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Clinicopathologic and prognostic relevance of miR-1256 in colorectal cancer: a preliminary clinical study

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) as a new class of biomarkers have been explored in recent studies. We investigate whether miR-1256 could be considered as powerful biomarkers for predicting the prognosis of colorectal cancer (CRC).

PATIENTS AND METHODS: The expression of miR-1256 in CRC was compared with matched normal tissue and using qRT-PCR. The correlation of miR-1256 expression with clinicopathological factors was statistically analyzed. Survival rate was determined with Kaplan-Meier and statistically analyzed with the log-rank method between groups. Univariate and multivariate Cox regression analysis was used to identify the independent risk factors for CRC.

RESULTS: We found that miR-1256 level in CRC tissues is notably reduced compared to matched non-cancerous specimens ($p < 0.01$), and the expression of miR-1256 was significantly correlated with TNM stage ($p = 0.000$) and lymph node metastasis ($p < 0.07$). Kaplan-Meier analysis showed that CRC patients with low miR-1256 expression level had distinctly shorter overall survival ($p = 0.004$) and disease-free survival ($p < 0.001$) than patients with high miR-1256 expression level. Finally, Cox regression analyses showed that low miR-1256 expression might be an independent prognostic parameter to predict poor prognosis of CRC patients.

CONCLUSIONS: We firstly provided evidence that low miR-1256 expression was associated with the progression of CRC and could be used as a prognostic biomarker for breast cancer. Further studies are needed.

Key Words:
miR-1256, Colorectal cancer, Prognosis.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies and causes large mortalities around the world each year[1]. In China, CRC is the fifth most prevalent malignancy in men and the third most prevalent malignancy in women[2]. Although considerable progress in CRC diagnosis and therapy has been achieved during the last few decades, the survival rate of CRC patients still remains unsatisfied[3,4]. About 60% of colorectal cancer patients are first diagnosed at an advanced stage with an expected 5-year survival rate of 5%[5]. The early detection of CRC is significantly beneficial to improve the probability of survival[6]. The identification of predictive and/or prognostic biomarkers able to guide treatment decision will supply an arm for improving diagnosis and management of human CRC. MicroRNAs (miRNAs) are a class of endogenous, noncoding, short (18-25 nucleotides in length), single-stranded RNA molecules that post-transcriptionally regulate the expression levels of genes and act as important regulator in various biological processes, including proliferation, differentiation and apoptosis[7-9]. It is now clear that the dysfunction or deregulation of miRNAs is involved in the processes of carcinogenesis, progression, and metastasis of various human malignancies including CRC[10-12]. They function either as oncogenes or as tumor suppressors depending on the role of their target mRNAs[13,14]. Recently, more and more evidence suggest that the miRNA expression profiles are capable of classifying human cancers efficiently, implying that miRNAs could become potential diagnostic and prognostic biomarkers[15-17]. However, the detail biological function and clinical significance of miRNA s in the tumorigenesis of CRC are still remaining largely unknown. MiR-1256, located in 1p36.12, was a newly identified miRNA which was firstly reported to be dysregulated in prostate cancer by Li et al[18]. Then, the dysregulation of miR-1256 was also confirmed in

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non-small cell lung cancer and human nasopharyngeal carcinoma[19,20]. These findings indicated that miR-1256 may act as an important regulator in malignancy. However, the expression, potential function and clinical significance of miR-1256 in CRC have not been reported. In this study, for the first time, we provided important clinical evidence that miR-1256 expression was downregulated in CRC and associated with poor prognosis of CRC patients, suggesting that miR-1256 may act as a novel diagnostic and prognostic marker for CRC patients.

Patients and Methods

Patients and Tissue Samples

The present study was approved by the Research Ethics Committee of Jining No.1 People's Hospital. A written informed consent was obtained from all the participating patients. A total of 178 pairs of CRC and matched adjacent non-tumor tissue samples were obtained from the Jining No.1 People's Hospital between July 2009 and January 2013. The colorectal cancer diagnosis was confirmed by an experienced pathologist. All samples were frozen immediately in liquid nitrogen and stored at -80°C until analysis. None of the patients received radiotherapy, chemotherapy, or other anticancer treatment before surgery. TNM stage was defined according to the 6th version of American Joint Committee on Cancer (AJCC) staging Manual. The follow-up periods ranged from 6 months to 5 years, with a mean of 3 years. Patients characteristics are described in Table II.

