

Expression of miR-21 in Colorectal Cancer and Its Relationship with Clinicopathological Characteristics of Colorectal Cancer

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ABSTRACT

Objective: To investigate the expression of miR-21 in colorectal cancer cells, and to analyze the relationship between the level of miR-21 and the clinicopathological characteristics of colorectal cancer patients. **Methods:** 210 patients with colorectal cancer treated in our hospital from January 2016 to June 2019 were selected. Cancer tissue specimens (study group) and adjacent normal tissue specimens (control group) were surgically collected, and the quantitative PCR was used to detect and compare the miR-21 expression of the two groups. **Results:** The expression of miR-21 in the study group was higher than that in the control group, and the difference was statistically significant ($P < 0.05$). There were significant differences among patients with early and intermediate TNM, patients with low differentiation and patients with moderate to high differentiation, patients with lymph node metastasis and patients without lymph node metastasis, patients with high infiltration and patients with low infiltration, patients with high CEA levels, and patients with low CEA, and the difference was statistically significant ($P < 0.05$). **Conclusion:** In colorectal cancer, miR-21 is highly expressed, which is closely related to stage and differentiation, and can be used to reflect the patient's condition.

1. Introduction

Colorectal cancer is very common in gastrointestinal tumors, and its incidence has always been high. The main causes are: family genetic factors; benign adenomas of the intestine; malignant changes in the intestinal tract; lack of sperm fibers in the diet; too much fat and carbohydrates. It is not conducive to defeca-

tion, causing a large amount of carcinogens to stay in the intestine, causing irritation to the intestinal mucosa and causing it to become cancerous; rectal polyps or ulcerative colon cancer are induced^[1]. Although patients can perform colorectal cancer through a healthy diet and regular inspection, colorectal cancer has the characteristics of early symptoms concealment of most cancers, so it is difficult to detect cancerous changes in the clinic. Imaging is also

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a common method for the diagnosis of colorectal cancer, such as MRI, CT, ultrasound, etc. are widely used in the diagnosis of colorectal cancer, and its diagnostic accuracy is relatively high, however, after all, these methods obtain images indirectly, and do not directly observe pathological results, so there may be missed or misdiagnosed results. With the development of science and technology and medical technology, clinical studies have found that dysregulated microRNA expression is the most common feature of colorectal cancer. Clinically, it is possible to understand the occurrence and development of tumors by detecting the level of tumor specimens. The miR-21 is one of the most common oncogenes, and its residues are responsible for the occurrence, development, and metastasis of colorectal cancer. The clinical test results are often used as a reference basis for screening colorectal cancer and evaluating the treatment effect of colorectal cancer.

2. Materials and Methods

2.1 General Information

A total of 210 patients with colorectal cancer treated in our hospital from January 2016 to June 2019 were selected. The clinical data are as follows: ① Gender composition: 116 males and 94 females; ② Age distribution: the youngest is 30 years old and the largest The patients were 84 years old, with an average age of (57.29 ± 12.34) years. ③ TNM staging: 6 cases of stage I, 108 cases of stage II, 70 cases of stage III, 26 cases of stage IV; ④ lymph node metastasis: 92 cases with metastasis, 118 cases without metastasis; Level: 124 cases above 3.4ng / mL, 86 cases below 3.4ng / mL; ⑥ degree of local infiltration: 110 cases in T4, 68 cases in T3, 30 cases in T2, 2 cases in T1; ⑦ poor differentiation 60 cases, 150 cases of moderate and high differentiation.

2.2 Methods

Cancer tissue specimens (study group) and adjacent normal tissue specimens (control group) were surgically collected. After the specimens were removed from the patient, they were stored in RNA sample preservation solution and quickly frozen with liquid nitrogen, and was then stored at -80°C for future use.

Take about 1g of tissue from the frozen specimen, grind it into a powder by liquid nitrogen grinding method, drip 1mL of Trizol reagent, and use TRizol reagent to extract total RNA from cancer tissue and normal tissue adjacent to the cancer. For extraction method and operation steps, please refer to the instruction manual. After extraction, the purity and concentration of RNA were measured by a UV spectrophotometer, and the integrity of RNA was detected

by agarose gel electrophoresis.

CDNA was synthesized according to the relevant requirements of the kit. The RT primers with specific stem and loop in the structure were derived from the quantitative PCR kit. The primer sequences were:

5'-GTCGTATCCAGTGGCTGGGTCCGAGTGATTCCG-CACTGGATACGACTCAACATC-3'

&

5'-GTCGTATCCAGTGGCTGGGTCCGAGTGATTCCG-CACTGGATACGACTCACAAGT-3'

Ribonuclease, reverse transcription primer is 50nmol / L, RNA template is 2.5 μL , heated to 70°C , heat denatured, given 5 minutes after ice bath, after cooling for 2 minutes, add 1 μL of 10mmol / L dNTP mixture / 0.5 Reverse transcription reaction was performed under the reaction conditions of 16°C for 30 minutes, 42°C for 20 minutes, and 85°C for 5 minutes.

