

miR-873 inhibits colorectal cancer cell proliferation by targeting TRAF5 and TAB1

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Abstract. MicroRNA-873 (miR-873) has been reported to be dysregulated in a variety of malignancies, however, the biological function and underlying molecular mechanism of miR-873 in colorectal cancer (CRC) remain unclear. In the present study we found that the expression levels of miR-873 were markedly decreased in CRC cell lines and tissues from patients. Statistical analysis revealed that miR-873 expression was inversely correlated with the disease stage of CRC. Kaplan-Meier survival analysis revealed that patients with CRC with lower miR-873 expression had shorter overall survival rates. Additionally, downregulation of miR-873 enhanced the proliferation of CRC cells, while upregulation of miR-873 reduced this proliferation. Furthermore, we found that tumor necrosis factor (TNF) receptor-associated factor 5 (TRAF5) and TGF- β activated kinase 1 (MAP3K7) binding protein 1 (TAB1) were direct targets of miR-873 in CRC cells. A luciferase assay revealed that ectopic expression of miR-873 significantly reduced nuclear factor κ B (NF- κ B) luciferase activity, while ectopic expression of miR-873 inhibitor enhanced luciferase activity, suggesting that downregulation of miR-873 can activate NF- κ B signaling. Therefore, our findings established a tumor-suppressive role for miR-873 in the inhibition of CRC progression, which may be employed as a novel prognostic marker and as an effective therapeutic target for CRC.

Introduction

Colorectal cancer (CRC), one of the most common malignancies of the gastrointestinal tract, is a major cause of

tumor-associated morbidity and mortality worldwide (1). Its incidence rate continues to rise (2). Over 1 million new cases of CRC are diagnosed globally each year, resulting in about 0.5 million deaths annually (3,4). The poor prognosis for CRC is mainly attributable to its insidious onset, atypical symptoms, and aggressive malignancy. Most patients with CRC are diagnosed at an advanced stage. Approximately 25% of patients with CRC present with liver metastases at the time of the initial diagnosis (5). Despite overall advances in the treatment of this disease, the overall cure rate of CRC has not markedly improved, and the overall 5-year survival rate remains at approximately 60% in Asia (6).

Nuclear factor κ B (NF- κ B) is a family of dimeric transcription factors required to coordinate numerous physiological and pathological processes, such as immunity, inflammation, and tumorigenesis (7,8). Notably, constitutively activated NF- κ B signaling has been demonstrated to play vital roles in the development and progression of a large array of malignancies, including CRC (9,10). Stimulatory factors, such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), or pathogen-derived components that include bacterial lipopolysaccharides (LPS), bind to their respective receptors, leading to the rapid recruitment of tumor necrosis factor receptor type 1-associated DEATH domain (TRADD), cellular inhibitor of apoptosis protein 1 (cIAP1), baculoviral IAP repeat-containing protein 3 (cIAP2), and TNF receptor-associated factors (TRAFs), including TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6. As molecular activators for the NF- κ B signaling pathway, TRAFs function as E3 ubiquitin ligases that induce the K63 polyubiquitination of receptor-interacting protein 1 (RIP1), resulting in activation of the transforming growth factor-activated kinase-1 (TAK1)/TAB2/3/ TGF- β activated kinase 1 (MAP3K7) binding protein 1 (TAB1) complex. During this process, TAB1, TAB2, and TAB3 form a complex with TAK1, which phosphorylates and activates the inhibitor of the NF- κ B kinase (IKK)- $\alpha/\beta/\gamma$ kinase complex, leading to nuclear translocation and activation of NF- κ B (11-13). Thus, further investigations into the mechanism of regulation of the NF- κ B pathway key components, such as TRAF2, TRAF5, TAK1, TAB1, and TAB3, would increase our knowledge of the mechanisms underpinning the constitutive activation of NF- κ B in cancer.

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MicroRNAs (miRNAs), a class of small non-coding RNAs, function as negative regulators of gene expression by interacting with the 3' untranslated region (3'-UTR) of their target mRNAs (14). These miRNAs, which are approximately 20-25 nucleotides in length, play important roles in a variety of physiological and pathological processes, such as development, cell proliferation, differentiation, and senescence (15). Previous research has revealed that miRNAs are involved in carcinogenesis via regulation of several key cellular processes, including cell proliferation, apoptosis, migration, invasion, and angiogenesis (16). Numerous miRNAs have been reported to be upregulated or downregulated in various types of cancer, demonstrating their potential roles as oncogenes or tumor suppressors (17-20). The altered expression of miRNAs in cancers suggests that they may serve as potential diagnostic or prognostic biomarkers for cancer (21-23). For instance, in glioma, ovarian cancer, and breast cancer, miR-873 is downregulated and may act as tumor suppressor (24-28). However, miR-873 has been revealed to be upregulated in lung adenocarcinoma, where it promotes tumor cell proliferation and migration (29).

