

## Original Article

# Expression of miR-221 in colon cancer correlates with prognosis

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**Abstract:** MicroRNAs are increasingly important in the study of cancer because of their ability to down regulate the expression of tumor suppressors and promote tumorigenesis. Here, miR-221, which is dysregulated in various tumors, was investigated for its expression in colon cancer tissues and its correlation with patient prognosis. Colon cancer tissue samples were obtained from 182 individuals who underwent surgical resection in our hospital from June 2008 to September 2009. Real-time PCR was used to detect the expression of miR-221 in these tissues. Patient survival was determined by telephone interview, and survival curves were plotted by using the Kaplan-Meier method and compared by the Log-rank test. Statistical methods also included  $X^2$  test and Cox proportional hazard regression model. Differences in the expression of miR-221 by gender, pathology, and pathological staging were not statistically significant ( $P>0.05$ ), but differences in the expression of miR-221 among age groups were statistically significant ( $P<0.05$ ). A survival analysis indicated that high expression of miR-221 was closely associated with a shorter survival time ( $P<0.05$ ). Further, later p-TNM (hazard ratio, HR=2.973, 95% confidence interval, CI: 1.329-6.519,  $P=0.003$ ) and high expression of miR-221 (HR=2.394, 95% CI: 1.210-4.910,  $P=0.006$ ) were identified as risk factors for colon cancer prognosis. Thus, high miRNA-221 expression might be a prognostic marker of colon cancer patients. The high expression of miRNA-221 was associated with poor prognosis of patients with colon cancer.

**Keywords:** miRNA-221, colon cancer, prognosis

### Introduction

Colon cancer occurs in the digestive tract, typically at the junction of the rectum and the sigmoid colon. One of the most common malignancies in China, the overall incidence has displayed a marked upward trend in recent years [1]. The current surgical and chemo- or radiotherapy treatment approaches enable approximately 60% of colon cancer patients to survive 5 years after diagnosis, but many colon cancers remain undiagnosed until the disease has progressed to a late stage. Therefore, identifying new diagnostic and prognostic markers may enable earlier detection and better treatment of the disease.

MicroRNAs (miRNAs) have emerging importance as potential cancer biomarkers [3]. These small, non-protein-coding, single-stranded, short RNAs bind to the 3'-untranslated region of target mRNAs to regulate their expression. This regulation leads to downstream effects on diverse biological processes including early

embryonic development and cell proliferation, differentiation, and apoptosis [4-6]. Further, miRNAs regulate the expression of various genes involved in tumorigenesis, including oncogenes and tumor suppressors, and tumors often display aberrant miRNA expression profiles [7-10]. miRNA-221 has demonstrated higher expression in multiple tumors including colon cancer [11, 12]. It is hypothesized that upregulation of this miRNA can contribute to disease initiation or progression. To better understand the contribution of miR-221 to colon cancer, in this study, the clinical data of 182 colon cancer patients were analyzed retrospectively to investigate the correlation between patient survival time and miRNA-221 expression.

### Participants and methods

#### Participants

Paraffin-embedded colon cancer specimens were collected and stored from 182 patients who underwent surgical resection between June 2008 and September 2009 and were con-

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**Table 1.** Relation between miRNA-221 expression and clinical characteristics in patients with colon cancer

Variables	miRNA-221 expression*		X <sup>2</sup>	P
	Low [n (%)]	High [n (%)]		
Gender			0.111	0.740
Male (73)	35 (47.95)	38 (52.05)		
Female (109)	55 (50.46)	54 (49.54)		
Age (years)			8.423	0.004
<45 (81)	29 (35.80)	52 (64.20)		
≥45 (101)	58 (57.43)	43 (42.57)		
Histology			0.712	0.399
SCC (70)	37 (52.86)	33 (47.14)		
ADC (112)	52 (46.43)	60 (53.57)		
Stage -TNM			0.145	0.704
I (67)	34 (50.75)	33 (49.25)		
II-IIIa (115)	55 (47.83)	60 (52.17)		

\*Relative expression of miR-221 was determined using the 2<sup>-ΔΔCT</sup> method, and the median of 2<sup>-ΔΔCT</sup> was used to divide all patients into groups based on high expression of miRNA-221 or low expression of miRNA-221. SCC = squamous cell carcinoma; ADC = adenocarcinoma.

firmed by pathologists to have colon cancer. Before surgery, none of the patients received anti-cancer treatment involving chemotherapy, radiotherapy, or biologicals. The pathological classification and staging of colon cancer was in accordance with the AJCC TNM Staging System (7th edition, 2010). This study was approved by the Hospital Ethics Committee, and informed consent was obtained from all participants.

### Polymerase chain reaction (PCR)

The human pre-miR-221 sequence was obtained from the miRBase ([www.mirbase.org](http://www.mirbase.org)), which was 5'-AGCUACAUUGUCUG CUGGGUUUC-3'.

