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The predictive value and correlation of β-catenin, CMTM6, and PD-L1 expression in colorectal cancer

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CMTM6 is a major regulator of PD-L1 expression. Aberrant Wnt pathway signaling occurs in most sporadic colorectal cancers (CRC). However, the significance and correlation of β-catenin, CMTM6, and PD-L1 immunohistochemical expression in CRC is still unknown and need to be further verified. We evaluated the expression levels of β-catenin, CMTM6, PD-L1, and MMR (mismatch repair) proteins by immunohistochemistry in CRC tissue microarray (TMA), and evaluated the association among β-catenin, CMTM6, PD-L1 expression, MMR status, and clinicopathological features in 704 CRC patients. Positive expression of PD-L1 in tumor cells (TC) is associated with more frequent dMMR (mismatch repair deficient) status, CMTM6 expression, right colon, and younger CRC patients. The expression of PD-L1 in tumor-infiltrating immune cells (IC) is associated with a higher frequency of adenocarcinoma, β-catenin, and CMTM6 expression. In univariate analysis, age, histological subtype, histologic grade, lymphatic metastasis, TNM stage, MMR status, and expression of PD-L1 protein in IC were significantly associated with the overall survival. In multivariate analysis, age, histologic grade, TNM stage, MMR status, and expression of PD-L1 protein in IC were independent prognostic factors. The overall survival of the adjuvant chemotherapy group was significantly higher than those non-chemotherapy in TNM stage III-IV CRC patients, but no significant overall survival improvement was found in the positive PD-L1 in TC, positive PD-L1 in IC, positive CMTM6, low β-catenin expression, or dMMR status subgroups. Expression of CMTM6 and PD-L1 in CRC are positively associated with β-catenin and reliable biomarkers for the prediction of responding to chemotherapy. The expression of β-catenin/CMTM6/PD-L1 and MMR status may be valuable biomarkers for guiding different treatment strategies in CRC patients.

Key words: CMTM6; PD-L1; Wnt/β-catenin; chemotherapy; colorectal cancer

Colorectal cancer (CRC) is the third most common cancer and second in terms of mortality [1]. Presently, immune checkpoint inhibitors (ICPI) that block the interaction of the programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) checkpoint proteins have been successfully applied to the treatment of various malignant tumors such as melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, urothelial carcinoma, and metastatic CRC (mCRC), which opens a new era of immunotherapy for malignant tumors and significantly improves the patient's life quality [2–7]. In recent years, expression of PD-1/PD-L1 protein in tumor cell (TC) and tumor-infiltrating immune cell (IC), measured by immunohistochemistry (IHC) using various antibodies and protocols, has become one of the most important and promising biomarkers to predict the response to ICPI. However, due to the intratumoral staining heterogeneity, lack of standardized scoring criteria, and antibody cloning, the clinical application of PD-L1 IHC in immunotherapy of malignancies has been limited [8, 9].

Recently, many studies have shown that CKLF-like MARVEL transmembrane domain-containing protein 6 (CMTM6) is a major regulator of constitutive cell surface PD-L1 expression in cell lines of melanoma, colorectal, and lung cancer, diseases that respond to ICPI [10–13]. However, the significance and correlation of CMTM6 and PD-L1 protein expression in CRC tissues are still unknown. Moreover, recent studies described the role of β-catenin, the main oncoprotein in CRC, in regulating immune cell infiltration of the tumor microenvironment, given its potential impact on immunotherapy treatments [14, 15]. We believe that further study on the correlation and significance of β-catenin and PD-L1 will enrich the practices of CRC

immunotherapy. In this study, we examined the clinical significance and correlation of β-catenin, CMTM6, and PD-L1 expressions with clinicopathological parameters and MMR (mismatch repair) status in CRC.

Patients and methods

Patients and tissue samples. Tumor samples were obtained from 781 patients who had undergone surgical resection for CRC and no preoperative chemotherapy or radiotherapy at the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital from 2005 to 2015. The median age at surgery was 62 years ranging from 17 to 93 years. The clinicopathological diagnoses of CRC were conducted by two pathologists. Patients with colorectal cancer were followed up after surgery and every six months by outpatient review, hospitalization, and telephone. Patients with CRC received adjuvant chemotherapy after surgery with fluorouracil or fluorouracil combined with oxaliplatin, excluding immunotherapy. This study was approved by the Committee on Human Research at the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, and written consent was obtained from CRC patients.

