Expression and clinical significance of ATM and PUMA gene in patients with colorectal cancer

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Abstract. The expression of ataxia-telangiectasia mutated (ATM) and p53 upregulated modulator of apoptosis (PUMA) genes in patients with colorectal cancer were investigated, to explore the correlation between the expression of ATM and PUMA and tumor development, to evaluate the clinical significance of ATM and PUMA in the treatment of colorectal cancer. Quantitative real-time PCR was used to detect the expression of ATM and PUMA in tumor tissue and adjacent healthy tissue of 67 patients with colorectal cancer and in normal colorectal tissue of 33 patients with colorectal polyps at mRNA level. The expression level of ATM mRNA in colorectal cancer tissues was significantly higher than that in normal mucosa tissues and adjacent non-cancerous tissue ($P \leq 0.05$), while no significant differences in expression level of ATM mRNA were found between normal mucosa tissues and adjacent noncancerous tissue ($P=0.07$). There was a negative correlation between the expression of ATM mRNA and the degree of differentiation of colorectal cancer ($r= -0.312$, $P=0.013$), while expression level of ATM mRNA was not significantly correlated with the age, sex, tumor invasion, lymph node metastasis or clinical stage ($P>0.05$). Expression levels of PUMA mRNA in colorectal cancer tissues, adjacent noncancerous tissue and normal tissues were 0.68±0.07, 0.88±0.04 and 1.76±0.06, respectively. Expression level of PUMA mRNA in colorectal cancer tissues and adjacent noncancerous tissue was significantly lower than that in normal colorectal tissues ($P<0.05$). The results showed that ATM mRNA is expressed abnormally in colorectal cancer tissues. Expression of PUMA gene in colorectal carcinoma is downregulated, and is negatively correlated with the occurrence of cancer.

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Introduction

With the improvement of people's living standards and changes in eating habits, morbidity and mortality rate of colorectal cancer has increased significantly in China in recent years. At present, surgical resection supplemented by chemotherapy and radiotherapy is the main treatment of colorectal cancer. Although short-term effect is satisfactory, the application of this treatment is still challenged by the adverse side effects, serious body injury, and high recurrence rate. In addition, treatment outcomes of patients with tumor metastasis are usually poor. Therefore, the development of safer and more effective treatment is always needed. The development of colorectal cancer is a long-term and complex process with various factors involved, such as diet, environmental factors, genetic factors, disease and other factors. The occurrence of colorectal cancer involves the activation of multiple proto-oncogenes and the inactivation of tumor suppressor genes (1), and mutations or deletions of tumor suppressor genes ATM and PUMA are major molecular biological changes in the development of colorectal cancer (2,3). However, the expression and the clinical significance of ATM and PUMA in colorectal cancer have not been well studied. This study aimed to detect the expression of ATM and PUMA in colorectal cancer tissue by qRT-PCR, and to explore the roles of ATM and PUMA in the development and progression of colorectal cancer, so as to provide new insights for the treatment of colorectal cancer.

Materials and methods

General information. A total of 67 patients with colorectal cancer were selected in the First Affiliated Hospital of Nanchang University, from September 2015 to September 2016 to serve as observation group. The patients include 33 males and 34 females, and the age ranged from 45 to 79 years with an average age of 69.1±2.4 years. Pathological staging: T1 in 15 cases, T2 in 19 cases, T3 in 23 cases and T4 in 10 cases; lymph node metastasis was found in 35 cases; low differentiation was found in 31 cases and high differentiation in 36 cases; mucosal and myometrial infiltration was found in 38 cases and outer layer infiltration in 29 cases; None of the patients received any treatment before the study. Inclusion criteria: patients with colorectal cancer diagnosed by histopathological

examination; age <80 years; patients willing to participate in the biopsy for pathological examination of this study; patients with complete clinical, laboratory and imaging data; patients who signed informed consent. Exclusion criteria: patients with other types of primary malignant tumor; patients with congenital malformations; patients with acute infectious diseases; patients with severe liver and kidney dysfunction and coagulation dysfunction; patients failed to cooperate with researchers. At the same time, a total of 33 patients with colorectal polyps were selected as control group. The patients include 16 males and 17 females, and the age ranged from 50 to 78 years with an average age of 65.3±3.6 years. All patients were diagnosed with colorectal polyps by pathological examination and all patients received high frequency electric coagulation trap exsection. No significant differences were found in general information between the two groups (P>0.05). The study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University and informed consents were signed by the patients and/or guardians.

