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Original Article

Clinicopathologic and prognostic significance of CMTM6 and PD-L1 expression in cervical squamous cell carcinoma

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Abstract: It has been demonstrated that interfering with the expression of chemokine-like factor-like MARVEL transmembrane domain-containing family member 6 (CMTM6) results in impaired programmed death 1 ligand 1 (PD-L1) protein expression in human tumor cells. PD-L1 relies on CMTM6 to inhibit T cell responses and promote tumor cell proliferation. The aim of the present study was to investigate the expression of CMTM6 and PD-L1 in cervical cancer and their clinical significance. Immunohistochemistry was used to detect the expression of CMTM6 and PD-L1 in 50 normal cervical tissues and 102 cervical cancer tissue samples. The results showed that CMTM6 and PD-L1 expression was associated with clinical staging, lymph node metastasis, distant metastasis, and tumor differentiation. In addition, there was a positive association between the expression of CMTM6 and that of PD-L1 in cervical cancer tissue. Survival analysis results showed that high expression of CMTM6 and PD-L1 was positively correlated with poor prognosis in patients. Univariate analysis showed that lymph node metastasis was associated with the prognosis of cervical cancer patients. Cox analysis indicated that PD-L1 is a risk factor affecting the survival time of cervical cancer patients. In conclusion, the expression of CMTM6 and PD-L1 is elevated in cervical cancer tissue and closely related to poor prognosis. Therefore, CMTM6 and PD-L1 may be new molecular targets for the treatment of cervical cancer.

Keywords: CMTM6, PD-L1, cervical squamous cell carcinoma, metastasis, prognosis

Introduction

Cervical cancer is one of the most common malignant tumors in women worldwide [1]. In China, the incidence of cervical cancer is second only to breast cancer and seriously endangers women's health [2]. It is estimated that ~90,000 people are diagnosed with cervical cancer every year in China, and the number of deaths is ~30,000 [3]. In the latest global cancer burden data released by the International Cancer Research Institute of the World Health Organization in December 2020, the global incidence rate of cervical cancer is the seventh and its mortality rate is the ninth [4]. The inactivation of tumor suppressor genes and the activation of oncogenes play an important role in the pathogenesis of cervical cancer [5]. Therefore, searching for molecular targets for the diagnosis and treatment of cervical cancer has practical significance for the early diagnosis, prognosis, and improvement of the quality of life of patients with this disease.

Certain growth factors, cytokines, and their receptors also play a role in the occurrence and development of cervical cancer. The human chemokine-like factor superfamily (CKLFSF) was first identified in 2001. The members of this family have different spliceosomes, and each gene has at least one spliceosome encoding a product with a MARVEL domain. In 2005, the CKLFSF1-8 genes were renamed CMTM1-8 by the International Human Gene Nomenclature Committee [6]. The proteins encoded by the CMTM family can affect the proliferation and

invasion of tumor cells and participate in the regulation of cell viability. This indicates that the CMTM family plays an important role in tumorigenesis and development [7, 8].

The CMTM6 gene is located in the p22 region of chromosome 3. The CMTM6 protein is composed of 183 amino acids and has a molecular weight of 20.4 kDa. It is a type-III transmembrane protein with a MARVEL-like domain and is primarily expressed on the plasma membrane [9]. Genome-wide CRISPR-Cas9 studies have suggested that CMTM6 may be a key regulator of programmed death 1 ligand 1 (PD-L1) in several cancer cell types; indeed, CMTM6 binds to PD-L1 and maintains its expression on the cell surface, protecting PD-L1 from lysosome-mediated degradation [10, 11]. PD-L1 binds to PD-L1 on the surface of T cells, inhibiting T cell activation, proliferation, cytokine secretion, and cell survival, thereby reducing tumor specific CD8+ T cell activity, inhibiting T cell-mediated immune response, hindering T cell killing of tumor cells, and assisting in tumor immune escape [12]. However, it has been observed that the overexpression of CMTM6 protein does not affect the proliferation of cervical cancer cells [13]. This suggests that CMTM6 may serve different biologil functions in different tumor types.

The aim of the present study was to evaluate the expression of CMTM6 and PD-L1 in cervical cancer tissue. The expression of CMTM6 and PD-L1 in cervical cancer and normal cervical tissue was examined using immunohistochemistry. In addition, the relationship between CMTM6 or PD-L1 expression and the clinicopathologic data of patients with cervical cancer was also analyzed.

Materials and methods

Collection of patient samples. Cervical squamous cell carcinoma (n=102) and normal cervical tissue (n=41) samples were collected by surgical resection in Cangzhou People's Hospital (China) from January 2018 to January 2023. All patients were female, and their age range was 26-78 years. Among the patients with cervical squamous cell carcinoma, 90 cases had HPV infection. Conduct telephone follow-up on all patients and organize clinical case data. Patients were excluded i) if they had received chemotherapy, radiotherapy or adjuvant therapy prior to surgery; ii) if they were diagnosed with cervical adenocarcinoma or iii) if they were diagnosed with squamous cell carcinoma metastasized to the cervix. The research protocol was approved by The Ethics Committee of Cangzhou People's Hospital (K2020-073(6.19)). Written informed consent was obtained from all participants.

