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Original Article

MicroRNA-135b is associated with tumor progression in colorectal cancer

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Abstract: Background: MicroRNA-135b (miR-135b) functions as an oncogene in several malignant tumors, including colorectal cancer. In the present study, we investigated the clinical significance of miR-135b in colorectal cancer. Methods: Paired tissue specimens (tumor and adjacent normal mucosa) from 105 patients with colorectal cancer were obtained from November 2007 to December 2014. The relationship between miR-135b expression and the clinicopathologic features of colorectal cancer was analyzed using the χ^2 test. The Kaplan-Meier method was used to estimate survival, and the survival differences between the subgroups were examined using the log-rank test. A Cox regression model was applied for the univariate analysis and multivariate analysis of prognostic factors. Results: The miR-135b was significantly upregulated in colorectal cancer tissues compared with the adjacent noncancerous tissues (P<0.001). High miR-135b expression was significantly associated with tumor differentiation (P=0.027), lymph node metastasis (P=0.002), distant metastasis (P<0.001) and TNM stage (P<0.001). We found that colorectal cancer patients with high miR-135b expression level had distinctly shorter overall survival than patients with low miR-135b expression level (P=0.021). Further multivariate COX regression analysis indicated that miR-135b expression served as predictors of poor prognosis (HR=2.549, CI: 1.293-9.823, P=0.009). Conclusions: Our findings suggested that miR-135b was associated with tumor progression in colorectal cancer and it could be used as a prognostic factor for colorectal cancer.

Keywords: MicroRNA-135b, expression, colorectal cancer, clinical significance

Introduction

Colorectal cancer is the third most common cancer and the fourth most common cause of cancer deaths worldwide [1]. Identification of molecule associated with the prognosis of patients with colorectal cancer may not only shed light on elucidation of the underlying biologic mechanisms involved in the development or progression of the disease, but also provide the opportunity to identify novel targets for colorectal cancer therapy.

MicroRNAs (miRNAs) are a class of short (about 22 nucleotides in length), endogenous, single-stranded, non-protein-coding RNAs that directly bind to the 3'-untranslated regions (3'-UTRs) of target messenger RNAs (mRNAs), leading to mRNA degradation or translational suppression [2]. miRNAs were shown to be associated with diverse biological processes, including development, tumorigenesis, cell differentiation, proliferation, apoptosis, and autophagy [3, 4]. Some miRNAs has been shown to act as tumor suppressors or oncogenes, and this functionality makes them useful biomarkers in cancer diagnosis and prognosis [5].

MicroRNA-135b (miR-135b) functions as an oncogene in several malignant tumors, including gastric cancer, cutaneous squamous cell carcinoma, breast cancer, prostate cancer, hepatocellular carcinoma, and osteoscarcoma [6-10], suggesting that it might be a target for malignant tumor therapy. Previously, Valeri et al. showed that miR-135b overexpression was triggered in mice and humans by APC loss, PTEN/PI3K pathway deregulation, and SRC overexpression and promoted tumor transformation and progression. Inhibition of miR-135b in colorectal cancer mouse models reduced tumor growth by controlling genes involved in proliferation, invasion, and apoptosis [11]. In the present study, we investigated the clinical significance of miR-135b in colorectal cancer.

Table 1. The relationship between miR-135b expression and clinicopathologic characteristics in 105 colorectal cancer patients

Variables	Case (n)	miR-135b expression level		*P* value
		High (n=54)	Low (n=51)	
Gender				
Male	67	31 (46.27%)	36 (53.73%)	0.223
Female	38	23 (60.53%)	15 (39.47%)	
Age				
≤60	47	22 (46.81%)	25 (53.19%)	0.436
>60	58	32 (55.17%)	26 (44.83%)	
Tumor size (cm)				
≤2	44	19 (43.18%)	25 (56.82%)	0.170
>2	61	35 (57.38%)	26 (42.62%)	
Lymph node metastasis				
Absent	68	27 (39.71%)	41 (60.29%)	0.002
Present	37	27 (72.97%)	10 (27.03)	
Vascular invasion				
Absent	70	34 (48.57%)	36 (51.43%)	0.535
Present	35	20 (57.14%)	15 (42.86%)	
Distant metastasis				
Absent	99	49 (49.49%)	50 (50.51%)	<0.001
Present	6	5 (83.33%)	1 (16.67%)	
Differentiation				
Well/Moderate	64	27 (42.19%)	37 (57.81%)	0.027
Poor	41	27 (65.85%)	14 (34.15%)	
Stage				
I/II	60	20 (33.33%)	40 (66.67%)	<0.001
III/IV	45	34 (75.56%)	11 (24.44%)	