In the present study we revealed that miR-873 was downregulated in CRC and this was correlated with a poor prognosis for patients with CRC. Furthermore, we determined that downregulation of miR-873 enhanced CRC cell proliferation by directly targeting TRAF5 and TAB1, leading to activation of NF- κ B signaling. These results demonstrated that miR-873 served as a tumor-suppressive miRNA in the development and progression of CRC.

Materials and methods

Cells. The CRC cell lines (SW620, SW480, DLD1, HCT116, LoVo, and HT-29) were purchased from the American Type Culture Collection (Manassas, VA, USA). The normal human colon mucosal epithelial cell line (NCM460) and the CRC cell line (KM12) were purchased from the BeNa Culture Collection (Beijing, China). The normal human colon mucosal epithelial cell line (NCM460) and seven CRC cell lines (SW620, SW480, DLD1, HCT116, KM12, LoVo, and HT-29) were grown in Dulbecco's modified Eagle's medium (Invitrogen; Thermo Fisher Scientific, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific) and 100 units of penicillin-streptomycin.

Patient information and tissue specimens. This study was conducted on a total of 125 paraffin-embedded and archived CRC samples, which were diagnosed histopathologically at the Affiliated Shenzhen Sixth Hospital of Guangdong Medical University from 2003 to 2012. Informed patient consent and approval from the Institutional Research Ethics Committee of the Affiliated Shenzhen Sixth Hospital of Guangdong Medical University was obtained for use of these clinical materials for research purposes. Clinical information regarding the samples is summarized in Table I. Ten CRC tissue samples and their matched adjacent non-cancerous colorectal tissues were frozen and stored in liquid nitrogen until further use.

Plasmids and transfection. The 3'-UTR regions of human *TRAF5* (from 1801 to 2211 nt, containing a predicted conserved

miR-873 binding site) and *TAB1* (from 3288 to 3700 nt, containing a predicted conserved miR-873 binding site) were generated by PCR and cloned into the modified pGL3-control luciferase reporter plasmid (Promega Corporation, Madison, WI, USA). Primer sequences were as follows: TRAF5-3'UTR sense, 5'-ACTCCGCGGATCCCAGATGATTAATT-3' and antisense, 5'-CTAACTGCAGTTCCCTTGTCTGGGATCAC-3'; TAB1-3'UTR sense, 5'-ACTCCGCGGCGGAGGTCCTGGCCCTCAG-3' and antisense, 5'-CTAACTGCAGCCATGGAGGAAACAACAGGGAG-3'. Point mutations in the putative miR-873-binding seed regions of the TRAF5-3'-UTR and TAB1-3'-UTR constructs were created using a Stratagene QuikChange Mutagenesis kit (Stratagene; Agilent Technologies Inc., La Jolla, CA, USA). A miR-873 mimic, miR-873 inhibitor, and the negative control (NC) were purchased from Guangzhou RiboBio Co., Ltd. (Guangzhou, China). The sequences were as follows: miR-873 mimic, 5' GCAGGAACUUGUGAGUCUCCU-3'; miR-873-NC sense, 5'-UUUGUACUACACAAAAGUACUG-3'; miR-873 inhibitor, 5'-AGGAGACUCACAAGUCCUGC-3'. The miR-873 mimic or miR-873 inhibitor (the miR-873 inhibitor is a locked nucleic acid (LNA)/O-Methyl oligo (OMe) modified antisense oligonucleotide designed specifically to bind to and inhibit the endogenous miR-873 molecule) was transfected into cells using the Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific) according to the manufacturer's instructions.