RNA Extraction, Reverse Transcription, and Quantitative RT-PCR

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and cDNA was synthesized with PrimeScript reverse transcriptase (TaKaRa, Otsu, Shiga, Japan) and oligo (dT) following the manufacturer's instructions. Real-time PCR was performed with a Taqman MicroRNA Assay Kit (Applied Biosystems, Foster City, CA, USA) on ABI7500 Real-time PCR detection system. Quantitative PCR was performed at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene. The relative degrees of expression of the genes tested were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers of miR-1256 was listed in Table I.

Statistical Analysis

All statistical analyses were carried out using SPSS 19.0 (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA). Counting data were reported as means ± standard deviation (SD). Differences between two groups of miR-1256 were analyzed by two-tailed Student's *t*-test. The association between miR-1256 and clinicopathologic features was tested using the x^2-test. Survival analysis was performed by Kaplan-Meier method with the log-rank test. A Cox regression model was applied for the univariate analysis and multivariate analysis of prognostic factors. $p < 0.05$ was considered statistically significant.

Results

miR-1256 was Down-Regulated in CRC Tissues

In order to explore the effect of miRT-1256 in progression of CRC, qRT-PCR assay was performed to detect the expression of miR-1256 in 183 CRC tissues and corresponding non-tumor tissues. As shown in Figure 1A, we found that the levels of miR-1256 in CRC tissues were significantly lower than those in matched noncancerous tissues ($p < 0.01$), indicating that miR-1256 may be involved in the carcinogenesis of CRC.

Correlations of miR-1256 Expression With Clinicopathological Features of CRC Patients

After we demonstrated down-regulation of miR-1256 in CRC patients, we further investigate the association of miR-1256 with clinicopathological features of CRC patients. Using the median expression level as the cutoff value, our patient cohort was assigned into high miR-1256 expres-

Table I. The primers for PCR.

Gene	Sequences (5′-3′)
miR-1256 (Forward)	GGCGCGATTTTAGTTTATC
miR-1256 (Reverse)	TTT AATTAC CAACCGAATACG
GAPDH (Forward)	GGGAGCCAAAAGGGTCAT
GAPDH (Reverse)	GAGTCCTTCCACGATACCAA

Table II. Association of miR-1256 expression with clinicopathological features of CRC.

Variables	Cases (N)	circ-NT5C2 expression		*p*-value
		High	Low	
Age				NS
≤ 50	91	41	50	
> 50	87	49	38	
Sex				NS
Male	99	51	48	
Female	79	39	40	
Tumor size				NS
≤ 5 cm	103	58	45	
> 5 cm	75	32	43	
Histological grade				NS
Well/moderate	93	53	40	
Poor	85	37	48	
Site				NS
Colon	108	58	50	
Rectum	70	32	38	
TNM stage				0.000
I and II	125	78	47	
III and IV	63	22	41	
Lymph node metastasis				0.007
Absence	122	70	52	
Presence	56	20	36	

sion group (above the median, n=90) and low miR-1256 expression group (below the median, n = 78). As shown in Table II, we observed that the expression of miR-1256 was significantly correlated with TNM stage (p = 0.000) and lymph node metastasis (p < 0.07). However, there were no significant correlations between miR-1256 expression and other clinical features including patient's age, sex, tumor size, histological grade and site (p > 0.05). Taken together, our results revealed that miR-1256 downregulation may be associated with aggressive progression in CRC.

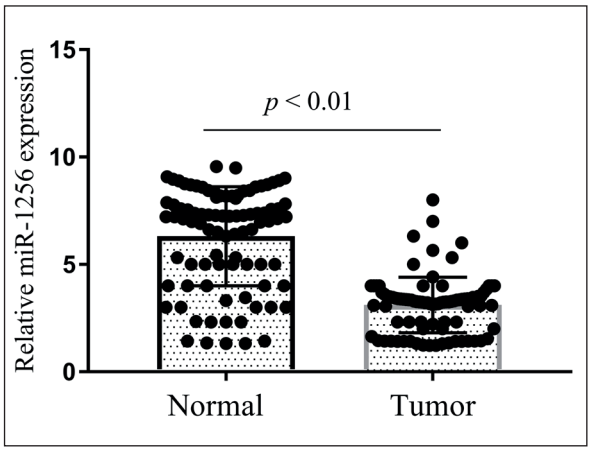

Figure 1. The relative expression levels of miR-1256 in CRC tissues and adjacent non-cancerous tissues by RT-PCR. miR-1256 expression was significantly higher in CRC tissues than in the corresponding adjacent non-cancerous tissues (p < 0.01).