Methods

Tissue samples

Paired tissue specimens (tumor and adjacent normal mucosa) from 105 patients with colorectal cancer were obtained and histologically confirmed by a pathologist at Zaozhuang Municipal Hospital from November 2007 to December 2014. All samples were derived from patients who had not received adjuvant treatment including radiotherapy or chemotherapy prior to surgery. Clinicopathologic information, including age, gender, tumor size, histological type, depth of invasion, tumor-node-metastasis classification, location, lymph node metastasis, lymph node invasion, and distant metastasis, was available for all patients. This study was approved by the Research Ethics Committee of Zaozhuang Municipal Hospital. Written informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards. All the clinicopathologic characteristics were shown in **Table 1**.

RNA extraction and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from clinical specimens with Trizol reagent according to the manufacturer's instructions. For miRNA expression, cDNA was synthesized using gene-specific primers (Ribobio, Guangzhou, China) and the M-MLV RT kit (Invitrogen, Carlsbad, CA, USA) in a 20-ml reaction volume. The RT reaction reagents contained 1 mg RNA template, 1 ml 10 mM dNTP mix, 2 ml 0.1 M DTT, 4 ml 56 first-strand buffer, and 1 ml 40 U/ml RNase inhibitor. The volume was adjusted with RNA-free H_2O. The reverse-transcription reaction was performed in triplicate to remove any outliers. MiRNA expression was assessed using qRT-PCR and an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). The relative amount of miR-135b to small nuclear U6 RNA was calculated using the equation $2^{-\Delta CT}$, where ΔCT = (CT miR-135b-CT U6).

Statistical analysis

Differences between groups were assessed using Student's t-test or Fisher's exact test. The relationship between miR-135b expression and the clinicopathologic features of colorectal cancer was analyzed using the χ^2 test. The Kaplan-Meier method was used to estimate survival, and the survival differences between the subgroups were examined using the log-rank test. A Cox regression model was applied for the univariate analysis and multivariate analysis of prognostic factors. *P* values of <0.05 were interpreted as statistically significant. The collected data were analyzed by using the SPSS version18.0 (SPSS Inc., Chicago, IL, USA).

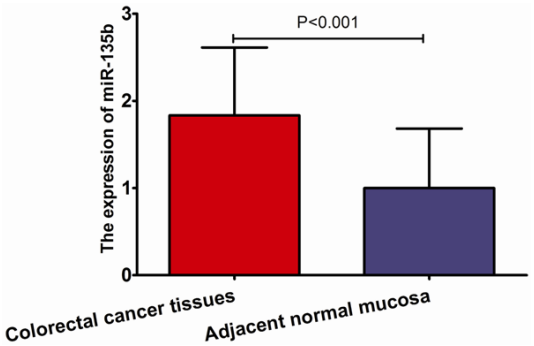

Figure 1. The relative expression of miR-135b in colorectal cancer tissues and adjacent noncancerous tissues.

Results

The relative expression of miR-135b in colorectal cancer

We quantified miR-135b in 105 pairs of colorectal cancer tissues and paracancerous tissues using qRT-PCR. The miR-135b was significantly upregulated in colorectal cancer tissues compared with the adjacent noncancerous tissues (P<0.001, shown in **Figure 1**). We divided the 105 samples into high (n=54) and low (n=51) miR-135b expression groups according to the median value of miR-135b level.

Relationship between miR-135b expression and clinicopathological parameters in patients with colorectal cancer

To evaluate the relationship between miR-135b expression and colorectal cancer progression, we analyzed the correlation between miR-135b overexpression and clinicopathological features of colorectal cancer patients. High miR-135b expression was significantly associated with tumor differentiation (P=0.027), lymph node metastasis (P=0.002), distant metastasis (P<0.001) and TNM stage (P<0.001). However, miR-135b expression was not found to be associated with age, gender, tumor size, and vascular invasion (all P>0.05, shown in **Table 1**).

