Expression and Clinical Significance of CMTM6 and PD-L1 in Colorectal Cancer

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Abstract: *Objective:* To study the expression of CMTM6 in colorectal cancer tissues and explore its relationship with tumor stage, lymph node metastasis, and prognosis. *Methods:* All patients underwent surgical resection and histopathological examination, and the collected tissue specimens were pathologically classified and divided into 41 cases in the cancer group and 75 cases in the paracancerous group to observe and analyze the expression of CMTM6 and PD-L1. *Results:* There was no statistically significant difference in the comparison of gender, age, disease duration, and other data between the two groups ($P > 0.05$); the high expression rate of CMTM6 in the tissues of the cancer group and the 2 groups of the paracarcinoma group was 82.93% and 1.33%, respectively, with statistically significant differences ($P < 0.05$), and the high expression rate of PD-L1 in the tissues of the cancer group and the 2 groups of the paracarcinoma group were 85.37% and 8.00%, respectively, with statistically significant differences ($P < 0.05$). The relationship between high expression of CMTM6 and PD-L1 and tumor diameter, differentiation degree, distant metastasis, and lymphatic metastasis was not statistically significant when compared between groups ($P > 0.05$). *Conclusion:* CMTM6 and PD-L1 are one of the factors predicting poor prognosis of colorectal cancer, and can be used as one of the reference indexes for treatment selection of colorectal cancer patients.

Keywords: CMTM6; PD-L1; Colorectal cancer

1. Introduction

Colorectal cancer is one of the common malignant tumors, which seriously threatens human health. According to statistics, it ranks third in the incidence rate of all cancers in China. With the continuous emergence of tumor molecularly targeted therapeutic drugs and the rapid development of immunotherapy strategies, its mortality rate has been decreasing year by year, and the 5-year survival rate has increased from 24% in 2003 to 69.1% at present. In recent years, more and more studies have found that tumor cells can express a variety of chemokine receptors (CCRs), mainly including CCL5, CCL7, and CXCL8 [1]. Among them, CCL5 is expressed at high levels in a variety of tumors and can attract macrophages to migrate to the tumor site. However, it has also been reported that CCL5 is not expressed in colorectal cancer cell lines, suggesting that it may be involved

in the process of tumorigenesis and development [2]. Meanwhile, both CCL7 and CCL8 were found to induce transplantation tumor formation in mice [3]. In addition, CCL7 has been shown to activate NK cells and promote the body's anti-tumor immune response. In addition, it has been shown that CCL8 causes human colorectal cancer cells to express PD-L1, which in turn promotes the proliferation and invasive metastatic ability of tumor cells [3]. Therefore, CCL5, CCL7, and CCL8 can all be potential biomarkers. There are fewer research reports on chemokine receptors in colorectal tumors, but with deeper research in this field, it is found that they may play an important role in tumorigenesis and development. Among them, CMTM6, as a member of the CCL5 family, its expression level in tumor tissues is affected by external stimuli in a time-dependent manner. This study investigated the expression and role of CMTM6 in colorectal cancer, aiming to provide a reference for further understanding of the pathogenesis of colorectal cancer. In this study, the expression of CMTM6 and PD-L1 in colorectal cancer tissues and normal tissues around the cancer were detected to analyze their relationship with the clinical characteristics and prognosis of patients, and the mechanism of CMTM6 and PD-L1 in colorectal cancer was further explored to provide a basis for the understanding of the role of CMTM6 in colorectal cancer, and to provide a new way of thinking for the clinical treatment of colorectal cancer patients.

2. Materials and methods

2.1. General information

In this study, 116 patients with colorectal cancer who were surgically treated in the Affiliated Hospital of Hebei Engineering University from August 2022 to August 2023 were included, including 72 males and 44 females, with an age range of 35–80 years, with a mean age of 60.31 ± 11.33 years. All patients underwent surgical resection and histopathological examination, and the collected tissue specimens were pathologically classified and divided into 41 cases in the cancer group and 75 cases in the paraneoplastic group. The cancer group included malignant tumor lesion tissues and non-tumor lesion tissues; the paracancer group included normal tissues of the colonic mucosa, muscularis propria, or plasma layer.

2.2. Methods

(1) Collection method: (a) Take fresh tissue specimens from patients; (b) Collect lymph nodes that have not been dislodged during the operation, and then send them to the Department of Pathology for consultation after rinsing clean with saline; (c) Collect the results of patients' early postoperative pathological examinations and make pathological diagnosis in parallel.

