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Increased expression of miR-552 acts as a potential predictor biomarker for poor prognosis of colorectal cancer

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Abstract. – OBJECTIVE: MiR-552 has recently been identified to be involved in tumorigenesis of colorectal cancer (CRC). The aim of this study was to determine if miR-552 could serve as a prognosis indicator for patients with CRC.

PATIENTS AND METHODS: MiR-552 expression levels were detected in 183 pairs of primary CRC and their matched non-tumor tissues by qPCR. The association between miR-552 expression and clinicopathological parameters was analyzed. The survival curves were calculated by the Kaplan-Meier method. Univariate and multivariate analyses were performed to explore the prognostic significance of miR-552 expression.

RESULTS: We found that miR-552 expression was upregulated in CRC tumor tissues compared to their adjacent normal tissues ($p < 0.01$). Increased miR-552 expression was significantly associated with histological grade ($p = 0.000$), lymph nodes metastasis ($p = 0.022$) and TNM stage ($p = 0.002$). In addition, the patients with high miR-552 expression had a significantly worse 5-year overall survival rate than those with low miR-552 expression ($p = 0.0036$). Furthermore, according to univariate and multivariate analysis, elevated miR-552 was confirmed to be an independent prognostic factor for worse survival.

CONCLUSIONS: The current results demonstrated that high miR-552 expression was associated with poor outcomes in patients with CRC. MiR-552 may be a potential prognostic biomarker and therapeutic target in CRC patients.

Key Words:
MiR-552, Colorectal cancer, Prognosis.

Introduction

Colorectal cancer (CRC) is one of the most frequent cancers in both men and women, with a worldwide incidence estimated at more than 1 million cases annually[1]. The major therapeutic approaches for CRC are surgery, neoadjuvant radiotherapy, and adjuvant chemotherapy[2]. When diagnosed early with only localized disease, surgery can often be curative. Unfortunately, CRC is often diagnosed at an advanced clinical stage[3,4]. The metastatic diseases are the main cause for the high mortality rates in CRC patients[5]. Thus, identification of new biomarkers for the diagnosis, prognosis, and treatment of CRC is essential for improving patient survival. MicroRNAs (miRNAs) were identified as an abundant class of small non-coding RNAs that regulates gene expression post-transcriptionally[6]. They are highly conserved and ubiquitously expressed in all species. MiRNAs-mediated gene regulation is thought to play a crucial role in in various biological processes, including cell proliferation, apoptosis, and tumorigenesis of cancer[7,8]. Growing evidence indicated that miRNAs were involved in development and progression, serving as either oncogenes or tumor suppressors[9,10]. Notably, miRNAs have been reported to be useful in the diagnosis and prognosis assessment of CRC[11,12]. Thus, identifying more accurate predictive miRNAs is of great clinical value.

MiR-552, which is located at 1p34.3, is a newly identified miRNA. Its function in tumors progression remains largely unknown. A recent study[13] reported that the expression levels of miR-552 were significantly upregulated in CRC tissues. In addition, functional assay confirmed that miR-552 serve as a tumor promoter in CRC[14]. The aim of this work was to investigate the clinical significance of miR-552 in human CRC.

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Table I. Clinicopathological features and miR-552 expression in CRC.

Parameters	Group	Total	MiR-552 expression		*p*-value
			High	Low	
Gender	Male	123	59	64	0.638
	Female	60	31	29	
Age (years)	< 60	96	50	46	0.409
	≥ 60	87	40	47	
Tumor size (cm)	< 5	115	52	63	0.163
	≥ 5	68	38	30	
Local invasion	T1-T2	86	37	49	0.117
	T3-T4	97	53	44	
Histological grade	Well and moderately	91	31	60	0.000
	Poorly	92	59	33	
Lymph nodes metastasis	Negative	95	39	56	0.022
	Positive	88	51	37	
TNM stage	I-II	92	35	57	0.002
	III-IV	91	55	36	

Patients and Methods

Patients and Specimens

Tumor tissues with paired adjacent normal tissues were obtained from 183 CRC patients at the Department of Gastroenterology, Fourth Hospital of Hebei Medical University from 2008 to 2011. The histopathological diagnosis of all samples was diagnosed by two pathologists. None of the patients had received neoadjuvant chemotherapy or radiotherapy prior to surgery. All tissue samples were frozen in liquid nitrogen immediately after resection and stored at -80°C. The overall survival time was calculated from the date of surgery to the date of death or to the last follow-up. The patient information is summarized in Table I. All protocols of this study were approved by the Ethics Committee of Fourth Hospital of Hebei Medical University. All participants were informed and gave written consent.