### RNA extraction

Total RNA of paraffin-embedded colon cancer tissues was extracted according to the instructions in the Recover All Total Nucleic Acid Isolation Kit (Applied Biosystems). The extracted RNA was diluted with 50 μL of DEPC-treated water and was then stored at -80°C for use. A NanoDrop 1000 Micro-volume UV-Vis Spectrophotometer (Tripbiotech, Shanghai, China) was used to measure the purity of total RNA, i.e., the ratio of OD260/OD280.

### Reverse transcription

Reverse transcription was performed strictly according to the instructions in the Real-time Reverse Transcription Kit (Invitrogen). In brief, 0.5 μg of RNA was added with the following reagents: 1 μL of total RNA, 1 μL of PrimeScript RT Enzyme Mix I, 5 mL of 5 × PrimeScript Buffer, 2 μL of stem-loop RT primer, and 20 μL of DEPC-treated water. The reaction conditions were: 42°C for 15 min, 85°C for 5 s and 4°C for 15 min. After the reaction, the mixture was stored at -80°C.

### Real-time PCR

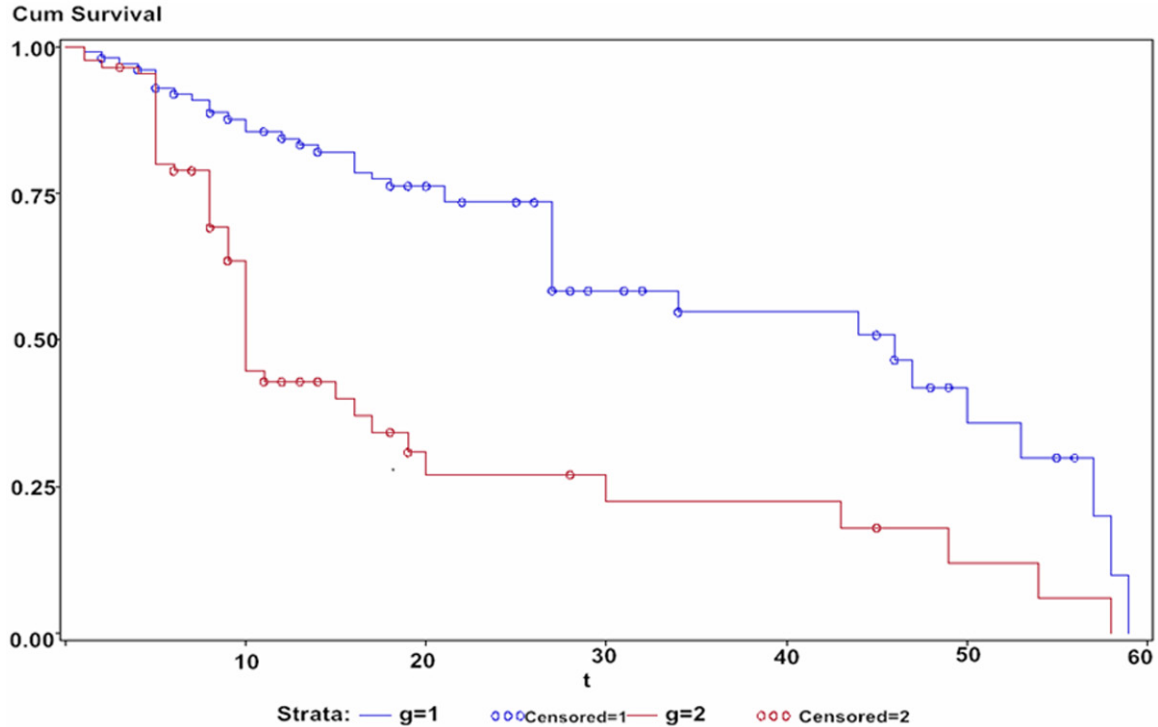
The PCR reaction system comprised a total volume of 20 μL, containing 10 μL of SYBR Green Real time PCR Master Mix (Mingyangkehua Bio Technology Co. Ltd., Beijing), 1 μL of downstream primer and 1 μL of upstream primer [RNU6B primer and has-miRNA-221 primer (Generay Biotech, Shanghai) as internal controls], 2 μL of cDNA and added DEPC-treated water. The 7300 Real-time PCR System was used under following conditions in a 9700 Thermal Cycler: denaturation at 95°C for 10 min, 95°C for 15 s and 1min for 60°C, for 40 cycles. The process above was repeated three times.

### Follow-up

To determine patient survival time, telephone follow-up was performed for all patients from whom a paraffin-embedded tissue sample was used. The deadline for completing the interviews was Dec. 2013, and the median follow-up time was 30 months (ranging from 5 to 6 months).

### Statistical analysis

Relative expression of miR-221 was determined using the 2<sup>-ΔΔCT</sup> method [13]. The median of 2<sup>-ΔΔCT</sup> was used as the demarcation value to divide all patients into groups with highly-expressed miRNA-221 or lowly-expressed miRNA-221. Statistical methods included a chi-squared test, Kaplan-Meier method, Log-rank test, and Cox proportional hazards model. P<0.05 was considered to indicate the difference was statistically significant.