RNA extraction, reverse transcription (RT), and quantitative PCR (qPCR). Total RNA from the indicated tissues or cells was extracted using the TRIzol reagent (Life Technologies; Thermo Fisher Scientific, Carlsbad, CA, USA), according to the manufacturer's instructions. Complementary DNA (cDNA) was amplified and quantified on an ABI PRISM 7500 Sequence Detection system (Applied Biosystems; Thermo Fisher Scientific, Foster City, CA, USA) using SYBR Green I (Roche Diagnostics, Grenzach-Wyhlen, Germany). miRNA quantification was determined using Bulge-loop™ miRNA quantitative reverse transcription PCR (qRT-PCR) Primer Set (one RT primer and a pair of qPCR primers for each set) specific for U6 and miR-873 that were designed and synthesized by Guangzhou RiboBio Co., Ltd. The catalog numbers of these primers were as follows: miR-873 RT primer (ssD809230648), miR-873 forward primer (ssD090525061), miR-873 reverse primer (ssD089261711), U6 RT primer (ssD0904071008), U6 forward primer (ssD0904071006), and U6 reverse primer (ssD0904071007). The quantitative PCR (qPCR) conditions were as follows: incubation at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec, and 60°C for 1 min. The expression of miR-873 was defined based on the quantification cycle (Cq), and the relative fold changes between normal human colon mucosal epithelial cell line and the CRC cell lines, and between CRC tissues and their tumor-adjacent tissues (TATs), were calculated according to the formula $2^{-[(Cq \text{ of miR-873}) - (Cq \text{ of U6})]}$ after normalization to the expression of U6 small nuclear RNA as a reference (30). REST-MCS beta software version 2 was used to further analyze the qPCR data.

MTT assays. Cells (2×10^3 /well) were seeded into 96-well plates. Transfection was performed 12 h later. At the indicated time-points, 100 μ l of sterile 3-(4,5-Dimethyl-2-thiazolyl)-2,

Table I. Clinicopathological characteristics of studied patients and expression of mir-873 in CRC.

Factors	No.	(%)
Sex		
Male	68	54.4
Female	57	45.6
Age (years)		
≤62	64	51.2
>62	61	48.8
Tumor site		
Colon	61	48.8
Rectal	64	51.2
Dukes' stage		
A	17	13.6
B	36	28.8
C	45	36.0
D	27	21.6
Clinical stage		
I	17	13.6
II	37	29.6
III	44	35.2
IV	27	21.6
T classification		
T ₁	2	1.6
T ₂	30	24.0
T ₃	42	33.6
T ₄	51	40.8
N classification		
N ₀	63	50.4
N ₁	39	31.2
N ₂	23	18.4
M classification		
M ₀	97	77.6
M ₁	28	22.4
Histological differentiation		
Well	29	23.2
Moderate	65	52.0
Poor	31	24.8
Vital status		
Alive	49	39.2
Dead	76	60.8
Expression of mir-873		
Low	62	49.6
High	63	50.4

CRC, colorectal cancer; T, tumor; N, node; M, metastasis.

5-diphenyl-2H-tetrazoliumbromide (MTT) dye (0.5 mg/ml; Sigma-Aldrich, St. Louis, MI, USA) was added and incubated for 4 h at 37°C. The culture medium was subsequently removed, and 150 μ l of dimethyl sulfoxide (DMSO; Sigma-Aldrich) was added. The absorbance was assessed at a wavelength of 490 nm. All experiments were performed in triplicate.

Colony formation assay. Cells (8×10^2 /well) were seeded into 6-well plates and cultured for 10 days. The colonies were stained with 1% crystal violet for 30 sec after incubation with 10% formaldehyde for 5 min. Colonies were counted only if they contained more than 50 cells, according to the established criteria for colony formation (31-33).

Western blot analysis. Cell lysates were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). The membranes were probed with antibodies against TRAF5 (anti-TRAF5 antibody; 1:1,000; mouse, polyclonal; cat. no. SAB1409766) and TAB1 (anti-TAB1 antibody; 1:500; rabbit, polyclonal; cat. no. SAB4301002; both from Sigma-Aldrich) overnight at 4°C, followed by incubation with horseradish peroxidase-conjugated secondary antibodies (1:2,000; goat; cat. no. 7074 and 1:2,000; horse; cat. no. 7076; Cell Signaling Technology, Danvers, MA, USA) for 1 h at 20°C. The membranes were stripped and re-probed with an anti- α -tubulin antibody (Sigma-Aldrich) as a loading control.

Luciferase assay. Cells (2×10^4 /well) were seeded in triplicate in 48-well plates and allowed to settle for 24 h. Next, 100 ng of the luciferase reporter plasmids or the control plasmid, both with 1 ng of pRL-TK *Renilla* plasmid (Promega Corporation), were transfected into cells using the Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific), according to the manufacturer's recommendation. Luciferase and *Renilla* signals were assessed 24 h after transfection using a Dual Luciferase Reporter assay kit (Promega Corporation), according to the manufacturer's instructions.

