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≤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 tissue. Expression of PUMA gene in colorectal carcinoma is downregulated, and is negatively correlated with the occurrence of cancer.

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
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'-ATCTGCGGTCAACTAGAA-3' (upstream) and 5'-GATCTCGAATCGGCCTTAA-3' (downstream) for ATM; 5'-GCCAGATTTGTGAGACAAGAGG-3' (upstream) and 5'-CAGGCCACCTAATTTGGGTCT-3' (downstream) for PUMA. β-actin was used as endogenous control. Data were processed using 2^−ΔΔCt 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 (%), and comparison between groups were performed using χ² 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.

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.
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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