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Original Article

MicroRNA-139-3p indicates a poor prognosis of colon cancer

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Abstract: MicroRNAs (miRNAs) play an important role in the regulation of gene expression and are involved in almost biological procession. Recently, miR-139-5p has been reported to be downregulated in some types of cancer, and inhibits cancer cell invasion and metastasis. However, there are few reports on the role of miR-139-3p in cancer. In this study, we examined the expression level of miR-139-3p in 63 pairs of colon cancer and adjacent paracancerous tissues using quantitative reverse transcription PCR. The levels of miR-139-3p in colon cancer tissues were significantly lower than those in adjacent noncancerous tissues. There was an inverse correlation between the level of miR-139-3p and patient's age. Lower level of miR-139-3p was significantly associated with poor overall survival, especially in patients with TNM stages I and II. In conclusion, miR-139-3p has potential as a prognostic biomarker for colon cancer. Further prospective studies are required to validate this result.

Keywords: MicroRNA, miR-139-3p, colon cancer, prognosis, miR-139

Introduction

Colorectal cancer (CRC) is one of the most frequently diagnosed cancer and the fourth most common cause of cancer diagnosed in males and the third in females. It was estimated that approximately 96,830 new cases and 39,590 deaths from CRC will occur in the United States in 2014 [1]. In China, CRC has become the fifth malignancy and its incidence has shown an obvious increasing trend over the decade [2, 3]. CRC patients with early stage can be treated successfully with surgical resection, and are most likely to be completely curative. However, patients with advanced stage is refractory to existing therapies and have a poor prognosis. Therefore, effective diagnosis is vital to the treatment and prognosis of CRC. Increasing studies have shown that the dysregulation of microRNAs (miRNAs) is closely related to the occurrence and development of CRC. This offers new insight in the diagnosis, targeted therapy and prognosis for CRC.

miRNAs are a highly abundant class of conserved, endogenous, noncoding RNAs of 18-25 nucleotides in length that play important regulatory roles in diverse physiological and pathological processes through regulating the expressions of thousands of target genes at the post-translational level [4]. Most miRNA genes are located on introns and are transcribed together with their host genes. Abnormal expressed miRNAs are found in a wide range of human cancers, including lung cancer and CRC [5-7]. Some miRNAs function as an oncogene and/or a tumor suppressor in a context-dependent manner during the development and progression of cancer [8]. Dysregulation of miRNAs not only affects cancer initiation, invasion, and metastasis, but also contributes to the resistance to anticancer drugs [6, 9-11]. miRNAs are novel

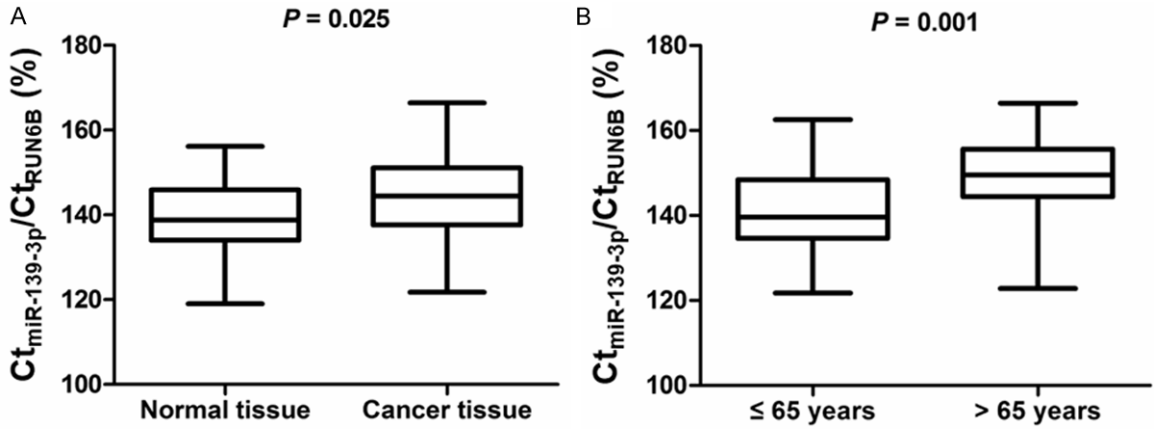

Figure 1. The expression level of miR-139-3p in colon cancer. A. The level of miR-139-3p in cancer tissues is higher than that in corresponding adjacent tissues. B. Patients whose age was older than 65 years had a higher levels of miR-139-3p compared with younger patients.