miR-1256 Downregulation Associates With Poor Prognosis in Patients With Human CRC

Kaplan-Meier analyses were performed to investigate the association between the miR-1256 expression and the prognosis of patients with CRC. From the Kaplan-Meier survival curves, the results showed that patients in the high miR-1256 expression group had better overall survival than those in the low miR-1256 expression group (p = 0.004, Figure 2). Moreover, we also found that patients with lower miR-1256 expression showed lower disease-free survival rate (p < 0.000, Figure 3). Univariate and multivariate analyses were performed to estimate the prognostic value of miR-1256 using Cox regression analysis. The result of univariate analysis showed that TNM stage, lymph node metastasis and miR-1256 expression were associated with both overall survival (Table III) and disease-free survival (Table IV) in CRC patients. More importantly, multivariate analysis indicated that miR-1256 expression level was an independent prognostic factor for predicting the 5-year overall survival and disease-free survival of CRC patients (Table III and IV).

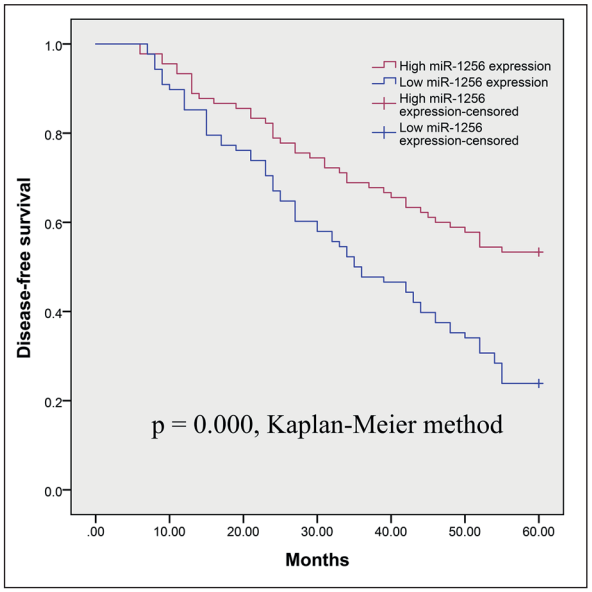

Figure 2. Kaplan-Meier curves for overall survival of miR-1256 in CRC patients, divided according to miR-1256 expression levels. Low miR-1256 expression was significantly associated with poor survival ($p = 0.004$, log-rank test).

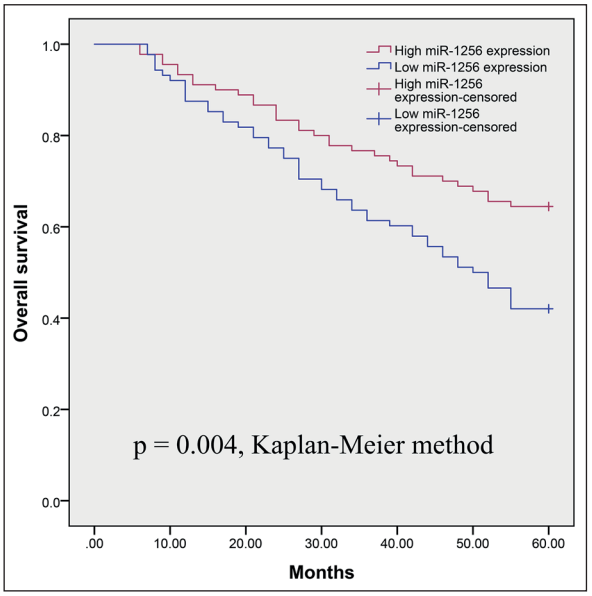

Figure 3. Kaplan-Meier curves for disease-free survival of miR-1256 in CRC patients, divided according to miR-1256 expression levels. Low miR-1256 expression was significantly associated with poor survival ($p < 0.000$, log-rank test).

Table III. Summary of overall survival analyses by univariate and multivariate Cox regression analysis.