Immunohistochemistry (IHC)

Immunohistochemistry was carried out on consecutive 4-μm-thick sections of paraffin-embedded tissue samples. The slides were deparaffinized with xylene three times, rehydrated with alcohol, and subjected to antigen retrieval by heating in target retrieval solution EDTA at 92-98°C for 20 min in a microwave oven. The sections were quenched with 3% hydrogen peroxide for 5 min to block endogenous peroxidase activity. Non-specific binding was blocked by adding 5% bovine serum albumin (Beijing ComWin Biotech Co., Ltd.) for 37°C 5 min. The sections were incubated with anti-CMTM6 (Abcam; 1:100; ab264067) and anti-PD-L1 (Abcam; 1:100; ab205921) antibodies for 1 h at room temperature, then anti-rabbit (Abcam; 1:200; ab996979) for 1 h at 37°C. After each incubation, all sections were washed three times with TBST (0.5 ml/l), and chromogen detection was carried out using DAB. After counterstaining with hematoxylin for 2 min at 37°C, the sections were dehydrated, cleared and mounted.

Two independent observers (SS and HM) randomly selected five representative fields from each section. Any discrepancies were checked by both observers until a consensus was reached. CMTM6 and PD-L1 positivity (frequency of CMTM6+ or PD-L1+ cells) was graded as follows: i) Negative, 0; ii) 1-50%, 1; iii) 50-74%, 2; and iv) ≥75%, 3. The staining intensity score was graded as follows: i) Weak, 1; ii) Intermediate, 2; and iii) Strong, 3. The scores for expression positivity and staining intensity were multiplied to obtain a final score categorized as: i) 0, -; ii) 1-2, +; iii) 3-5, ++; and iv) 6-9, +++.

Kaplan-Meier plotter analysis

The prognostic significance of CMTM6 and PD-L1 (CD274) mRNA was also analyzed in cer-

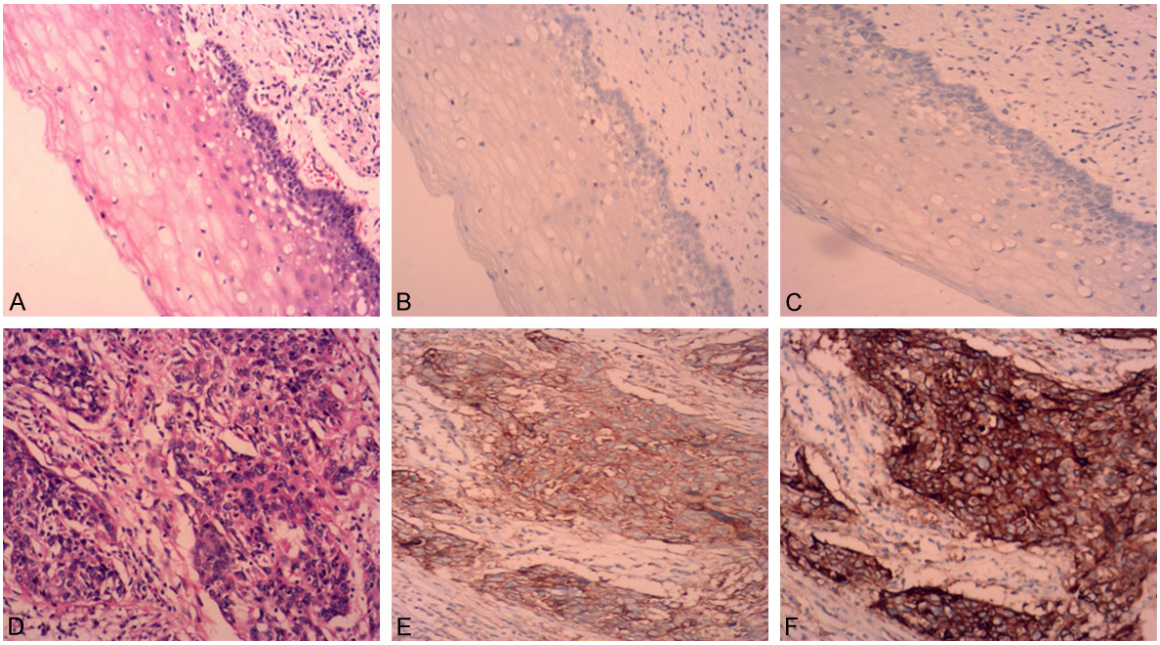

Figure 1. CMTM6 and PD-L1 expression in normal and cervical cancer tissue. A. Hematoxylin and eosin staining of normal tissue. B and C. Expression of CMTM6 and PD-L1 in normal cervical tissue. D. Hematoxylin and eosin staining of cervical cancer tissue. E and F. Expression of CMTM6 and PD-L1 in cervical cancer. Magnification, ×200. CMTM6, chemokine-like factor-like MARVEL transmembrane domain-containing family member 6; PD-L1, programmed death 1 ligand 1.

vical cancer using Kaplan-Meier plotter (http://kmplot.com).