Immunohistochemistry (IHC) and hematoxylin and eosin (H&E) staining. Histology was performed to quantify Ki67 expression in 10 paraffin-embedded human CRC samples. IHC was performed on sections using an anti-Ki67 antibody (1:1; mouse, monoclonal; cat. no. IR62661-2; Dako; Agilent Technologies, Inc., Glostrup, Denmark). H&E staining was performed using Mayer's hematoxylin solution. Immunostaining of the sections was quantified and scored independently by two observers based on both the proportion of positively-stained tumor cells and the intensity of staining. The proportion of tumor cells enriched for Ki67 was scored as follows: 0 (no positive tumor cells), 1 (<10% positive tumor cells), 2 (10-50% positive tumor cells), and 3 (>50% positive tumor cells). The intensity of staining was scored according to the following criteria: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow brown), and 3 (strong staining, brown). The staining index (SI) was calculated as the proportion of positive tumor cells x the staining intensity score. Using this scoring method, we evaluated the expression of Ki67 by determining the SI, which was scored as 0, 1, 2, 3, 4, 6 and 9.

Statistical analysis. All values are presented as the means \pm standard deviation (SD). Student's t-test was used to determine the statistical differences. The Chi-square test was used to analyze the relationship between miR-873 expression and clinicopathological characteristics. Multivariate statistical analysis was performed using a Cox regression model. Survival curves

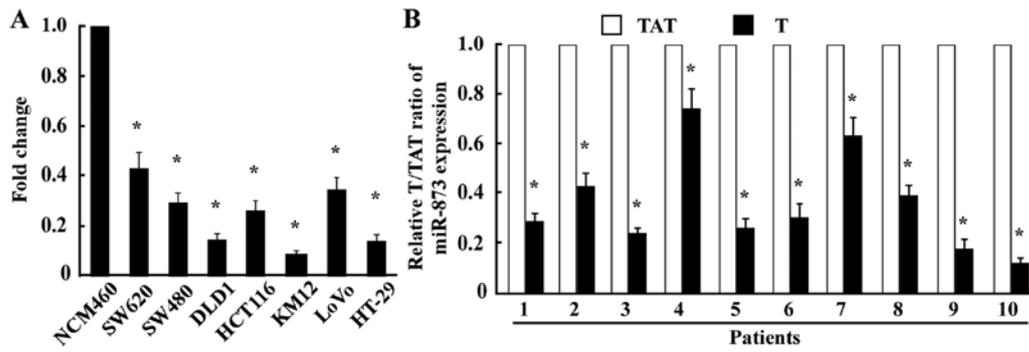


Figure 1. miR-873 expression is reduced in CRC cell lines and CRC tissues. (A) qPCR analysis of miR-873 expression in normal colon mucosal epithelial cell line NCM460 and seven cultured CRC cell lines (SW620, SW480, DLD1, HCT116, KM12, LoVo, and HT-29). (B) qPCR analysis of miR-873 expression in ten pairs of CRC samples (T) and TATs. Transcript levels were normalized to *U6* expression. * $P < 0.05$. CRC, colorectal cancer; qPCR, quantitative PCR; TATs, tumor-adjacent tissues.

