Serum miR-218 is a Potential Biomarker in the Diagnosis of Colorectal Cancer

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Abstract

Background: Our study was designed to explore the diagnostic role of serum microRNA-218 (miR-218) in colorectal cancer (CRC).

Methods: Serum level of miR-218 was measured in 117 CRC samples and 88 normal controls using quantitative reverse transcription (qRT-PCR). Chi-square test was performed to assess the relationships between miR-218 expression and clinical characteristics of CRC patients. The receiver operating characteristic (ROC) analysis was established to investigate the diagnostic significance of miR-218 in CRC with the area under ROC curve (AUC).

Results: MiR-218 was found to be weekly expressed in CRC serum samples compared with healthy controls ($P < 0.001$). And the down-regulation of miR-218 shared close relationships with lymph node metastasis ($P = 0.013$), vascular invasion ($P = 0.020$) and TNM stage ($P = 0.031$). However, age, tumor size and gender had no significant influence on miR-218 expression ($P > 0.05$). According to the ROC curve, miR-218 yielded an AUC value of 0.897 and an optimal cutoff point value of 0.021, providing a 83.8% sensitivity and a 78.4% specificity.

Conclusion: In summary, decreased expression of serum miR-218 was detected in CRC patients and the expression of miR-218 was a diagnostic marker in CRC.

Background

Colorectal cancer (CRC) has been reported to rank the third among all types of tumors in men and be the second most frequent malignancy among women all around the world [1, 2]. Though people have raised the awareness about CRC, its mortality and morbidity are still high worldwide [3]. It has been reported that CRC is responsible for more than 600,000 deaths every year according to the World Health Organization (WTO), accounting for about 10% of all cancer types [4, 5]. Nonetheless, certain investigations have demonstrated that the death rate of CRC has been reduced recently in developed countries resulting from early diagnosis and better treatments [6]. Therefore, it is important to early diagnose CRC for improving patients' survival. Current screening and detection methods for CRC are always invasive and presented with unsatisfactory specificity and sensitivity globally, such as colonoscopy [7, 8]. In addition, CRC patients at early stages usually have no obvious symptoms, which leads to the advanced stage at diagnosis time. Thus, it is urgently needed to find biomarkers to early diagnose CRC.

MicroRNAs (miRNAs) are a group of endogenous non-coding short RNAs that exist in a large number of eukaryoic cells with high conservatism [9, 10]. The miRNAs are all single stranded and consist of about 19–25 nucleotides [11]. MiRNAs can regulate the expression of numerous genes at posttranscription level through binding to the 3'-untranslated region (3'-UTR) of target mRNA [12, 13]. A growing number of studies have demonstrated that miRNAs are involved in lots of biological progresses, such as cell proliferation, differentiation, apoptosis and tumor dissemination [14–16]. MicroRNA-218 (miR-218) is reported to be a vertebrate-specific miRNA that plays critical roles in tumorigenesis and tumor

development [17]. A growing number of studies have proved that abnormal expression of miR-218 is now frequently observed in various cancers, which might be related with tumor development and progression, such as breast cancer and prostate cancer [18, 19]. In addition, Li et al. revealed that miR-218 down-regulation in CRC could predict the outcomes of patients [20].

In the present study, we would like to investigate the expression of serum miR-218 in CRC patients and then assess its diagnostic significance in CRC.

Methods

Patients and specimens

A total of 117 CRC patients who were pathologically diagnosed in Southwest Hospital, Army Medical University were enrolled in our study, including 46 females and 71 males. In addition, 88 healthy blood donors were also enrolled in the study as controls. An aliquot of 5 ml peripheral blood was collected from each participant and then put into EDTA-tubes. Serum samples were prepared through centrifugation within 6 hours after blood collection, 2000 × g for 10 min, 4 ℃ and finally stored at -80 ℃ for further studies. This pilot study was authorized by the Ethics Committee of Southwest Hospital, Army Medical University. All participants had provided the written informed consents in advance.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated from CRC serum samples and healthy controls using miRNeasy Mini Kit (Qiagen, Hilden, Germany) following the protocols. Then the first strand of cDNA was generated with total RNA using PrimeScript™ RT Master Mix Perfect Real Time (TaKaRa, Dalian, China). Finally quantitative real-time PCR was performed with SYBR Premix Dimer Eraser (TaKaRa). U6 was taken as an internal reference. The expression of miR-218 was calculated by the $2^{-\Delta\Delta Ct}$ method. All data were collected from three independent parallel experiments.

Statistical analysis

All data in the study were statistically analyzed by SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA) software. The difference of miR-218 expression between CRC and control groups was compared using student's t-test. The associations between miR-218 expression and clinical characteristics were described by Chi-square test. The receiver operating characteristics (ROC) curve was generated to evaluate the diagnostic value of serum miR-218 in CRC. Besides, the sensitivity, specificity and area under the curve (AUC) were also parameters assessing the diagnostic role of serum miR-218 in CRC. Results were of great significance when *P* values were less than 0.05.