Statistical analysis

SPSS 17.0 software was used to analyze the data. χ^2 test was used to analyze the association between CMTM6 and PD-L1 expression in cervical cancer tissue samples. The association between the expression of CMTM6 and PD-L1 and clinicopathologic data was analyzed using Spearman analysis. Survival analysis was conducted using the Kaplan Meier method, and risk factor analysis was conducted using Cox proportional risk regression. $P<0.05$ was considered a significant difference.

Results

CMTM6 and PD-L1 expression in cervical cancer tissue

CMTM6 and PD-L1 were expressed on the cell membrane. As shown in **Figure 1A** and **1D**, we performed H&E staining on cervical cancer tissue and normal cervical tissue. The expression of CMTM6 in cervical cancer tissue (**Figure 1E**) is higher than that in normal cervical tissue (**Figure 1B**). Expression of PD-L1 is lower in normal cervical tissue (**Figure 1C**) than cervical cancer tissue (**Figure 1F**). The positive rates of CMTM6 in cervical cancer and normal cervical tissue were 68.6% (70/102) and 20.0% (10/50), respectively. CMTM6 was upregulated in cervical cancer tissue ($P<0.05$; **Table 1**). The positive rate for PD-L1 expression in cervical cancer tissue was 30.4% (31/102). The expression of CMTM6 was associated with that of PD-L1 in cervical cancer tissue ($P<0.001$; **Table 2**).

Association between CMTM6 and PD-L1 expression with the clinicopathologic parameters of patients with cervical cancer

The clinicopathologic data of the patients with cervical cancer (n=76) were collected, including age, tumor size, clinical staging, lymph node metastasis, distant metastasis, tumor differentiation, vascular infiltration, HPV infection and nerve invasion. As shown in **Table 3**, CMTM6 expression was associated with clinical staging, lymph node metastasis, distant metastasis, tumor differentiation and HPV infection ($P<0.05$, **Table 3**). PD-L1 expression was associated with the clinical staging, lymph node

Table 1. CMTM6 and PD-L1 expression in normal tissue and cervical cancer tissue

Group	n	CMTM6 expression				PD-L1 expression			
		-	+	PR (%)	P	-	+	PR (%)	P
Normal	50	40	10	20.0	<0.0001	48	2	4.0	<0.0001
Cancer	102	32	70	68.6		71	31	30.4	

PR, positive rate; CMTM6, chemokine-like factor-like MARVEL transmembrane domain-containing family member 6; PD-L1, programmed death ligand 1.

Table 2. Correlation analysis of CMTM6 and PD-L1 in cervical cancer

CMTM6	PD-L1		*Total*	*p* value
	Positive	Negative		
Positive	40	30	70	<0.001
Negative	9	23	32	
Total	49	53	102	

CMTM6, chemokine-like factor-like MARVEL transmembrane domain-containing family member 6; PD-L1, programmed death ligand 1.

metastasis, distant metastasis, tumor differentiation and Nerve invasion (P<0.05, **Table 3**). Survival analysis results show that high expression of CMTM6 and PD-L1 is positively correlated with poor prognosis in patients (P<0.05, **Figure 2A** and **2B**, P<0.05). The results of univariate analysis indicate that lymph node metastasis is associated with the prognosis of cervical cancer patients with high expression of CMTM6 (P<0.05, **Table 4**). Simultaneously, it was found that high expression of PD-L1 is closely related to distant metastasis (P<0.05, **Table 4**). Cox analysis indicates that PD-L1 is a risk factor affecting the survival time of cervical cancer patients (P<0.05, **Table 5**).

Clinicopathologic and prognostic significances of CMTM6 and PD-L1 (CD274) mRNA expression in cervical cancer

According to Kaplan-Meier plotter database, we found that higher CMTM6 expression was negatively correlated with overall survival rate of Grade 3, mesenchymal stem cells enriched, and natural killer T-cells enriched (**Figure 3A-C**, P<0.05). A higher CD274 expression was positively correlated with overall survival rate of Type 1 T-helper cells enriched, White and Mutation burden high (**Figure 3D-F**, P<0.05). According to Kaplan-Meier plotter, we found that a higher CD274 expression was positively correlated with relapse-free survival rate (**Figure 4A**, P<0.05). It was the same for the patients with Grade 3, Females, Eosinophils enriched, Macrophages enriched, Basophils enriched, B-cells enriched, CD4+ memory T-cells enriched, CD8+ T-cells enriched, Natural killer T-cells enriched, Type 1 T-helper cells enriched, Type 2 T-helper cells enriched, Mesenchymal stem cells decreased, and Regulatory T-cells decreased (**Figure 4B-N**, P<0.05).

Discussion

Chemokines play an important role in cancer, autoimmune diseases, and other processes. With improved understanding of the structure and function of each member of the CMTM family, evidence suggests that CMTM proteins serve an important role in the occurrence and development of diseases, including cancer [8]. Moreover, CMTM proteins may be used as prognostic indicators for a variety of tumors and can mediate antitumor immunity by regulating the cell cycle and various signaling pathways. For example, inactivation or methylation of the CMTM3 allele can inhibit its ability to negatively regulate cell proliferation. CMTM3 reduces EGFR expression by enhancing Rab5 activity, promotes EGFR degradation, and inhibits EGF-mediated tumorigenicity of gastric cancer cells [14, 15].

