment is imperative to promote the management of mCRC.

Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is involved in the progression of several tumors by regulating the malignant behaviors of tumor cells (18,19). For instance, downregulation of MALT1 suppresses proliferation and promotes apoptosis of prostate cancer cells *in vivo* and *in vitro* (20). Furthermore, knockdown of MALT1 inhibits the proliferation and migration of CRC cells (21). In addition, it has also been reported that MALT1 is able to regulate the tumor immune escape and inactivation of $CD8^+$ T cells, which are viewed as crucial processes for the antitumor activity of PD-1 inhibitors (22). Overall, it can be deduced that MALT1 may have the potential to influence the PD-1 inhibitor response in patients with mCRC; however, related data are scarce.

Thus, the present study aimed to explore the association of blood MALT1 levels with the efficacy of PD-1 inhibitor-based treatment among patients with mCRC.

Patients and methods

Patients. A total of 75 patients with unresectable mCRC who received PD-1 inhibitor treatment at Wuhan No. 8 Hospital (Wuhan, China) between January 2019 and March 2022 were consecutively enrolled in this prospective, cohort study. The major criteria for inclusion were as follows: i) Confirmed as CRC by pathological results; ii) diagnosis of unresectable mCRC; iii) >18 years old; iv) Eastern Cooperative Oncology Group performance status (ECOG PS) score within the scope of 0-2 (23); v) at least one assessable lesion; and vi) planned to receive PD-1 inhibitor alone or combined with other treatments. Meanwhile, the major criteria for exclusion were as follows: i) Pregnancy or lactation; ii) autoimmune or immunodeficiency diseases; and iii) other malignancies. In addition, 20 healthy subjects were also included in the present study as health controls (HCs). All individuals provided written informed consent, and the study was approved by the Ethics Committee of Wuhan No. 8 Hospital.

Clinical data and sample collection. Peripheral blood and other baseline characteristics were collected from all patients with mCRC before the initiation of PD-1 inhibitor-based treatment. After 2 cycles (6 weeks) of treatment (treatment regimen described below), peripheral blood was again collected from the patients. Additionally, peripheral blood was collected from the HCs after enrollment. Following the sample collections, peripheral blood mononuclear cells (PBMCs) were extracted from the peripheral blood with gradient density centrifugation using Ficoll PM400 (Cytiva), and then the PBMCs were used to detect MALT1 expression by reverse transcription-quantitative PCR (RT-qPCR).

RT-qPCR. RT-qPCR was conducted for the quantitative analysis of MALT1 mRNA expression. In brief, total RNA was extracted by QIAamp RNA Blood Mini Kit (Qiagen GmbH) and reverse transcribed using a QuantiNova Reverse Transcription Kit (Qiagen GmbH) according to the manufacturer's protocol. Meanwhile, qPCR was performed with a QuantiNova SYBR Green PCR Kit (Qiagen GmbH). The thermocycling conditions were as follows: 1 cycle of 95˚C for 60 sec, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec. The relative expression was calculated using the $2^{-\Delta\Delta Cq}$ method and GAPDH was used as the internal reference (24). The primers used were as follows: MALT1 foward, 5'-GATGCGTAATGCTGTGGATG-3' and reverse, 5'-GGTATCATCGTAGTCATTTCTTTTCC-3'; and GAPDH forward, 5'-GCCAAGGTCATCCATGACAACTTTGG-3' and reverse, 5'-GCCTGCTTCACCACCTTCTTGATGTC-3'.

Treatment. All patients with mCRC received PD-1 inhibitor-based treatment, which mainly included three regimens: i) PD-1 inhibitor (camrelizumab or sintilimab) plus chemotherapy (CapeOx or FOLFOX6); ii) PD-1 inhibitor plus bevacizumab/apatinib; or iii) PD-1 inhibitor plus bevacizumab/apatinib and chemotherapy (CapeOx or FOLFOX6). To be specific, camrelizumab or sintilimab were administrated at 200 mg once for every 3-week cycle. Bevacizumab was administered at 7.5 mg/kg once for every 2 or 3-week cycle [combined with CapeOx once for every 3-week cycle or FOLFOX6 once for every 2-week cycle; the detailed administration of CapeOx and FOLFOX6 took a preceding study for reference (25)]. Apatinib was administered at 375 or 500 mg/day (the administration could be adjusted to 250 mg/day if patients were not tolerant of the original regimen).

Assessment. Patients in the present study were followed up continuously, with radiographic progression assessed every 4-6 weeks for the first 3 months and then every 2 months until disease progression or death. Treatment response at the third month was obtained. The progression-free survival (PFS) and overall survival (OS) were calculated accordingly based on the follow-up data. PFS was defined as the time from treatment initiation to disease progression or death. OS was defined as the time from treatment initiation to death.