Results

Down-regulation of serum miR-218 in CRC cases

The expression of miR-218 in 117 CRC serum specimens and 88 healthy controls was determined using qRT-PCR. As shown in Fig. 1, the expression of serum miR-218 was significantly lower in CRC patients than that in healthy controls (0.013 ± 0.007 vs. 0.026 ± 0.007, mean ± SD, $P < 0.001$).

Association between miR-218 expression and clinical parameters

We analyzed the correlations between miR-218 expression and diverse clinical features of CRC patients with Chi-square (Table 1). It was apparent that miR-218 lowexpression was positively correlated with lymph node metastasis ($P = 0.013$), vascular invasion ($P = 0.020$) and TNM stage ($P = 0.031$). However, there was no close relationship between miR-218 expression and age ($P = 0.117$), tumor site ($P = 0.078$) and gender ($P = 0.111$).

Table 1
Relationship of miR-218 expression and clinical parameters

Clinical characteristics	Case	Expression		χ^2	*P* value
	NO.	Low	High		
Age (year)				2.462	0.117
> 55	62	36	26		
≦ 55	55	23	32		
Tumor site				3.096	0.078
Colon	57	34	23		
Rectum	60	25	35		
Gender				2.539	0.111
Male	71	42	29		
Female	46	17	29		
Lymph node metastasis				6.233	0.013
Positive	63	39	24		
Negative	54	20	34		
Vascular invasion				5.370	0.020
Yes	56	35	21		
No	61	24	37		
TNM stage				4.649	0.031
I, II	65	27	38		
III, IV	52	32	20		

Diagnostic performance of serum miR-218 in CRC

The potential use of miR-218 in CRC diagnosis was explored by ROC analysis. As shown in Fig. 2, the optimal cutoff point was 2.33, accompanied by the sensitivity and specificity of 83.8% and 78.4%, respectively. Besides, the AUC was 0.882, indicating miR-218 could discriminate between CRC patients and healthy controls.

Discussion

CRC is one of the most frequent malignancies in the digestive tract and contributes to part of cancer-related deaths worldwide. Though great progress has been achieved in CRC diagnosis, new and efficient

method for early detection of CRC is still in urgent need owing to the detects of the existing screening methods [21]. Nowadays, with the development of biotechnology, more and more investigations are focusing on biomarkers that could detect and predict CRC to improve the outcomes of CRC patients. It was claimed by Toiyama et al. that *ANGPTL2* was linked to the migration of CRC and was a detector of CRC recurrence and diagnosis [22]. Besides, Xiao et al. revealed that methylated *NDRG4* acted as a diagnostic biomarker in CRC [23]. However, this is far from enough to precisely diagnose CRC.

MiRNAs have been demonstrated to be significantly associated with the development and progression of various cancers as tumor suppressors or oncogenes [24, 25]. The potential roles of miRNAs in cancers make them promising biomarkers for prognosis and diagnosis of cancers. The aberrant expression of miRNAs has been detected in various cancers, such as miR-195 in non-small cell lung cancer, miR-372 in renal cell carcinoma and miR-128 in prostate cancer [26–28]. What's more, current screening modalities for cancers mainly depends on tissue biopsy, magnetic resonance imaging (MRI) and computed tomography (CT), which are invasive and money consuming for patients. Therefore, more and more investigations are paying attention on serum detection, which is frugal and convenient. In the study of Jiang et al., down-regulation of serum miR-218 could act as a promising candidate in early diagnosis of esophageal cancer [29]. Besides, Yu et al. observed that serum miR-218 levels were reduced in CRC patients compared healthy individuals, which represented a poor prognosis for patients [30]. Therefore, in this study, we attempted to explore the relationship between miR-218 expression and CRC diagnosis.

We first compared the expression of serum miR-218 in CRC patients and healthy controls using qRT-PCR. The expression profile showed that the level of serum miR-218 was significantly lower in CRC cases than healthy controls, which was in accordance with the previous results, indicating miR-218 might be related to CRC progression. Then Chi-square test was adopted to analyze the relationship between miR-218 expression and clinical characteristics and miR-218 expression was influenced by lymph node metastasis, vascular invasion and TNM stage. Based on the above results, we hypothesized that miR-218 might play an important role in the diagnosis of CRC. Thereby, the ROC curve was established to validate our conjecture. And the result demonstrated miR-218 could serve as a diagnostic marker for CRC with high specificity and sensitivity.