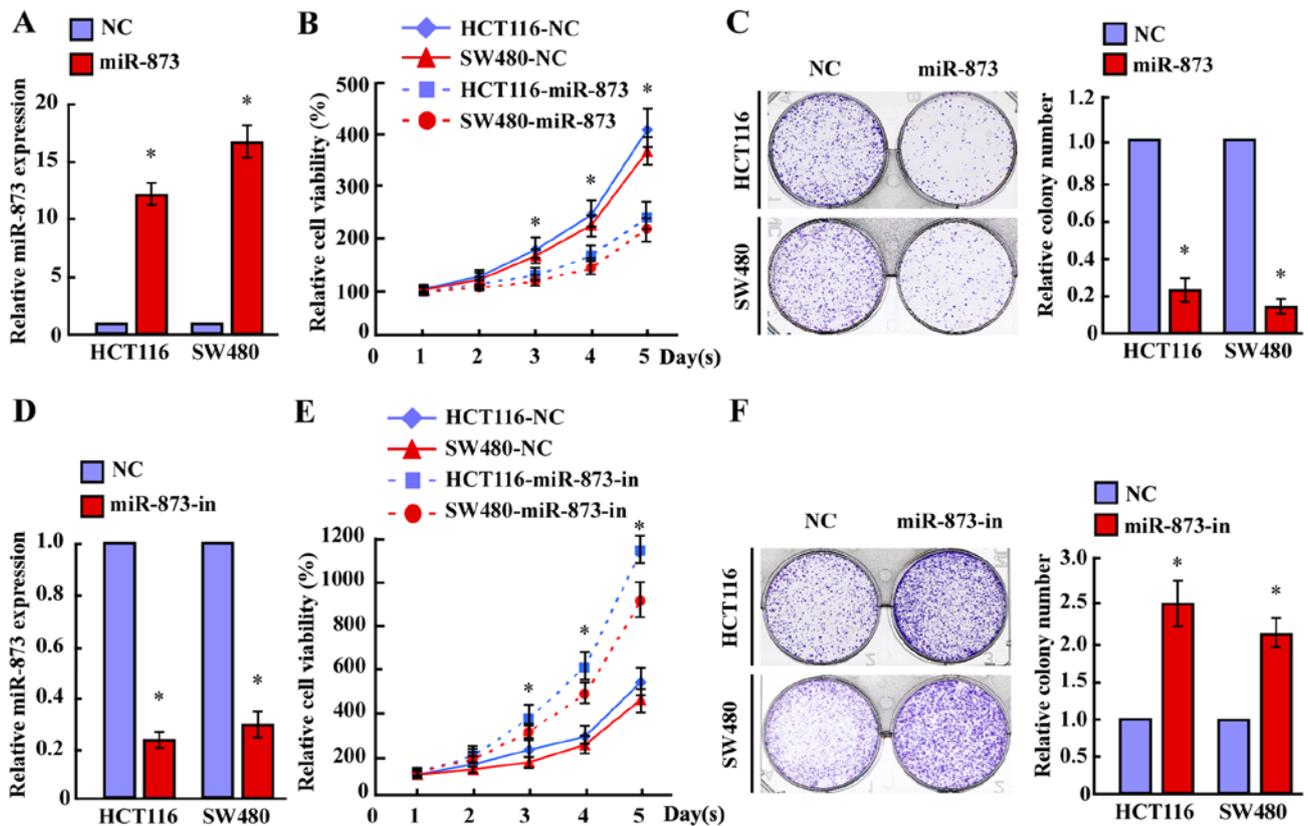


Figure 2. miR-873 inhibits proliferation of CRC cells. (A and D) The expression of miR-873 in HCT116 and SW480 cells transfected with (A) miR-873 mimic (miR-873) or (D) miR-873 inhibitor (miR-873-in) as assessed by qPCR. (B and E) The effects of miR-873 overexpression on the growth of CRC cells HCT116 and SW480. MTT assays revealed that (B) miR-873 reduced cell proliferation, whereas (E) miR-873 inhibitor-transfected HCT116 and SW480 cells proliferated more rapidly than the control cells. (C and F) Representative images (left) and quantification (right) of colonies for the indicated cells, as determined by a colony formation assay. * $P < 0.05$. CRC, colorectal cancer; qPCR, quantitative PCR.

were plotted using the Kaplan-Meier method and compared using the log-rank test. Statistical analyses were performed using the SPSS 19.0 software (SPSS, Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant result.

Results

miR-873 is downregulated in CRC cell lines and CRC tissues. To examine the expression levels of miR-873 expression in CRC, we conducted qPCR in one normal human colon

mucosal epithelial cell line, seven CRC cell lines, and ten pairs of CRC tissues and their TATs. The results revealed that miR-873 was markedly decreased in all seven CRC cell lines (SW620, SW480, DLD1, HCT116, KM12, LoVo, and HT-29) compared with the normal human colon mucosal epithelial cell line NCM460 (Fig. 1A). Consistent with the results obtained from the cell lines, miR-873 expression in the ten CRC tissue samples was significantly lower compared with that in their TATs (Fig. 1B), indicating that the expression of miR-873 was downregulated in CRC.

Table II. Correlation between clinicopathological features of CRC patients and expression of miR-873.