Real-time PCR quantitative detection using a fluorescent PCR instrument and related kits, the reaction system is 0.03mL, 3 μL of 10x buffer, 1 μL of cDNA, 3 μL of 25mmol / L magnesium chloride solution, upstream primer 0.33 μmol / L and downstream primer 0.33 μmol / L, 3.6 μL of 2.5 mmol / L dNTP, 1.5 U of rYaq polymerase. The reaction conditions were: 40 cycles, predenaturation at 94°C for 3 minutes, 94°C for 20 seconds, and 60°C for 40 seconds. U6 was selected as the internal reference, and the relative expression of miR-21 in colon cancer cells was determined by the $2^{-\Delta\Delta\text{Ct}}$ method. $-\Delta\Delta\text{Ct}$ is (miR-21 • Ct-U6 • Ct) cancer tissue- (miR-21 • Ct-U6 • Ct) normal tissue adjacent to the cancer^[2].

2.3 Observation Indicators

(1) Compare the PCR results of miR-21 in two groups of specimens;

(2) Compare the results of miR-21 detection in patients with different clinicopathological characteristics.

2.4 Statistical Methods

The clinical data of all subjects in this study were included in the SPSS version 19.0 statistical software. Count data were expressed as n (%), χ^2 test was performed, measurement data was expressed as $(\bar{x} \pm s)$, and t test was used for statistical analysis. If the latter result is $P < 0.05$, it means that the difference is statistically significant.

3. Results

3.1 Expressions of miR-21

The expression of miR-21 in the study group was (5.16 ± 1.07) , which was higher than that in the control group $(0.68$

± 0.13), and the difference was statistically significant ($t = 60.231$, $P = 0.000 < 0.05$).

3.2 Relationship between miR-21 Expression and Clinicopathological Characteristics

Among patients with early and intermediate TNM, patients with low differentiation and patients with moderate to high differentiation, patients with lymph node metastasis and patients without lymph node metastasis, patients with high infiltration and patients with low infiltration, patients with high CEA levels, and patients with low CEA, their miR-21 expressions were significantly different, and the difference was statistically significant ($P < 0.05$). See Table 1.

Table 1. Relationship between miR-21 expression and clinicopathological characteristics (n, %)

Clinicopathological features	Cases	miR-21 Expressions	P
Early TNM (I, II)	114	1.69	0.000
TNM mid-late stage (III, IV)	96	7.22	
Poorly differentiated	60	7.54	0.005
Medium to high differentiation	150	2.88	
Lymph node metastasis	92	7.22	0.000
No lymph node metastasis	118	1.75	
Infiltration T1 ~ T3	100	1.72	0.006
Infiltrated T4	110	5.46	
CEA above 3.4ng / mL	124	0.39	0.000
CEA below 3.4ng / mL	86	6.32	

4. Discussion

Colorectal cancer is a malignant tumor with a very high incidence both at home and abroad. The pathological process is more complicated, involving multiple factors, going through multiple stages, and related to multiple genetic changes. Many studies have shown that the occurrence and development of colorectal cancer are related to inactivation of tumor suppressor genes and activation of oncogenes in genetics, and that the abnormal regulation of MiRNA is the main reason for inactivation of tumor suppressor genes and activation of oncogenes. As a type of non-coding small molecule RNA that can regulate gene function, MicroRNA has specific recognition effect on the 3'-terminal untranslated region in mRNA, which can be combined with it in a targeted manner to degrade mRNA or inhibit translation. After transcription, it will effectively regulate the expression of target genes. Clinically found that MicroRNA has a regulatory effect on a variety of human diseases, and has abnormal expression in a variety of tumor tissues. It is either the presence of tumor suppressor genes or the presence

of oncogenes, with high or low expression, and is involved in the generation, proliferation, and differentiation of tumor cells. The research object miR-21 in this paper is a kind of miRNA with carcinogenic properties. It is the only miRNA that is found to be highly expressed in non-solid tumors and solid tumors at this stage, miR-21 plays a role very similar to oncogenes, and the high expression of miR-21 means the occurrence, development, proliferation, invasion, and metastasis of tumors, and even it has a very important impact on angiogenesis and drug resistance, which promotes a series of biological behaviors of tumors.

As a kind of MicroRNA, miR-21 has very distinct active properties, and has high expression in breast cancer, pancreatic cancer, lung cancer, and colorectal cancer, and its expression level is closely related to tumor development and patient prognosis. In this paper, miR-21 expression was detected for colorectal cancer tissues and adjacent normal tissues. The results showed that the expression of miR-21 in colorectal cancer tissues was higher than that of normal adjacent tissues ($P < 0.05$), which undoubtedly proves that miR-21 high expression is closely related to canceration. At the same time, existing studies have pointed out that the level of miR-21 is closely related to pathological features such as lymph node metastasis, clinical stage, and differentiation. This paper also compared the expression of miR-21 in patients with different pathological characteristics, and the results showed that, patients with early TNM are lower than patients with advanced stage, patients with lower differentiation are lower than patients with moderate to high differentiation, patients with lymph node metastasis are higher than patients without lymph node metastasis, patients with high degree of infiltration are higher than patients with low degree of infiltration, and patients with high CEA level are lower than CEA Patients ($P < 0.05$), which undoubtedly proves the close relationship between the high expression of miR-21 and the occurrence and development of colorectal cancer.

It is worth noting that, in view of the regulatory effect of miR-21 expression on colorectal cancer, clinical treatment can be adopted for patients to improve the prognosis of patients.

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