In the present study, we stated a significant relationship between miR-218 expression and CRC diagnosis for the first time. However, to our best knowledge, the precise mechanism of miR-218 on CRC development and progression is still dismal. Wang et al. explained that miR-218 could suppress the proliferation, metastasis and EMT of gastric cancer cells through targeting *WASF3* [31]. Song et al. claimed that miR-218 impeded lung cancer growth via regulating *MEF2D* [32]. In the study of Llm et al., miR-218 proved to regulate the expression of *MACC1*, which is a tumor suppressor in CRC [33]. Moreover, evidence has indicated that a single miRNA might target on thousands of mRNAs [34]. Thus, we conjectured miR-218 might function on CRC occurrence and development through targeting *WASF3*, *MEF2D* and *MACC1*, which needs verifying with more and further investigations in future.

Conclusion

In conclusion, our study observed a significantly low miR-218 expression in serum of CRC patients compared with healthy controls, which was induced by lymph node metastasis, vascular invasion and TNM stage. Furthermore, the reduced expression of serum miR-218 could be regarded as a diagnostic marker in CRC according to ROC analysis with high accuracy.

Abbreviations

microRNA-218 (miR-218)

colorectal cancer (CRC)

quantitative reverse transcription (qRT-PCR)

receiver operating characteristic (ROC)

area under ROC curve (AUC)

MicroRNAs (miRNAs)

3’-untranslated region (3’-UTR)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Southwest Hospital, Army Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Data availability All data generated or analysed during this study are included in this published article.

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Competing interests The authors declare that they have no competing interests.

Authors’ contributions K.Z., T.M. design of the work; Z.H., X.W. the acquisition, analysis, Y.P., Y.C. interpretation of data; Y.D., Z.R. the creation of new software used in the work; Z.W. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

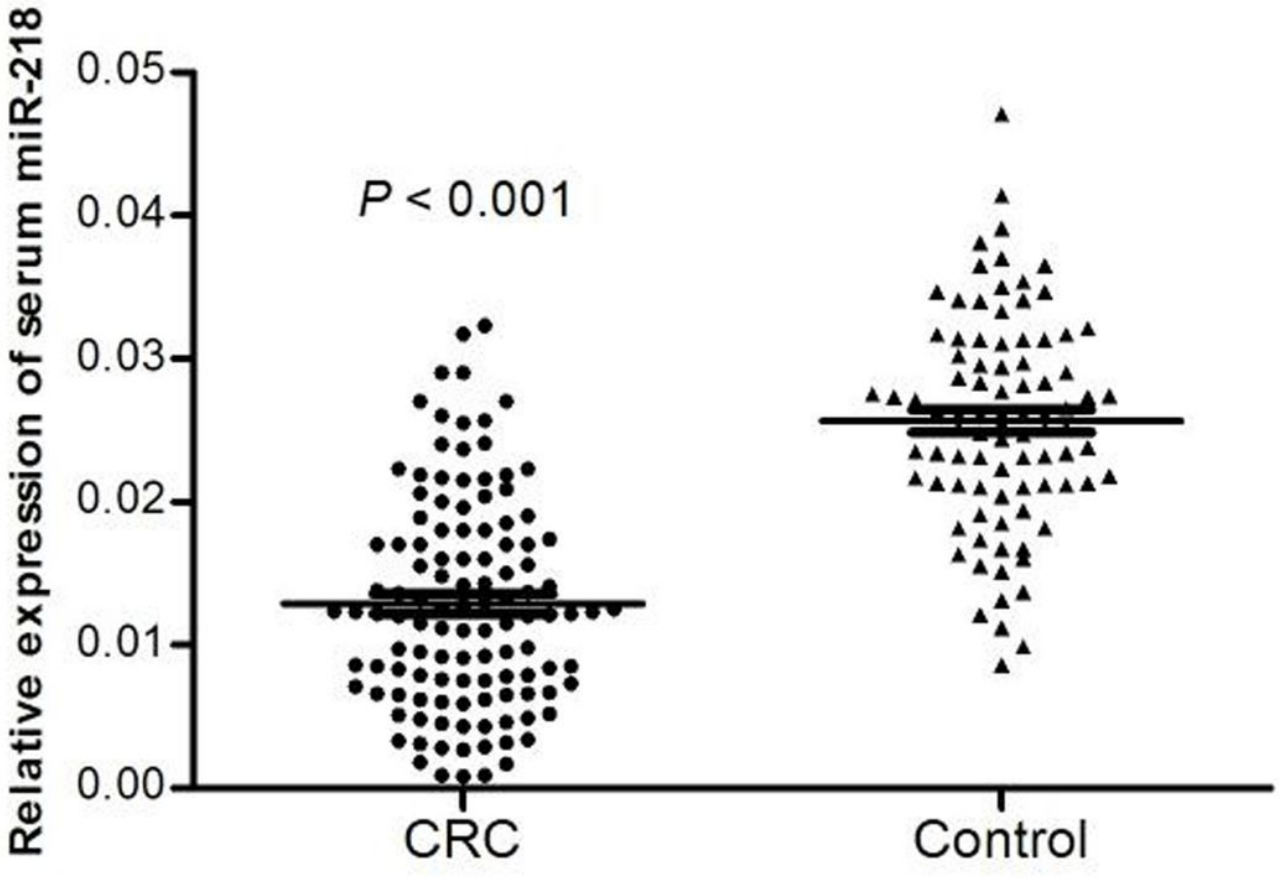

Figure 1

Expression of miR-218 in CRC serum samples and healthy controls was measured using qRT-PCR. The result showed a high level of serum miR-218 in CRC patients (P < 0.001).