Patient characteristics	miR-873 expression		P-value
	Low or none (%)	High (%)	
Sex			
Male	31 (24.8)	37 (29.6)	0.372
Female	31 (24.8)	26 (20.8)	
Age (years)			
≤62	31 (24.8)	33 (26.4)	0.859
>62	31 (24.8)	30 (24.0)	
Dukes' stage			
A	6 (4.8)	11 (8.8)	0.005
B	11 (8.8)	25 (20.0)	
C	26 (20.8)	19 (15.2)	
D	19 (15.2)	8 (6.4)	
Clinical stage			
I	6 (4.80)	11 (8.80)	0.002
II	11 (8.80)	26 (20.8)	
III	25 (20.0)	19 (15.2)	
IV	20 (16.0)	7 (5.6)	
T classification			
T1	0 (0.0)	2 (1.6)	0.036
T2	16 (12.8)	14 (11.2)	
T3	15 (12.0)	27 (21.6)	
T4	31 (24.8)	20 (16.0)	
N classification			
N ₀	23 (18.4)	40 (32.0)	0.001
N ₁	20 (16.0)	19 (15.2)	
N ₂	19 (15.2)	4 (3.2)	
M classification			
M ₀	42 (33.6)	55 (44.0)	0.010
M ₁	20 (16.0)	8 (6.4)	
Histological differentiation			
Well	21 (16.8)	8 (6.4)	0.014
Moderate	26 (20.8)	39 (31.2)	
Poor	15 (12.0)	16 (12.8)	
Vital status			
Alive	15 (12.0)	34 (27.2)	0.001
Dead	47 (37.6)	29 (23.2)	

CRC, colorectal cancer; T, tumor; N, node; M, metastasis.

ectopic expression of miR-873 in HCT116 and SW480 cells decreased the levels of the TRAF5 and TAB1 proteins, whereas ectopic expression of the miR-873 inhibitor increased their levels (Fig. 3B-D). However, we determined that the mRNA expression levels of *TRAF5* and *TAB1* did not exhibit evident alterations in the miR-873 dysregulated cells (data not shown), suggesting that miR-873 negatively regulated the expression of these proteins at the translation level. To further test this,

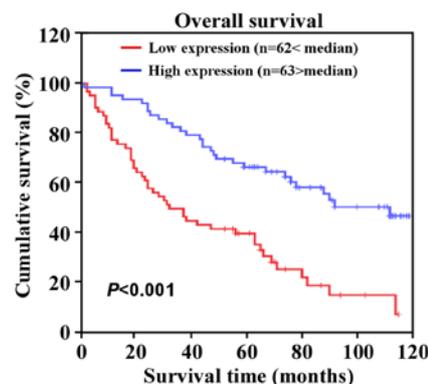


Figure 4. Expression of miR-873 is correlated with CRC prognosis. Kaplan-Meier analysis of the correlation between the miR-873 expression levels and the overall survival rates of patients with CRC with high (> the median, n=63) and low (< the median, n=62) miR-873 expression.

we subcloned the 3'-UTRs of *TRAF5* and *TAB1* into the pGL3 luciferase reporter. Transfection of miR-873 consistently attenuated the luciferase activity of the TRAF5-3'-UTR and TAB1-3'-UTR luciferase reporter in both HCT116 and SW480 cells, whereas transfection of the miR-873 inhibitor rescued luciferase suppression. However, dysregulation of miR-873 did not result in the alteration of the reporter activities driven by the 3'-UTRs of *TRAF5* and *TAB1* mutated within the miR-873-binding seed regions (Fig. 3E). Collectively, these results further supported the view that *TRAF5* and *TAB1* are genuine targets of miR-873.

Furthermore, the luciferase assay revealed that ectopic expression of miR-873 in HCT116 and SW480 cells significantly reduced NF- κ B luciferase activity, while ectopic expression of the miR-873 inhibitor enhanced NF- κ B luciferase activity (Fig. 3F). This suggested an important role for NF- κ B signaling in the CRC cell proliferation induced by miR-873 downregulation.

miR-873 expression is correlated with clinical features and prognosis of patients with CRC. To further evaluate whether miR-873 downregulation was associated with the clinical features or prognosis of CRC, we examined the expression of miR-873 in a large cohort of clinical CRC samples using qPCR and performed a correlation analysis between the clinicopathological features and the expression of miR-873. The data revealed that miR-873 expression was inversely correlated with Dukes' stage (P=0.005), clinical stage (P=0.002), tumor-node-metastasis (TNM) classification (T, P=0.036; N, P=0.001; M, P=0.010), and histological differentiation (P=0.014) (Table II). Additionally, Kaplan-Meier survival analysis revealed that patients with CRC with lower miR-873 expression levels had shorter overall survival (Fig. 4). Moreover, univariate and multivariate analysis indicated that miR-873 expression levels were an independent prognostic factor for CRC (Table III). Collectively, these results indicated a possible link between miR-873 downregulation and CRC progression.

Clinical relevance of miR-873 downregulation and cell proliferation in CRC. Finally, we examined whether the reduction in miR-873 expression induced cell proliferation in CRC

Table III. Univariate and multivariate analysis of different prognostic parameters in patients with CRC by Cox-regression analysis.