**Figure 1.** Survival analysis of colon cancer patients based on miRNA-221 expression. Note: g=1: Low expression; Censored =1: Low expression-censored; g=2: High expression; Censored=2: High expression-censored.

## Results

### *miR-221 expression is associated with clinicopathological features in patients with colon cancer*

Of the 182 colon cancer patients, 73 were male and 109 were female (mean age,  $52 \pm 6.25$  years). Pathological assessment indicated that 112 cases had adenocarcinoma, and 70 cases had squamous cell carcinoma; additionally, 67 cases had stage I cancer, and 115 cases had stage II-IIIa.

miR-221 expression was detected in paraffin-embedded tissues by qRT-PCR. Patients were then stratified by the level of relative expression (high or low) of miR-221 in their colon cancer tissue samples (**Table 1**). Differences in miR-221 expression were not statistically significant by sex, pathological type, or tumor staging. In contrast, differences in miR-221 expression were statistically significant by patient age ( $P < 0.05$ ).

### *miR-221 expression is associated with the survival time of colon cancer patients*

A Kaplan-Meier survival analysis was performed on the 182 colon cancer patients with

stratification by miR-221 expression. In the group with highly-expressed miR-211, the median survival time of colon cancer patients was 39.6 months; in the group with low miR-211 expression had a median survival of 53.9 months (**Figure 1**). The difference between these groups was statistically significant (Log-rank test:  $P = 0.004$ ). A Cox proportional hazards model for single factor analysis showed that both late (II-IIIa) TNM staging for colon cancer [hazard ratio (HR)=3.014, 95% confidence interval (CI): 1.389-6.421,  $P = 0.002$ ] and the high expression of miR-211 (HR=2.185, 95% CI: 1.108-4.430,  $P = 0.009$ ) were correlated with poorer prognosis of colon cancer patients (**Table 2**). Similarly, a multivariate Cox regression analysis showed that late TNM staging (HR=2.973, 95% CI: 1.329-6.519,  $P = 0.003$ ) and the high expression of miR-211 (HR=2.394, 95% CI: 1.210-4.910,  $P = 0.006$ ) were risk factors for the prognosis of colon cancer patients.

## Discussion

With the third highest cancer incidence, colon cancer poses a significant health threat [14]. The investigation of miRNAs as contributors to colon cancer pathogenesis offers the potential to uncover new therapeutic targets [15, 16].

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**Table 2.** Univariate and multivariate analyses of overall survival (OS) of colon cancer patients

variable	Univariate analysis		Mutivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Age				
<45	1	1	1	1
≥45	1.164 (0.435-2.011)	0.620	1.219 (0.397-2.103)	0.607
Gender				
Male	1	1	1	1
Female	0.852 (0.283-1.726)	0.538	0.981 (0.374-2.206)	0.841
Histology				
SCC	1	1	1	1
ADC	0.895 (0.399-1.507)	0.611	0.742 (0.320-1.497)	0.540
p-TNM stage				
I	1	1	1	1
II-IIIa	3.014 (1.389-6.421)	0.002	2.973 (1.329-6.519)	0.003
MiR221 expression				
Low				
High	2.185 (1.108-4.430)	0.009	2.394 (1.210-4.910)	0.006

miR-221 plays a role similar to that of oncogenes in tumor initiation and progression, and is correlated with multiple cancers including ovarian, breast, lung, and papillary thyroid cancer [17-20]. In tumor cells, miR-221 tends to be highly expressed, and overexpressed miR-221 can target tumor suppressor genes such as *P57*, *BIM*, *PUMA*, *TIMP3*, and *PTEN*. Inhibition of these target genes thereby activates the AKT-induced bypass, initiates the cell cycle, inhibits related apoptosis-inducing ligands, and contributes to tumor proliferation. Similarly, high expression of miR-221 in colon cancer can inhibit *TIMP3*, thereby activating the AKT-induced bypass and promoting the proliferation and infiltration of colon cancer cells [12, 15]. Thus, miR-221 may play a key role in colon cancer.

The results of the current study support the hypothesis that miR-221 may contribute to the etiology of colon cancer. Interestingly, although differences among patients in the level of miR-221 expression in colon cancer samples were not meaningful by patient gender or by tumor type or staging, miR-221 expression did differ by patient age.

Further, the difference in miR-221 expression was significantly associated with survival time. The Cox multivariate analysis indicated that highly-expressed miR-221 is a risk factor for poorer prognosis of colon cancer patients.

Indeed, the miR-221 expression showed a negative correlation with the overall survival time of patients, i.e., the lower the miR-221 expression level, the longer the overall survival time, and vice versa.

In summary, in colon cancer patients with low expression of miR-221, the survival time was longer; in contrast, patients with high expression of miR-221 had shorter survival. Thus, miR-221 could serve as a molecular marker for the prognosis of colon cancer

patients. Because this study was a retrospective study with inadequate clinical data, further prospective clinical studies should be conducted in future to further confirm the reliability of the findings.

### Disclosure of conflict of interest

None.

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