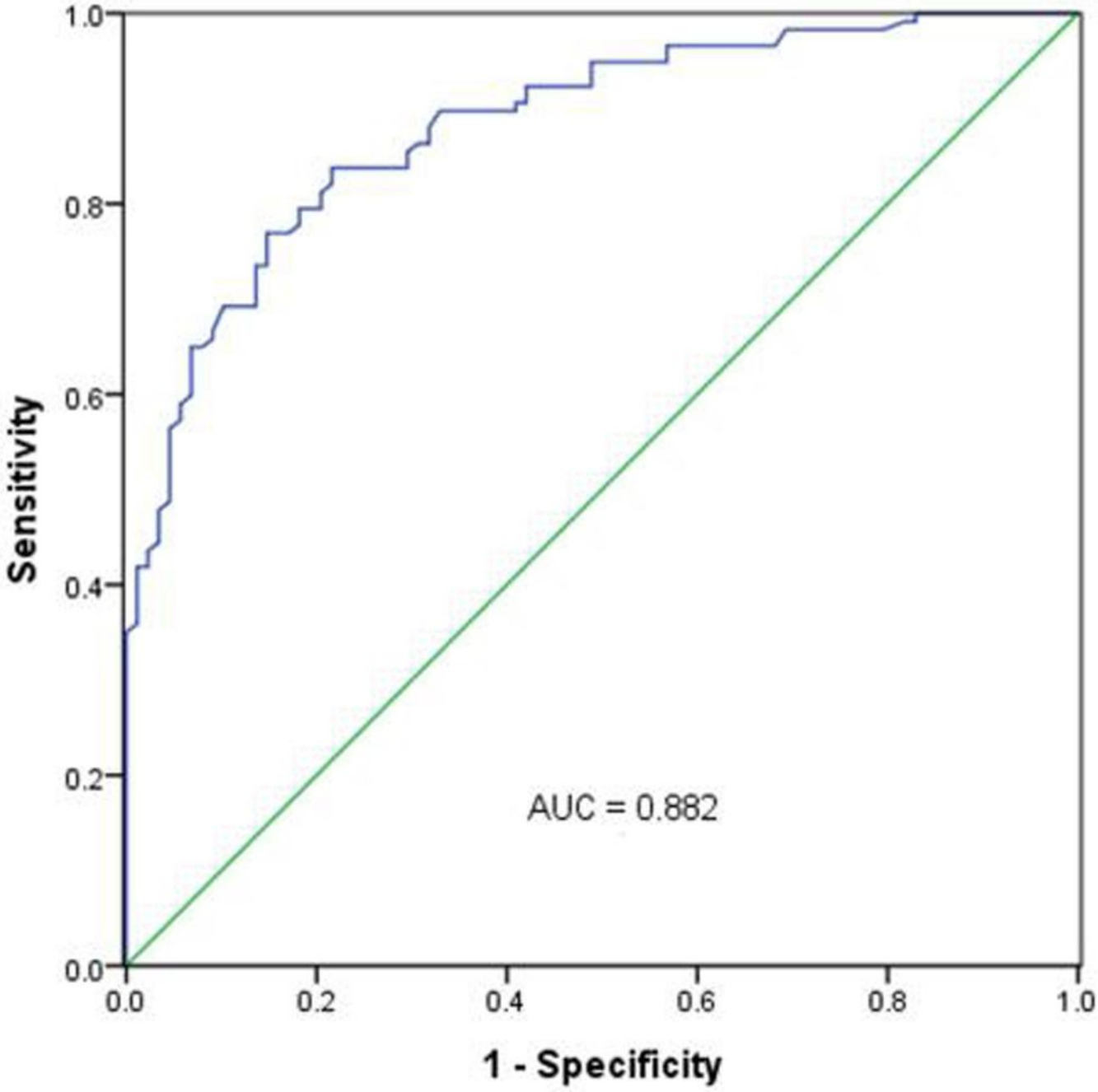

Figure 2

The ROC curve was plotted to describe the diagnostic significance of serum miR-218 in CRC. The curve provided a high diagnostic value of miR-218 in CRC (AUC = 0.882).