Methods. Tissue (50-100 mg) was ground in TRIzol (1 ml) on ice. The suspension was transferred into 1.5 ml centrifuge tubes and kept at room temperature for 5 min for complete dissociation to extract total RNA. Reverse transcription was then performed: 1) a DEPC-treated 200 μl PCR tube was placed on ice, and total RNA, primer (0.5 μg/μl) and RNase-free water were added and mixed, followed by centrifugation for 30 sec (1,409 x g); 2) denaturing at 70˚C for 5 min in PCR machine, followed by cooling on ice; 5X RT Buffer, RNase inhibitor (20 U/μl) and 10 mM dNTP Mix were added and centrifuged (1,409 x g) for 30 sec; 4) incubation at 25˚C in PCR instrument for 5 min, followed by cooling on ice; 5) 1 μl (200 U/μl) of M-MuLV reverse transcriptase and water was added to a final volume of 20 μl; 6) 25˚C for 10 min, 42˚C for 60 min and 70˚C for 10 min in PCR instrument, followed by cooling on ice. PCR reaction was then performed: 1) 2X PCR Master Mix, cDNA, upstream and downstream primers, and sterilized and deionized water were added into a 200 μl PCR tube, followed by centrifugation at 1,409 x g for 30 sec; 2) PCR reaction was performed according to the operation instructions: 94˚C for 5 min, followed by 30-35 cycles of 94˚C for 30 sec, 55-60˚C for 45 sec, and 72˚C for 45 sec, and 72˚C for 7 min.

Detection of the expression of ATM and PUMA at mRNA level: qRT-PCR was performed using a kit. Primers used in PCR reaction were: 5'-ATCTGCCGTCAACTAGAA-3' (upstream) and 5'-GATCTCGAATCAGGCGCTTAAA-3' (downstream) for ATM; 5'-GCCAGATTTGTGAGACAAG AGG-3' (upstream) and 5'-CAGGCACCTAATTGGGCTC-3' (downstream) for PUMA. β-actin was used as endogenous control. Data were processed using $2^{-\Delta\Delta Ct}$ method.

Observation indicators. Expression of ATM and PUMA in colorectal cancer tissues, adjacent noncancerous tissues and normal colorectal tissues were compared; correlations between expression of ATM mRNA in colorectal cancer tissues and clinicopathological features were analyzed.

Statistical analysis. Data were analyzed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Countable data are expressed as rate (n%), and comparison between groups were performed using χ^2 test. Measurement data were expressed as mean ± standard deviation, and comparisons between groups were performed by t-test. P<0.05 was considered to indicate a statistically significant difference.

Table I. Expression of ATM mRNA in different tissues (mean ± standard deviation).

ATM	Colorectal cancer tissue	Adjacent noncancerous tissue	Normal mucosa tissue
ATM mRNA	5.63±0.02	2.47±0.06[a]	2.31±0.04[a]
t-value		4.534	
P-value		0.07	

[a]P<0.05 compared with colorectal cancer tissues. ATM, ataxia-telangiectasia mutated.

Results

Expression of ATM mRNA in colorectal cancer tissues, adjacent noncancerous tissue and normal colorectal tissues. Expression level of ATM in colorectal cancer tissues was significantly higher than that in normal mucosa and adjacent noncancerous tissues (P≤0.05) at mRNA level. However, there was no significant difference in ATM mRNA expression between normal mucosa tissue and adjacent noncancerous tissue (P=0.07) (Table I).

Expression of ATM mRNA in colorectal cancer and the correlation with clinicopathological features. Expression level of ATM mRNA was negatively correlated with the degree of colorectal cancer differentiation (r= -0.312, P=0.013); expression of ATM mRNA in colorectal cancer tissues was not correlated with age, sex, depth of tumor invasion, lymph node metastasis or clinical stage (P>0.05) (Table II).

Expression of PUMA mRNA in colorectal cancer tissues, adjacent noncancerous tissues and normal colorectal tissues. Expression levels of PUMA mRNA in colorectal cancer tissues, adjacent noncancerous tissues and normal colorectal tissues were 0.68±0.07, 0.88±0.04 and 1.76±0.06, respectively. Expression level of PUMA mRNA in colorectal cancer tissues and adjacent noncancerous tissues was significantly lower than that in normal colorectal tissues (P<0.05) (Table III).

Discussion

Colorectal cancer is the third most common cancer in the world and is a leading cause of cancer-related death. Surgery is the primary treatment of patients with early stage of colorectal cancer. However, most patients are diagnosed with advanced stages and distant metastasis is also very common. Drug treatment is challenged by drug resistance and high recurrence rate. At molecular level, colorectal cancer is a heterogeneous disease, and approximately 30% of cases are caused by genetic factors.

Table II. Correlation between expression level of ATM mRNA and clinicopathological features.