Table 1. Association of serum miR-139-3p expression with clinicopathologic parameters

Characteristics	miR-139-3p expression		*P* value
	Low	High	
Age (years)			
> 65	26	13	0.002
≤ 65	6	18	
Sex			
male	19	14	0.190
female	1	17	
Histologic grade			
well	2	3	0.300
moderate	17	21	
poor	13	7	
Tumor size (cm)			
> 6	14	10	0.459
≤ 6	18	19	
TNM			
I + II	19	21	0.596
III + IV	12	10	
LNM			
Yes	12	10	0.663
No	20	21	

potential biomarkers for cancer detection and prognosis.

miR-139 is located in the second intron of PDE2A gene on chromosome 11q13.4. Previous studies have reported the abnormal expression of miR-139 in some type of human cancers, such as breast cancer, gastric cancer, hepatocellular cancer and CRC [12-16]. Over-expression of miR-139 inhibits cancer cell proliferation, invasion, and metastasis [11, 17, 18]. However, there are few reports on the relationship between miR-139-3p, one form of miR-139, and cancer. In this study, we aimed to determine the potential roles of miR-139-3p as a molecular biomarker for colon cancer.

Materials and methods

Patients

All specimens from 63 patients with pathologically diagnosed primary colon cancer were stored in the Biobank of National Engineer Center for Biochip at Shanghai. All patients did not receive anticancer treatment, including chemotherapy, radiotherapy and biotherapy, prior to surgery resection. The end point of the study was overall survival. The protocol was approved by the Ethics Committee of National Engineer Center for Biochip at Shanghai, and the study was carried out according to the provisions of the Helsinki Declaration. All patients signed the informed consent form before surgery resection. Colon cancer staging relies on the TNM system designed jointly by the UICC (Union International Against Cancer) and the AJCC (American Joint Committee on Cancer) [19].

RNA extraction

Total RNA was isolated from the FFPE tissues by using the RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. The con-