Statistics. Statistical analysis and graph making were conducted using SPSS 24.0 (IBM Corp.) and GraphPad Prism 9.0 (Dotmatics). Wilcoxon's rank sum test was used for the comparison analyses of MALT1 expression between patients with mCRC and HCs. The capability of MALT1 expression level in discriminating patients with mCRC from HCs was measured via the receiver operating characteristic (ROC) curve. Wilcoxon's rank sum test was used for the comparison analyses of MALT1 expression in patients with mCRC with different characteristics (such as diagnosis, number of metastatic sites, lung metastasis, liver metastasis, peritoneal metastasis, other metastases and KRAS expression). Spearman's rank correlation test was utilized to evaluate the correlation of MALT1 expression with ECOG PS score and tumor differentiation. Wilcoxon's rank sum test was also used for the comparison analyses of MALT1 expression between objective response rate (ORR) patients [patients who achieved complete response (CR) and partial response (PR)] and non-ORR patients, as well as between disease control rate (DCR) patients (patients who achieved CR, PR and stable disease) and non-DCR patients. Beyond that, Wilcoxon's signed rank test was applied to analyze the high and low of MALT1 expression levels before and after PD-1

inhibitor-based treatment. The high and low MALT1 expression levels were determined according to the median value (2.529) of MALT1 expression in all patients with mCRC. Meanwhile, if MALT1 expression declined >30% after 2 cycles of treatment [(MALT1 expression at baseline-MALT1 expression after 2 cycles of treatment)/MALT1 expression at baseline >30%], this was defined as MALT1 decline >30%. Kaplan-Meier curves and the log-rank test were also utilized to evaluate the PFS and OS between patients with mCRC with different MALT1 expression levels. Stepwise forward regression method was used for multiple regression without artificial selection of variables. Specifically, the stepwise regression method introduced the independent variables into the model successively according to the probability of the score test, and then the likelihood ratio probability test was conducted based on the assumed parameters to eliminate the independent variables that were no longer statistically significant in the model. Such steps were repeated until the end, when there were no more variables outside the model that had a significant impact on the dependent variable, and there were no more variables in the model that could be eliminated that were not significant on the dependent variable. Forward stepwise multivariate Cox's proportional hazards regression analyses were used to screen the independent prognostic factors for PFS and OS. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of patients with mCRC. Among the 75 patients with mCRC (mean age 58.0±7.7 years; age range, 40-79 years) there were 44 (58.7%) patients <60 years and 31 (41.3%) patients ≥60 years. There were 25 (33.3%) females and 50 (66.7%) males. With respect to the ECOG PS score, 34 (45.3%), 39 (52.0%) and 2 (2.7%) patients had a score of 0, 1 and 2, respectively. Furthermore, there were 8 (10.7%), 28 (37.3%) and 39 (52.0%) patients with well, moderately and poorly differentiated tumors, respectively. Moreover, 47 (62.7%), 42 (56.0%), 21 (28.0%) and 27 (36.0%) patients possessed lung, liver, peritoneal and other metastases, respectively. As for microsatellite instability (MSI) status, 68 (90.7%) patients were MSI-low/microsatellite stable and 7 (9.3%) patients were MSI-high. Regarding treatments, there were 25 (33.3%), 31 (41.3%) and 19 (25.3%) patients who received PD-1 inhibitor plus chemotherapy, PD-1 inhibitor plus bevacizumab/apatinib and PD-1 inhibitor plus bevacizumab/apatinib and chemotherapy, respectively (Table I).

Table I. Characteristics of the 75 patients with metastatic colorectal cancer.

Patient characteristics	Value
Age, years	
Mean ± SD	58.0±7.7
<60, n (%)	44 (58.7)
≥60, n (%)	31 (41.3)
Sex, n (%)	
Female	25 (33.3)
Male	50 (66.7)
ECOG PS score, n (%)	
0	34 (45.3)
1	39 (52.0)
2	2 (2.7)
Diagnosis, n (%)	
Rectum	19 (25.3)
Colon	56 (74.7)
Differentiation, n (%)	
Well	8 (10.7)
Moderate	28 (37.3)
Poor	39 (52.0)
Metastatic sites, n (%)	
Single	29 (38.7)
Multiple	46 (61.3)
Lung metastasis, n (%)	47 (62.7)
Liver metastasis, n (%)	42 (56.0)
Peritoneal metastasis, n (%)	21 (28.0)
Other metastases, n (%)	27 (36.0)
KRAS, n (%)	
Wide-type	54 (72.0)
Mutation	21 (28.0)
MSI status, n (%)	
MSI-L/MSS	68 (90.7)
MSI-H	7 (9.3)
Treatments, n (%)	
PD-1 inhibitor plus chemotherapy	25 (33.3)
PD-1 inhibitor plus bevacizumab/apatinib	31 (41.3)
PD-1 inhibitor plus bevacizumab/apatinib and chemotherapy	19 (25.3)
Treatment lines, n (%)	
1st	0 (0.0)
2nd	37 (49.3)
3rd	27 (36.0)
≥4th	11 (14.7)

MSI, microsatellite instability; MSI-L MSI low; MSS, microsatellite stable; MSI-H, MSI high; PD-1, programmed cell death protein-1; ECOG PS, Eastern Cooperative Oncology Group performance status.

