EXPRESSION OF TIPE2 IN COLON CANCER AND ITS CORRELATION WITH CLINICOPATHOLOGICAL FEATURES AND PROGNOSIS

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ABSTRACT

Objective: *To investigate the expression of tumor necrosis factor α induced protein 8-like factor 2 (TIPE2) in colon cancer and its correlation with clinicopathological features and prognosis.*

Methods: *The cancer tissues and paracancerous tissues of 56 cases of colon cancer patients treated in our hospital from January 2018 to March 2019 will be included in this study. Immunohistochemical method was used to detect the positive expression of TIPE2 in colon cancer and its paracancerous tissues, and the correlation between its expression level and clinicopathological features and prognosis of colon cancer patients was analyzed in depth.*

Results: *The positive expression rate of TIPE2 in colon cancer tissues was 73.21% (41/56), and that in paracancerous tissues was 16.07% (9/56). The difference of positive expression rate between the two groups was statistically significant (P<0.05). TIPE2 expression level was associated with lymph node metastasis and Dukes stage of colon cancer (P<0.05), but not with age, gender and differentiation degree (P>0.05). The 5-year survival rate of patients with positive TIPE2 expression was 63.41 months, and that of patients with negative TIPE2 expression was 80.00 months. There was no statistically significant difference in the 5-year survival rate between the two groups (P>0.05). Lymph node metastasis and Dukes stage were independent risk factors for prognosis of colon cancer patients.*

Conclusion: *The expression level of TIPE2 in colon cancer tissues was increased, and its expression level was correlated with the clinicopathological features of colon cancer patients.*

Keywords: *TIPE2, colon cancer, lymph node metastasis, dukes stage.*

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Introduction

Colon cancer is one of the nine common tumors in China. In recent years, its incidence tends to be higher than that of rectal cancer[(1)]. Relevant investigation showed that the 5-year survival rate of colon cancer patients was about 65%, while the 5-year survival rate of patients with metastasis was only 10%[(2)]. Therefore, early diagnosis and effective treatment is an important way to improve the 5-year survival rate of colon cancer patients. The onset and progression of colon cancer is related to genetic and environmental factors, and it is a complex process involving multiple genes and steps. In addition, it is also closely related to apoptosis dysfunction[(3)].

Tumor necrosis factor α induced protein 8-like factor 2 (TIPE2) has the functions of regulating inflammatory response and immune balance. Clinical studies have confirmed that knockout of TIPE2 gene in mice can lead to inflammatory responses in multiple organs and premature death in mice[(4)]. Other studies have confirmed that mice

with TIPE2 defect are prone to septic shock. In addition, cells with TIPE2 defect may be extremely sensitive to Toll-like receptor activation[5]. Studies on systemic lupus erythematosus have shown that the expression level of TIPE2 in peripheral blood mononuclear cells of patients with systemic lupus erythematosus is significantly lower than that of the normal population[6]. Because TIPE2 is an important regulator of inflammatory response and immune balance, we hypothesized that it may play an important role in the pathogenesis of colon cancer[7].

However, there are few reports on the relationship between TIPE2 and colon cancer. Therefore, this study will further reveal the correlation between TIPE2 expression level and clinicopathological features and prognosis of colon cancer patients by detecting TIPE2 expression level in colon cancer and its paracancerous tissues.

Methods

General information

The cancer tissues and paracancerous tissues of 56 cases of colon cancer patients treated in our hospital from January 2018 to March 2019 will be included in this study. Gender: there were 32 male patients and 24 female patients. Age: 34 cases with age equal to or older than 60 years old and 22 cases with age younger than 60 years old. Degree of differentiation: 31 cases with high/medium differentiation, 25 cases with low differentiation. Lymph node metastasis: there were 30 cases with lymph node metastasis and 26 cases without lymph node metastasis. Dukes stage: 13 cases of grade A, 14 cases of Grade B, 15 cases of Grade C, 14 cases of Grade D.