RNA Extraction and RT-qPCR

According to the manufacturer's instructions, total RNA was isolated from tissues using the miRNeasy kit (TaKaRa, Otsu, Shiga, Japan). cDNA synthesis was performed with 2 μg total RNA using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (TaKaRa, Otsu, Shiga, Japan). The expression levels of miR-552 were quantified using miRNA-specific TaqMan miRNA assay kit (Biosystems, Foster City, CA, USA). The expression level of GAPDH was used as a reference gene. The primers for the examined genes are presented in Table II. The relative expression levels of interest gene were calculated by the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

The significance of differences between groups was estimated by Student's *t*-test and x^2 test. The survival curves of the patients were plotted and analyzed by Kaplan-Meier method and the survival rates were compared by the log-rank test. The Cox proportional hazards model was used for univariate and multivariate regression analyses. All *p*-values were obtained using the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA).

Results

Increased Expression of miR-552 in CRC Tissues

To investigate the function of miR-552 in the development of human CRC, we detected expression of miR-552 in paired 183 CRC tissues and adjacent non-cancer tissues. As shown in Figure 1, the results indicated that the expression levels of miR-

Table II. Sequence of the primers used in this study.

Genes	Primer sequences (5′ → 3′)
MiR-552	Forward: CCGCACAGGTGACTGGTTAGA Reverse: GTGCAGGGTCCGAGGT
GAPDH	Forward: GCGAGATCGCACTCATCATCT Reverse: TCAGTGGTGGACCTGACC

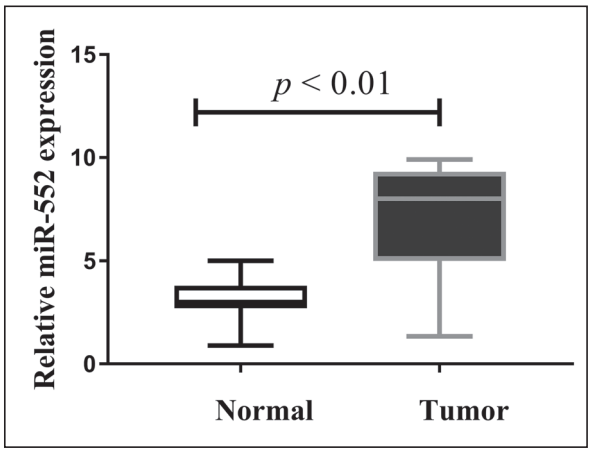

Figure 1. The expression level of miR-552 in CRC patients. The results showed that miR-552 expression was significantly higher in CRC tissues than in the corresponding noncancerous tissues ($p < 0.01$).

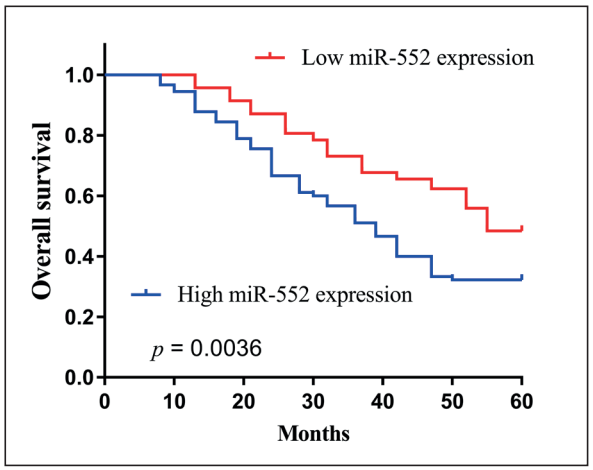

Figure 2. Kaplan-Meier survival analysis of association between miR-552 expression level and overall survival of 183 CRC patients.

552 in CRC tissues were significantly lower than those in corresponding noncancerous tissues ($p < 0.01$). The data revealed that abnormal miR-552 expression may be related to CRC pathogenesis.

Relationship Between miR-552 Expression and the Clinicopathologic Parameters

To further investigate clinicopathological significance of miR-552 expression in CRC patients, we divided CRC patients divided into two groups based on the mean value (3.23) of relative miR-552 expression. As shown in Table II, high miR-552 level was significantly positively correlated with histological grade ($p = 0.000$), lymph nodes metastasis ($p = 0.022$) and TNM stage ($p = 0.002$). However, miR-552 expression was not correlated with gender, age, tumor size and local invasion ($p > 0.05$).

