miR-124 Functions as A Marker in Diagnosing Colorectal Cancer

Yang Yan
Chinese PLA General Hospital
Xiaohui Du (gkikhm@126.com)
Chinese PLA General Hospital https://orcid.org/0000-0001-7102-602X
Shaoyou Xia
Chinese PLA General Hospital
Songyan Li
Chinese PLA General Hospital
Da Teng
Chinese PLA General Hospital
Shidong Hu
Chinese PLA General Hospital
Yufeng Wang
Chinese PLA General Hospital
Rong Li
Chinese PLA General Hospital

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Abstract

Background: *MicroRNA-124* (*miR-124*) regulates cell growth, angiogenesis, invasion, and apoptosis of many human tumors. Our objective was to evaluate the levels of *miR-124* in serum samples of colorectal cancer (CRC) patients to explore whether *miR-124* in serum can be used as a biomarker for the detection of CRC.

Methods: Serum *miR-124* level was measured in 85 patients with CRC and 60 healthy control subjects using reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR). The correlations between *miR-124* levels and clinicopathologic factors were analyzed. Diagnostic performance of serum *miR-124* level was calculated by using the receiver operating characteristic (ROC) curve.

Results: The expression level of *miR-124* in serum samples was significantly lower in CRC patients than in healthy controls (P<0.001). A positive correlation between *miR-124* level and tumor size (P=0.010), invasion depth (P=0.021), lymph node metastasis (P=0.015), and TNM stage (P=0.004) was observed. The serum *miR-124* yielded an AUC (areas under the ROC curve) of 0.832 with a sensitivity of 80.0% and a specificity of 81.7%, and the optimal cutoff point of *miR-124* was 1.61.

Conclusions: Serum *miR-124* may have a potential as a novel biomarker for the detection of CRC.

Background

Colorectal cancer (CRC) is the most common cancer worldwide, and it is the second cause of cancer death just behind lung cancer [1, 2]. It causes 655,000 deaths worldwide every year [3]. Although the true etiology of CRC is still not clear, but there is an in-depth study for the risk factors of CRC, including environment, diet, and the synergy of lifestyle and genetic factors. Despite great progresses have been made in therapy of CRC, 30–40% patients still died of relapse and metastasis, among which liver metastasis is the leading cause of death [4–6]. Several CRC screening tests, including fecal occult-blood testing and colonoscopy, have been available for years and have aided in reducing the mortality associated with this disease [7–10]. However, the prognosis effect of patients with CRC is not ideal. Therefore, it is urgent to develop an effective noninvasive assay to screen patients of CRC at early stage.

MiRNAs are single-strand small RNA molecules that are between 19 and 22 nucleotides in length. They participate in various biological processes, such as cell proliferation, differentiation, apoptosis, metabolism and tumorigenesis through inhibition of RNA translation or degradation of target messenger RNA by binding to 3’ untranslated region (UTR) of the target genes [11]. Studies confirmed that miRNAs play an important adjustment function in the process of the tumor development by regulating proto-oncogenes or tumor suppressor genes [12]. Recent studies have shown that miRNAs abnormally expressed in peripheral blood of CRC patients. Besides, miRNAs can participate in the progress of CRC, and have the potential function that serve as biomarkers for human cancers [13–15]. *MicroRNA-124* (*miR-124*), a brain-enriched miRNA, was first found to be involved in stem cell regulation and neuro-development [16, 17]. Studies showed that *miR-124* expression is down-regulated in a variety of cancers,

including breast cancer, non-small cell lung cancer, and colorectal cancer [18–20]. However, the role of *miR-124* in the diagnosis and prognosis of CRC has not been reported.

In the present study, we determined the serum *miR-124* expression in CRC specimens, investigated the correlations between *miR-124* expression and clinicopathological parameters, and further detected the diagnostic value of *miR-124* for CRC.

Methods

Patients and sample collection.

