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Original Article

Expression of miR-192-5p in colon cancer serum and its relationship with clinicopathologic features

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Abstract: Colon cancer has a poor prognosis due to a lack of biomarkers for early diagnosis and prognosis. The present study analyzed serum miR-192-5p expression levels in colon cancer patients and their correlations with clinicopathologic features. Relative mRNA expression was assessed by real-time fluorescence-based quantitative PCR in the serum of 164 colon cancer patients and 60 healthy controls. Patients were enrolled in a high or low miR-192-5p group according to the cutoff value determined by ROC curve analysis. The Kaplan-Meier method and univariate and multivariate Cox regression models were applied to analyze the risk factors influencing the postoperative survival of colon cancer patients. miR-192-5p mRNA expression in the colon cancer group was significantly reduced compared with the control group (P<0.01). Low miR-192-5p expression was significantly associated with a poor differentiation degree, lymphatic metastasis, vascular invasion, and high TNM stage (P=0.027, 0.001, 0.010, and <0.001, respectively). Colon cancer patients in the low miR-192-5p group exhibited a low survival rate (P<0.001). The independent risk factors for postoperative survival included lymphatic metastasis, a high TNM stage, and miR-192-5p<1.16 (P=0.017, 0.025, and 0.008, respectively). miR-192-5p may represent a promising biomarker for early diagnosis and prognosis in colon cancer patients.

Keywords: Colon cancer, miR-192-5p, biomarker, diagnosis, prognosis

Introduction

Colon cancer is the most frequent digestive system malignant tumor, killing approximately 700,000 people every year and accounting for approximately 10% of all diagnosed cancers worldwide [1, 2]. Its incidence and mortality are rapidly increasing and pose a serious threat to human health. With the continual progress of medical standards, surgical operation and drug therapy have improved colon cancer diagnosis and treatment. However, the prognosis of colon cancer remains unsatisfactory given that the primary site of tumor growth is often unclear and patients present with obscure symptoms; thus, early diagnosis of colon cancer is difficult. Consequently, it is of great importance to identify a reliable biomarker for early diagnosis and prognostic evaluation. In recent years, numerous studies have demonstrated that microRNAs (miRNAs) play a key role in the formation and pathogenesis of cancer and are involved in cell proliferation, differentiation, apoptosis and metabolism [3-6]. miRNAs are considered promising candidate biomarkers for cancers. Recent research findings indicated that miR-192-5p is abnormally expressed in gastrointestinal tumors and participates in their pathogenesis. No studies that focus on the relationship between miR-192-5p expression levels and colon cancer prognosis have been reported to date. In the current study, we investigated serum miR-192-5p expression levels and the value of this biomarker in the early diagnosis and prognosis of colon cancer patients.

Methods

Study population and sample collection

All colon cancer patients (n=164) were admitted to the People's Hospital of Danzhou from January 2013 to June 2020. All diagnoses of colon cancer cases were confirmed by patho-

Table 1. Comparison of clinical features between the high miR-192-5p group and the low miR-192-5p group

Clinical feature	N	miR-192-5p group		χ^2	P
		Low	High		
Age (years)					
<60	102	59	43	0.053	0.817
≥60	62	37	25		
Gender					
Male	89	53	36	0.082	0.774
Female	75	43	32		
Pathologic type					
Tubular adenocarcinoma	120	68	52	0.644	0.422
Mucous adenocarcinoma	44	28	16		
Signet ring adenocarcinoma	0	/	/		
Squamous cell carcinomas	0	/	/		
TNM stages					
I-II	55	21	34	14.126	<0.001
III-IV	109	75	34		
Primary site					
Left hemicolorectal	91	51	40	0.523	0.469
Right hemicolorectal	73	45	28		
Differentiation degree					
Well-differentiated	38	21	17	4.912	0.027
Intermediately differentiated	40	25	15		
Poorlydifferentiated	86	65	21		
Tumor size					
<5 cm	71	32	39	9.354	0.002
≥5 cm	93	64	29		
Lymphatic metastasis					
Yes	51	39	12	9.808	0.001
No	113	57	56		
Vascular invasion					
Yes	65	46	19	6.639	0.010
No	99	50	49		

Poorly, intermediately and well-differentiated adenocarcinoma.

logic examinations. Before surgery, no subjects had received radiotherapy, chemotherapy, or other treatments. In addition, no distant metastases were found in these patients by imaging examination. Three pathologists reviewed and assessed pathologic features, including age, sex, pathologic type, lymphatic metastasis, TNM stage, differentiated degree, primary site and vascular invasion. Healthy persons who came to our hospital for physical examination were selected as the control population (n=60). Detailed information on the clinical features is listed in **Table 1**. The present investigation was approved by the Ethics Committee of People's Hospital of Danzhou (Danzhou, China). All patients, control subjects, and their family members provided informed consent. All materials and methods conformed to correlative policies and regulations.