CMTM6 is expressed in tumor tissue and is involved in epigenetic regulation, embryonic development, and tumorigenesis. Previous studies have suggested that CMTM6 can act as a key regulator of PD-L1 in tumor cells, binding to PD-L1 to maintain its expression on the cell surface. Indeed, CMTM6 is co-expressed with PD-L1 in cells and can prevent PD-L1 from becoming a target of lysosome-mediated degradation [16]. In non-small cell lung cancer and oral squamous cell carcinoma, a positive correlation between CMTM6 and PD-L1 protein levels has been observed [17, 18]. CMTM6 gene knockout result in downregulation of

Table 3. Relationship between expression of CMTM6 and PD-L1 protein and clinicopathologic features of cervical cancer

Feature	n	Expression							
		CMTM6				PDL-1			
		-	+	PR (%)	*P* value	-	+	PR (%)	*P* value
Age									
≤50	60	16	44	73.3	0.225	44	16	26.7	0.472
>50	62	36	26	41.9		48	14	22.6	
Size									
<3	44	15	29	65.9	0.611	35	9	20.5	0.085
≥3	58	17	41	70.7		37	21	36.2	
Lymph node metastasis									
-	79	29	50	63.3	0.031	64	15	19.0	<0.001
+	23	3	20	87.0		8	15	65.2	
Distant metastasis									
-	94	32	62	66.0	0.047	70	24	25.5	0.003
+	8	0	8	100.0		2	6	75.0	
Clinical stage									
I	67	26	41	61.2	0.025	54	13	19.4	0.002
II-III	35	6	29	82.9		18	17	48.6	
Vascular infiltration									
-	44	14	30	68.2	0.834	33	11	25.0	0.473
+	58	18	40	69.0		39	19	32.8	
Nerve invasion									
-	86	30	56	65.1	0.185	68	18	20.9	<0.001
+	16	2	14	87.5		4	12	75.0	
Differentiation									
Well	13	9	4	30.8	0.001	13	0	0.00	0.012
Moderately-Poorly	89	23	66	74.2		59	30	33.7	
HPV									
-	12	9	3	25.0	0.002	11	1	8.3	0.120
+	90	23	67	74.4		61	29	32.2	

PR, positive rate; CMTM6, chemokine-like factor-like MARVEL transmembrane domain-containing family member 6; PD-L1, programmed death 1 ligand 1.

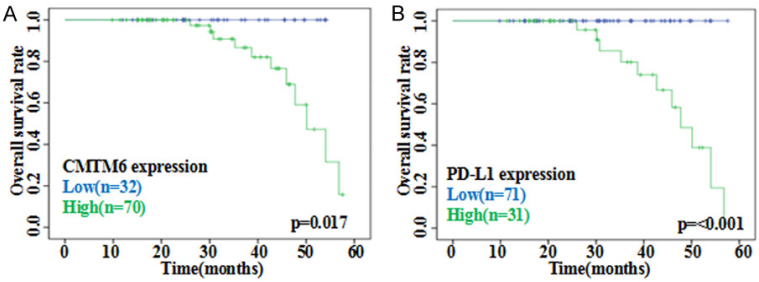

Figure 2. Relationship between the expression of CMTM6 and PD-L1 and the prognosis of cervical cancer patients. Kaplan-Meier analysis of overall survival associated with CMTM6 (A) and PD-L1 (B).

PD-L1 protein on the cell surface, but does not reduce the levels of PD-L1 mRNA, indicating that CMTM6 regulates the PD-L1 expression post-transcriptionally [10, 11]. In our study, the expression of CMTM6 was associated with that of PD-L1 in cervical cancer tissue samples. In hepatocellular carcinoma, the level of CMTM6 protein was significantly lower than that of adjacent tissue [12]. However, in the present study, the expression of CMTM6 in cervical cancer was significantly higher than that of normal tissue. This indicates that CMTM6 is expressed at different levels in

Table 4. Univariate analysis of prognostic risk factors in the patients with cervical cancer

Characteristic	CMTM6		PD-L1	
	Relative Risk (95% CI)	*p* Value	Relative Risk (95% CI)	*p* Value
Age				
≤50	1.376 (0.419-4.519)	0.599	1.342 (0.356-5.054)	0.664
>50				
Size				
<3	0.810 (0.204-3.220)	0.765	0.372 (0.085-1.625)	0.189
≥3				
Lymph node metastasis				
-	5.217 (1.330-20.456)	0.018	3.061 (0.761-12.304)	0.115
+				
Distant metastasis				
-	2.750 (0.562-13.453)	0.212	5.260 (0.947-29.231)	0.050
+				
Clinical stage				
I	1.667 (0.487-5.770)	0.412	0.561 (0.139-2.262)	0.417
II-III				
Vascular infiltration				
-	1.423 (0.404-5.019)	0.583	2.079 (0.551-7.849)	0.280
+				
Nerve invasion				
-	1.643 (0.484-5.572)	0.426	1.610 (0.468-5.537)	0.450
+				
Differentiation				
Well	4.936 (0.001-2.235*10^4)	0.581	23.371 (0-2.157*10^6)	0.589
Moderately-Poorly				
HPV				
-	0.138 (0.015-1.248)	0.078	0.336 (0.038-2.952)	0.325
+				

CI, confidence interval.