High miR-552 Expression is Associated with Poor Prognosis of Patients with CRC

Then, we performed Kaplan-Meier survival analysis to investigate the association between the miR-552 expression and the prognosis of CRC patients. As shown in Figure 2, patients with higher miR-552 expression had significantly worse overall survival compared with patients with lower miR-552 expression (log-rank test, $p < 0.0036$). In Cox regression univariate analysis, histological grade ($p = 0.001$), lymph nodes metastasis ($p = 0.014$), TNM stage ($p = 0.003$) and miR-552 expression ($p = 0.002$) was associated with overall survival in CRC patients (Table III). Then, multivariate analysis indicated that miR-552 expression ($p = 0.007$) were an independent prognostic factor for patients with CRC (Table II).

Table III. Univariate and multivariate analyses for prognostic factors in patients with CRC.

	Univariate analysis			Multivariate analysis		
Parameter	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Gender (male *vs.* female)	0.933	0.672-1.774	0.421	-	-	-
Age (< 60 *vs.* ≥ 6 0)	1.342	0.732-1.932	0.218	-	-	-
Tumor size (< 5 *vs.* ≥ 5)	1.394	0.699-2.138	0.114	-	-	-
Local invasion (T1-T2 *vs.* T3-T4)	1.632	0.832-2.216	0.093	-	-	-
Histological grade (well and moderately *vs.* poorly)	4.856	1.672-8.933	0.001	4.131	1.372-7.121	0.001
Lymph nodes metastasis (negative *vs.* positive)	2.321	1.112-3.893	0.014	1.876	1.004-2.893	0.019
TNM stage (I-II *vs.* III-IV)	3.783	1.832-6.452	0.003	2.932	1.562-5.342	0.005
MiR-552 (low *vs.* high)	3.562	1.792-7.442	0.002	3.121	1.452-6.114	0.007

Discussion

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world. Thus, it is of great clinical significance to identify new molecular targets for the diagnosis, prognosis, and treatment of CRC[15]. Up to date, even though much effort has been made to identify the prognostic factors for CRC, these biomarkers alone are not sufficient to predict prognosis of CRC patients[16,17]. Thus, finding biomarkers with high specificity and sensitivity for predicting the prognosis of CRC is important.

MiRNAs have been shown to have critical regulatory role in progression of various tumors, including CRC. For instance, Wang et al[18] reported that miR-187 suppressed tumor growth and invasion by directly targeting CD276 in CRC. In addition, they confirmed that decreased miR-187 levels were associated with poor prognosis in patients with CRC. Deng et al[19] found that over-expression of miR-203 suppressed CRC cell proliferation, migration, and invasion. Moreover, they identified EIF5A2 as a targeting gene of miR-203. Zhang et al[20] showed that miR-340 inhibited tumor growth and enhanced chemosensitivity of CRC by targeting RLIP76. Given the importance of miRNAs in CRC, more and more studies focused on the effect of miRNAs as novel biomarkers, which can predict clinical outcome of CRC. Indeed, several miRNAs, such as miR-320e[21], miR-193a-3p[22], and miR-1260b[23], have been identified as independent prognostic factors for CRC patients. Recently, miR-552 attracted our attention. As a newly identified miRNA, its role in tumors remains largely unknown. A previous study by Cao et al[24] found that miR-552 was significantly up-regulated in CRC tissues. Functional assay indicated that miR-552 served as a tumor promoter in CRC by directly targeting DACH1 via the Wnt/β-catenin signaling pathway. Wang et al[14] also showed the similar carcinogenic effect of miR-552 in CRC. However, to our best knowledge, the prognostic value of miR-552 in CRC patients has not been investigated.

In this work, we investigated the clinical significance of miR-552 in CRC patients. The results indicated that miR-552 expression was significantly higher in CRC tissues as compared to matched normal tissues. Then, we further investigate the correlations between miR-552 expression and clinicopathologic features. Notably, miR-552 upregulation was correlated with histological grade, lymph nodes metastasis, and TNM stage. Moreover, by Kaplan-Meier analysis, we found that high miR-552 expression was associated with poorer overall survival in patients with CRC. In addition, our data confirmed that miR-552 was an independent prognostic factor for CRC patients by multivariate analysis.

Conclusions

We firstly identified miR-552 as a prognostic biomarker for CRC. Further studies are needed to confirm this correlation.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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