Specimen collection

Approximately 5-ml venous blood samples were drawn from colon cancer patients and healthy controls on an empty stomach in the morning using a heparin centrifuge tube (Becton, Dickinson and Company, Shanghai, China). The heparin centrifuge tubes were inverted several times at room temperature and then centrifuged at 3,000×g for 15 min using a centrifuge (Baiyang, Beijing, China). Finally, the supernatant was obtained using a pipettor and transferred into an RNase-free tube. All RNase-free tubes were stored at -70°C together with TRIzol.

Reagents and equipment

TRIzol reagent for RNA extraction was purchased from Thermo Electron Corporation (Waltham, MA, USA). A PE480 reverse transcription PCR machine was purchased from Perkin-Elmer Corporation (Waltham, MA, USA). The NanoDrop 2000c ultramicro spectrophotometer was obtained from Thermo Fisher Scientific Corporation (Waltham, MA, USA). Other instruments, including a fluorescence quantitative PCR machine, were obtained from ABI Corp. (Foster City, CA, USA).

Real-time fluorescence-based quantitative PCR analysis

U6 served as an internal reference. The forward primer, reverse primer and U6 primer sequences were designed. Detailed information on the primer sequences can be found in **Table 2**. Total RNA extraction and amplification were conducted using a total RNA rapid extraction kit and an ABI 7500 fluorescent quantitation

Table 2. miR-192-5p and U6 primer sequences

Gene	Forward primers	Reverse primers
miR-192-5p	5'-ATACAGGATAACGATTGACG-3'	5'-GCTCTAGAGATCACATAG-3'
U6	5'-AATCCTTCATTCCACCGG-3'	5'-AACGCTTCACGAATTTGCGT-3'

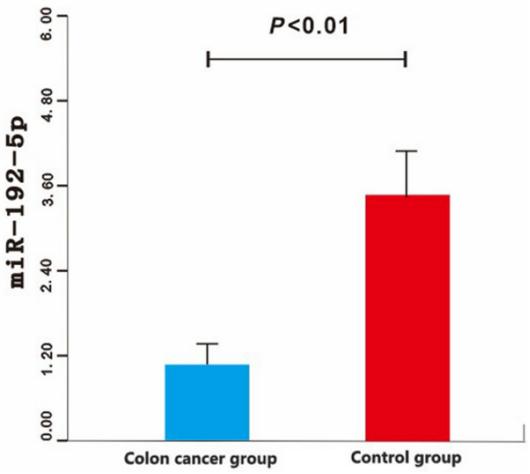

Figure 1. Comparison of miR-192-5p miRNA expression levels between the colon cancer group and the control group.

PCR machine. The 15-μL miRNA reverse transcription reaction system included the following components: 5 μL RNA template, 3 μL U6 and miRNA-specific stem loop primer, 0.15 μL dNTPs (100 mmol/L), 1 μL reverse transcriptase (50 U/L), 1.5 μL 10* reverse transcription buffer, 0.19 μL RNase inhibitor (20 U/L) and 4.16 μL tri-distilled water. The reaction was performed in three steps: 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min. The PCR amplification system included 20: 1 μL primer and probe mix (20*), 10 μL TaqMan general mixed solution (2*), 1.33 μL reverse transcription product cDNA, and 7.67 μL nuclease-free water. The reaction was performed in three steps (45 cycles), including 95°C for 10 min, 95°C for 15 seconds, and 60°C for 60 seconds. We used the $2^{-\Delta\Delta Ct}$ calculation formula to determine the relative expression level of miR-192-5p as follows: $\Delta Ct=C_T$ value of target gene (miR-192-5p)-C_T value of U6.

Statistical analysis

SPSS20.0 software was used to perform all statistical analyses. Measurement data exhibiting a normal distribution are represented by x±S. The independent sample T test was applied to analyze comparisons between groups. The χ^2 test was used for enumeration data comparisons between groups. Kaplan-Meier, log-rank and Cox regression analyses were applied for single-factor survival analysis and multivariate survival analysis. P<0.05 was considered significant.

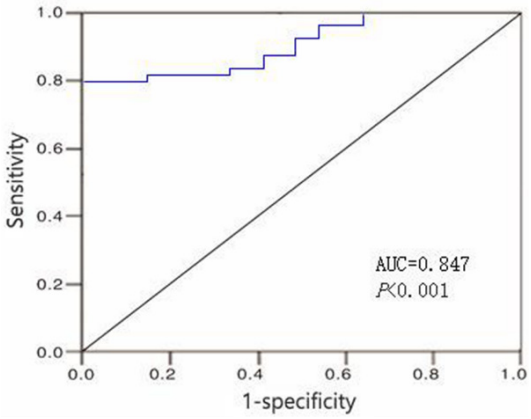

Figure 2. miR-192-5p ROC curve of postoperative survival in colon cancer patients.