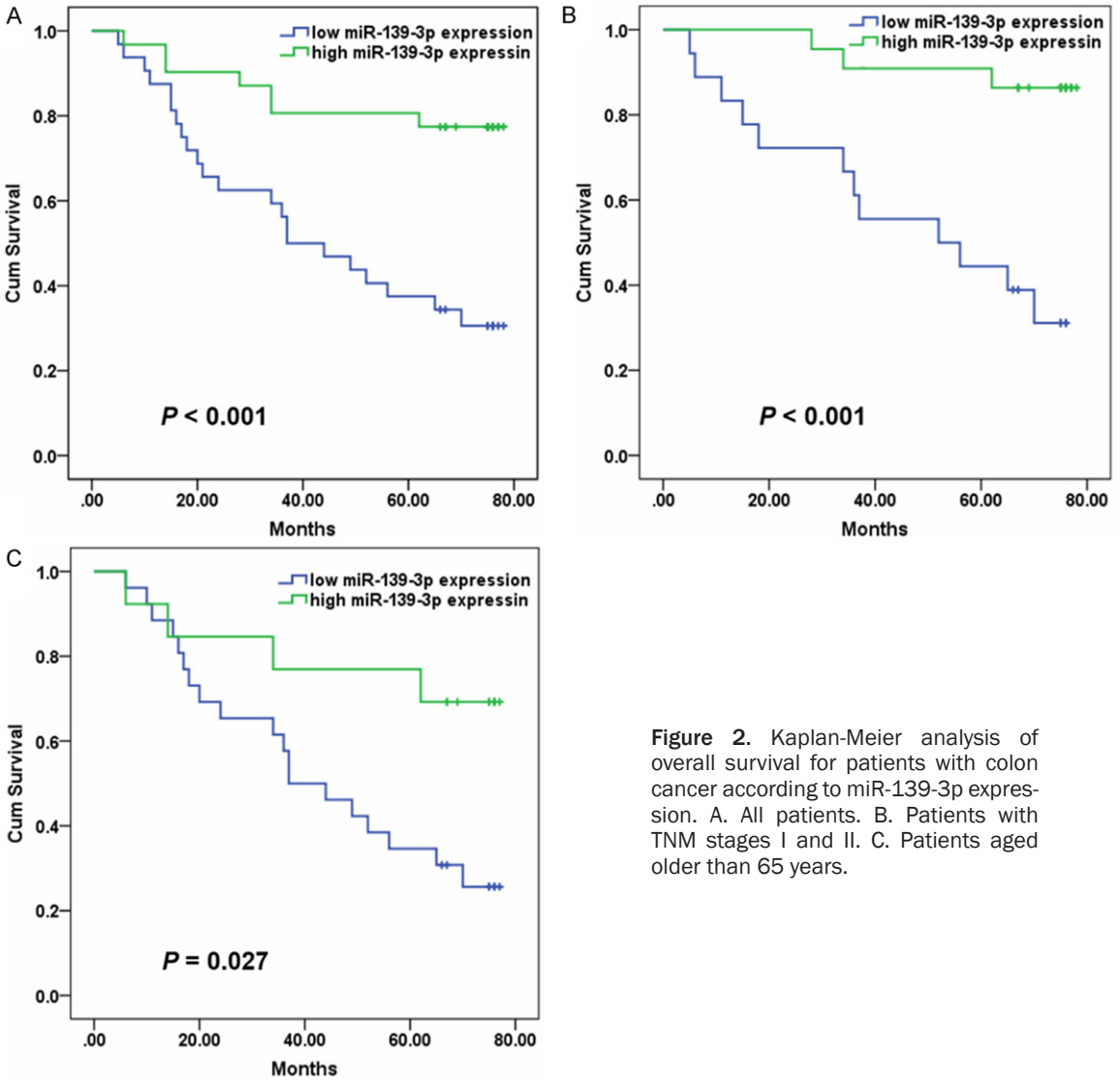

Figure 2. Kaplan-Meier analysis of overall survival for patients with colon cancer according to miR-139-3p expression. A. All patients. B. Patients with TNM stages I and II. C. Patients aged older than 65 years.

centration and quality of the isolated RNA were assessed on a NanoDrop ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE, USA). RNA samples were stored at -80°C until later use.

Quantitative reverse transcription PCR (qRT-PCR)

The First Strand cDNA was synthesized by using the miScript Reverse Transcription Kit (Qiagen, Hilden, Germany) in a final volume of 20 μl containing 6 μl of total RNA, 4 μl of 5 × miScript RT buffer and 1 μl of miScript Reverse Transcriptase Mix. The reaction mixtures were incubated at 37°C for 60 min, at 95°C for 5 min, and then held at 4°C. Quantitative RT-PCR was performed as previously described [20]. The relative expression level of miR-139-3p were normalized to the endogenous control RNU6B, and were calculated with the formula $2^{-\Delta\Delta Ct}$ [21] .

Statistical analyses

All statistical analyses were performed using SPSS v19.0 software (SPSS, Inc., Chicago, IL, USA). The expression level of miR-139-3p was compared using Mann-Whitney U test. Chi-square test or Fisher's exact test were performed to determine the relationship between miR-139-3p level and clinicopathological parameters. Kaplan-Meier and log-rank testing were performed to evaluate the effect of miR-139-3p on survival of colon cancer. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated by Cox regres-

Table 2. Univariate and multivariate analysis for overall survival

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (year), > 65 vs ≤ 65	2.786 (1.132-6.867)	0.026	1.735 (0.600-5.020)	0.310
Sex, male vs female	1.312 (0.626-2.748)	0.472		
Histologic grade, poor vs. well, moderate	1.585 (0.748-3.360)	0.229		
Tumor size (cm), > 6 vs. ≤ 6	0.555 (0.267-1.150)	0.113		
TNM, III + IV vs I + II	2.334 (1.121-4.859)	0.023	2.465 (0.606-10.024)	0.208
LNM, positive vs. negative	2.404 (1.155-5.006)	0.019	1.345 (0.231-7.831)	0.741
miR-139-3p, high vs. low	4.164 (1.770-9.793)	0.001	2.793 (1.005-7.760)	0.049

sion models. A *P* value < 0.05 were considered statistically significant.

Results

Expression level of miR-139-3p in colon cancer

To examine the expression level of miR-139-3p in colon cancer, we used qRT-PCR to measure its expression in 63 pairs of colon cancer and adjacent non-cancerous tissues. The levels of miR-139-3p in cancer tissues were significantly increased compared with those in adjacent tissues (*P* = 0.025, **Figure 1A**). In addition, an inverse correlation was observed between the level of miR-139-3p and age (*P* = 0.003). The levels of miR-139-3p in patients aged 65 years and younger were higher than those in patients aged old than 65 years (*P* = 0.001, **Figure 1B**).