Tissue microarray and immunohistochemistry. Tissue microarray (TMA) was constructed using the specimens from paraffin-embedded blocks of CRC primary tumors. Each case in the TMA was represented in 5 cores, with 1 mm in size for each core. The immunostained slides were scored by two pathologists who were blinded to the clinical data. Antibodies against PD-L1 (IHC411) and CMTM6 (Clone1) were purchased from Genomeme Lab Inc. (Richmond, BC, Canada). Antibodies against PD-L1 (SP142), β-catenin (UMAB15), MSH2 (RED2), MSH6 (EP49), PMS2 (EP51), and MLH1 (ES05) were purchased from ZSBIO (Beijing, China). Antigen-antibody reactions were visualized using a Ventana OptiView™ Amplification kit, followed by a Ventana OptiView™ DAB IHC Detection Kit (Ventana Medical Systems, Inc.). Counterstaining was performed by Ventana Hematoxylin II, followed by a bluing reagent. Immunohistochemistry was performed by BenchMark® ULTRA (Ventana Medical Systems, Inc.). Positive and negative controls were stained concurrently and showed appropriate immunostaining.

Immunohistochemistry was used to detect PD-L1 expression on tumor cells and tumor-infiltrating immune cells, respectively. Immunohistochemical staining of PD-L1 using SP142 in tumor cells (TC) and tumor-infiltrating immune cells (IC) was considered as positive (+) if ≥1% of TC or IC showed convincing cell membrane or cell cytoplasm staining. Immunohistochemical staining of PD-L1 using IHC411 in TC was considered as positive (+) if ≥1% of TC showed convincing cell membrane or cell cytoplasm staining. A positive (+) expression of PD-L1 using IHC411 in IC was given if ≥5% of IC showed convincing cell membrane or cell cytoplasm staining. TC or IC that had both positive/negative expressions on these two antibodies SP142 and IHC411 were defined as the real positive/negative expressions of PD-L1. After screening, 704 cases of a total of 781 CRC patients met the criteria according to the results of IHC. Data on the following clinical parameters of these 704 CRC patients were collected: age, gender, TNM stage, tumor location, histological subtype, histologic grade, and lymphatic metastasis (Table 1).

Immunohistochemical staining of CMTM6 using Clone1 in CRC cells has been considered as positive (+) if ≥1% of TC showed convincing cell membrane or cell cytoplasm staining.

Intensity of β-catenin expression (absent, weak, moderate, strong) was scored as IHC 0, 1, 2, and 3, and the percentage of positively stained cells at different levels (0–5%, 6–25%, 26–50%, >50%) was scored as IHC 0, 1, 2, and 3. After adding the intensity scores to percentage scores, samples with final scores ≤4 were defined as low expression β-catenin while samples with scores >4 were defined as high expression β-catenin.

Immunohistochemical staining of MSH2, MSH6, PMS2, and MLH1 was regarded as proficient (+) or deficient (–). Surrounding normal cells (stroma, epithelium, lymphocytes) were used as a control variable. A proficient score was given if ≥1% of TC showed convincing nuclear staining. CRC patients with MMR proficient protein expression were regarded as pMMR, while CRC patients with MMR deficient protein expression were regarded as dMMR.

Statistical analyses. Statistical analyses were performed with SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). Associations of two parameters were evaluated with Pearson's Chi-squared test or Fisher's exact test. Overall survival was defined by the Kaplan-Meier method. Survival analyses were performed using the log-rank test. A p-value <0.05 was considered significant.

Results

PD-L1, CMTM6, and β-catenin expression, MMR status, and clinicopathological characteristics. IHC staining of β-catenin, CMTM6, PD-L1, and MMR proteins in CRC TMA is shown in Figure 1. After screening, 704 out of 781 CRC patients met our criteria for the real positive/negative expression of PD-L1. Therefore, we used these 704 CRC cases for our subsequent analysis. The positive expression rate of PD-L1 in tumor cells was 11.36% (80/704), in tumor-infiltrating immune cells was 29.26% (206/704), respectively. The age of 704 CRC patients ranged from 17 to 93 years (median 62 years). Clinicopathological characteristics of 704 CRC patients are shown in Table 1.