Variables	Univariate analysis			Multivariate analysis		
	p-value	HR	CI (95%)	*p*-value	HR	CI (95%)
Age	0.217	1.213	0.774-1.966	-	-	-
Sex	0.167	1.114	0.815-1.672	-	-	-
Tumor size	0.113	0.893	0.366-1.556	-	-	-
Histological grade	0.234	1.233	0.673-2.317	-	-	-
Site	0.366	1.436	0.563-1.994	-	-	-
TNM stage	0.000	6.653	3.226-9.964	0.000	5.466	2.846-8.452
Lymph node metastasis	0.004	3.673	1.325-5.563	0.008	3.015	1.113-4.679
miR-1256 expression	0.001	4.368	1.558-6.674	0.004	3.457	1.231-5.452

Table IV. Summary of overall survival analyses by univariate and multivariate Cox regression analysis.

Variables	Univariate analysis			Multivariate analysis		
	p-value	HR	CI (95%)	*p*-value	HR	CI (95%)
Age	0.178	1.342	0.648-1.793	-	-	-
Sex	0.234	1.221	0.941-2.215	-	-	-
Tumor size	0.345	0.795	0.554-1.894	-	-	-
Histological grade	0.327	1.338	0.891-2.442	-	-	-
Site	0.245	1.313	0.667-2.034	-	-	-
TNM stage	0.000	7.673	3.445-11.321	0.000	5.783	2.994-9.997
Lymph node metastasis	0.004	3.423	1.226-5.781	0.012	2.342	1.241-4.884
miR-1256 expression	0.001	4.783	1.418-7.342	0.001	3.893	1.136-6.569

Discussion

CRC, a serious health problem, is associated with a high mortality rate due to its rapid progression and advanced tumor presentation at the time of diagnosis[21]. In China, more than 376,300 new cases (including rectum cancer) and 191,000 deaths occurred in 2015[22]. Approaches to improve diagnosis or prognosis in patients with CRC may help to establish individualized tailored therapy[23]. Up to date, several clinical clinicopathologic factors, such as TNM stage, histological grade or lymph node metastasis, are used for the prognosis prediction of CRC patients[24]. Nevertheless, they may not accurately estimate outcome because of heterogeneity in the patient population. Thus, it is necessary to search for novel diagnostic and prognostic markers for CRC. Recently, growing studies indicated that miRNAs are extremely stable and significantly dysregulated in several disease, indicating the great potentiality of circulating miRNAs as candidate biomarkers for multiple diseases including cancer[25,26]. MiRNAs are a group of small non-coding RNAs that regulate genes expression by targeting mRNAs for translational repression[27]. Because some mRNAs served as tumor suppressor or oncogenes, miRNAs were involved in the development and progression of tumors[28]. In addition, several miRNAs, such as miR-944[29], miR-10b[30] and miR-552[31], were reported to be associated with prognosis of CRC patients. Recently, the expression pattern and biological function of miR-1256 have reported in several tumors. For instance, in prostate cancer, it was reported that epigenetic deregulation of miR-1256 contributes to the inhibition of prostate cancer cell growth and invasion[18]. In lung cancer, it was found that miR-1256 was highly expressed in non-small cell lung cancer tissues and its overexpression suppresses proliferation and migration of non-small cell lung cancer cells via regulating TCTN1[19]. Those findings revealed that miR-1256 may be a tumor suppressor miRNA in tumors. However, whether miR-1256 is abnormally expressed in CRC, and its biological function in CRC has not been reported. In this study, we firstly detect the expression of miR-1256 in CRC tissues and matched normal tissues; we found that miR-1256 expression was significantly downregulated in CRC tissues compared with matched normal tissues. Then, by analyzing clinical samples, we observed that decreased miR-1256 expression was associated with advanced TNM stage and lymph node metastasis, indicating that miR-1256 down-regulation may promote an aggressive phenotype of CRC. In addition, Kaplan-Meier analysis showed that decreased miR-1256 expression levels were correlated with poor overall survival and disease-free survival of CRC patients. Importantly, the univariate and multivariate survival analyses showed that down-regulation of miR-1256 was an independent factor for predicting both overall survival and disease-free survival in CRC patients, demonstrating that miR-1256 might function as a potential prognostic biomarker for CRC patients.

Conclusions

We first revealed that miR-1256 expression was upregulated in CRC tissues and its down-regulation was associated with prognosis of CRC patients. Our study provides a new perspective for miR-1256 acting as a non-coding oncogene in CRC tumorigenesis and, therefore, it is a novel early diagnostic and prognostic marker of CRC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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