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The relationship between miR-219-5p Gene Polymorphisms and the prognosis of colorectal cancer

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Research article

Keywords: MiR-219-5p, Prognosis, Colorectal cancer

Posted Date: June 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-30253/v1

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Abstract

Background

Colorectal cancer (CRC) is one of the most common malignant tumors, its morbidity and mortality are increasing year by year, it is a serious threat to people's health. Some studies have reported that miR-219-5p acts as a tumor suppressor in some malignant tumors. So the purpose of this study was to investigate the prognostic value of miR-219-5p expression in CRC patients.

Methods

QRT-PCR was used to detect the expression levels of miR-219-5p in CRC tissues and corresponding normal tissues ($P < 0.001$). The prognostic value of miR-219-5p in CRC was analyzed by Kaplan-Meier and Cox regression analysis.

Results

The results indicated that the expression of miR-219-5p was significantly lower in CRC tissues, and its expression was closely correlated with tumor differentiation, TNM staging and lymph node metastasis (all $P < 0.05$). Moreover, Kaplan Meier survival analysis showed that the patients with low expression of miR-219-5p had worse overall survival rates ($P < 0.05$). Cox regression analysis further demonstrated that miR-219-5p expression was an independent prognostic factor for survival time in CRC patients ($P = 0.018$, HR = 2.026 and 95%CI: 1.127–3.643).

Conclusions

All the results suggest that miR-219-5p expression can be used as a potential prognostic biomarker for CRC patients.

Background

Colorectal cancer (CRC) is one of the most common malignant tumors in the clinic [1]. In recent years, with the improvement of people's living standard and changes of living habits, the incidence of CRC is increased gradually, and has risen to third in the incidence of all malignant tumors, its mortality rate is the second most important cause of death in malignant tumors [2]. At present, the treatment of CRC is mainly surgery, supplemented by radiotherapy and chemotherapy, but the curative effect is poor, the 5 year survival rate is still only 50–70%, and the treatment of middle and advanced CRC is worse [3–5]. As far as we know, at present, no specific and known biomarkers have been found to provide reliable information for the prognosis of CRC patients [6]. Therefore, it is urgent to search a novel and reliable biomarker to help evaluate the clinicopathological features and prognosis of CRC.

MicroRNAs (MiRNAs) are non protein encoded small molecule RNA family of about 21–25 nucleotides in plants and animals. The mature miRNA can be specifically combined with the 3’ untranslated region (3'-UTR) of target gene mRNAs, which regulates the target gene through interaction, and it is mainly characterized by the negative regulation of protein expression [7–9]. Studies have shown that miRNA plays an important role in the development of tumors, so miRNAs has become another hot spot in tumor research, attracting many scholars' attention [10, 11]. MiR-219-5p is one of the few studies currently in miRNAs, and acts as a tumor suppressor in gliomas [12]. But the role of miR-219-5p in CRC and its prognostic value have been rarely reported.

Therefore, in this study, we investigated the expression level of miR-219-5p in clinical CRC tissues and adjacent normal tissues. The relationship between miR-219-5p expression and clinicopathological characteristics of CRC patients was also investigated. The aim of this study was to investigate the prognostic value of miR-219-5p in CRC patients.

Methods

Patients and specimens collection

The study was conducted at the First Medical Center, Chinese PLA General Hospital, and was approved by the Ethics Committee of this hospital. From October 2015 to May 2019, we screened 138 patients who had been diagnosed as CRC by pathological diagnosis in the hospital. All patients signed the written informed consent. CRC tissues and corresponding normal tissue samples were collected from all the patients during surgery, and to ensure that all patients did not receive any radiation or chemotherapy prior to the operation. All fresh tissue samples immediately frozen in liquid nitrogen and stored at -80 °C for RNA extraction. All the patients were followed up from 3 to 60 months after surgery, and the clinical pathological features were recorded and shown in Table 1.

Table 1
Association of miR-219-5p expression with clinicopathological features of CRC patients

Clinicopathologic features	Number of cases	miR-219-5p expression		χ^2	*P* Value
		low	high		
Total	138	74	64		
Age (years)				0.275	0.600
< 55	68	38	30		
≥ 55	70	36	34		
Tumor size				0.402	0.526
< 5 cm	65	33	32		
≥ 5 cm	73	41	32		
Gender				0.226	0.634
Male	72	40	32		
Female	66	34	32		
Tumor differentiation				4.678	0.031
Poor	74	46	28		
Well/Moderate	64	28	36		
TNM stage				4.520	0.034
Stage I-II	60	26	34		
Stage III-IV	78	48	30		
Lymph node metastasis				5.691	0.017
Negative	80	36	44		
Positive	58	38	20		

RNA extraction and quantitative real-time RT-PCR (qRT-PCR)

The total RNA in all tissue samples were isolated by using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and then measured their concentration and quality using NanoDrop ND-1000 Spectrophotometer (Agilent, USA). The qualified RNA was used to synthesize the first-strand cDNA through reverse transcription. Thereafter, the expression of miR-219-5p were detected by qRT-PCR reaction in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). U6 was

used as endogenous control. MiR-219-5p expression levels was calculated by the $2^{-\Delta\Delta Ct}$ method. This experiment was repeated at least three times.