Table 5. Multivariate analysis of clinicopathological variables for the survival of the patients with cervical cancer

Clinicopathological Parameters	CMTM6	PD-L1
	P	*P*
CMTM6/PD-L1 expression (-/+)	0.963	0.010
Age (≥50 years)	0.243	0.095
Size (<3/≥3)	0.849	0.200
Differentiation (well/moderateld-poorly)	0.987	0.992
Lymph node metastasis (-/+)	0.158	0.079
Distant metastasis (-/+)	0.186	0.036
Clinical stage (I/III-IV)	0.144	0.903
Vascular infiltration (-/+)	0.455	0.090
Nerve invasion (-/+)	0.452	0.479
HPV (-/+)	0.182	0.502

CI, confidence interval.

different tumor tissue types. Moreover, CMTM6 protein expression is associated with pathologic grade, tumor metastasis, and α-fetoprotein level in hepatocellular carcinoma [12]. However, in breast cancer, the expression levels of CMTM6 are significantly higher than those of adjacent tissue and are associated with pathologic stage and HER2 expression [19]. In the present study, CMTM6 expression was associated with clinical staging, lymph node metastasis, distant metastasis, tumor differentiation, and HPV infection. There was no relationship between CMTM6 expression and other clinicopathologic features of the patients with cervical cancer. PD-L1 expression was associated with the clinical staging, lymph node metastasis, dis-

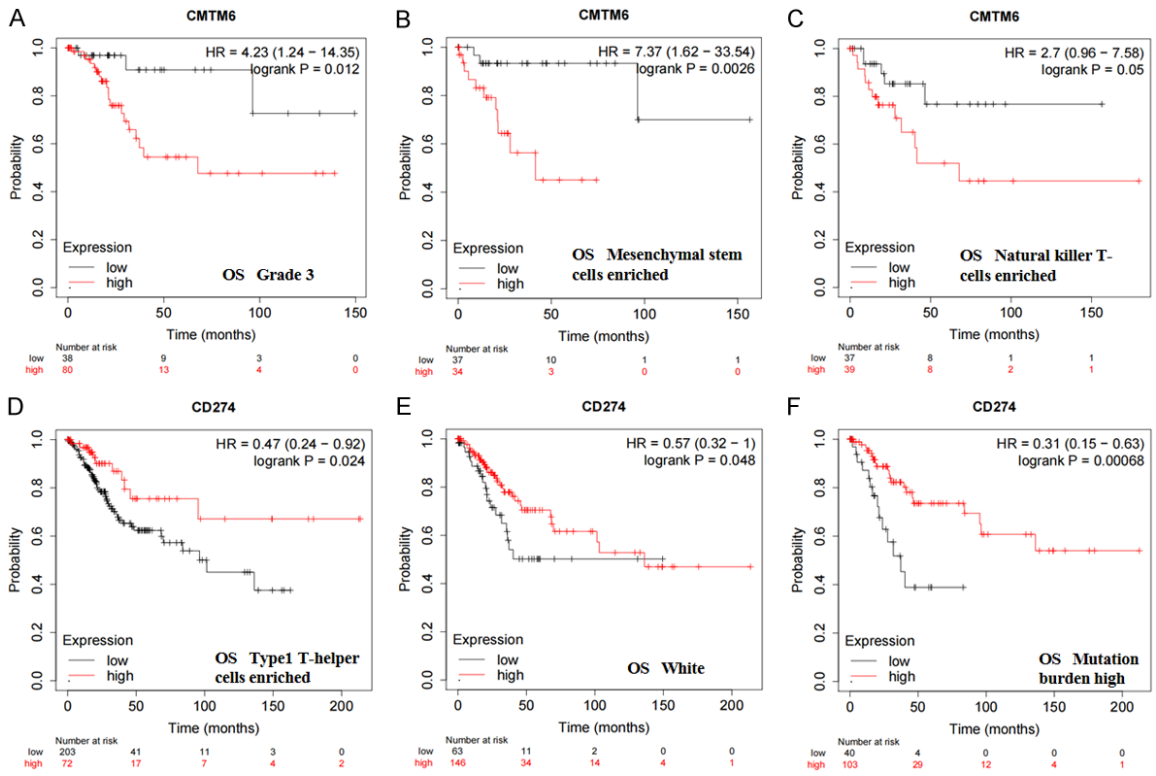

Figure 3. According to Kaplan-Meier plotter database, the expression of CMTM6 and PD-L1 and overall survival rate of patients. CMTM6 with Grade 3 (A), mesenchymal stem cells enriched (B), and natural killer T-cells enriched (C). PD-L1 with Type 1 T-helper cells enriched (D), White (E) and Mutation burden high (F).

tant metastasis, tumor differentiation and nerve invasion.