Correlation of PD-L1, CMTM6, and β-catenin expression, MMR status, and clinicopathological parameters. The association of PD-L1 expression in TC with clinicopathologic parameters is summarized in Table 1. Notably, positive expression of PD-L1 in TC is associated with a

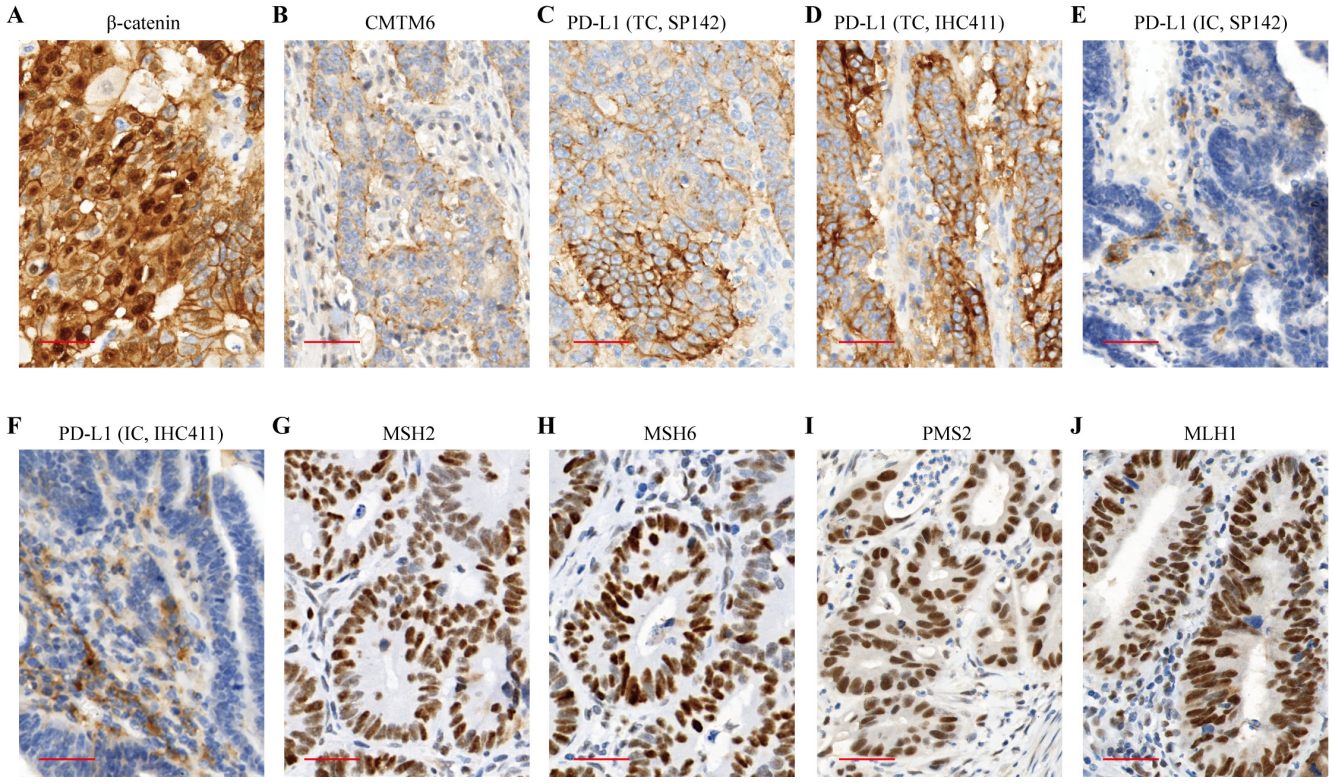

Figure 1. IHC staining on CRC TMA. β-catenin (A) and CMTM6 (B) expression in tumor cells. PD-L1 expression in TC (C, D) and IC (E, F). Mismatch repair proteins MSH2 (G), MSH6 (H), PMS2 (I), and MLH1 (J) expressions in tumor cells. Scale bar, 40 μm.

higher frequency of CMTM6 expression, dMMR status, right colon, and younger CRC patients. There was no significant relationship among PD-L1 expression in TC and histological subtype, histologic grade, lymphatic metastasis, TNM stage, and β-catenin expression (Table 1).