Comparison of MALT1 expression levels between patients with mCRC and HCs. MALT1 expression was elevated in patients with mCRC compared with that in HCs [median (interquartile range (IQR)), 2.529 (1.945-3.864) vs. 0.974 (0.782-1.546), respectively; $P<0.001$; Fig. 1A]. In addition, the ROC curve demonstrated that MALT1 expression level had a good capability of distinguishing patients with mCRC from HCs, with an area under the curve and 95% confidence interval (CI) of 0.887 and 0.812-0.962, respectively (Fig. 1B).

Correlation of MALT1 expression with clinical characteristics of patients with mCRC. Elevated MALT1 expression was correlated with multiple metastatic sites ($P=0.032$) and peritoneal metastasis ($P=0.029$), while it was not correlated with other clinical and pathological characteristics, including ECOG PS score, diagnosis, differentiation, lung metastasis,

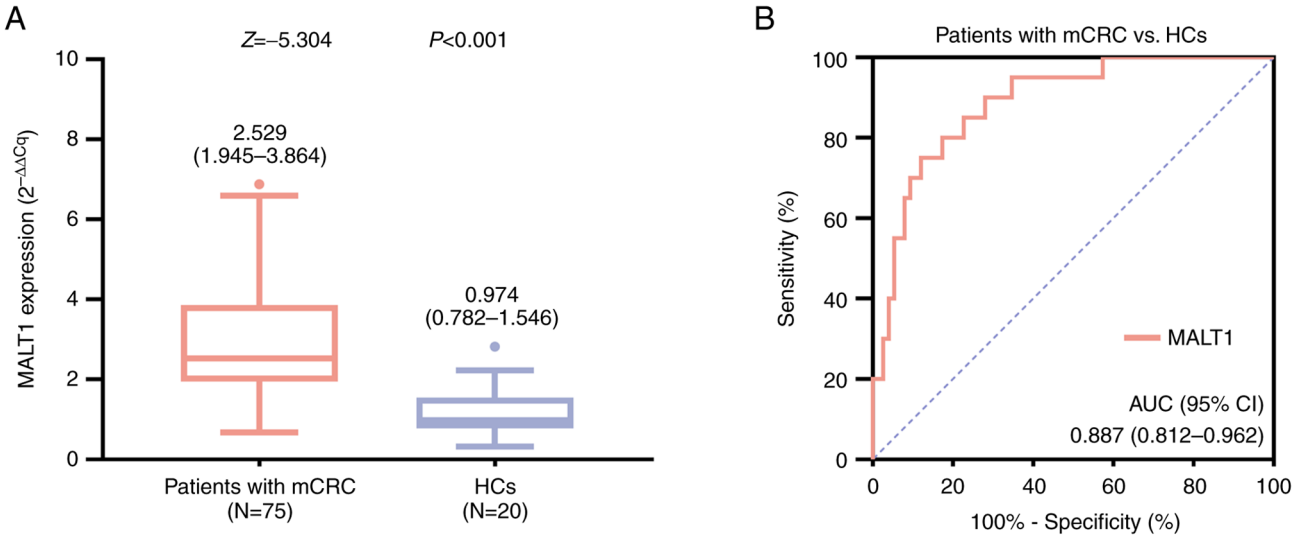

Figure 1. MALT1 is elevated in patients with mCRC vs. HCs. (A) Comparison of MALT1 expression level in patients with mCRC vs. HCs. The numbers above the bars represent the median (confidence interval). (B) The capability of MALT1 to discriminate patients with mCRC from HCs. AUC, area under curve; CI, confidence interval; HCs, healthy controls; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; mCRC, metastatic colorectal cancer.

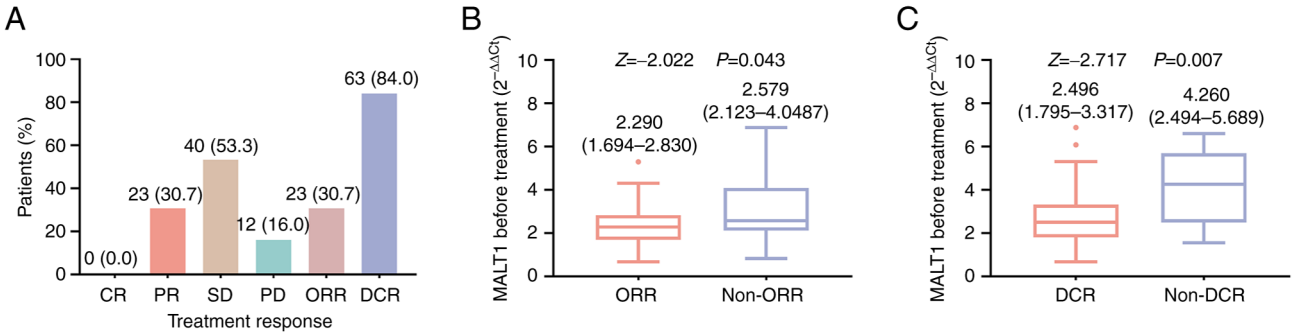

Figure 2. MALT1 expression levels before treatment are decreased in response vs. non-response patients withmCRC. (A) Treatment response of patients. The numbers above the bars represent the number of patients (%). (B) Comparison of MALT1 expression level before treatment in ORR patients vs. non-ORR patients, and in (C) DCR patients vs. non-DCR patients. The numbers above the bars in (B) and (C) represent the median (confidence interval). CR, complete response; DCR, disease control rate; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.

liver metastasis, other metastases, KRAS mutation or MSI status in patients with mCRC (all P>0.05) (Table II).