Mamesier et al. analyzed survival data from 403 patients with primary pancreatic ductal adenocarcinoma using databases such as TCGA and GEO (Gene Expression Omnibus). The results showed that high CMTM6 levels in tumor tissue were associated with lower 2-year overall survival rate [20]. Our study found a positive correlation between high expression of CMTM6 and poor prognosis of cervical cancer, and lymph node metastasis is associated with patient prognosis. In triple negative breast cancer, the high expression of PD-L1 is positively correlated with the patient's neural invasion, WHO high grade, and Ki67 high index, and WHO high grade is an independent influencing factor of PD-L1 expression [21]. We found that high levels of PD-L1 were positively correlated with poor prognosis in cervical cancer patients, and high expression of PD-L1 and distant metastasis were independent risk factors affecting patient prognosis.

Guan et al. showed through a genome-wide association study that CMTM6 can positively regulate and partially inhibit the process of T cells, and can also negatively regulate the differentiation of cytotoxic T cells and the role of T cells in killing tumor cells, suggesting that CMTM6 may participate in the genesis and development of tumors in a variety of ways [22]. Additionally, the expression of CMTM6 gene is highly positively correlated with the infiltration of CD8+ T cells, macrophages, neutrophils, and dendritic cells in human lung squamous cell carcinoma, but highly negatively correlated with the infiltration level of CD4+ T cells [23]. Our study found a higher CMTM6 expression was negatively correlated with overall survival rate of mesenchymal stem cells enriched and natural killer T-cells enriched. PD-L1 is expressed in activated T cells, B cells, dendritic cells (DC), monocytes, and macrophages [24]. PD-L1, as the ligand of PD-1, inhibits the activation, proliferation, cytokine secretion and cell survival of T cells by combining with PD-1 on the surface of T cells, thereby reducing the activity

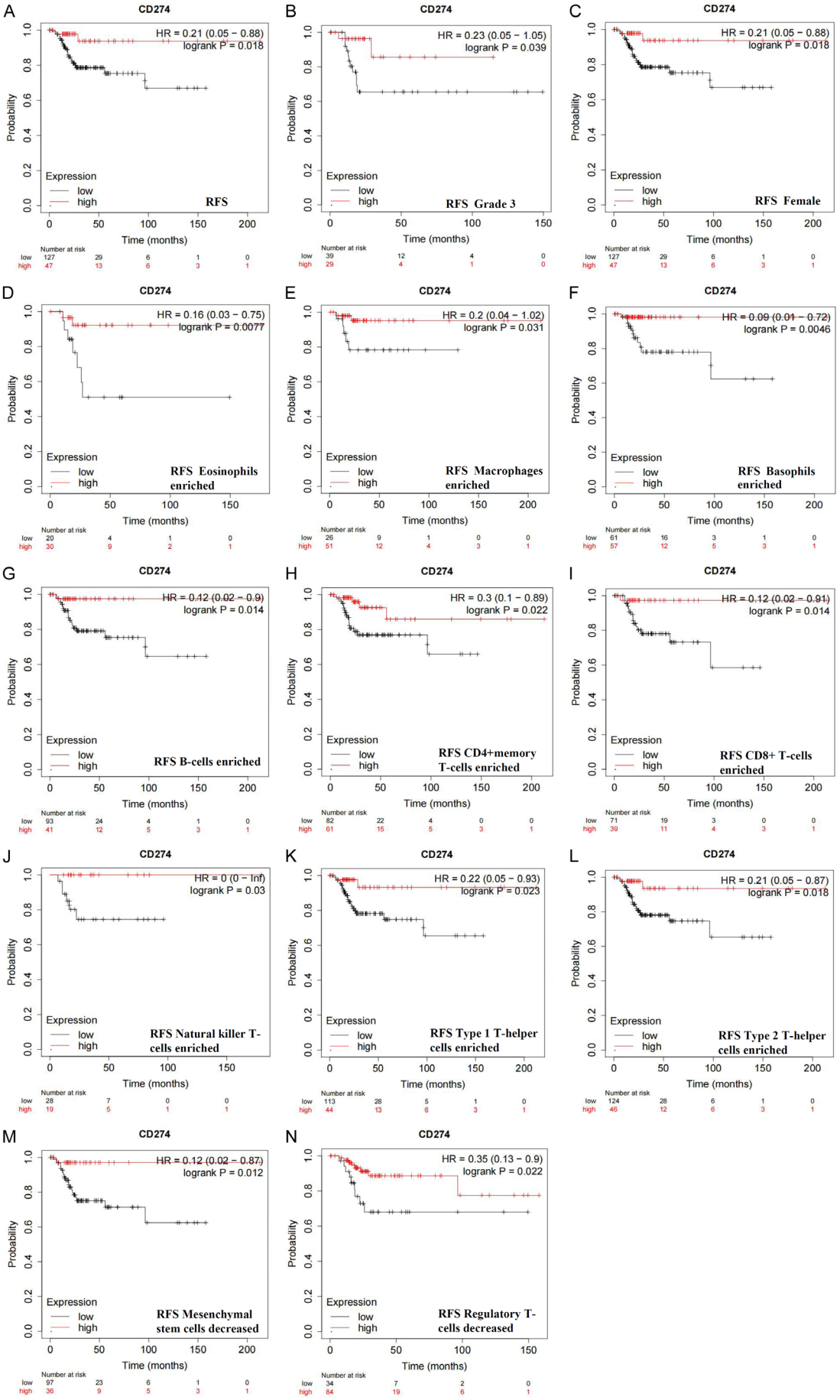
A
CD274
HR = 0.21 (0.05 − 0.88)
logrank P = 0.018
RFS
B
CD274
HR = 0.23 (0.05 − 1.05)
logrank P = 0.039
RFS Grade 3
C
CD274
HR = 0.21 (0.05 − 0.88)
logrank P = 0.018
RFS Female
D
CD274
HR = 0.16 (0.03 − 0.75)
logrank P = 0.0077
RFS Eosinophils enriched
E
CD274
HR = 0.2 (0.04 − 1.02)
logrank P = 0.031
RFS Macrophages enriched
F
CD274
HR = 0.09 (0.01 − 0.72)
logrank P = 0.0046
RFS Basophils enriched
G
CD274
HR = 0.12 (0.02 − 0.9)
logrank P = 0.014
RFS B-cells enriched
H
CD274
HR = 0.3 (0.1 − 0.89)
logrank P = 0.022
RFS CD4+memory T-cells enriched
I
CD274
HR = 0.12 (0.02 − 0.91)
logrank P = 0.014
RFS CD8+ T-cells enriched
J
CD274
HR = 0 (0 − Inf)
logrank P = 0.03
RFS Natural killer T-cells enriched
K
CD274
HR = 0.22 (0.05 − 0.93)
logrank P = 0.023
RFS Type 1 T-helper cells enriched
L
CD274
HR = 0.21 (0.05 − 0.87)
logrank P = 0.018
RFS Type 2 T-helper cells enriched
M
CD274
HR = 0.12 (0.02 − 0.87)
logrank P = 0.012
RFS Mesenchymal stem cells decreased
N
CD274
HR = 0.35 (0.13 − 0.9)
logrank P = 0.022
RFS Regulatory T-cells decreased
Probability
Expression
low
high
Time (months)
Number at risk