Results

Clinical data

The 164 colon cancer cases included 89 males and 75 females with ages ranging from 32 to 75 years old (55.70±8.53). The 60 healthy controls consisted of 36 males and 24 females with ages ranging from 34 to 76 years old (56.84±8.37). In the present study, the miR-192-5p expression level of the colon cancer group was significantly reduced compared with that of the controls (P<0.05). Detailed information is presented in **Figure 1**.

MiR-192-5p expression

A receiver operating characteristic curve (ROC) was applied to evaluate the cutoff value of the miR-192-5p level as a predictive factor of colon cancer prognosis. When the value of miR-192-5p was 1.16, the greatest Youden index was obtained. Therefore, 1.16 was regarded as the optimum cutoff value and applied to evaluate the postoperative survival of colon cancer patients. The sensitivity and specificity values were 84.6% and 79.2%, respectively. The area under the ROC was 0.847. Detailed information is presented in **Figure 2**. No significant differ-

Table 3. Univariate and multivariate Cox regression analysis of patients with colon cancer after surgery

Clinical feature	Univariate survival analysis			Multivariate survival analysis		
	HR	95% CI	P	HR	95% CI	P
TNM stage	2.816	1.924-4.105	0.017	2.390	1.648-3.572	0.025
Differentiation degree	1.985	1.305-2.912	0.038	1.304	0.891-1.904	0.151
Tumor size	1.804	1.193-2.675	0.042	1.104	0.826-1.617	0.193
Lymphatic metastasis	3.152	2.148-5.270	0.006	2.725	1.904-4.103	0.017
Vascular invasion	2.438	1.327-3.265	0.034	1.527	0.973-2.016	0.094
miR-192-5p<1.16	4.416	3.802-9.853	<0.001	3.627	2.984-7.260	0.008

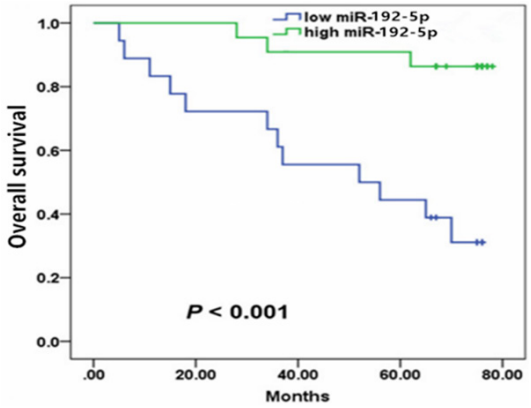

Figure 3. Comparison of survival time curves of colon cancer patients between the high miR-192-5p group and the low miR-192-5p group.

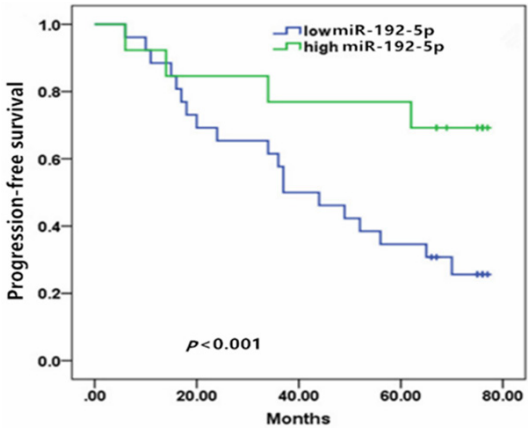

Figure 4. Comparison of progression-free survival time of colon cancer patients between the high miR-192-5p group and the low miR-192-5p group.

ences in age, sex, pathologic type, or primary site were noted between the high miR-192-5p group and the low miR-192-5p group. Nevertheless, significant differences in lymphatic metastasis, TNM stages, differentiation degrees, and vascular invasion were noted. Detailed information can be found in **Table 3**.

Relationship between miR-192-5p expression levels and postoperative survival of colon cancer patients

Among 164 colon cancer patients, four patients were lost to follow-up. The follow-up visit rate is 97.6% (160/164). The follow-up time ranged from 10 to 71 months, and the median follow-up time was 31 months. Survival analysis showed that the overall survival rate of patients in the low miR-192-5p group was significantly reduced compared with that of patients in the high miR-192-5p group (P<0.01). Similarly, the progression-free survival rate in patients in the low miR-192-5p group was significantly lower than that in the high miR-192-5p group (P<0.01). Detailed information can be found in **Figures 3** and **4**. COX regression analysis showed that lymphatic metastasis, TNM stage, and miR-192-5p<1.16 were three independent prognostic factors that affected the postoperative survival of colon cancer patients.

