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Original Article

Correlation between microRNA-107 expression level and prognosis in patients with colorectal cancer

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Abstract: Colorectal cancer is one of the most common cancers in the world. This study aimed to investigate the correlation between microRNA-107 (miR-107) expression level and the prognosis in colorectal cancer patients with its clinical significance. 80 cases of cancer tissues and 15 cases of adjacent cancer tissues were collected from colorectal cancer patients treated with surgery from February 2006 to January 2010. The expression of miR-107 was detected by real-time PCR. The correlation between miR-107 expression and clinic pathological factors and survival time of patients was statistically analyzed. The expression level of miR-107 in cancer tissues (0.0213 ± 0.0096) was significantly higher than that in adjacent tissues (0.0355 ± 0.0487). The expressions of miR-107 in patients with different TNM stages, Dukes stages, and lymph node metastasis rates were significantly different ($P < 0.05$). Cox proportional hazards regression model showed that miR-107 may be an independent factor affecting the prognosis of colorectal cancer patients ($P < 0.05$). The hazard ratio (HR) was 5.165. MiR-107 is highly expressed in colorectal cancer tissues and is closely related to the pathogenesis, progression, and metastasis of colorectal cancer. MiR-107 is expected to become a new molecular marker to assist the diagnosis, treatment effect and prognosis evaluation of colorectal cancer, and may also become a new target for colorectal cancer biotherapy.

Keywords: MicroRNA-107, colorectal cancer, cox regression analysis, prognosis

Instruction

Colorectal cancer is the third most common cancer in men and the fourth most common in women in the world. Data from Global Cancer Observatory in 2018 showed that the mortality rate of colorectal cancer (9.2%) ranks the second among malignant tumors [1]. Although the mortality rate of colorectal cancer patients has been decreased due to great advances in radiotherapy, chemotherapy, and surgical treatment, the prognosis of patients suffering from colorectal cancer is still not ideal. At present, some factors affecting the survival time of colorectal cancer patients, such as clinical stages of tumor and lymph node metastasis, are still difficult to explain. For example, some patients with early tumors still undergo recurrence and metastasis after radical surgery, which affects the prognosis. Therefore, it is necessary to search for a new index with high sensitivity to predict the prognosis of colorectal cancer.

MicroRNAs are a class of endogenous, small (18-24 nucleotides), non-coding RNAs presented in eukaryotic cells. It is often combined with mRNAs to regulate the expression of genes. MicroRNA-107 (miR-107) is widely expressed in various tissues of the human, and its dysregulation is involved in a variety of diseases. For example, miR-107 can promote the secretion of amyloid precursors and regulate inflammation and apoptosis, thereby accelerating Alzheimer's disease, the progression of silent disease, and Parkinson's disease [2, 3]. Through regulating the expression level of miR-107, intestinal flora can maintain intestinal homeostasis.

Widely studied is the function of miR-107 as an oncogene or anti-oncogene, which can have a certain impact on the occurrence, development, recurrence, and metastasis of various tumors [4, 5]. It is worth mentioning that miR-107 can play different roles in different tumor tissues. For example, in non-small cell lung can-

cer, it can inhibit the progress of tumors by targeting the expression of TGFβR2 and play its role as a anti-oncogene [6]. Whereas miR-107 highly expressed in liver cancer tissues, enhances the proliferation of liver cancer cells, and plays its role as an oncogene [7].

Multiple studies suggest that the expression of miR-107 in gastric cancer tissues is significantly higher than that in normal tissues adjacent to cancer, and down-regulation of miR-107 inhibits the proliferation, migration, and invasion of gastric cancer cells [8-10]. MiR-107 can also promote proliferation and inhibit the apoptosis of colorectal cancer cells through targeting PAR4 [11]. However, its role in colorectal cancer is not fully revealed. In our study, we intend to explore the correlation between miR-107 expression and prognosis of colorectal cancer.