The study was approved by the Ethics Committee of Chinese PLA General Hospital, and all the patients provided written informed consent in advance. Serum sample collection was performed between Chinese PLA General Hospital. 85 patients with CRC and 60 healthy controls were included in this study. None of the healthy controls had formerly been diagnosed with any malignancy. The clinicopathological parameters of patients were summarized in Table 1. Blood samples were obtained prior to surgery. Serum was separated after centrifugation (3,000 rpm, 10 min) and stored at -80˚C.

Table 1
Association between serum *miR-124* expression and clinicopathological features in CRC patients

Features	No.	*miR-124* expression		*P* values
	N = 85	High (n = 38)	Low (n = 47)	
Age (years)				
< 60	41	16	25	0.309
≧ 60	44	22	22	
Gender				
Male	38	15	23	0.383
Female	47	23	24	
Tumor location				
Colon	43	20	23	0.735
Rectum	42	18	24	
Tumor size				
< 4.7 cm	45	26	19	0.010
≧ 4.7 cm	40	12	28	
Histology grade				
Well	19	10	9	0.473
Moderate	42	16	26	
Poor	24	12	12	
Invasion depth				
T1-T2	51	28	23	0.021
T3-T4	34	10	24	
Lymph node metastasis				
Absent	48	27	21	0.015
Present	37	11	26	
TNM stage				
I-II	39	24	15	0.004
III-IV	46	14	32	

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from serum samples using MicroMini Kit (Qiagen) according to manufacturer's protocol. The RNA concentration and purity were controlled by UV spectrophotometry using a Nanodrop ND-1000 (Thermo Scientific). The RNA specimens were stored at -80℃ until reverse transcription. The reverse transcription reaction was carried out with miScript Reverse Transcription Kit (Qiagen). For synthesis of cDNA, the reaction mixtures were incubated at 37℃ for 60 min, at 95℃ for 5 min and then held at 4℃. The cDNA specimens were stored at -20℃ until PCR. The amounts of miRNAs were quantified by qPCR using the miScript SYBR Green PCR kit (Qiagen). Quantitative PCR was run on a 7500 Real-Time PCR system (Applied Biosystems). The reaction mixtures were incubated at 95℃ for 10 min, followed by 40 cycles of 95℃ for 15 s, 56℃ 30 s and 72℃ 35 s. The relative expression level (fold change) of *miR-124* was determined by the $2^{-\Delta\Delta Ct}$ method using *U6* as the endogenous control to normalize the data.

Statistical analysis

Statistical analyses were performed using SPSS 21.0 software (SPSS, Inc, Chicago, Illinois). Data were expressed as mean ± SD. The independent sample t-test was utilized to determine the statistical difference between CRC patients and healthy controls. The relationship between clinicopathologic parameters and *miR-124* levels was examined using the χ^2 test. Sensitivity, specificity and area under curve (AUC) for serum *miR-124* levels were determined using receiver operator characteristic (ROC) analysis. *P*-values of < 0.05 were considered to represent statistical significance.

Results

Serum *miR-124* down-regulation in blood samples of CRC.

The expression levels of serum *miR-124* in CRC samples and healthy samples were detected by qRT-PCR. The results indicated that serum *miR-124* expression in the CRC samples was significantly down-regulated in comparison to that in the healthy control samples ($P < 0.001$, **Figure. 1**).

Relationship between *miR-124* and clinicopathological characteristics of CRC patients

Table 1. summarized the associations of serum *miR-124* expression with various clinicopathological parameters of CRC patients. A significant relationship was present between *miR-124* expression and the tumor size ($P = 0.010$), invasion depth ($P = 0.021$), lymph node metastasis ($P = 0.015$), and TNM stage ($P = 0.004$). However, there was no association between *miR-124* expression and age, gender, tumor location, or histology grade (all $P > 0.05$).

Diagnostic values of *miR-124* for CRC

Receiver operating characteristic (ROC) curve analysis was performed to discriminate CRC patients from healthy controls. Serum *miR-124* yielded an AUC (areas under the ROC curve) of 0.832 (95% CI: 0.760–0.904) with a sensitivity of 80.0% and a specificity of 81.7% (**Figure. 2**).