Association of MALT1 level before treatment with treatment response in patients with mCRC. No patients achieved a complete response. Meanwhile, the rates of partial response, stable disease and progressive disease were 30.7, 53.3 and 16.0%, respectively, which led to an ORR and DCR of 30.7 and 84.0%, respectively (Fig. 2A). In addition, MALT1 expression before treatment was lower in ORR patients compared with that in non-ORR patients [median (IQR), 2.290 (1.694-2.830) vs. 2.579 (2.123-4.087); P=0.043; Fig. 2B], and it was also lower in DCR patients compared with that in non-DCR patients [median (IQR), 2.496 (1.795-3.317) vs. 4.260 (2.494-5.689); P=0.007; Fig. 2C].

MALT1 expression level after treatment, and its association with treatment response in patients with mCRC. In all patients, the MALT1 expression level after treatment was lower compared with that before treatment (P<0.001; Fig. 3A). In ORR patients (P<0.001; Fig. 3B), non-ORR patients (P<0.001; Fig. 3C) and DCR patients (P<0.001; Fig. 3D), but not in non-DCR patients (P=0.060; Fig. 3E), the MALT1 expression level after treatment was lower compared with that before treatment. Additionally, the MALT1 expression level after treatment was lower in ORR patients compared with that in non-ORR patients [median (IQR), 1.183 (0.647-1.905) vs. 2.157 (1.480-3.560); P<0.001; Fig. 3F] and was also lower in DCR patients compared with that in non-DCR patients [median (IQR), 1.761 (0.976-2.494) vs. 3.783 (2.121-4.688); P<0.001; Fig. 3G].

Association of MALT1 with survival of patients with mCRC. Low MALT1 expression before treatment was associated with longer PFS (P=0.030; Fig. 4A) and OS (P=0.025; Fig. 4B) times. Meanwhile, low MALT1 expression after treatment was significantly associated with prolonged PFS (P=0.010; Fig. 4C) and OS (P=0.005; Fig. 4D) times. In addition, a

Table II. Correlation of MALT1 expression level with the clinical and pathological characteristics of patients with metastatic colorectal cancer.

Patient characteristics	Median MALT1 expression level before treatment ($2^{-\Delta\Delta Ct}$) (IQR)	Z/ρ-value	P-value
ECOG PS score		-0.003[a]	0.979
0	2.577 (1.998-3.794)		
1	2.505 (1.795-3.864)		
2	4.020 (2.210-NA)		
Diagnosis		-0.280[b]	0.779
Rectal cancer	2.578 (1.501-4.300)		
Colon cancer	2.519 (2.118-3.571)		
Differentiation		0.143[a]	0.221
Well	2.577 (1.703-3.528)		
Moderate	2.484 (1.630-3.530)		
Poor	2.768 (2.034-4.300)		
Metastatic sites		-2.143[b]	0.032
Single	2.344 (1.527-3.117)		
Multiple	2.554 (2.197-4.197)		
Lung metastasis		-0.088[b]	0.930
No	2.556 (2.195-3.687)		
Yes	2.508 (1.795-3.864)		
Liver metastasis		-0.096[b]	0.923
No	2.823 (1.610-3.872)		
Yes	2.501 (2.086-3.872)		
Peritoneal metastasis		-2.183[b]	0.029
No	2.486 (1.721-3.498)		
Yes	2.828 (2.445-4.666)		
Other metastases		-0.806[b]	0.420
No	2.507 (1.820-3.861)		
Yes	2.578 (2.103-3.864)		
KRAS		-0.873[b]	0.383
Wide-type	2.579 (1.932-3.907)		
Mutation	2.407 (1.873-3.146)		
MSI status		-1.220[b]	0.222
MSI-L/MSS	2.556 (1.963-3.887)		
MSI-H	2.290 (1.501-2.828)		

MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not available; MSI, microsatellite instability; MSI-L, low MSI; MSS, microsatellite stable; MSI-H, high MSI. [a]Spearman's rank correlation test with P-value; [b]Wilcoxon's rank sum test with Z-value.

MALT1 expression decline of >30% was significantly associated with longer PFS (P=0.001; Fig. 4E) and OS (P<0.001; Fig. 4F) times.