Materials and methods

Clinical data

80 cancer tissue samples and 15 adjacent tissue samples were collected from patients with colorectal cancer who underwent colorectal cancer resection from February 2006 to January 2010. Patients' general clinic pathological data, including gender, age, tumor pathological grade, pathological classification, depth of invasion, lymph node metastasis, clinical stage, etc., were recorded. Telephones and outpatient visits were used as the follow-up method to collect data on the patients' survival or death time. Follow-up was conducted every 3 months within 2 years after operation, and every 6 months from the third year on. The follow-up was up to September 2015. Complete data from the dead patients was taken, and the survivors were censored data. The overall survival (OS) time was defined as the first day after surgery to the date of death or follow-up deadline. Of the 80 patients with colorectal cancer, 47 were male and 33 were female. They were 33 to 85 years old, with an average age of 64 ± 12 years old. By the end of follow-up, 44 patients had died.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients with newly diagnosed colorectal cancer who were treated with radical surgery, without radiation or chemotherapy before the surgery and (2) The postoperative pathology of the patients was confirmed to be colorectal cancer.

Exclusion criteria: (1) Colorectal cancer patients with severe cardiopulmonary diseases which may be the leading cause of death during follow-up; (2) Patients with a history of liver and kidney diseases; (3) Patients with autoimmune diseases and long-term immunosuppressive therapy before surgery.

Materials

MiRcute miRNA cDNA reverse transcription kit (KR201) and miRcute miRNA fluorescence quantitative detection kit (FP411-02) was purchased from Tiangen (Beijing, China). PrimeScript real-time PCR kits were purchased from TAKARA (Shiga, Japan). Axygen Plate Max Ultraclear Sealing Film (UC-500) was obtained from Millipore (Bedford, MA, USA). The miR-107 specific primers and primers for internal reference U6 were synthesized by Shanghai Yingweijieji Company (Shanghai, China). The PCR instrument (Roche Light Cycler 480 II) and Plate centrifuge (Xiangyi L-530) were involved in this study. Trizol Reagents were purchased from Invitrogen (Carlsbad, CA, USA).

Quantitative real-time PCR

Total RNA from colorectal cancer tissues and adjacent tissues was extracted using Trizol Reagent according to the protocol. RNA samples with an A260/A280 value between 1.8 and 2.0 were reverse transcripted using PrimeScript real-time PCR kit. Following the instruction, the level of miR-107 was measured in triplicate using real-time quantitative PCR with a SYBR Premix PCR kit. The relative expression level of miR-107 was normalized to U6 snRNA and calculated using $2^{-\Delta CT}$ method.

Statistical analysis

SPSS 17.0 statistical software was used for statistical analysis. Two samples were compared using independent sample *t* test. Chi-square test was used for rate comparison. The survival time distribution of patients was described by life table method. Log Rank test was used for single factor analysis of survival time. $P < 0.05$ was considered as statistically significant. Multi-factor analysis of survival time was carried out using the forward Cox risk regression model. $\alpha = 0.05$ indicated that the variable was within the multi-factor Cox model formula.

Table 1. Expression of miR-107 in colorectal cancer tissues and adjacent tissues

	Case number	miR-107 expression (2-ΔCT)	F	t	P
Cancer tissues	80	0.0213 ± 0.0096			
Adjacent tissues	15	0.0355 ± 0.0487	40.130	-2.420	0.017

Table 2. Correlation between the expression of miR-107 in colorectal cancer and clinic pathological characteristics

Clinicopathological parameters	miR-107 (n, %)		χ^2	P
	< median	> median		
Age				
< 60 (n = 29)	18 (62.07)	11 (37.93)		
> 60 (n = 51)	24 (47.06)	27 (52.94)	1.670	0.196
Gender				
Male (n = 47)	24 (51.06)	23 (48.94)		
Female (n = 33)	18 (54.54)	15 (45.45)	0.094	0.759
Tumor location				
Left colon (n = 44)	25 (56.82)	19 (43.18)		
Right colon (n = 36)	17 (47.22)	19 (52.78)	0.731	0.393
Pathological type				
Mucinous carcinoma (n = 6)	4 (66.67)	2 (33.33)		
Adenocarcinoma (n = 62)	33 (53.23)	29 (46.77)		
Mixed carcinoma (n = 12)	5 (41.67)	7 (58.33)	1.072	0.671
Pathological grade				
I/II (n = 60)	31 (51.67)	29 (48.33)		
III/IV (n = 19)	11 (57.89)	8 (42.11)	0.225	0.635
TNM staging				
I/II (n = 34)	28 (82.35)	6 (17.64)		
III/IV (n = 44)	13 (29.55)	31 (70.45)	21.450	0.000
Dukes staging				
I/II (n = 38)	31 (81.58)	7 (18.42)		
III/IV (n = 35)	7 (20)	28 (80)	27.681	0.000
Lymph node metastasis rate				
0 (n = 40)	31 (77.50)	9 (22.50)		
< 50% (n = 29)	8 (27.59)	21 (72.41)		
> 50% (n = 6)	0 (0)	6 (100)	24.25	0.000