Discussion

Colorectal cancer remains a significant cause of mortality worldwide. The CRC incidence and mortality are actually increasing despite recent advances in surgery, radiotherapy and chemotherapy [21]. And despite curative surgical resection of the primary tumor, 40–50% of the patients ultimately die of metastasis [22]. Due to fact that CRC usually appear obvious symptoms until it progresses to advanced stages, so the implementation of screening programs aimed at early detection is essential to reduce incidence and mortality rates. Currently, The most common clinical imaging screening methods is colonoscopy. However, there were some limitations for this method such as invasion character, and causing abdominal pain [23]. In addition, the sensitivity and specificity of the laboratory detection methods, such as stool test, occult blood immunology and tumor markers detection are poor [24]. Therefore, looking for a novel biomarker for CRC non-invasive examination is of important clinical significance.

Recent studies indicated that miRNAs, which are non-protein-coding small RNAs, are involved in cancer progression and metastasis. MiRNA expression signatures have been shown to be promising biomarkers for understanding the tumorgenesis of a wide array of human cancers [25, 26]. Accordingly, studies speculated that some tumors secretion circulation miRNAs in serum or plasma could be molecular markers for clinical diagnosis of CRC. Ng et al. found that the high expression of *miR-17-3p* and *miR-92a* was closely related to CRC, and the expression levels of plasma *miR-17-3p* and *miR-92a* decreased significantly after surgery [27]. The results confirmed abnormal expression of miRNAs in the plasma circulation. Huang et al. further confirmed the diagnosis potential of *miR-92a*, indicated that the plasma of patients with CRC contains high levels *miR-92a*, and its diagnostic sensitivity and specificity are consistent with the results of Ng et al. [28].

Previous research confirmed that *miR-124* was a tumor suppressor in various types of cancers, including glioblastoma, hepatocellular carcinoma, medulloblastoma, and gastric cancer [29, 30]. Xie et al. [30] reported that *miR-124* was down-regulated in gastric cancer cells and specimens and it inhibited cancer cell proliferation and induced apoptosis by targeting enhancer of zeste homolog 2 in gastric cancer. The expression level and mechanism of *miR-124* have also been investigated in breast cancer. Han et al. [31] found that *miR-124* played a critical role in inhibiting the invasive and metastatic potential of breast cancer cells. Moreover, the previous study also reported the function of *miR-124* in colorectal cancer, it was significantly downregulated in CRC and interacted with ROCK1 to regulate the tumor cell proliferation [20]. However, the diagnostic value of *miR-124* in CRC was not studied.

This study is the first to demonstrate the potential role of serum *miR-124* in the early detection of CRC. The data showed that patients with CRC had significantly lower serum *miR-124* levels compared with healthy control subjects. To evaluate the relationship between serum *miR-124* and CRC progression, we further analyzed the correlation between *miR-124* expression and clinical pathological features of CRC. The results demonstrated that there is a strong correlation between serum *miR-124* levels and tumor size, invasion depth, lymph node metastasis, and TNM stage. These observations suggested a correlation

between increased expression of *miR-124* and clinical progression in CRC. Furthermore, we established the ROC curve to evaluate the diagnostic value of *miR-124* in CRC. These results indicate that down-regulation of serum *miR-124* may occur in the early stage of CRC and can serve as a potential biomarker of early diagnosis in CRC.

Conclusions

In conclusion, the present study provides evidence that serum levels of *miR-124* could be used to predict CRC disease progression. However, further investigations to determine the clinical values of *miR-124* as a predictor for the patients with CRC are needed.

Abbreviations

MicroRNA-124 (*miR-124*)

colorectal cancer (CRC)

quantitative real-time polymerase chain reaction (qRT-PCR)

receiver operating characteristic (ROC)

untranslated region (UTR)

area under curve (AUC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Chinese PLA General Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

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

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y.Y. and S.X. conceived and designed the experiments; S.L. and D.T. conceived and performed the experiments; S.H. and Y.W. prepared figures. R.L. and X.D. wrote the main manuscript text. All authors reviewed the manuscript.