(2) Reagents and instruments: The qRT-PCR kit was purchased from Beijing Qingrun Gene Technology Co., Ltd, and the WB detection kit was purchased from Thermo, USA.

(3) Extraction and testing methods: (a) Total cellular RNA was extracted and reverse transcribed into cDNA. The amplified product was detected by 1% agarose gel electrophoresis to detect the fragment size and concentration, select the appropriate primers, and carry out PCR amplification by PCR instrument; (b) The tissue samples were diluted with ddH_2O for protein quantification. The WB method was used to detect the expression level of CMTM6. The specific operation steps were as follows: a small amount of tissue was taken into 4% neutral buffered saline for fixation and washed. Quantitative saline, phosphate buffer, and anti-CMTM6 primary antibody (1:400) were added sequentially and mixed well. Subsequently, it was incubated in the refrigerator at 4°C overnight and removed the next day. The secondary antibody (1:1000) was then added and incubated for 1 h at room temperature on a shaker protected from light, after which horseradish peroxidase-labeled goat serum (HRP-Goat Anti-Rabbit IgG) dilution (1:1000) was added. Finally, the precipitate was washed and the absorbance value

was determined on a fully automated enzyme marker.

2.3. Observation index

Firstly, the whole section was observed under a low-power microscope, and the area with the most expression was selected. Positive staining was shown when a yellow to tan color was presented in the cytoplasm or cell membrane, and the results were judged by the criteria [5]. The staining intensity of the positive cells was classified as follows: 0 for no staining, 1 for light yellow, 2 for tan, and 3 for brownish color. The grading of positive cell density is as follows: the number of positive cells < 10% is 1 point, 10%–50% is 2 points, > 50% is 3 points, and the result of multiplying two scores ≥ 3 is positive, i.e., high expression.

2.4. Statistical methods

All data were statistically analyzed using SPSS 21.0 software. The chi-squared test and t-test were used for data analysis, and $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Comparison of clinical data

There is no statistically significant difference in the comparison of gender, age, disease duration, and other information between the two groups ($P > 0.05$), as shown in **Table 1**.

Table 1. Comparison of clinical data between the two groups

Group	Gender [n (%)]	Age (mean ± SD)	Disease duration (mean ± SD)
Cancer group (n = 41)	M 28 (68.29); F 13 (31.71)	58.23 ± 10.09	2.13 ± 0.69
Paracancer group (n = 75)	M 44 (58.67); F 31 (41.33)	58.31 ± 11.02	2.01 ± 0.78
χ^2 / t	1.043	0.039	0.824
P	> 0.05	> 0.05	> 0.05

3.2. Expression of CMTM6 and PD-L1 in tissues of 2 groups

The high expression rate of CMTM6 in the tissues of the 2 groups of cancer group and paracancer group was 82.93% and 1.33%, respectively, with statistically significant differences ($P < 0.05$), and the high expression rate of PD-L1 in the tissues of the 2 groups of cancer group and paracancer group was 85.37% and 8.00%, respectively, with statistically significant differences ($P < 0.05$), as shown in **Table 2**.

Table 2. Expression of CMTM6 and PD-L1 in tissues of 2 groups [n (%)]

Group	CMTM6		PD-L1	
	High expression	Low expression	High expression	Low expression
Cancer group (n = 41)	34 (82.93)	7 (17.07)	35 (85.37)	6 (14.63)
Paracancer group (n = 75)	1 (1.33)	74 (98.67)	6 (8.00)	69 (92.00)
χ^2	83.765		69.432	
P	< 0.05		< 0.05	

3.3. Relationship between high expression of CMTM6, PD-L1, and tumor diameter, differentiation degree, distant metastasis, lymphatic metastasis

The relationship between CMTM6, PD-L1 high expression and tumor diameter, differentiation degree, distant metastasis, and lymphatic metastasis was not statistically significant when compared between groups ($P > 0.05$), as shown in **Table 3**.