Figure 4. According to Kaplan-Meier plotter, the expression of and relapse-free survival rate of patients. Relapse-free survival rate (A). PD-L1 with Grade 3 (B), Females (C), Eosinophils enriched (D), Macrophages enriched (E), Basophils enriched (F), B-cells enriched (G), CD4+ memory T-cells enriched (H), CD8+ T-cells enriched (I), Natural killer T-cells enriched (J), Type 1 T-helper cells enriched (K), Type 2 T-helper cells enriched (L), Mesenchymal stem cells decreased (M), and Regulatory T-cells decreased (N).

of tumor specific CD8+ T cells, inhibiting the immune response mediated by T cells, impeding the killing of T cells to tumor cells, and assisting tumor immune escape [12]. According to Kaplan Meier plotter, we found that the higher expression of PD-L1 (CD274) was positively correlated with eosinophil enrichment, macrophage enrichment, basophil enrichment, B cell enrichment, CD4+ memory T cell enrichment, CD8+ T cell enrichment, natural killer T cell enrichment, type 1 T helper cell enrichment, type 2 helper cell enrichment, mesenchymal stem cell reduction, regulatory T cell reduction. Therefore, PD-L1/PD-1 has become a prominent molecule in tumor immune targeted therapy.

In summary, the expression of CMTM6 and PD-L1 is closely related in cervical cancer and positively correlated with poor prognosis in cervical cancer patients. CMTM6 and PD-L1 play important roles in tumor immunity, which will provide new ideas and strategies for improving the efficacy of immune checkpoints. However, further research is needed to determine whether CMTM6 and PD-L1 can become molecular targets for cervical cancer. In fact, the sample size of this study is very small, which may affect the validity of the results. Whether CMTM6 and PD-L1 can serve as biomarkers for invasion, metastasis, and prognosis of cervical cancer still needs to be experimentally validated in larger cohorts, such as through cell experiments to verify the mechanism of action of CMTM6 and PD-L1. The mechanism of action of CMTM6 and PD-L1 in cervical cancer needs further research.

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Written informed consent was obtained from all participants.

Disclosure of conflict of interest

None.

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References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

[2] Del Carmen MG and Rice LW. Management of menopausal symptoms in women with gynecologic cancers. Gynecol Oncol 2017; 146: 427-435.

[3] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-32.

[4] Sharma R. Global, regional, national burden of breast cancer in 185 countries: evidence from GLOBOCAN 2018. Breast Cancer Res Treat 2021; 187: 557-567.

[5] Ma LJ, He JC and Chai ZY. Expression of inhibitor of differentiation-1 in cervical cancer tissues and its correlation with Ki-67. The Chinese Journal of Clinical Pharmacology 2015; 627-630.

[6] Han W, Ding P, Xu M, Wang L, Rui M, Shi S, Liu Y, Zheng Y, Chen Y, Yang T and Ma D. Identification of eight genes encoding chemokine-like factor superfamily members 1-8 (CKLFSF1-8) by in silico cloning and experimental validation. Genomics 2003; 81: 609-17.