The association of PD-L1 expression in IC with clinicopathological parameters is summarized in Table 1. Notably, positive expression of PD-L1 in IC is associated with a higher frequency of adenocarcinoma, β-catenin, and CMTM6 expression. There was no significant relationship among PD-L1 expression in IC and age, gender, MMR status, tumor location, histologic grade, and lymphatic metastasis (Table 1). In addition, there was no significant relationship between PD-L1 expression in IC and TNM stage (p=0.053), however, a trend between these two exists.

The association of CMTM6 expression in CRC cells with clinicopathological parameters is summarized in Table 1. Notably, positive expression of CMTM6 in CRC cells is associated with a higher frequency of pMMR status, β-catenin, and PD-L1 TC and IC expressions. There was no significant relationship among CMTM6 expression and age, gender, tumor location, histological subtype, histologic grade, lymphatic metastasis, and TNM stage (Table 1).

Prognostic significance of PD-L1, CMTM6, and β-catenin expression, MMR status, and clinicopathological parameters in CRC. In this study, the follow-up rate was 73.2% (515/704). The 5-year overall survival rate was 65%. Age, histological subtype, histologic grade, lymphatic metastasis, TNM stage, MMR status, and expression of PD-L1 protein in IC were significantly associated with the overall survival (Table 2, Figures 2A–2E). In multivariate analysis, age, histologic grade, TNM stage, MMR status, and expression of PD-L1 protein in IC were independent factors of prognostic (Table 2).

In TNM stage III–IV CRC patients, the survival curves with respect to the overall survival of the adjuvant chemotherapy group were significantly higher than that of non-chemotherapy (Figure 3A, p=0.005). But no significant improvement of survival curve of the chemotherapy was found in the positive expression of PD-L1 in TC (p>0.05), positive expression of PD-L1 in IC (p>0.05), positive expression of CMTM6 (p>0.05), low β-catenin expression (p>0.05), or dMMR subgroups (p>0.05), respectively. Significant improvement of survival curves of the chemotherapy was only found in the negative expression of PD-L1 in TC (Figure 3B, p=0.041), negative expression of PD-L1 in IC (Figure 3C, p=0.023), high β-catenin expression (Figure 3D, p=0.001), negative expression of CMTM6 (Figure 3E, p=0.018), and pMMR (Figure 3F, p=0.011) subgroups, respectively.

Table 1. Correlation of PD-L1, CMTM6, and β-catenin expression and clinicopathological parameters.

Clinicopathological parameters	n	PD-L1 (TC)[a]	p-value	PD-L1 (IC)[b]	p-value	CMTM6	p-value
Age							
≤60	322	48 (14.9%)	0.007	103 (32%)	0.144	79 (24.5%)	0.334
>60	382	32 (8.4%)		103 (27%)		106 (27.7%)	
Gender							
Male	396	42 (10.6%)	0.473	108 (27.3%)	0.188	114 (28.8%)	0.086
Female	308	38 (12.3%)		98 (31.8%)		71 (23.1%)	
Tumor location							
Left-sided	529	49 (9.3%)	0.002	156 (29.5%)	0.817	147 (27.8%)	0.114
Right-sided	175	31 (17.7%)		50 (28.6%)		38 (21.7%)	
Histological subtype							
Adenocarcinoma	674	80 (11.9%)	0.087	204 (30.3%)	0.005	179 (26.6%)	0.425
Mucinous	30	0 (0%)		2 (6.7%)		6 (20.0%)	
Histologic grade							
Low	638	71 (11.1%)	0.541	187 (29.3%)	0.929	166 (26.0%)	0.627
High	66	9 (13.6%)		19 (28.8%)		19 (28.8%)	
Lymphatic metastasis							
No	379	43 (11.3%)	0.987	118 (31.1%)	0.238	95 (25.1%)	0.430
Yes	325	37 (11.4%)		88 (27.1%)		90 (27.7%)	
TNM stage							
I–II (1+2)	309	38 (12.3%)	0.490	102 (33%)	0.053	81 (26.2%)	0.972
III–IV (3+4)	395	42 (10.6%)		104 (26.3%)		104 (26.3%)	
MMR proteins							
pMMR	608	61 (10.0%)	0.005	175 (28.8%)	0.483	169 (27.8%)	**0.021**
dMMR	96	19 (19.8%)		31 (32.3%)		16 (16.7%)	
β-catenin							
High	435	57 (13.1%)	0.064	154 (35.4%)	<0.001	140 (32.2%)	<0.001
Low	269	23 (8.6%)		52 (19.3%)		45 (16.7%)	
CMTM6							
Positive	185	32 (17.3%)	0.003	65 (35.1%)	0.041		
Negative	519	48 (9.2%)		141 (27.2%)			

Notes: [a]PD-L1 expression in tumor cells; [b]PD-L1 expression in tumor-infiltrating immune cells

Table 2. Univariate and multivariate analyses of factors associated with overall survival (OS) in CRC patients.