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Figures

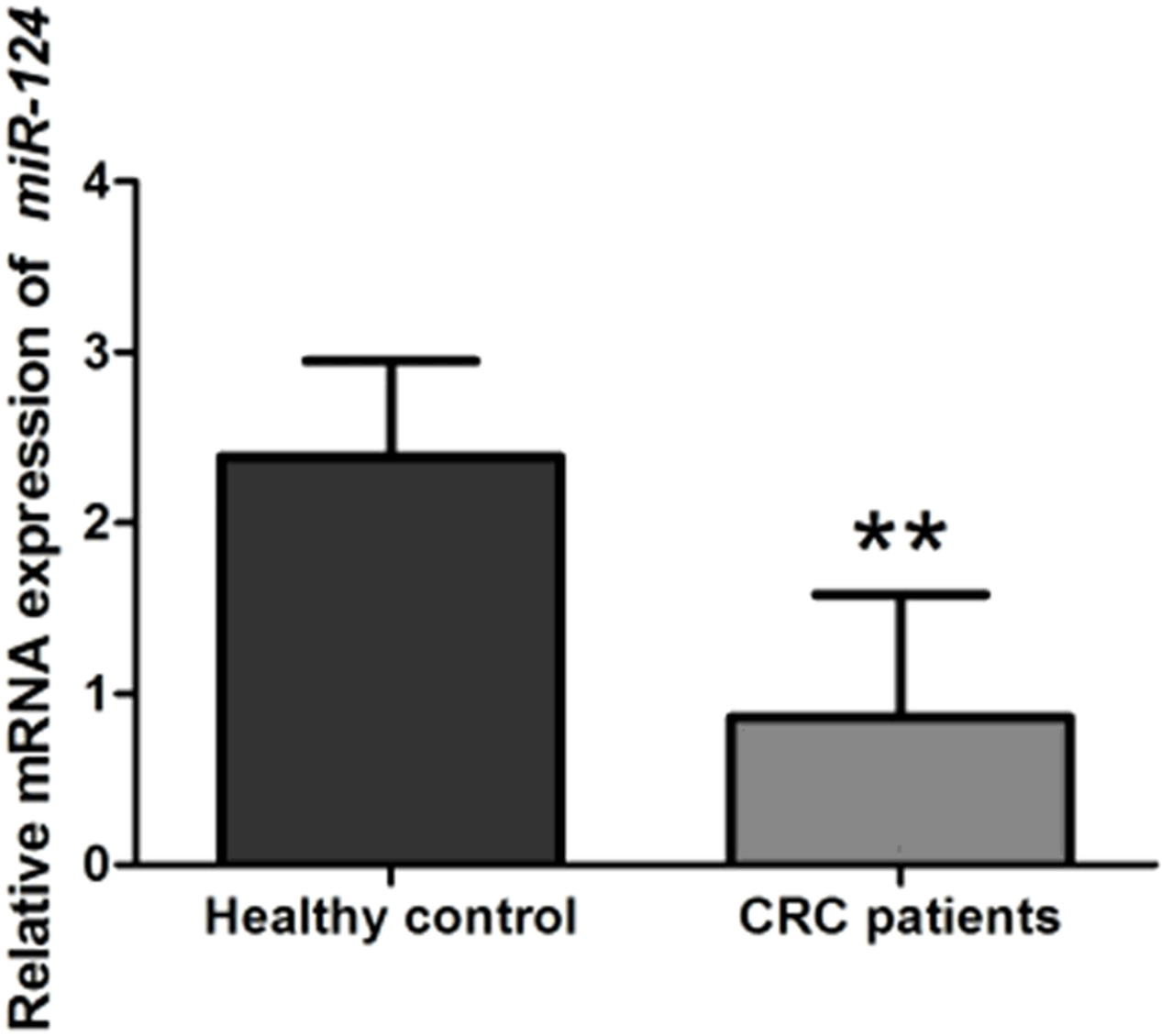

Figure 1

Serum miR-124 expression in CRC patients (n=85) and healthy volunteers (n=60). MiR-124 expression was significantly lower in patients with CRC than that in healthy controls (**, P<0.001).