Factors associated with PFS in patients with mCRC. Univariate Cox's proportional hazards regression analysis demonstrated that MALT1 expression decline (>30 vs. ≤30%) was associated with prolonged PFS time [hazard ratio (HR), 0.429; P=0.002], while MALT1 expression before treatment (high vs. low), MALT1 expression after treatment (high vs. low), higher ECOG PS score, worse differentiation, multiple (vs. single) metastatic, lung metastasis (yes vs. no), peritoneal metastasis (yes vs. no) and higher treatment lines (all P<0.05) were associated with declined PFS. Forward stepwise multivariate Cox's proportional hazards regression analysis demonstrated that MALT1 expression decline (>30 vs. ≤30%; HR, 0.410; P=0.001) independently predicted prolonged PFS time, while MALT1 expression before treatment (high vs. low), worse differentiation and higher treatment lines were independently associated with shorter PFS times (all P<0.05) (Table III).

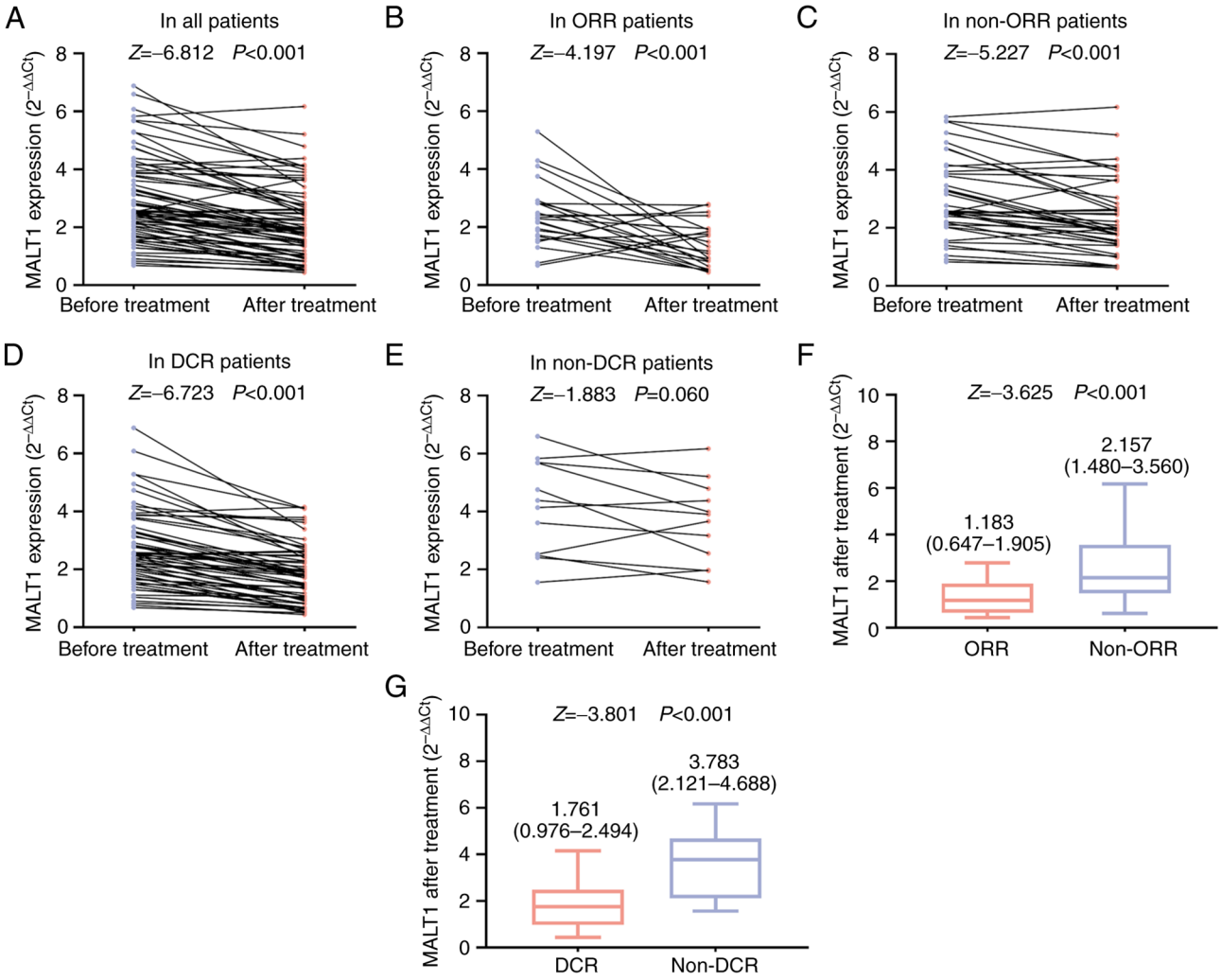

Figure 3. MALT1 expression decreases after treatment in patients with mCRC. Comparison of MALT1 expression level before treatment vs. after treatment in (A) all patients, (B) ORR patients, (C) non-ORR patients, (D) DCR patients (E) and non-DCR patients. (F) Comparison of MALT1 expression level after treatment in ORR patients vs. non-ORR patients, and in (G) DCR patients vs. non-DCR patients. The numbers above the bars in (F) and (G) represent the median (confidence interval). DCR, disease control rate; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; ORR, objective response rate.