Table 3. Relationship between high expression of CMTM6, PD-L1, and tumor diameter, differentiation degree, distant metastasis, lymphatic metastasis

Group	Tumor diameter (cm)	Clinical staging		Degree of differentiation		Distance metastasis		Lymphatic node transfer	
		I–II	III–IV	Low polarization	Medium and high polarization	Yes	No	Yes	No
Cancer group (n = 41)	2.31 ± 0.79	21 (65.63)	13 (34.37)	11 (34.37)	23 (65.63)	10 (31.25)	24 (68.75)	8 (25.00)	26 (75.00)
Paracancer group n = 75	2.47 ± 1.01	17 (40.48)	18 (59.52)	9 (69.05)	26 (30.95)	12 (76.19)	23 (23.81)	11 (73.81)	24 (26.19)
χ^2 / t	0.732	1.213		0.369		0.189		0.539	
P	> 0.05	> 0.05		> 0.05		> 0.05		> 0.05	

4. Discussion

At present, the clinical treatment of colorectal cancer mainly consists of surgical resection and radiotherapy, however, the radicality of surgery and postoperative recurrence and metastasis are important reasons for the survival and survival rate of tumor patients. Recent studies have shown that tumor cells can escape from the body's immune surveillance and suppress autoimmune responses in various ways, among which the phenomenon of "immune escape" plays a key role in the development of tumors [6,7]. Therefore, the search for immune escape mechanisms and their related molecular markers has become a hot research topic.

He *et al.* [8] compared the expression of CMTM6 in patients with different stages of colorectal cancer and found that CMTM6 was highly expressed in colorectal cancer, especially in patients with advanced colorectal cancer, and this high expression correlated with poor prognosis, which suggests that CMTM6 may be a new predictor of prognosis, whereas the expression of PD-L1 was negative, which may be related to the infiltration of immune cells. immune cell infiltration. Meanwhile, Dong *et al.* [9] concluded that the expression level of PD-L1 was positively correlated with the degree of tumor malignancy, i.e., those with high expression of PD-L1 tended to have higher clinical stage as well as poorer prognosis. In addition, Dan *et al.* [10] reported that tumor cells can induce exosome secretion, and exosomes can carry a variety of protein molecules, including miRNAs, to the extracellular space and participate in the malignant behavior of tumor cells. In addition to exosomes, TGF-β1 is also positively expressed in cancer tissues and is closely correlated with the TNM stage of patients, which may suggest that the high expression of TGF-β1 promotes tumor progression and leads to poor prognosis. However, there has not been any study on what kind of protein molecules can be carried by exosomes to the extracellular space, which will provide a direction for subsequent research.

In this study, we found that the high expression rate of CMTM6 in the tissues of the cancer group and the 2 groups of the paracancer group was 82.93% and 1.33%, respectively, with statistically significant differences ($P < 0.05$), and the high expression rate of PD-L1 in the tissues of the cancer group and the 2 groups of the paracancer group was 85.37% and 8.00%, respectively, with statistically significant differences ($P < 0.05$),

and the relationship between the high CMTM6 and PD-L1 CMTM6 and PD-L1 were closely related to each other in colorectal cancer, and they may be jointly involved in the immune escape process of colorectal cancer. CMTM6 may affect the interaction between tumor cells and immune cells by regulating the expression and function of PD-L1, thus realizing the immune escape process against colorectal cancer. interactions between tumor cells and immune cells, thus realizing the regulation of colorectal cancer progression. CMTM6 may be involved in colorectal cancer development and progression by regulating the biological behaviors of tumor cells such as proliferation, apoptosis, and invasion. In addition, CMTM6 may also affect the immune escape of tumor cells by binding to receptors on the surface of immune cells and inhibiting the activity of immune cells, thus promoting the immune escape of tumor cells.PD-L1, a member of the B7 family, is mainly expressed on the surface of tumor cells and antigen-presenting cells, and inhibits the activity of T cells by binding to the PD-1 receptor on the surface of T cells In colorectal cancer, PD-1 is a member of the PD-1 family. In colorectal cancer, the expression level of PD-L1 is also closely related to the poor prognosis of patients. It was found that the expression level of PD-L1 in colorectal cancer tissues was significantly higher than that in normal colonic mucosal tissues, and its high expression was closely related to the degree of malignancy of the tumor, the depth of infiltration, lymph node metastasis, and other clinicopathological features. The high expression of PD-L1 may allow tumor cells to evade the recognition and attack of the immune system, leading to immune escape of tumor cells. Therefore, immunotherapy against PD-L1 has become one of the new strategies for colorectal cancer treatment.

In summary, the expression of CMTM6 and PD-L1 in colorectal cancer tissues correlates with its stage, which can be used as one of the indicators for determining the prognosis of the tumor, and the patients with high expression of CMTM6 have certain advantages in the treatment.

Disclosure statement

The authors declare no conflict of interest.

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