Results

The mean $2^{-\Delta CT}$ of miR-107 expression was 0.0213 ± 0.0096 in 80 colorectal cancer tissues and 0.0355 ± 0.0487 in 15 adjacent tissues. The expression level of miR-107 in cancer tissues was significantly higher than that in paracancerous tissues (**Table 1**, P = 0.044).

There was no significant difference in the miR-107 expression between different ages, genders, tumor locations, pathological types, and pathological grades. The rate of miR-107 higher-expression in patients with TNM stage I/II was 17.64%, which was significantly lower than that in patients with TNM stage III/IV (70.45%). The rate of miR-107 higher-expression in dukes stage III/IV patients (80%) was significantly higher than that in stage I/II patients (18.42%). The rate of lymph node metastasis was positively correlated with the expression rate of miR-107. The rate of miR-107 higher-expression without lymph node metastasis was the lowest (22.5%). The rate of miR-107 higher-expression with lymph node metastasis rate exceeding 50% is 100%. The differences in each group were statistically significant, with a P < 0.05 (**Table 2**).

In 80 cases of colorectal cancer, 38 cases had higher expression of miR-107, 42 cases had lower miR-107 expression, and the rate of higher-expression was 47.5%. Among the higher miR-107 expression patients, there were 32 deaths as of the follow-up date. The median survival time was estimated to be 46.64 (35.95-57.34) months, and the mortality rate was 84.21%. 12 patients with lower miR-107 expression died, the median survival time was estimated to be 89.11 (77.17-101.06) months, and the mortality rate was 28.57%, which was significantly lower than that of patients with higher miR-107 expression (**Table 3**, P < 0.05). The survival curve also suggests that patients with lower miR-107 expression may have a longer survival time than patients with higher miR-107 expression (**Figure 1**).

All indicators were included in the Cox proportional hazard model. The forward stepwise (conditional LR) method was used for multi-

Table 3. Correlation between miR-107 level and survival time (months) in patients with colorectal cancer

miR-107	N	Deaths	CENSORED		Median survival time	95% CI	χ^2	P
			N	Median survival time				
< median	42	12	30	71.4	89.11	77.17~101.06		
> median	38	32	6	15.8	46.64	35.95~57.34	23.72	0.00

Data were analyzed with Log-Rank method.

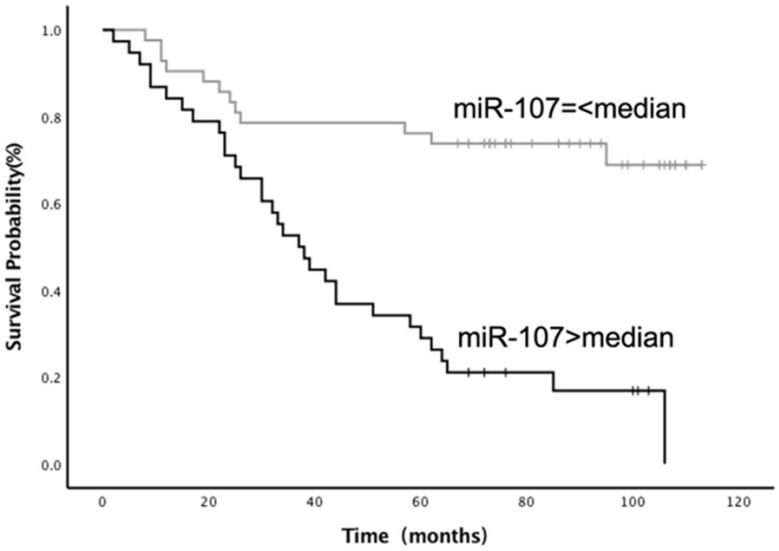

Figure 1. Survival curves of colorectal cancer patients with high and low expression of miR-107.