Statistical analysis

SPSS software ver. 21.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze all the statistical data in the study. The data were presented as the mean ± standard deviation (SD). Student's t-test was performed to assess the difference between miR-219-5p expression in CRC tissues and corresponding adjacent normal tissues. Chi-square test was used to analye the association of miR-219-5p expression and clinical features of patients. The overall survival rate of these 138 CRC patients were calculated and compared via Kaplan–Meier method with log-rank tests. The analysis of prognostic value was carried out using Cox regression analysis. $P < 0.05$ was considered statistically significant.

Results

The expression of miR-219-5p was down-regulated in CRC tissues

The expression of miR-219-5p in 138 patients' CRC tissues and adjacent normal tissues were detected by qRT-PCR, the result showed that miR-219-5p expression was significantly lower in CRC tissues compared with that in adjacent normal tissues (Fig. 1, $P < 0.001$).

The relationship between miR-219-5p expression and clinical features of CRC patients

The relationship between miR-219-5p expression and clinical features of CRC patients was analyzed, and the results were shown in Table 1. We could find that miR-219-5p expression was closely associated with tumor differentiation ($P = 0.031$), TNM stage ($P = 0.034$) and lymph node metastasis ($P = 0.017$), but it has no association with other features (all $P > 0.05$; Table 1).

The relationship between miR-219-5p expression and prognosis of CRC patients

Kaplan-Meier method with log-rank tests were carried out to calculate and compare the overall survival rates of all the patients. We found that the patients with low expression of miR-219-5p had poorer overall survival than those with high miR-219-5p expression (log-rank test, $P = 0.005$, Fig. 2).

Prognostic values of miR-219-5p expression in colorectal cancer

In this study, we evaluated the prognostic value of miR-219-5p expression in CRC patients by Cox regression analysis. The results indicated that low expression of miR-219-5p was detrimental to the

survival of CRC patients ($P = 0.018$), and miR-219-5p could be used as an independent prognostic factor in CRC patients, with the value of HR and 95% CI was 2.026 and 1.127–3.643, respectively (Table 2)..

Table 2
Multivariate Cox regression analyses for miR-219-5p in CRC patients

Variables	*P*-Value	HR	95%CI
miR-219-5p	0.018	2.026	1.127–3.643
Age	0.862	-	-
Tumor size	0.667	-	-
Gender	0.905	-	-
Tumor differentiation	0.781	-	-
TNM stage	0.751	-	-
Lymph node metastasis	0.733	-	-

Discussion

CRC is one of the most frequently malignancies in the digestive system. From the analysis of relevant statistics, the number of CRC in China reached 25159 cases, the number of death reached 12161 cases in 2009. And its morbidity and mortality increased year by year, which suggest that the situation of CRC in our country is relatively severe [13, 14].

At present, the principle of clinical treatment for CRC is mainly comprehensive treatment based on surgical resection. In the clinical process, in spite of the surgical technique and methods for treatment of CRC developed rapidly, as well as radiotherapy, chemotherapy, neoadjuvant radiochemotherapy, immunization, targeted therapy and other comprehensive means have been widely used in clinical treatment, however, the prognosis of CRC patients still poor. The recurrence and metastasis of CRC remain the main cause of the poor 5 year survival and mortality [15, 16]. According to the statistics, 25% newly diagnosed CRC patients were with distant metastasis, about 40%-50% of patients with primary CRC who have not yet been diagnosed with metastasis will eventually develop distant metastases after diagnosis and treatment. The patients with advanced metastatic CRC, their median survival was only 5–6 months if not treated in time. Therefore, it is very important to find an effective CRC screening method to improve the detection rate of early CRC, so as to establish appropriate treatment plans and improve the cure rate and prognosis of CRC.

In recent years, more and more studies show that miRNAs participate in many important life activities, such as cell growth, proliferation, differentiation, apoptosis and metabolism [17, 18]. MiRNA was almost involved in the occurrence and development process of all human tumors, and its expression in tumors with timing, tissue differences, ethnic differences, individual differences. It has a certain commonality in

different tissue sources and different stages of tumor, which resulting in that it can be used as an important marker for differential diagnosis, early diagnosis, classification and individualized treatment [19]. In 2003, Michael et al. first reported the differential expression of miRNA in human CRC tissues and normal colorectal mucosa, and a total of 28 miRNAs expression changes were detected, including miR-320, miR-321, miR-200c, miR-143, miR-145, and so on [20]. Subsequently, the studies of differential expression of miRNA in CRC have been reported, more and more miRNAs have been proved to participate in the occurrence and development of CRC, and it is also closely related to the diagnosis, staging, prognosis and therapeutic sensitivity of CRC [21, 22]. The role of miRNAs in CRC have attracted more attention of scholars.