Variable	OS (Univariate analysis)			OS (Multivariate analysis)		
	HR	95% CI	p-value	HR	95% CI	p-value
Age/year (≤60 vs. >60)	2.029	1.514–2.719	**<0.001**	2.146	1.598–2.881	**<0.001**
Gender (male vs. female)	0.853	0.646–1.126	0.262	0.883	0.664–1.175	0.394
Tumor location (left-sided vs. right-sided)	1.138	0.833–1.553	0.417	1.190	0.855–1.657	0.301
Histological subtype (adenocarcinoma vs. mucinous)	2.121	1.231–3.654	**0.007**	1.071	0.433–2.648	0.881
Histologic grade (low vs. high)	1.653	1.061–2.575	**0.026**	1.812	1.152–2.851	**0.010**
Lymphatic metastasis (no vs. yes)	2.293	1.733–3.033	**<0.001**	0.757	0.508–1.127	0.171
TNM stage (I+II vs. III+IV)	3.296	2.402–4.522	**<0.001**	3.353	2.438–4.612	**<0.001**
Expression of MMR proteins (pMMR vs. dMMR)	0.383	0.218–0.672	**0.001**	0.374	0.210–0.664	**0.001**
Expression of β-catenin protein (high vs. low)	0.822	0.623–1.084	0.165	0.922	0.684–1.242	0.592
Expression of CMTM6 protein (positive vs. negative)	1.107	0.812–1.509	0.520	1.192	0.865–1.647	0.288
Expression of PD-L1 protein in TC[a] (positive vs. negative)	1.703	0.989–2.933	0.055	1.261	0.705–2.258	0.434
Expression of PD-L1 protein in IC[b] (positive vs. negative)	1.700	1.202–2.403	**0.003**	1.486	1.049–2.104	**0.026**

Notes: [a]PD-L1 expression in tumor cells; [b]PD-L1 expression in tumor-infiltrating immune cells. Abbreviations: HR-Hazard ratio; CI-Confidence interval