Table 4. Cox regression analysis of prognostic factors in patients with colorectal cancer

Prognostic factors	β	SE	Wald	P	HR	95% CI
miR-107 > median	1.642	0.374	19.282	0.000	5.165	2.482~10.749

factor analysis. Our results showed that the higher the miR-107 expression level, the shorter the survival time. The hazard ratio (HR) is 5.165 (95% CI 2.482~10.749) (**Table 4**, P < 0.05), indicating that miR-107 may be an independent factor affecting the prognosis of colorectal cancer patients.

Discussion

MiRNAs have been proven to be closely related to tumor progression, diagnosis, and prognosis by many studies. As one miRNA can regulate hundreds of downstream target genes, even the same miRNA may play completely opposite roles in different tumor tissues due to this complexity. Recently, two studies have confirmed that miR-107 can promote the proliferation and tumorigenic ability of colorectal cancer cells *in vitro* and inhibit the apoptosis of colorectal cancer cells [11, 12]. The results of Zhang *et al.* suggest that high expression of miR-107 may contribute to the proliferation and invasion of tumor cells [13]. Our study examined the expression level of miR-107 in colorectal cancer tissues and normal tissues adjacent to the cancer. The results showed that the expression level of miR-107 in cancer tissues was higher than that in adjacent tissues. In addition, there was no significant difference in miR-107 expression between different ages, genders, tumor locations, pathological types, and pathological grades. Difference in the rate of higher miR-107 expression in patients with different TNM stages, Dukes stage, and lymph node metastasis was significant, indicating that miR-107 may act as an oncomiR in colorectal cancer.

In the survival curve and Cox regression analysis, the expression level of miR-107 was significantly related to the survival time of colorectal cancer patients. The higher the expression level, the shorter the survival time. It is revealed that the higher expression of miR-107 is closely related to the condition and prognosis of colorectal cancer patients. In addition, the expression level of miR-107 is closely related to lymph node metastasis and distant metastasis of colorectal cancer and may be used to

evaluate the chemotherapy effect and prognosis of patients [14]. However, the mechanism of miR-107 is still not all clear, and its different expression levels in different tumors also suggest that its regulatory effects on tumors may be tissue or cell specific [15]. Hsin-Yi Chen *et al.* found that high expression of miR-107 is closely related to the invasion of colorectal cancer cell lines and the poor prognosis of patients. The reason may be that miR-107 mediates the down-regulation of death-associated protein kinase (DAPK) and Krüppe-like factors 4 (KLF4) expression [16]. Studies show that miR-107 can directly inhibit the expression of prostate apoptosis response protein 4 (PAR-4), and then play a role in promoting tumor cell proliferation and inhibiting tumor cell apoptosis [11]. In our study, the pathological type of tumor, TNM stage, dukes classification and lymph node metastasis rate did not affect the survival time of colorectal cancer patients, which is not consistent with related researches [17-20]. The possible reason is that there is a certain deviation in the retrospective research. It may also be related to the source of sample, the sample size, the geographical life of the population, and the socioeconomic situation. Thus, further investigation is still needed.

In summary, miR-107 is highly expressed in colorectal cancer tissues, which is closely related to the occurrence, development, and prognosis of colorectal cancer. MiR-107 is expected to become a new molecular marker to assist the diagnosis, treatment effect, and prognosis evaluation of colorectal cancer patients. It may also become a new target for the treatment of colorectal cancer. Our research still has some shortcomings, such as insufficient sample size, lack of *in vivo* and *in vitro* tests, and needs for further research of target genes.

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Disclosure of conflict of interest

None.

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