At present, there are few studies on miR-219-5p in CRC. In this study, we detected its expression levels in CRC tissues and adjacent normal tissues, and found that the expression of miR-219-5p in CRC tissues was obviously lower than that in adjacent normal tissues. The same expression was also found in other tumors, such as papillary thyroid carcinoma, hepatocellular carcinoma, glioblastoma and gastric cancer, miR-219-5p could inhibit the growth and proliferation of these tumor cells [12, 23–25]. In addition, we also analyzed the relationship between miR-219-5p expression and clinical features of NPC patients, the results showed that miR-219-5p expression was closely associated with tumor differentiation, TNM staging and lymph node metastasis. Besides, Kaplan Meier survival analysis indicated that its expression was observably related with the overall survival rates of all the CRC patients. And Cox regression analysis demonstrated that low expression of miR-219-5p was an independent prognostic factor affecting the survival time of CRC patients.

Conclusions

In conclusion, miR-219-5p is down-regulated in CRC tissues, and its expression is significantly correlated with the clinical characteristics of CRC patients. MiR-219-5p can be used as a potential prognostic biomarker for CRC patients.

Abbreviations

Colorectal cancer (CRC)

MicroRNAs (MiRNAs)

3’ untranslated region (3'-UTR)

quantitative real-time RT-PCR (qRT-PCR)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of First Medical Center, Chinese PLA General Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

Y.Y., S.X. design of the work; S.L., D.T. the acquisition, analysis, S.H., Y.W. interpretation of data; R.L. X.D. the creation of new software used in the work; X.D. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

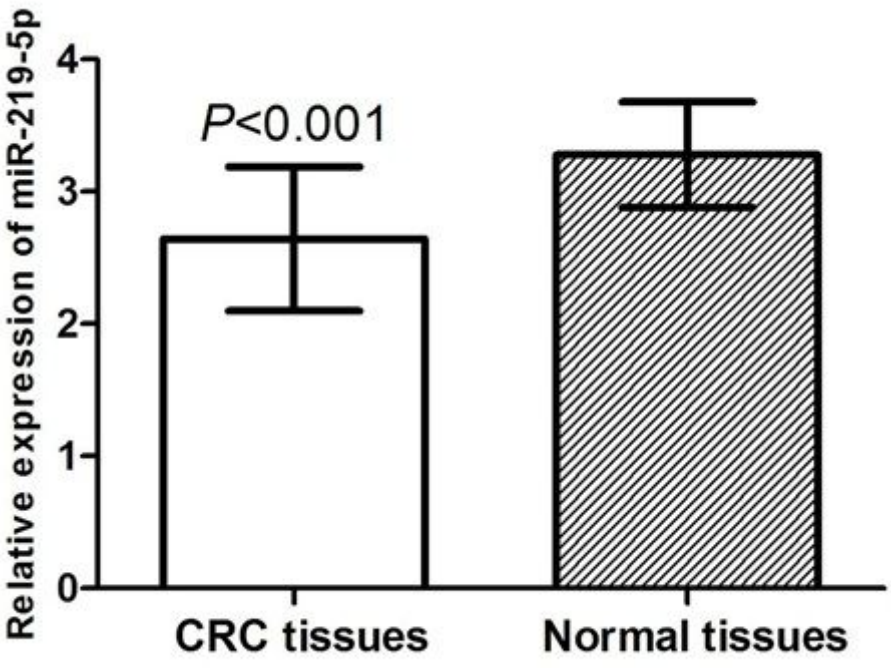

Figure 1

miR-219-5p expression levels in CRC tissues and matched noncancerous normal tissues. The expression of miR-219-5p was significantly lower in CRC tissues. P<0.001.

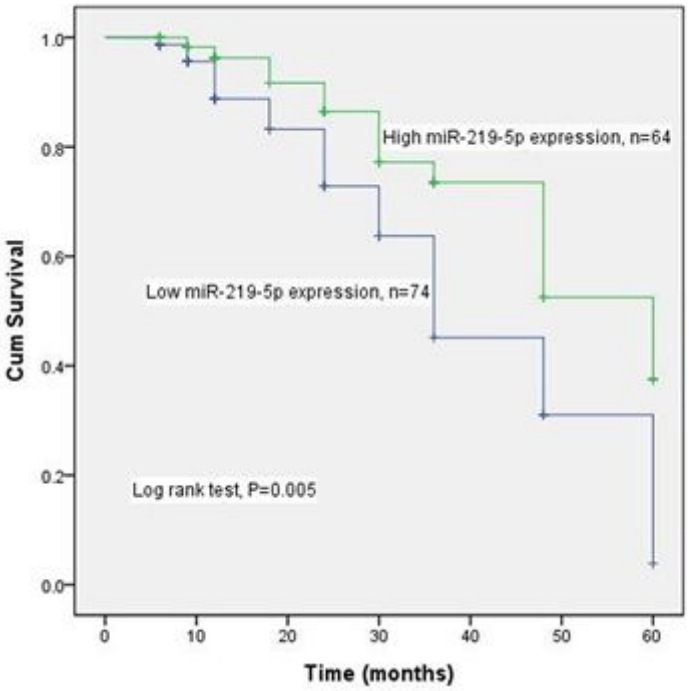

Figure 2

Kaplan-Meier analysis for CRC patients based on the expression of miR-219-5p. The CRC patients with low expression of miR-219-5p means have poor prognosis.