[7] Yuan W, Liu B, Wang X, Li T, Xue H, Mo X, Yang S, Ding S and Han W. CMTM3 decreases EGFR expression and EGF-mediated tumorigenicity by promoting Rab5 activity in gastric cancer. Cancer Lett 2017; 386: 77-86.

[8] Delic S, Thuy A, Schulze M, Proescholdt MA, Dietrich P, Bosserhoff AK and Riemenschneider MJ. Systematic investigation of CMTM family genes suggests relevance to glioblastoma pathogenesis and CMTM1 and CMTM3 as priority targets. Genes Chromosomes Cancer 2015; 54: 433-43.

[9] Mamessier E, Birnbaum DJ, Finetti P, Birnbaum D and Bertucci F. CMTM6 stabilizes PD-

L1 expression and refines its prognostic value in tumors. Ann Transl Med 2018; 6: 54.
[10] Burr ML, Sparbier CE, Chan YC, Williamson JC, Woods K, Beavis PA, Lam EYN, Henderson MA, Bell CC, Stolzenburg S, Gilan O, Bloor S, Noori T, Morgens DW, Bassik MC, Neeson PJ, Behren A, Darcy PK, Dawson SJ, Voskoboinik I, Trapani JA, Cebon J, Lehner PJ and Dawson MA. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. Nature 2017; 549: 101-105.
[11] Mezzadra R, Sun C, Jae LT, Gomez-Eerland R, de Vries E, Wu W, Logtenberg MEW, Slagter M, Rozeman EA, Hofland I, Broeks A, Horlings HM, Wessels LFA, Blank CU, Xiao Y, Heck AJR, Borst J, Brummelkamp TR and Schumacher TNM. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. Nature 2017; 549: 106-110.
[12] Scimeca M, Bonfiglio R, Urbano N, Cerroni C, Anemona L, Montanaro M, Fazi S, Schillaci O, Mauriello A and Bonanno E. Programmed death ligand 1 expression in prostate cancer cells is associated with deep changes of the tumor inflammatory infiltrate composition. Urol Oncol 2019; 37: 297.e19-297.e31.
[13] Li H, Li J, Su Y, Fan Y, Guo X, Li L, Su X, Rong R, Ying J, Mo X, Liu K, Zhang Z, Yang F, Jiang G, Wang J, Zhang Y, Ma D, Tao Q and Han W. A novel 3p22.3 gene CMTM7 represses oncogenic EGFR signaling and inhibits cancer cell growth. Oncogene 2014; 33: 3109-18.
[14] Ding Y and Jiang JT. Biological function and mechanism of CMTM family. Chin J Cancer Biother 2019; 26: 104-108.
[15] Yuan W, Liu B, Wang X, Li T, Xue H, Mo X, Yang S, Ding S and Han W. CMTM3 decreases EGFR expression and EGF-mediated tumorigenicity by promoting Rab5 activity in gastric cancer. Cancer Lett 2017; 386: 77-86.
[16] Zhao YF. PD1/PD-L1 inhibitors to treat tumors. Chinese Journal of Cancer Prevention and Treatment 2017; 24: 904-911.
[17] Koh YW, Han JH, Haam S, Jung J and Lee HW. Increased CMTM6 can predict the clinical response to PD-1 inhibitors in non-small cell lung cancer patients. Oncoimmunology 2019; 8: e1629261.
[18] Feng XB, Zhao JZ, Li W, Zhang YY and Zhang ST. Expression of PD-1, PD-L1 and CMTM6 in oral squamous cell carcinoma and its significance. Journal of Chinese Oncology 2019; 25: 577-580.
[19] Yang XJ, Wei L, Zheng X, Xu B, Wang Q, Liu YT, Zhang DC and Jiang JT. Expression of CMTM6 in breast cancer tissues and its correlation with patients' clinicopathological characteristics and prognosis. Chin J Cancer Biother 2020; 27: 59-63.
[20] Mamessier E, Birnbaum DJ, Finetti P, Birnbaum D and Bertucci F. CMTM6 stabilizes PD-L1 expression and refines its prognostic value in tumors. Ann Transl Med 2018; 6: 54.
[21] Meng Y, Wu HY, Yao YZ and Li R. Expression of progranmmed cell death ligand 1 in invasive breast cancer and its clinical significance. Cancer Res Clinic 2020; 32: 540-545.
[22] Guan X, Zhang C, Zhao J, Sun G, Song Q and Jia W. CMTM6 overexpression is associated with molecular and clinical characteristics of malignancy and predicts poor prognosis in gliomas. EBioMedicine 2018; 35: 233-243.
[23] Shang X, Li J, Wang H, Li Z, Lin J, Chen D and Wang H. CMTM6 is positively correlated with PD-L1 expression and immune cells infiltration in lung squamous carcinoma. Int Immunopharmacol 2020; 88: 106864.
[24] Su Y, Wang Q, Ru Y, Dong J, Li X and Zhang Z. Regulation of PD-L1 posttranslational modification and its application progress in tumor immunotherapy. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2022; 38: 1036-1043.