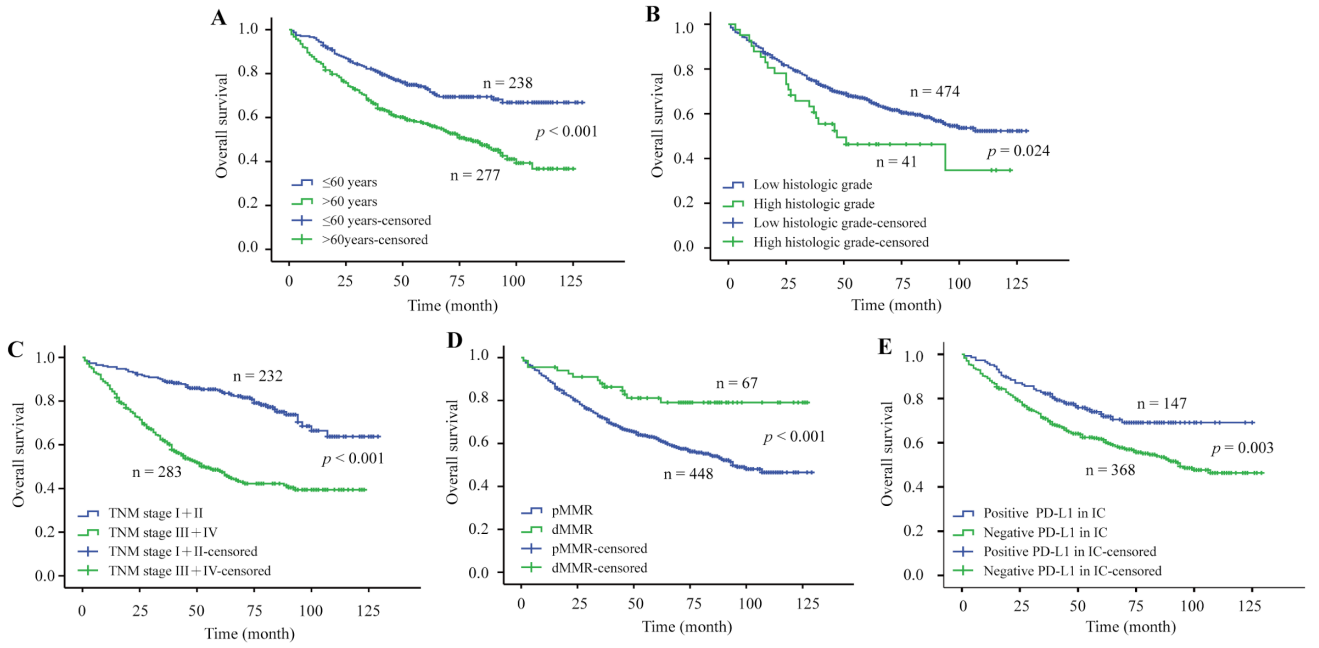

Figure 2. Kaplan-Meier curves of CRC patients. Kaplan-Meier survival curves for the overall survival of CRC patients (total n=515) according to age (A), histologic grade (B), TNM stage (C), MMR proteins (D), and PD-L1 expression in IC (E).

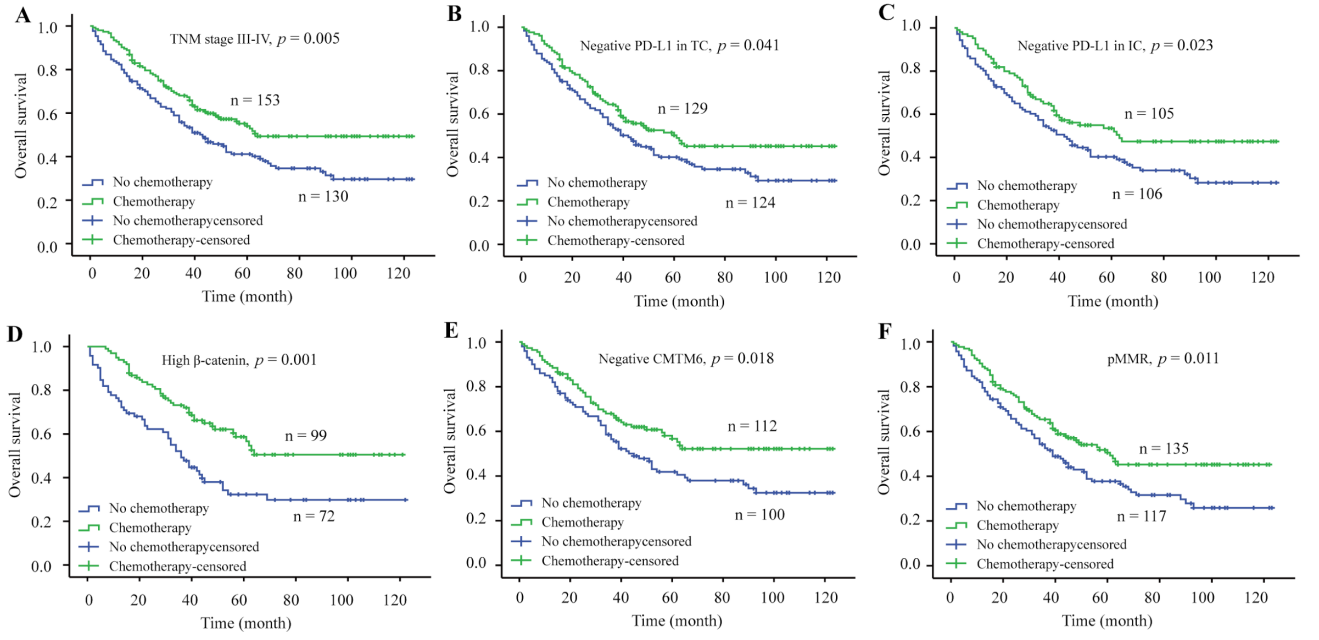

Figure 3. Kaplan-Meier survival curves for the overall survival for TNM stage III-IV CRC patients according to chemotherapy (A, total n=283) and the negative expression of PD-L1 in TC (B, total n=253), negative expression of PD-L1 in IC (C, total n=211), high β-catenin expression (D, total n=171), negative expression of CMTM6 (E, total n=212) and pMMR (F, total n=252) subgroups.