Characteristics	Cases	ATM mRNA	t-value	P-value
Sex				0.270
Male	33	4.32±0.07	2.396	
Female	34	3.94±0.02		
Age (years)				0.394
≤60	30	3.99±0.03	4.279	
>60	37	4.52±0.08		
Pathological stage				0.081
T1-T2 stage	34	5.29±0.01	3.385	
T3-T4 stage	33	4.96±0.05		
Differentiation degree				0.013
High	36	3.35±0.06	5.391	
Low	31	5.63±0.02		
Tumor infiltration				0.199
Mucosal and muscular	38	5.29±0.07	4.263	
Outer layer	29	5.54±0.09		
Lymph node metastasis				0.067
Yes	35	4.48±0.05	2.738	
No	32	4.37±0.02		

ATM, ataxia-telangiectasia mutated.

Table III. Expression of PUMA mRNA in different tissue (mean ± SD).

PUMA	Colorectal cancer tissue	Adjacent noncancerous tissue	Normal colorectal tissue
PUMA mRNA	0.68±0.07	0.88±0.04	1.76±0.06
t-value		1.629	2.011
P-value		0.009	0.043

PUMA, p53 upregulated modulator of apoptosis.

In the development of colorectal adenocarcinoma, deletions or mutations of a specific proto-oncogene or tumor suppressor gene in epithelial cells of gastrointestinal tract cause the onset, progression and metastasis of colorectal cancer. Studies on mutations of cancer-related gene can provide valuable information for the treatment and prognosis (4). Ataxia-telangiectasia mutated (ATM) is newly discovered gene that can initiate the repair of DNA double-strand breaks, and the expression level of ATM is closely correlated with the repair of damaged cells and the regulation of apoptosis cycle (5). Studies have shown that (6-8) abnormal expression of ATM plays pivotal roles in the occurrence of lung cancer, breast cancer, thyroid cancer and other types of cancers by regulating gene expression and angiogenesis. In addition, abnormal cell apoptosis is also a cause of the development and progression of tumor. The p53 upregulated modulator of apoptosis (PUMA) is a newly discovered pro-apoptotic gene. As a member of Bcl-2 family, PUMA can induce apoptosis through mitochondrial pathway (9). Some studies have shown that (10-12), expression of PUMA protein is closely correlated with the onset, lymph node metastasis and prognosis of liver cancer, gastric cancer and renal cell cancer.

In this study, expression of ATM and PUMA in patients with colorectal cancer was detected by qRT-PCR to explore the roles of the two genes in the development and progression of colorectal cancer. We found that, expression level of ATM was significantly higher in colorectal cancer than in normal mucosa tissues and adjacent noncancerous tissues (P≤0.05). While expression level of ATM in normal mucosa tissues was not significantly different from that in adjacent noncancerous tissues (P=0.07). In addition, expression of ATM at mRNA level was negatively correlated with the degree of colorectal cancer differentiation (r= -0.312, P=0.013), while expression of ATM mRNA in colorectal cancer tissues was not significantly correlated with age, sex, depth of tumor invasion, lymph node metastasis and clinical stage (P>0.05). Based on these results, we hypothesized that, as a DNA damage response gene, expression level of ATM can be upregulated after cancer cell damage caused by chemical drugs or radiation to initiate a self-repair mechanism or induce apoptosis of damaged cells, so as to promote the renewal of cancer cells and maintain the activity of tumor cells. This finding is consistent with previous studies that ATM-deficient cancer cells have high sensitivity to radiotherapy and chemotherapy (13,14).

In this study, we also found that expression levels of PUMA in colorectal cancer tissues, adjacent noncancerous tissues and normal mucosa tissues were 0.68±0.07, 0.88±0.04 and 1.76±0.06, respectively. Expression level of PUMA in colorectal cancer tissues and adjacent noncancerous tissues was lower than that in normal mucosa tissues. In addition, PUMA expression is negatively correlated with tumorigenesis (r= -0.312, P=0.013), which is consistent with previous studies (15). Apoptosis disorders play key roles in tumorigenesis and anticancer treatment. The reduced expression level of PUMA in cancer cells indicates its pro-apoptotic functions. Studies have shown that some drugs can increase the sensitivity of tumor cells to chemotherapy by upregulating the expression level of PUMA in cancer cells (16,17).

Recent studies found that the occurrence of mutations in ATM gene may be related to its phosphorylation and methylation, and the activated IL-8 by oxidative stress (18,19). Studies also reported that caffeine can inhibit apoptosis of cancer cells by inhibiting the activation of ATM-Chk2-p53-PUMA channel (20), indicating that ATM and PUMA may act on different sites of the same channel. This study is limited by the small sample size. The interactions between the two genes were not studied. Thus, further studies are still needed.

In conclusion, ATM mRNA was abnormally expressed in colorectal cancer tissue. PUMA gene expression in colorectal cancer tissue was downregulated, and is negatively correlated with the occurrence of cancer. With in-depth studies, the two genes may potentially be targets for the treatment of colorectal cancer.

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