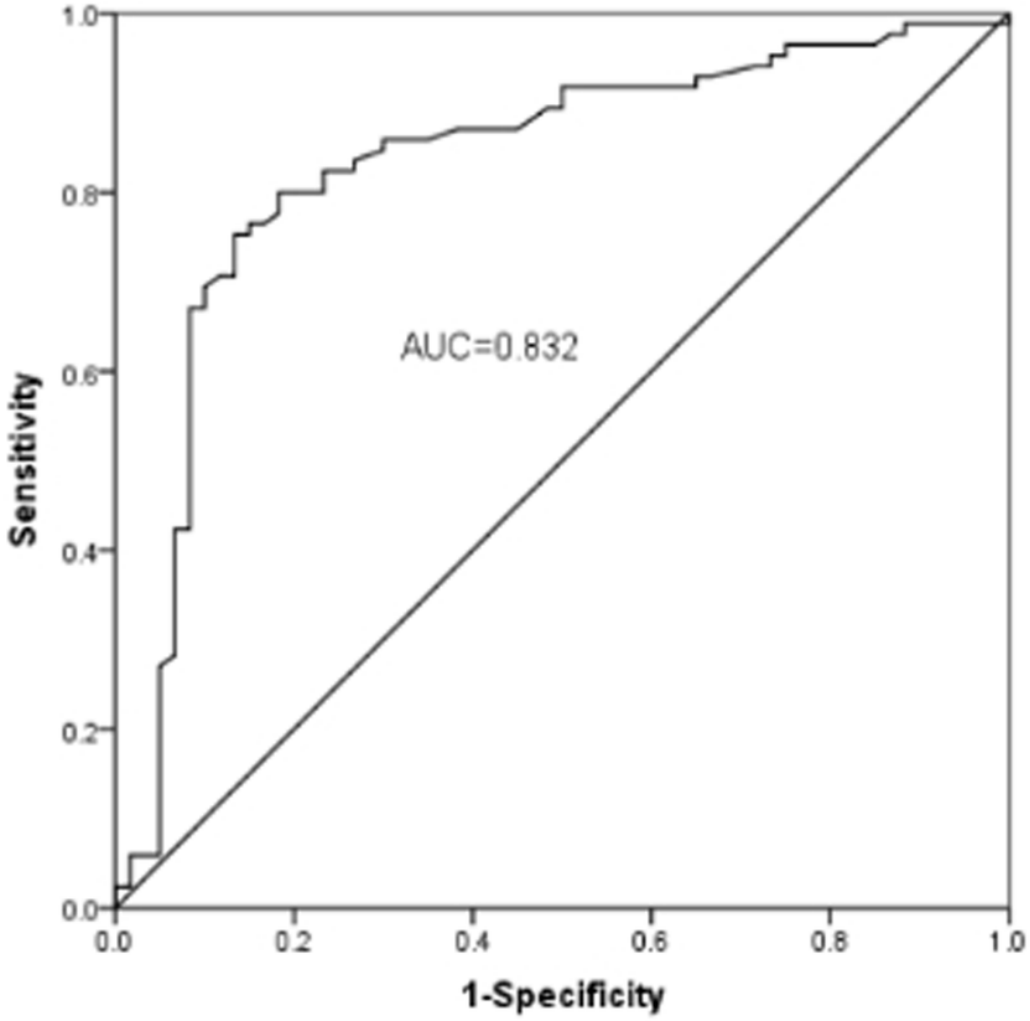

Figure 2

ROC curve for evaluation the accuracy of miR-124 to discriminate CRC patients from healthy controls.