Correlation between miR-135b expression and prognosis of colorectal cancer patients

Kaplan-Meier method and log-rank test were used to evaluate the differences of overall survival between low-expression group and high-expression group. We found that colorectal cancer patients with high miR-135b expression level had distinctly shorter overall survival than patients with low miR-135b expression level (P=0.021, shown in **Figure 2**). Further multivariate COX regression analysis indicated that miR-135b expression served as predictors of poor prognosis (HR=2.549, CI: 1.293-9.823, P=0.009, shown in **Table 2**).

Discussion

Despite the clinical implementation of numerous therapeutic strategies, colorectal cancer remains a leading cause of cancer-related deaths due to therapy resistance and metastasis [12]. Previous studies have demonstrated diverse genetic alterations in colorectal cancer, but the highly complex molecular mechanisms underlying colorectal cancer carcinogenesis and progression remain obscure. Therefore, it is necessary to search novel markers for colorectal cancer, which can accurately identify biological characteristics of tumors, improve therapeutic strategies, and predict clinical outcome.

There is increasing evidence that miRNAs are widely dysregulated in cancer, suggesting that they have potential applications for cancer diagnosis, prognosis, and treatment [13]. Specific microRNAs can act as either tumor suppressors or oncogenes depending on the cellular environment in which they are expressed. The expression of microRNAs is reproducibly altered in colorectal cancer, and their expression patterns are associated with diagnosis, prognosis, and therapeutic outcome in colorectal cancer. Studies have begun to examine the association of microRNA-related polymorphisms and their association with colorectal cancer incidence and prognosis as well as the possibility of using circulating microRNAs or fecal microRNA expression as noninvasive early detection biomarkers. These data suggest that microRNAs may be potential molecular classifiers, early detection biomarkers, and therapeutic targets for colorectal cancer [14].

miR-135b functions as an oncogene in several malignant tumors, including gastric cancer, cutaneous squamous cell carcinoma, breast cancer, prostate cancer, hepatocellular carcinoma (HCC), and osteoscarcoma, suggesting that it might be a target for malignant tumor therapy [6-10]. Previously, Valeri et al. showed