Inclusion criteria:

• The patients were diagnosed as colon cancer by relevant examination in the department of pathology of our hospital;

• All patients were sampled immediately during surgery;

• All paracancerous tissues were more than 5cm away from the cancer tissue, ensuring no residual cancer in the adjacent tissues;

• The patients had complete pathological and clinical data;

• Patients or their family members agreed to participate in this study and signed the informed consent;

• Approved by the Ethics Committee of our hospital.

Exclusion criteria:

• Patients who have been using non-steroidal drugs for a long time were excluded;

• Patients who had previously received antitumor therapy such as chemoradiotherapy were excluded;

• Patients with cardiovascular diseases such as acute myocardial infarction were excluded;

• Patients with liver, kidney, brain and other organ tissue disorders were excluded.

Main reagents and instruments

Reagents

Rabbit anti-TIPE2 polyclonal antibody was purchased from Beijing Taize Jiaye Technology Development Co., Ltd. Immunohistochemical kit was purchased from Changzhou Beiyuanxin Biotechnology Co., Ltd. Citric acid buffer was purchased from Beijing Xinhua Lvyuan Technology Co., Ltd. DAB color development kit was purchased from Jiangxi Jianglanchun Biological Reagent Co., Ltd.

Instruments

Automatic slicer was purchased from Beijing Jiayuan Xingye Technology Co., Ltd. Paraffin embedding machine was purchased from Shanghai Lianshuobao Biotechnology Co., Ltd. The microscope was purchased from Precision Medical Technology (Beijing) Co., Ltd. 37°C incubator was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd.

Methods

• Immunohistochemical method was used to detect the expression of TIPE2 in colon cancer and its paracancerous tissues. The cancer tissues and paracancerous tissues of 56 colon cancer patients were fixed in formaldehyde. After 12h, they were taken out, dehydrated with different concentrations of alcohol, embedded with xylene, and placed in an automatic slicer to continuously sliced, with the thickness was about 5cm. The wax was spread on the glass slide using adhesive, and baked in an oven at 60°C for 60min. After dewaxing with xylene and hydration with gradient alcohol, it was rinsed with distilled water for 3 times, with 5min each time.

The sections were placed in citric acid buffer for antigen repair at a temperature of 98 to 100°C. After 20min, normal goat serum working solution was added and placed in a constant temperature incubator at 37°C for 10min. After completion, excess working solution was removed from the sections. The working

solution of primary antibody was dropped to the sections and incubated at 4°C overnight. After being taken out and restored to natural room temperature, biotinylated secondary antibody was dropped and incubated at room temperature for 30min.

The sections were rinsed with PBS buffer for 3 times, with 5min each time. DAB staining was performed after washing, and its dynamic changes were observed under a microscope.

After the chromogenic reaction, hematoxylin staining, bluing, alcohol dehydration and xylene transparency were performed and then the sections were sealed with neutral gum. The positive colon cancer was known as positive control, and PBS buffer was used as negative control instead of primary antibody.

• All the enrolled patients were followed up for 5 years by telephone, outpatient review, etc.

Interpretation standard

TIPE2 was mainly localized in cytoplasm and was positively expressed when brownish yellow cell granules appear. Maruyama method and percentage of positive cells were used to evaluate the positive expression of patients.

Maruyama method:

• No staining or unclear staining: 0 point;
• Light yellow: 1 point;
• Brownish yellow: 2 points;
• Sepia: 3 points.

Percentage of positive cells:

• Below 5%: 0 point;
• 5% to 25%: 1 point;
• 25% to 75%: 2 points, over 75%: 3 points.

The two scoring criteria were added:

• 0 to 1 point: negative expression;
• 2 points and above: positive expression.

Statistical methods

Data in this study were analyzed and processed by SPSS23.0 software package. The correlation between the positive expression rate and expression level of TIPE2 in colon cancer and its paracancerous tissues and the clinicopathological features of patients was tested by χ^2 or Fisher's test.

Kalpan-meier method was used to draw the survival curve of colon cancer patients, and Log-rank test was used for comparison between groups. COX proportional hazards regression model was established to analyze the independent risk factors affecting the prognosis of colon cancer patients, and the test level was $P<0.05$.