Discussion

Colon cancer is the most frequent gastrointestinal tract malignancy. Its incidence is below only those of lung cancer and breast cancer. In recent years, the incidence and mortality of colon cancer have rapidly increased. The occurrence and development of colon cancer is a complicated process and is associated with numerous diverse factors, including polyp formation, inflammatory bowel disease, a high-fat diet, lack of exercise, alcohol, smoking, and obesity [7]. However, approximately half of colon cancer patients do not exhibit polyp formation, inflammatory bowel disease, and not everyone who has bad habits, such as a high-

fat diet, lack of exercise, alcohol, smoking and obesity, will develop colon cancer. These findings suggest that additional genetic or nongenetic factors that modulate colon cancer risk have yet to be discovered.

miRNAs are a cluster of approximately 21-25 nucleotides that regulate gene expression by targeting mRNAs. miRNAs are small, conserved, noncoding and endogenous [8, 9]. miRNAs are implicated in multiple biological and pathologic processes, including cell growth, signal transduction, cell development, proliferation, migration, and apoptosis [10]. Abnormal expression of miRNAs is ubiquitous. Extensive studies have shown that dysregulated miRNA expression is observed in body fluids of colon cancer patients, such as serum, plasma and feces [11, 12]. miRNAs have been recognized as diagnostic and prognostic biomarkers to evaluate the occurrence, development and prognosis of colon cancer. Different miRNAs exhibit dual roles of cancer inhibition and promotion. Furthermore, miRNAs play a nonnegligible role in colon cancer-related signaling pathways, such as Wnt/β-catenin, EGFR, TGF-β, and TP53 [13].

Previous studies demonstrated that miR-192-5pis a multifunctional regulator. It is not only the gene that P53 directly targets; it is also a regulatory gene that targets the P53 signaling pathway [14]. Hence, miR-192-5p plays a dominant role in disease physiology and pathology processes. MiR-192-5p regulates esophageal carcinoma cell apoptosis by targeting the BIM gene [15]. In addition, miR-192-5p participates in the caspase pathway by targeting RB1 to induce cell apoptosis [16]. Li et al reported that miR-192-5p was involved in hepatoma carcinoma cell proliferation and metastasis by targeting SEMA3A [17]. Cumulative evidence has demonstrated that miRNA dysfunction is implicated in the occurrence andpathological processes of various cancers, including gastric carcinoma, lung cancer, breast cancer and prostate cancer [8, 18-20]. Although miR-192-5p overexpression may play a potential role as a cancer suppressor, numerous studies have reported that miR-192-5p is downregulated in various malignant carcinomas [21, 22]. In the present study, we found low miR-192-5p expression in colon cancer patients, suggesting that miR-192-5p might act as a tumor suppressor gene and that low expression might be associated with colon cancer occurrence and development. Our results conform to the previous reports. Our results indicate that low miR-192-5p is significantly associated with poor differentiation degree, lymphatic metastasis, vascular invasion and high TNM stage, suggesting that low miR-192-5p expression is related to poor clinical features of colon cancer patients. This phenomenon may be explained by the notion that miR-192-5p acts as a tumor suppressor gene and that low expression of miR-192-5p contributes to colon cancer occurrence and progression through a series of overactive targeted oncogenes. To our knowledge, this is the first study to specifically investigate miR-192-5p expression levels in serum samples of colon cancer patients. We also discovered that colon cancer patients in the low miR-192-5p group exhibit a low survival rate. The independent risk factors for postoperative survival in colon cancer patients include lymphatic metastasis, high TNM stage and miR-192-5p<1.16. Recently, Li et al published a study that investigated miR-192-5p expression in colon cancer tissues and indicated that low miR-192-5p expression was associated with a low overall survival rate [23].

Although miR-192-5p represents a potential biomarker for early diagnosis and prognosis in colon cancer patients, more studies on miR-192-5p should be performed in the future. First, real-time fluorescence-based quantitative PCR analysis can be used to detect miR-192-5p expression levels in cancer cell lines and epithelial cell lines. Second, gene recombination technology can be applied to establish lentiviral vectors, which can be transferred into cancer cell lines to generate cell lines with reduced miR-192-5p expression.

In conclusion, miR-192-5p represents a promising biomarker for early diagnosis and prognosis in colon cancer patients. In the future, larger multicenter prospective studies are urgently needed to confirm our results.

Disclosure of conflict of interest

None.

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