Factors associated with OS in patients with mCRC. Univariate Cox's proportional hazards regression analysis demonstrated that MALT1 expression decline (>30 vs. ≤30%) was associated with prolonged OS time (HR, 0.321; P<0.001), while MALT1 expression before treatment (high vs. low), MALT1 expression after treatment (high vs. low), higher ECOG PS score, worse differentiation, multiple (vs. single) metastatic sites, peritoneal metastasis (yes vs. no) and higher treatment lines were associated with shorter OS times (all P<0.05). Forward stepwise multivariate Cox's proportional hazards regression analysis demonstrated that MALT1 expression decline (>30 vs. ≤30%; HR, 0.276; P<0.001), PD-1 inhibitor plus bevacizumab/apatinib (vs. PD-1 inhibitor plus chemotherapy; HR=0.138; P=0.001) independently predicted prolonged OS time, while age (≥60 vs. <60 years), worse differentiation and higher treatment lines were independently associated with shorter OS times (all P<0.05) (Table IV).

Discussion

To date, the association between MALT1 expression and disease risk in mCRC has been unclear. To the best of our knowledge, only one published study compared the difference between MALT1 expression in CRC tissue and adjacent tissue based on the Gene Expression Omnibus database and immunohistochemistry staining, which illustrated that MALT1 expression was markedly increased in CRC tissue compared with adjacent tissue (21), suggesting the potential association of MALT1 with CRC risk. In the present study, it was discovered that blood MALT1 was elevated in patients with mCRC compared with HCs, which also had the capability to discriminate patients with mCRC from HCs. A potential explanation for this observation may be that MALT1 could regulate several oncogenic signaling pathways of mCRC (such as the NF-κB and extracellular signal-regulated kinase/mitogen-activated protein kinase signal pathways) (21,26,27), which might accelerate the occurrence of CRC. In addition, the present study also demonstrated that MALT1 expression was positively correlated with multiple metastases and peritoneal metastasis. The possible reason for this observation may be that MALT1 can accelerate CRC cell invasion and migration, which may lead to multiple metastases and peritoneal metastases in mCRC (20,21).