Parameters	Univariate analysis			Multivariate analysis		
	No. of patients	P-value	Regression coefficient (SE)	P-value	Relative risk	95% confidence interval
N classification		<0.001	0.150	<0.001	1.746	1.306-2.336
N ₀	63					
N ₁	39					
N ₂	23					
M classification		<0.001	0.260	0.001	2.405	1.430-4.044
M ₀	97					
M ₁	28					
Expression of mir-873		<0.001	0.242	0.001	0.450	0.275-0.734
Low	62					
High	63					

CRC, colorectal cancer; N, node; M, metastasis.

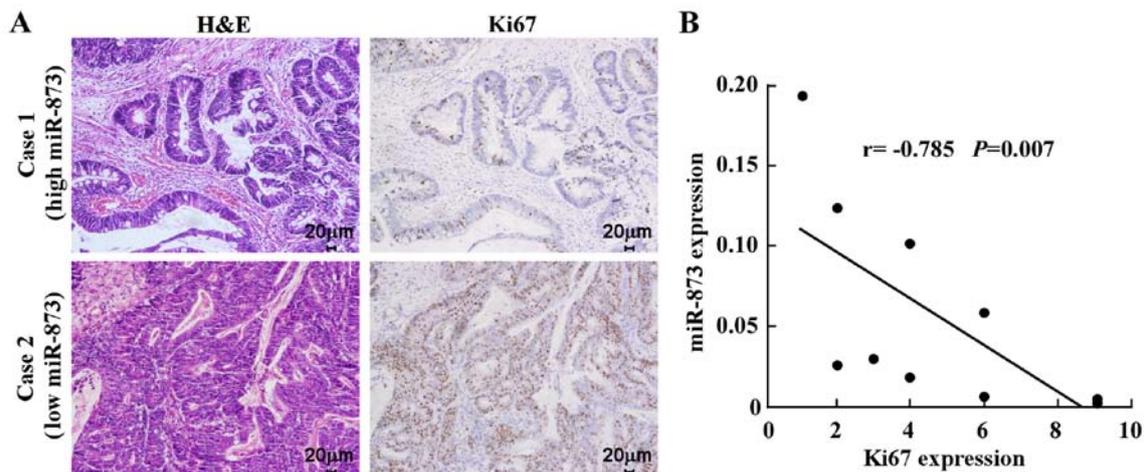


Figure 5. Clinical relevance of miR-873 downregulation and cell proliferation in CRC. (A) miR-873 levels were inversely correlated with Ki67 in ten primary CRC specimens. Two representative specimens for low and high levels of miR-873 are displayed. Original magnification, x200. (B) Analysis of the correlation between miR-873 and Ki67 expression in ten CRC specimens. The relative data of qPCR were used as the ordinate and the staining index of Ki67 as the abscissa. CRC, colorectal cancer; qPCR, quantitative PCR.

samples and whether this was clinically relevant. IHC analysis of ten CRC specimens revealed that low miR-873-expressed specimens had a higher proportion of cells expressing the proliferation marker Ki67. In contrast, high miR-873-expressed specimens displayed a small proportion of Ki67-positive cells among the ten CRC specimens (Fig. 5A). Correlation analysis revealed that miR-873 expression levels were inversely correlated with Ki67 in these CRC samples (Fig. 5B). Collectively, these results established that miR-873 suppressed CRC cell proliferation via inhibition of TRAF5 and TAB1, which are key components of the NF- κ B signaling pathway.

Discussion

The key finding of the current study is that miR-873 is a tumor-suppressive miRNA in CRC. Our data revealed that miR-873

was significantly downregulated in both CRC cell lines and primary CRC specimens. Furthermore, downregulation of miR-873 expression was associated with more advanced tumor stages and poor prognoses for patients with CRC. Ectopic expression of miR-873 inhibited CRC cell proliferation, whereas silencing of miR-873 promoted cell proliferation. In addition, we demonstrated that miR-873 suppressed the NF- κ B pathway by directly targeting *TRAF5* and *TAB1*, which encode vital components of this pathway. Collectively, our results demonstrated that miR-873 plays a critical role in the tumorigenesis and progression of CRC and may represent an important target for clinical intervention of CRC.