Results

Comparison of TIPE2 expression levels in colon cancer tissues and paracancerous tissues

The positive expression rate of TIPE2 in colon cancer tissues was 73.21% (41/56), and that in paracancerous tissues was 16.07% (9/56). The difference of positive expression rate between the two groups was statistically significant ($P<0.05$). The results were shown in Figure 1.

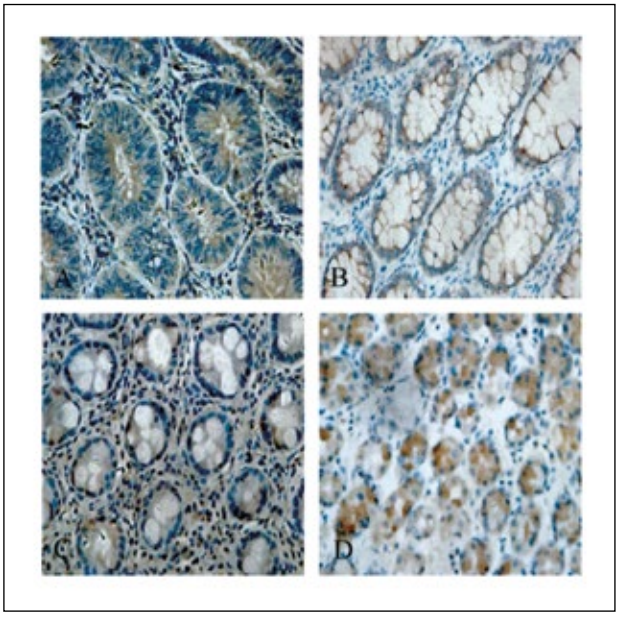

Figure 1: Comparison of TIPE2 expression levels in colon cancer tissues and paracancerous tissues.
Notes: Figure A: Positive expression of TIPE2 in colon cancer tissues. Figure B. Negative expression of TIPE2 in colon cancer tissues. Figure C: Negative expression of TIPE2 in paracancerous tissues. Figure D. Positive expression of TIPE2 in paracancerous tissues.

Correlation between TIPE2 expression level and clinicopathological features of colon cancer patients

TIPE2 expression level was associated with lymph node metastasis and Dukes stage of colon cancer ($P<0.05$), but not with age, gender and differentiation degree ($P>0.05$). The data were shown in Table 1.

Correlation between TIPE2 expression level and prognosis of colon cancer patients

The 5-year survival rate of patients with positive TIPE2 expression was 63.41 months, and

that of patients with negative TIPE2 expression was 80.00 months. There was no statistically significant difference in the 5-year survival rate between the two groups (P>0.05). The results were shown in Table 2.

Clinicopathological parameters	n	TIPE expression level		*P*
		Negative expression (n=15)	Positive expression (n=41)	
Gender				0.794
Male	32	9	23	
Female	24	6	18	
Age (years old)				0.494
≥60	34	8	26	
<60	22	7	15	
Differentiation degree				0.162
High/medium differentiation	31	6	25	
Low differentiation	25	9	16	
Lymph node metastasis				0.015
With	30	4	26	
Without	26	11	15	
Dukes staging				0.032
A	13	8	6	
B	14	3	11	
C	15	3	12	
D	14	2	12	

Table 1: Correlation between TIPE2 expression level and clinicopathological features of colon cancer patients.

Group	Survival date				
	12 months	24 months	36 months	48 months	60 months
TIPE2 Positive	96.89±1.20	88.59±1.35	81.31±1.33	76.99±1.22	69.98±1.32
TIPE2 Negative	97.34±1.30	89.33±1.32	81.91±1.36	77.34±1.21	70.11±1.32
χ^2	1.215	1.827	1.486	0.952	0.330
P	0.229	0.073	0.143	0.345	0.742

Table 2: Correlation between TIPE2 expression level and prognosis of colon cancer patients.