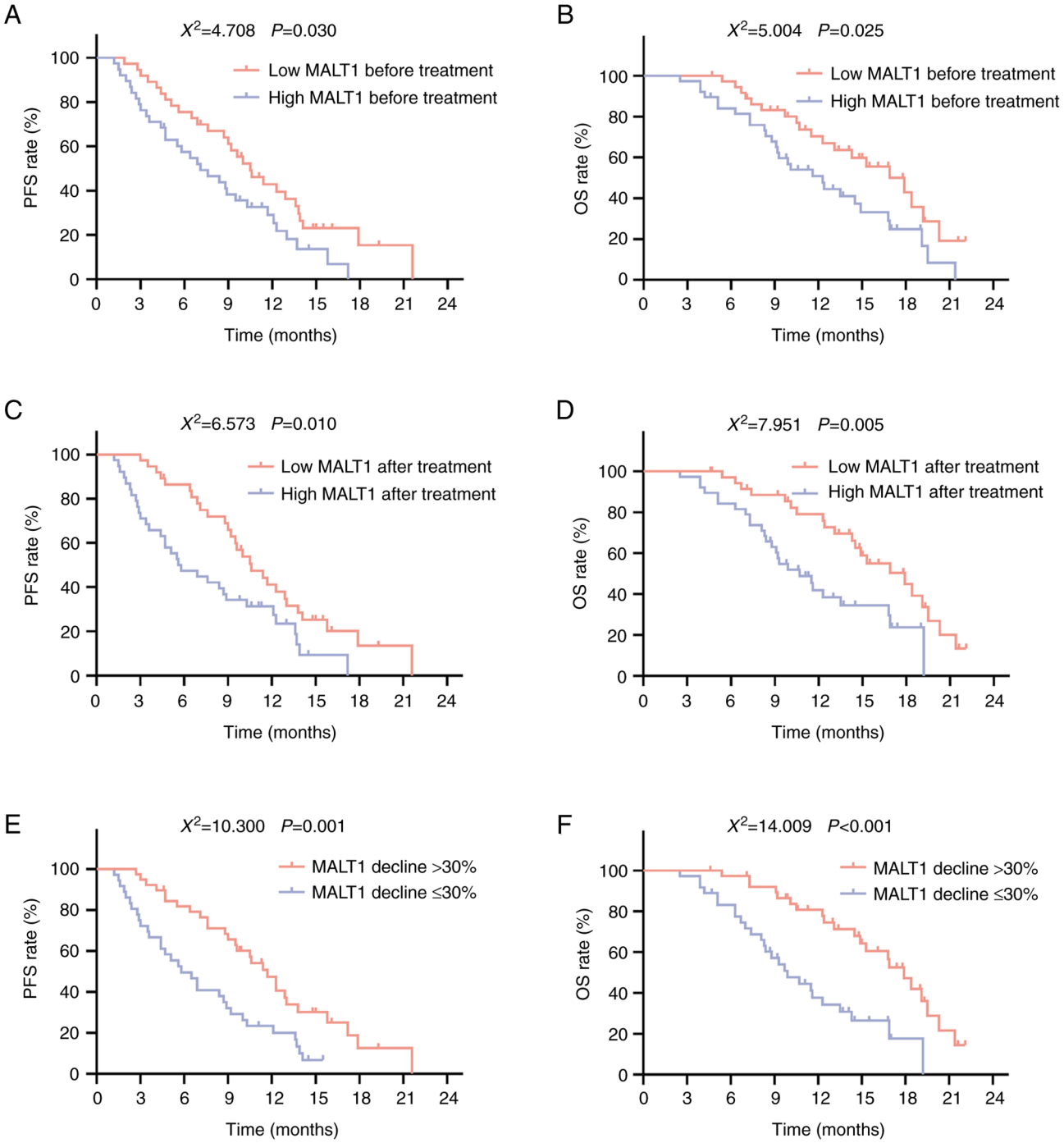

Figure 4. Low MALT1 expression is associated with prolonged survival times in patients with mCRC. Association of low MALT1 expression before treatment with (A) PFS and (B) OS times. Association of low MALT1 expression after treatment with (C) PFS and (D) OS times. Association of a MALT1 expression level decline of >30% with (E) PFS and (F) OS times. MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; OS, overall survival; PFS, progression-free survival.

PD-1 inhibitor therapy combined with systematic therapy and/or targeted therapy is widely applied in mCRC treatment (15,17). However, the low response rate of PD-1 inhibitor-based treatment is still a challenge for the treatment of mCRC (16,17). Although previous studies have discovered the predictors of treatment response of PD-1 inhibitor-based treatment among patients with mCRC (including pan-immune-inflammation value and microsatellite instability-high), the role of MALT1 in reflecting short-term efficacy in these patients remains unclear (28,29). The present study discovered that, before treatment, blood MALT1 levels were lower in patients with mCRC with a favorable treatment response, while after treatment, blood MALT1 was notably decreased, and its low post-treatment expression was significantly associated with an improved treatment response in patients with mCRC receiving PD-1 inhibitor-based treatment. This observation may be due to the suggestions that: i) MALT1 could regulate the proliferation of CRC cells, leading to CRC growth and, after treatment, the tumor growth was decreased; therefore, MALT1 expression

Table III. Cox's proportional hazards regression analysis for PFS.

A, Univariate Cox's proportional hazards regression analysis for PFS

			95% CI	
Patient characteristics	P-value	HR	Lower	Upper
MALT1 decline (>30 vs. ≤30%)	0.002	0.429	0.252	0.731
MALT1 expression before treatment (high vs. low)	0.033	1.769	1.049	2.983
MALT1 expression after treatment (high vs. low)	0.012	1.958	1.160	3.306
Age (≥60 vs. <60 years)	0.229	1.374	0.818	2.308
Sex (male vs. female)	0.236	1.407	0.800	2.473
Higher ECOG PS score	0.016	1.849	1.119	3.055
Diagnosis (colon cancer vs. rectal cancer)	0.898	1.043	0.549	1.981
Worse differentiation	0.007	1.813	1.181	2.782
Metastatic sites (multiple vs. single)	0.044	1.736	1.014	2.971
Lung metastasis (yes vs. no)	0.019	1.955	1.117	3.422
Liver metastasis (yes vs. no)	0.423	1.236	0.736	2.075
Peritoneum metastasis (yes vs. no)	<0.001	5.890	3.131	11.078
Other metastases (yes vs. no)	0.150	0.670	0.389	1.155
KRAS (mutation vs. wild-type)	0.287	1.384	0.760	2.520
MSI status (MSI-H vs. MSI-L/MSS)	0.188	0.500	0.178	1.403
Treatments				
PD-1 inhibitor plus chemotherapy	Reference			
PD-1 inhibitor plus bevacizumab/apatinib	0.293	1.379	0.757	2.512
PD-1 inhibitor plus bevacizumab/apatinib and chemotherapy	0.332	0.714	0.362	1.410
Higher treatment lines	0.002	1.834	1.255	2.678

B, Forward stepwise multivariate Cox's proportional hazards regression analysis for PFS

			95% CI	
Patient characteristics	P-value	HR	Lower	Upper
MALT1 decline (>30 vs. ≤30%)	0.001	0.410	0.238	0.707
MALT1 expression before treatment (high vs. low)	0.012	1.981	1.161	3.379
Worse differentiation	0.004	1.883	1.226	2.891
Higher treatment lines	<0.001	2.205	1.484	3.277

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; ECOG PS, Eastern Cooperative Oncology Group performance status; MSI, microsatellite instability; MSI-H, high MSI; MSI-L, low MSI; MSS, microsatellite stable; PD-1, programmed cell death protein-1.

was reduced after treatment (20,21); and ii) decrease in MALT1 before and after treatment could inhibit tumor immune escape and promote activation of $CD8^+$ T cells, which may lead to the elevated treatment response to PD-1 inhibitors (22,30).