Previous research has revealed that miRNAs are involved in tumor initiation, progression, metastasis, and response to chemotherapy in CRC. In 2003, the first study describing the roles of miRNAs in CRC was published, which revealed

that miR-143 and miR-145 were specifically dysregulated in CRC, and multiple other miRNAs were found to contribute to CRC by regulating critical target mRNAs (34). For example, several studies have revealed the dysregulation of a variety of tissue-specific miRNAs, e.g., miR-21, miR-181b, miR-155, miR-92a, and let-7, as well as some circulating miRNAs, e.g., miR-26a, miR-21, miR-126, and miR-203 in CRC (35,36). These miRNAs exert their effects by negatively regulating their targets, such as p53, c-Met, K-Ras, COX-2, Rb, and the Bcl-2 family (37). Accumulating evidence indicates that miRNAs may serve as targets for miRNA-based therapeutics of CRC. Inhibition of overexpressed oncogenic miRNAs or introduction of tumor-suppressive miRNAs into cancer cells may represent novel treatment strategies for CRC therapy in the future (38).

Previously, it was shown that miR-873 is dysregulated in a variety of malignancies. Notably, miR-873 was downregulated in glioblastoma, and inhibited tumorigenesis and metastasis by suppressing the expression of insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) (24,25). In addition, miR-873 enhanced the sensitivity of glioma cells to cisplatin by targeting Bcl-2 (26). It was also reported that overexpression of miR-873 increased the sensitivity of ovarian cancer cells to cisplatin and paclitaxel by targeting multidrug resistance protein 1 (MDR1) (27). However, there are also studies which have reported that cisplatin is not a P-glycoprotein substrate and multidrug resistance induced by cisplatin in ovarian carcinoma cell lines was not due to overexpression of MDR1 and MDR3, both of which are P-glycoproteins (39,40). Therefore, the controversial mechanisms of multidrug resistance induced by cisplatin in ovarian carcinoma warrant further investigation. In addition, miR-873 was downregulated in tamoxifen-resistant breast cancer cell lines, while overexpression of miR-873 reversed tamoxifen resistance by targeting cyclin-dependent kinase 3 (CDK3) (28). By contrast, however, Gao *et al* reported that miR-873 may act as an oncogene in lung adenocarcinoma since it increased tumor cell proliferation and migration via direct inhibition of SRCIN1 expression (29). Collectively, these findings indicated that miR-873 can act as either a tumor-suppressive or -promoting miRNA depending on the type of cancer. In this context, we demonstrated that miR-873 inhibited CRC cell proliferation and functioned as a tumor-suppressive miRNA in CRC. Meanwhile, the inversed clinical relevance of miR-873 reduction with higher Ki67 signaling further supported the suppressive effect of miR-873 on proliferation in CRC. However, the inhibitory effect of miR-873 on proliferation in CRC warrants further investigation using an in vivo mouse model. In addition, the expression and biological function of miR-873 in other gastrointestinal tract cancers also warrant further clarification.

NF- κ B is a family of transcription factors that controls the expression of a large number of genes related to inflammation, immune responses, development, survival, and proliferation (41). Since its discovery nearly three decades ago (42), numerous studies have reported that the NF- κ B signaling pathway is frequently activated in a variety of human cancers and it is associated with tumor initiation and progression (6,8). The NF- κ B signaling pathway plays critical roles in the physiological and pathological processes of CRC, and the relationship between CRC development and NF- κ B signaling

is becoming clear (43). Multiple research groups revealed that the constitutively activated form of NF- κ B was frequently expressed in CRC cells (44-46). NF- κ B may contribute to the progression of CRC by regulating the expression of diverse target genes that are involved in cell proliferation, angiogenesis, and metastasis (47). Therefore, the NF- κ B pathway and its upstream and downstream network constitute a potential druggable target for therapeutic interventions (48). Although IKK complex-mediated NF- κ B activation has been studied in great detail, the regulatory mechanism of the constitutive activation of NF- κ B in CRC remains largely unknown. Herein, we found that miR-873 significantly inhibited the NF- κ B pathway by directly targeting TRAF5 and TAB1, key components of the NF- κ B pathway. Thus, our results indicated that miR-873 plays a regulatory role in NF- κ B activation, and the effect of miR-873-induced activation of NF- κ B on invasion, angiogenesis, or metastasis of CRC warrant further investigation.

In conclusion, the present study reported that miR-873 was downregulated in CRC and the expression level of miR-873 was correlated with CRC progression and prognosis. We have demonstrated, for the first time, that the upregulation of miR-873 markedly inhibited CRC cell proliferation by inhibiting the expression of two key components (TRAF5 and TAB1) of the NF- κ B pathway. Therefore, our results demonstrated that miR-873 may play an important role in the progression of CRC and could represent a potential therapeutic target for CRC.

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