COX proportional hazard regression model analysis for colon cancer patients

Lymph node metastasis and Dukes stage were independent risk factors for prognosis of colon cancer patients. The data were shown in Table 3.

Clinicopathological parameters	*P*	Relative hazard value	95%CI	
			Upper limit	Lower limit
Gender	0.587	0.932	0.789	1.458
Age	0.272	1.035	0.986	1.021
Differentiation degree	0.056	0.921	0.663	1.369
Lymph node metastasis	<0.001	1.458	1.05	2.013
Dukes staging	0.019	1.589	1.282	2.102

Table 3: COX proportional hazard regression model analysis for colon cancer patients.

Discussion

At present, the incidence of colon cancer is increasing at an annual rate of about 4.2%, which seriously affects patients' life, health and quality of life. The incidence and mortality of colon cancer in developed countries occupy the third place in malignant tumors. With the changes in China's human living standard and diet structure, especially in large and medium-sized cities, the incidence and mortality of colon cancer patients rose from 5th and 6th in the 1980s to 4th and 5th, respectively, and the number of deaths from colon cancer every year exceeded 200,000[8-9]. It has been documented in clinical literature that liver metastasis is the main cause of treatment failure and death in colon cancer patients[10]. Clinical studies have confirmed that the 5-year survival rate of colon cancer patients with liver metastasis is significantly shorter than that of patients without liver metastasis. Many studies have been conducted on the pathogenesis of colon cancer, and some achievements have been made[11]. However, its pathogenesis has not been clear, so further explanation of its pathogenesis is of great significance for early disease assessment and prevention and treatment of colon cancer.

TIPE2 is a member of the tumor necrosis factor α induced protein 8 family. Studies have found that it is expressed in mouse thymus, spleen and other immune organs, but not in heart, liver, muscle and other organs[12]. In addition, TIPE2 mRNA was over-expressed in the spinal cord of the rat model of cerebrospinal meningitis, but was very low in the lungs, skin and other tissues[13]. Other studies have shown that TIPE2 was not detected in mouse embryonic fibroblast cell lines or myeloma cell lines, but it could be expressed in mouse embryonic fibroblast cell lines induced by tumor necrosis factor α[14]. TIPE2 can participate in inflammatory

reaction and apoptosis, and play an important role[15]. Clinical studies showed that TIPE2 could be linked to caspase-8 in the apoptotic staging state induced by fatty acid synthase (Fas), which blocked the activation of active protein 1 and nuclear factors, while inhibiting caspase-8 could significantly reduce the overreaction of TIPE2 deficient cells. Some scholars have suggested that the selective expression of TIPE2 can prevent overreaction. It has been reported that TIPE2, as a cytoplasmic protein, can be over-expressed in mononuclear macrophage-derived cancer cells and SK-OV-3 ovarian cancer cell lines, while it is weakly expressed in liver cancer cell lines. Other researchers found that the levels of interleukin-1, interleukin-6, IL-12 and tumor necrosis factor α in the blood of TIPE2 gene knockout mice were obviously higher than those of mice without TIPE2 gene knockout.

In this study, immunohistochemistry method was used to detect the positive expression of TIPE2 in colon cancer tissues and paracancerous tissues, and the results showed that the positive expression rate of TIPE2 in colon cancer tissues was 73.21% (41/56), significantly higher than that in paracancerous tissues by 16.07% (9/56), suggesting that TIPE2 may be involved in the onset and progression of colon cancer and play an important role. In addition, the correlation between TIPE2 expression level and clinicopathological features and prognosis of colon cancer patients was analyzed. It was found that TIPE2 expression level was associated with lymph node metastasis and Dukes stage of colon cancer, but there was no significant correlation between TIPE2 expression and prognosis of patients. By establishing the COX proportional hazard regression model, it was found that lymph node metastasis and Dukes stage were independent risk factors affecting the prognosis of colon cancer patients, but TIPE2 is not an independent risk factor for prognosis in colon cancer patients.

In conclusion, the expression level of TIPE2 in colon cancer tissues was increased, and its expression level was correlated with the clinicopathological features of colon cancer patients.

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