The survival profile of patients with mCRC is dismal (13-15). Thus, the exploration of potential biomarkers to predict survival time among patients with mCRC is imperative. In the present study, it was demonstrated that reduced blood MALT1 levels before treatment were associated with longer PFS and OS times. Meanwhile, lower blood MALT1 levels after treatment and a >30% decline in MALT1 level were significantly associated with prolonged PFS and OS times. The potential explanations for these observations may be that: i) Lower MALT1 expression was associated with better treatment response, thus lower MALT1 expression level was related to longer PFS and OS times among patients with mCRC; and ii) decreased MALT1 expression may inhibit the malignant behavior and immune escape of tumor cells, which may lead to less disease burden and consequently result in longer PFS and OS times (21,31).

Table IV. Cox's proportional hazards regression analysis for OS.

A, Univariate Cox's proportional hazards regression analysis for OS

Patient characteristics	P-value	HR	95% CI	
			Lower	Upper
MALT1 decline (>30 vs. ≤30%)	<0.001	0.321	0.172	0.600
MALT1 expression before treatment (high vs. low)	0.028	1.927	1.072	3.463
MALT1 expression after treatment (high vs. low)	0.006	2.370	1.277	4.397
Age (≥60 vs. <60 years)	0.121	1.589	0.885	2.853
Sex (male vs.. female)	0.170	1.597	0.818	3.116
Higher ECOG PS score	0.002	2.714	1.443	5.106
Diagnosis (colon cancer vs. rectal cancer)	0.632	0.833	0.395	1.758
Worse differentiation	0.007	2.018	1.215	3.354
Metastatic sites (multiple vs. single)	0.025	2.029	1.092	3.771
Lung metastasis (yes vs. no)	0.078	1.744	0.939	3.236
Liver metastasis (yes vs. no)	0.182	1.490	0.829	2.678
Peritoneum metastasis (yes vs. no)	<0.001	5.503	2.888	10.485
Other metastases (yes vs. no)	0.283	0.713	0.385	1.323
KRAS (mutation vs. wide-type)	0.171	1.641	0.808	3.335
MSI status (MSI-H vs. MSI-L/MSS)	0.218	0.409	0.099	1.695
Treatments				
PD-1 inhibitor plus chemotherapy	Reference			
PD-1 inhibitor plus bevacizumab/apatinib	0.627	1.179	0.607	2.290
PD-1 inhibitor plus bevacizumab/apatinib and chemotherapy	0.154	0.561	0.253	1.242
Higher treatment lines	0.003	1.884	1.232	2.881

B, Forward stepwise multivariate Cox's proportional hazards regression analysis for OS

Patient characteristics	P-value	HR	95% CI	
			Lower	Upper
MALT1 decline (>30 vs. ≤30%)	<0.001	0.276	0.142	0.534
Age (≥60 vs. <60 years)	0.026	2.397	1.112	5.167
Worse differentiation	<0.001	3.830	2.065	7.102
Treatments				
PD-1 inhibitor plus chemotherapy	Reference			
PD-1 inhibitor plus bevacizumab/apatinib	0.001	0.138	0.042	0.457
PD-1 inhibitor plus bevacizumab/apatinib and chemotherapy	0.101	0.497	0.216	1.145
Higher treatment lines	<0.001	5.222	2.342	11.646

OS, overall survival; HR, hazard ratio; CI, confidence interval; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; ECOG PS, Eastern Cooperative Oncology Group performance status; MSI, microsatellite instability; MSI-H, high MSI; MSI-L, low MSI; MSS, microsatellite stable; PD-1, programmed cell death protein-1.

The present study included two characteristics: i) MALT1 level from PBMCs among patients with mCRC was detected in the present study, which was convenient to acquire and, notably, the detection of accessible blood MALT1 levels may help clinicians promote the correct treatment management of patients with mCRC; and ii) considering that MALT1 regulates tumor immune escape and inactivation of $CD8^+$ T cells (22), the present study explored the association of blood MALT1 levels with the efficacy of PD-1 inhibitor-based treatment, while this association with PD-ligand 1 inhibitor-based treatment was not explored. Nevertheless, several limitations exist in the present study: i) The sample size was relatively small, which may lead to less generalization of discoveries in the study; ii) the association of

MALT1 expression levels with treatment response of other immunotherapy methods among patients with mCRC should be explored in a further study; and iii) the included patients were patients with unresectable mCRC, so the association of MALT1 expression level with the efficacy of PD-1 inhibitor-based treatment in patients with resectable CRC should be explored in the future.

In conclusion, blood MALT1 levels were decreased after PD-1 inhibitor-based treatment, and this decrease was associated with low disease risk, better treatment response and longer survival times of patients with mCRC.

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Availability of data and materials

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

Authors' contributions

WX contributed to the conception and the design of the study, project administration, resources and validation. CL and FY were responsible for the acquisition and analysis of data, methodology, interpretation of the data, and reviewing and editing of the manuscript. WX and CL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by The Ethics Committee of Wuhan No.8 Hospital (Wuhan, China). All individuals included in the study 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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