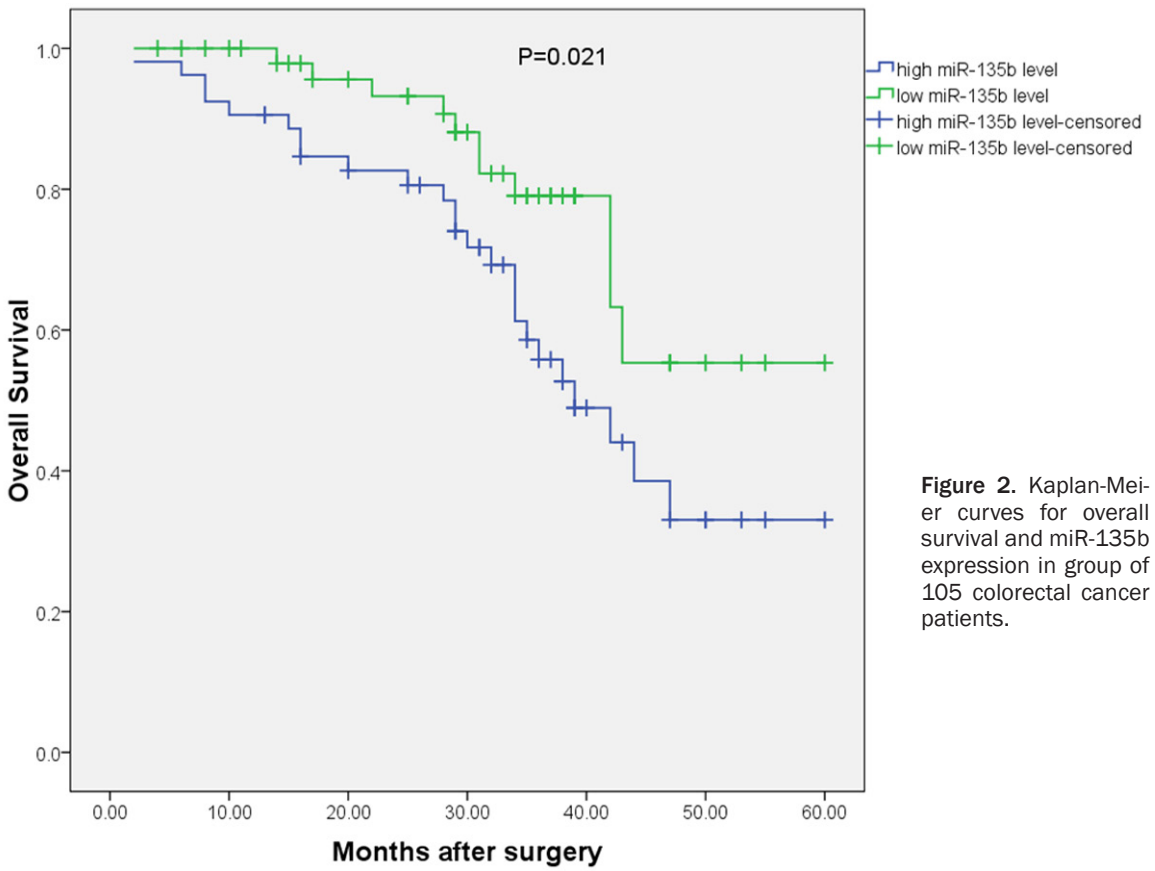

Figure 2. Kaplan-Meier curves for overall survival and miR-135b expression in group of 105 colorectal cancer patients.

Table 2. Multivariate analysis of clinicopathological factors for overall survival

Variable	Hazard ratio	95% CI	*P* value
Gender (male vs female)	0.819	0.273-1.926	0.728
Age (>60 y vs ≤60 y)	1.283	0.677-3.102	0.192
Tumor size (>2 cm vs ≤2 cm)	1.682	0.781-4.002	0.098
Lymph node metastasis (present vs absent)	3.772	1.893-7.116	0.011
Vascular invasion (present vs absent)	3.271	1.573-8.091	0.017
Distant metastasis (present vs absent)	4.921	1.999-14.921	0.001
Differentiation (poor vs well/moderate)	2.385	1.067-9.068	0.013
Stage (III/IV vs I/II)	3.192	1.823-10.092	0.007
miR-135b expression (high vs low)	2.549	1.293-9.823	0.009

that miR-135b overexpression was triggered in mice and humans by APC loss, PTEN/PI3K pathway deregulation, and SRC overexpression and promoted tumor transformation and progression. Inhibition of miR-135b in colorectal cancer mouse models reduced tumor growth by controlling genes involved in proliferation, invasion, and apoptosis [11]. In the study by He et al., they observed high levels of miR-135b in colorectal cancer cell lines and clinical tissues, compared to colorectal epithelium cell line and noncancerous tissues. Furthermore, enforced expression of miR-135b attenuated doxorubicin-induced apoptosis in colorectal cells. In elucidating the molecular mechanism by which miR-135b participate in the regulation of apoptosis and chemoresistance in colorectal cancer, they discovered that large tumor suppressor kinase 2 (LATS2) was a direct target of miR-135b. The role of miR-135b was confirmed in colorectal tumor xenograft models [15]. Wu et al. demonstrated that miR-135b downregulated metastasis suppressor-1 (MTSS1) expression and contributed to colorectal cancer cell invasion, indicating its involvement in colorectal cancer progression [16]. Li et al. found that miR-135b promoted cancer progression by tar-

geting transforming growth factor beta receptor II (TGFBR2) in colorectal cancer [17]. In the present study, we investigated the clinical significance of miR-135b in colorectal cancer. We quantified miR-135b in 105 pairs of colorectal cancer tissues and paracancerous tissues using qRT-PCR. The miR-135b was significantly upregulated in colorectal cancer tissues compared with the adjacent noncancerous tissues. To evaluate the relationship between miR-135b expression and colorectal cancer progression, we analyzed the correlation between miR-135b overexpression and clinicopathological features of colorectal cancer patients. High miR-135b expression was significantly associated with tumor differentiation, lymph node metastasis, distant metastasis and TNM stage. Kaplan-Meier method and log-rank test were used to evaluate the differences of overall survival between low-expression group and high-expression group. We found that colorectal cancer patients with high miR-135b expression level had distinctly shorter overall survival than patients with low miR-135b expression level. Further multivariate COX regression analysis indicated that miR-135b expression served as predictors of poor prognosis. In conclusion, our findings suggested that miR-135b was associated with tumor progression in colorectal cancer and it could be used as a prognostic factor for colorectal cancer.

Disclosure of conflict of interest

None.

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