Association of miR-139-3p expression with patient characteristics

We further evaluated the relationships of miR-139-3p expression with clinicopathological characteristics. Sex, age, tumor size, histologic grade, TNM stage, and LNM were include in our analysis, since these clinical features are considered as the key elements of the colon cancer patients prognosis [22]. There was a significant statistical difference between miR-139-3p and age (*P* = 0.002, **Table 1**). No association between miR-139-3p expression and other characteristics was observed.

Correlation of miR-139-3p expression with prognosis of colon cancer patients

Overall survival was calculated as the time from the date of surgery resection to the date of last contact or death. Among 63 colon cancer patients, 29 (46.0%) patients died as a result of disease progression during the follow-up. Colon cancer patients with high expression of miR-139-3p had a significantly longer survival time compared with those with low expression of miR-139-3p (log-rank *P* < 0.001, **Figure 2A**). In univariate analysis, older age (HR = 2.786, 95% CI: 1.132-6.867, *P* = 0.026), advanced TNM stage (HR = 2.334, 95% CI: 1.121-4.859, *P* = 0.023), LNM (HR = 2.404, 95% CI: 1.155-5.006, *P* = 0.019), and low expression of miR-139-3p (HR = 4.164, 95% CI: 1.770-9.793, *P* = 0.001) were associated with poor survival. In multivariate analysis, only miR-139-3p expression was an independent prognostic factor in colon cancer (HR = 2.793, 95% CI: 1.005-7.760, *P* = 0.049) (**Table 2**).

Stratified analyses further revealed that in TNM stages I and II group, patients with high expression of miR-139-3p was significantly associated with better prognosis compared with those with low expression of miR-139-3p (HR = 7.217, 95% CI: 2.023-25.746, *P* = 0.002, **Figure 2B**). In older age (> 65 years) group, univariate analysis showed that there was significant association between miR-139-3p expression and over survival of colon cancer patients (HR = 3.181, 95% CI: 1.076-9.407, *P* = 0.036, **Figure 2C**). However, this association disappeared after adjustment for LNM and TNM stage (HR = 2.146, 95% CI: 0.660-6.973, *P* = 0.204).

Discussion

There is mounting evidence demonstrating that miRNAs play an important role in the development of CRC, which exhibit oncogenic or tumor suppressive role by directly regulating oncogenes or tumor-suppressor genes [23, 24]. A better understanding of the changes in miRNA expression during CRC initiation and progression may facilitate better understanding of the

molecular mechanism of carcinogenesis, which could make it possible to improve the diagnosis and treatment of CRC [25].

A series of explorations have been underway for CRC, and some cancer-related miRNAs have been identified. For example, miR-21, miR-18a and miR-29a that function as promoters for cell proliferation, invasion and metastasis are significantly overexpressed in CRC [26, 27], whereas the down-regulation of miRNAs, such as miR-138 and miR-144, has also been implicated in CRC and predict a poor prognosis in CRC patients [9, 10]. miR-139 is downregulated in some types of human cancer, including CRC [15, 28, 29]. In this study, we found that miR-139-3p was downregulated in colon cancer and showed no significant difference with respect to tumor stage and lymph node status, which was in agreement with findings from previous study [11]. In addition, the level of miR-139-3p inversely correlated with age. It has been shown that some miRNAs are differentially expressed with human age [30, 31]. Different expression levels of miRNAs in different age groups were also observed in cancer patients [32, 33]. These indicate that different molecular mechanisms give rise to the different in the age at onset of cancers.

Guo et al. [15] reported that miR-139 inhibited CRC growth, and its level was inversely correlated with prognosis in CRC patients. Two recent studies revealed that miR-139-5p did not inhibit CRC growth but suppressed CRC invasion and metastasis by targeting AMFR, NOTCH1, matrix metalloproteinase 2 and type I insulin-like growth factor receptor, whereas miR-139-3p showed no significant effect on CRC cell growth and migration [11, 34]. However, Zhang et al. [24] reported an inhibitory effect of miR-139-5p on CRC growth. Furthermore, miR-139-5p expression was an independent prognostic factor for CRC [24]. In the present study, we found that low miR-139-3p expression was associated with older age (> 65 years) and poor prognosis. Further studies are required to elucidate the exact role of miR-139-3p in colon cancer.

In summary, our results provide first evidence that miR-139-3p is an independent poor prognostic factor in colon cancer patients. Because of small sample size, larger studies are warranted to validate this result.

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Disclosure of conflict of interest

None.

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