Discussion

With the in-depth exploration of new principles and methods of tumor immunology, immunotherapy of malignant tumors has become a new anti-tumor therapy with significant clinical effects and advantages after surgery, radiotherapy, and chemotherapy [16–18]. Studies have shown that different antibody clones require different criteria to determine the PD-L1 positivity [19, 20]. What's more, it seems the same antibody clone also requires different

criteria to determine the PD-L1 positivity in different cell types. For example, NSCLC tissue with 'high PD-L1 expression' using Ventana SP142 assay is defined as those with either ≥50% TC PD-L1 staining or ≥10% IC PD-L1 staining independent of TC PD-L1 staining [21, 22]. The Blueprint PD-L1 IHC comparability project assesses the feasibility of harmonizing the clinical use of five independently developed commercial PD-L1 IHC assays by using clinical lung cancer samples to overcome the detecting efficiency among different antibodies [23]. In this study, two independent PD-L1 antibodies, SP142 and IHC411 were used to access the expression of PD-L1 in TMA containing 781 CRC cases. We assessed the expression levels of PD-L1 in TC and IC by immunohistochemistry. TC or IC that had both positive/negative staining with two antibodies SP142 and IHC411 were defined as the real positive/negative expression of PD-L1 in 704 CRC cases. The positive expression rate of PD-L1 detected by two different antibodies in TC and IC was 11.36% and 29.26%, respectively.

After determining the expression of PD-L1 in CRC tissues, we evaluated the correlation between PD-L1 expressions and CRC clinicopathological parameters and prognosis. The expression of PD-L1 in TC is associated with younger CRC patients, right colon, and dMMR status. Recent studies also indicate that MSI gastrointestinal cancers harbor high PD-L1/PD-1 expression [24, 25]. CRC with dMMR/MSI status has a distinct phenotype characterized by the proximal colon, poor differentiation, and young patients [26]. Taken into consideration, these results suggested that the expression pattern of PD-L1 in TC closely correlates with the dMMR/MSI phenotype. In addition, the positive expression rate of PD-L1 in IC of adenocarcinoma is significantly higher than that in mucinous adenocarcinoma. These results also suggested that the correlation between PD-L1 protein expression in TC and IC and clinicopathological parameters is not fully consistent. Berntsson et al. also found that PD-L1 expression in immune cells and tumor cells carry different prognostic values and might be regulated by distinct mechanisms [27].

In univariate analysis, age, histological subtype, histologic grade, lymphatic metastasis, TNM stage, MMR status, and expression of PD-L1 protein in IC were significantly associated with the overall survival. In multivariate analysis, apart from age, histologic grade, TNM stage, MMR status, the classic prognostic indicators of CRC, we found that the positive expression of PD-L1 in IC is an independent prognostic factor. Interestingly, other independent studies have found similar results [27, 28]. Wyss et al. found that stromal PD-L1 expression was associated with less aggressive tumor behavior (lower frequency pT3-T4 tumors, lower frequency lymph node metastasis, lower frequency distant metastasis, and lower frequency stage IV tumors in colon cancer patients), which was translated into the better OS and disease-free survival [28]. In this study, we also found that positive expression of PD-L1 in IC is associated with a trend towards the earlier TNM stage (I–II) (p=0.053). Conversely, another study found that PD-L1 expression in tumor-infiltrating immune cells was associated with a poor outcome [29]. Therefore, the significance and mechanism of PD-L1 expression in IC of CRC still need further evaluation.

CMTM is the human chemokine-like factor superfamily, including nine member-chemokine-like factors CKLF and CMTM1-8. This family of genes plays an important role in regulating human immunity, reproduction, and hematopoiesis. Li et al. found that CMTM6 expression was significantly related to PD-L1 in gastric cancer cells [30]. In addition, Shang et al. found that CMTM6 was positively correlated with PD-L1 expression in lung squamous carcinoma cells and immune cells infiltration [31]. Peng et al. also found that CMTM6 expression in CRC cells was significantly higher in PD-L1 (tumor stroma)+ than in PD-L1 (tumor stroma)- of CRC. They also found that CMTM6 levels are positively correlated with the immune response in CRC cells [32]. Similarly, we found that the expression of PD-L1 in TC and IC were positively correlated with CMTM6 in CRC. Recent studies have shown that CMTM6 directly binds to PD-L1 protein on the cell membrane surface, reducing its ubiquitination and prolonging the half-life of PD-L1 protein. Meanwhile, CMTM6 inhibition downregulates the expression of PD-L1 protein in tumor cells including primary colorectal cancer cells [10–12]. Genetic mutations in the β-catenin destruction complex, lead to β-catenin over activation in most sporadic CRC [33–35]. Studies have shown that the abnormally activated Wnt/β-catenin signaling pathway reduces the immunotherapy effect by regulating T cell infiltration into tumor cells [14, 15]. What's more, clinical studies have shown that patients with Wnt-activated colorectal cancer have a low response rate to immunoassay inhibitors [36]. Tumor stem cells can stabilize and upregulate PD-L1 through the EMT/β-catenin/STT3/PD-L1 axis, thereby achieving the immune escape [37]. In this study, we used IHC to detect the expression of CMTM6 and β-catenin in CRC tissues and evaluated the correlation of PD-L1, CMTM6, and β-catenin expression. The findings of this study show that the expressions of PD-L1 and CMTM6 were closely correlated with β-catenin expression. Studies have found that abnormal activation of the Wnt/β-catenin signaling pathway plays an important role in the tumor microenvironment [38–41]. Taken into consideration together, the Wnt signaling pathway may affect PD-L1 expression by targeting β-catenin/CMTM6/PD-L1 axis, inhibition this oncogenesis axis may provide a new promising immunotherapy strategy for CRC patients.

5-Fu and its derivatives are commonly used in CRC chemotherapy. In this study, our findings suggested that the survival curves of the adjuvant chemotherapy group were significantly higher than that of the non-chemotherapy in TNM stage III–IV CRC patients. Interestingly, our study evaluated the predictive value of β-catenin/CMTM6/PD-L1 protein expressions of CRC in adjuvant chemotherapy. TNM stage III–IV CRC patients with negative expression of PD-L1 in TC, negative expression of PD-L1 in IC, high β-catenin

expression, negative expression of CMTM6, and pMMR subgroups obtain benefit from adjuvant chemotherapy. However, TNM stage III–IV CRC patients with positive PD-L1 expression both in TC and IC, positive CMTM6, low β-catenin expression, and dMMR status have not obtained benefit from adjuvant chemotherapy. These results lay the foundation for further research on the underlying mechanism of the β-catenin/CMTM6/PD-L1 axis in CRC chemotherapy resistance.

Being consistent with our results, many studies indicated that CRC patients with pMMR/MSS status obtain benefits from adjuvant 5-Fu chemotherapy [42, 43]. Other research results also indicated that CRC patients with MSI-H status have not obtained benefits from adjuvant 5-Fu chemotherapy [44]. In addition, many studies indicated that the Wnt/β-catenin pathway activation can enhance the drug resistance activity of CRC cells [45, 46]. In this study, we found that patients with high β-catenin expression in CRC tissues can benefit from adjuvant chemotherapy. However, this effect was not observed in CRC patients with low β-catenin expression. Interestingly, we found that patients with negative expression of PD-L1 and CMTM6 obtain benefits from adjuvant chemotherapy. Some published findings were similar to ours [47, 48]. It was reported that CMTM6 drives cisplatin resistance by interaction with membrane-bound enolase-1 stabilized its expression, leading to activation of Wnt signaling mediated by AKT-GSK-3β [47]. Philip et al. also found that patients with low expression of the PD-L1 gene significantly obtain benefits from adjuvant chemotherapy, patients in the higher PD-L1 subgroup have poorer RFS following treatment in CRC [48]. Meanwhile, ICPI that block the interaction of PD-1 and PD-L1 checkpoint proteins have been successfully applied to the treatment of mCRC with MSI-H/dMMR status and positive PD-L1 expression [34, 49]. Taken together, CRC patients with different β-catenin/CMTM6/PD-L1 expressions may diversion to different therapeutic strategy.

In conclusion, the expression of CMTM6 and PD-L1 in CRC is positively associated with β-catenin. The expressions of β-catenin, CMTM6, and PD-L1 show as a reliable biomarker for predicting chemotherapy response. β-catenin, CMTM6, and PD-L1 expression, and MMR status may be valuable biomarkers for guiding different treatment strategies in CRC patients.

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