Serum miR-663 expression and the diagnostic value in colorectal cancer

Ning Wanga, Liyi Zengb, Zhaoxia Lia, Yanfang Zhena and Huoming Chena

aDepartment of Oncology, the General Hospital of the PLA Rocket Force, Beijing, China; bDepartment of Infection Control, Zhuzhou Central Hospital and Affiliated Zhuzhou Hospital of Xiangya Medical College of Central South University, Zhuzhou, China

ABSTRACT
Colorectal cancer (CRC) is one of the most common digestive cancers leading to deaths worldwide. In this study, we aimed to investigate the diagnostic value of miR-663 in CRC. The expression of miR-663 was detected by quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR). The association between miR-663 and clinical parameters of subjects was evaluated by chi-square test. Additionally, ROC (receiver operating characteristic) analysis was performed to evaluate the diagnostic role of miR-663 in CRC. The expression of miR-663 in CRC patients was significantly upregulated compared with benign colorectal disease patients and healthy controls (p < .01). Besides, the expression of miR-663 was significantly associated with tumour differentiation, invasion, lymph node metastasis and TNM stage (p < .05). The cutoff value of miR-663 was 1.31, and the corresponding sensitivity and specificity were 83.1% and 73.8%, respectively. In ROC analysis, the area under the curve (AUC) was 0.806, which indicated that miR-663 could act as an independent diagnostic biomarker for CRC. In conclusion, miR-663 was up-regulated in CRC patients and may be an effective biomarker for CRC diagnosis.

Introduction
Colorectal cancer (CRC), also known as colon cancer, bowel cancer or rectal cancer, occurs in colon or rectum. It is one of the most common malignancies worldwide, with an estimated incidence of more than 1.2 million cases globally and 608,000 deaths annually [1]. High death rate results from abnormal growth of cells, which have the ability to invade or spread to other parts of the body through blood and lymph systems. About 35% of CRC patients are often in stage IV when diagnosed, among whom the 5-year survival rate is less than 10% [2,3]. Thus, it is necessary to find out specific and sensitive biomarkers for early CRC diagnosis, thus providing the patients with timely treatment.

MicroRNAs (miRNAs), small and noncoding RNAs (20–22 nucleotides), exert regulatory influences on gene expression by targeting sequences located in the 3′-untranslated region of mRNAs, and by inhibiting the translation or degradation [4–6]. Besides, miRNAs participate in cell proliferation, migration, differentiation and apoptosis [7,8]. Increasing evidence have displayed that miRNAs act as oncogenes or tumour suppressors in the pathogenesis of various cancers [9]. So miRNAs showed great potential to function as cancer biomarkers.

Similarly, miR-663 may function as either oncogenes or tumour suppressors in malignant progression. It can serve as an oncogene in many cancers such as non-small cell lung cancer [10], lung cancer [11], prostate cancer [12] and nasopharyngeal carcinoma [13], while acting as a suppressor against gastric cancer [14] and glioblastoma [15]. Currently, few studies have focused on the diagnostic value of miR-663 in CRC.

In this study, we aimed to detect the expression level of miR-663 in CRC patients, its relationship with clinical characteristics, and its diagnostic value in the disease.

Materials and methods

Patients and sample collection
CRC patients were recruited who were diagnosed in the General Hospital of the PLA Rocket Force. The present study was authorized by the Ethics Committee of the above hospital. Each of the 126 CRC patients, benign colorectal disease patients and healthy individuals were enrolled in this study. CRC cases contained 81 females and 45 males with an average age of 59.02 ± 9.43. Benign colorectal disease group and healthy controls were frequency-matched with CRC group in age and gender. All subjects were Chinese Han population without blood relationship and had signed written informed consents before collecting samples.

After fasting for 8–10 h, 10 ml peripheral blood of each participant was extracted in the morning, and coagulated for 1 h at room temperature. The serum was then separated through centrifugation. Serum samples with low levels of haemolysis (haemoglobin, 0.1 g/l) were aliquoted and stored at −80°C until use.
RNA extraction and quantitative real-time reverse transcriptase- polymerase chain reaction (qRT-PCR)

Total RNA was isolated from serum samples using the mirVana miRNA Isolation Kit (Ambion, Austin, TX). Then, 0.05 µg of total RNA was reverse-transcribed with Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The expression level of miR-663 was detected by qRT-PCR adopting Platinum SYBR Green aPCR SuperMix-UDG reagent (Invitrogen, Renfrew, Scotland) in the Applied Biosystems 7900 Fast Real-time PCR system under optimal conditions. Total RNA samples would be employed only when they reached an OD A260/A280 ratio close to 2.0, which indicated that RNA is pure. The relative expression level of miR-663 was normalized to U6 and calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analysis was conducted with SPSS version 13.0 (SPSS Inc., Chicago, IL) and Graphpad prism 5. The difference in the expression level of miR-663 was pairwise analyzed between CRC cases, colorectal adenomas patients and healthy controls through the t-test. The relationship between clinical factors and miR-663 expression was estimated by chi-square test. Receiver operating characteristic (ROC) curve was established to assess diagnostic value of miR-663 in CRC. $p$-values of less than .05 were considered statistically significant.

Results

Expression level of miR-663

As shown in Figure 1, the level of miR-663 was significantly higher in patients with CRC than in benign colorectal disease group and healthy controls ($p < .01$). Meanwhile, the level of miR-663 was higher in benign colorectal disease cases than in healthy controls ($p < .01$).

Diagnostic value of miR-663 in CRC

ROC curve was established to estimate the value of miR-663 in CRC diagnosis. The analysis suggested that the serum level of miR-663 was a potential biomarker in differentiating CRC patients from benign cases and healthy controls, with an area under curve (AUC) of 0.806. The cutoff value for miR-663 was 1.31, accompanied by a sensitivity and specificity of 83.1 and 73.8%, respectively (Figure 2).

Discussion

CRC is one of the most frequent malignant neoplasms [16] and the second leading cause of cancer-related deaths in developed countries [17]. It is urgent to improve CRC diagnosis level for its prevention and better prognosis. Currently, the most common and efficient method in CRC diagnostics is endoscopy, but this invasive method brings pain and other adverse effects. Screening examination adopting tumor markers and intervention for early stages of CRC may significantly decrease the mortality rate of patients [16]. Moreover, tumour markers in blood, urine and body [16] are

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Table 1. The relationship between clinical factors and expression level of miR-663.

| Clinical factors          | No. of cases | High | Low  | $P$-value |
|---------------------------|--------------|------|------|------------|
| **Age (year)**            |              |      |      |            |
| $\leq 60$                  | 74           | 42   | 32   | .110       |
| $>60$                     | 52           | 22   | 30   | .750       |
| **Gender**                |              |      |      |            |
| female                    | 81           | 40   | 41   | .021       |
| male                      | 45           | 22   | 23   |            |
| **Differentiation**       |              |      |      |            |
| Poor                      | 39           | 29   | 10   | .005       |
| Moderate                  | 50           | 27   | 23   |            |
| Well                      | 37           | 16   | 25   |            |
| **Invasion**              |              |      |      |            |
| T1–T2                     | 42           | 14   | 28   | .006       |
| T3–T4                     | 84           | 50   | 34   |            |
| **Lymph node metastasis** |              |      |      |            |
| Negative                  | 78           | 32   | 46   | .001       |
| Positive                  | 48           | 32   | 16   |            |
| **TNM stage**             |              |      |      |            |
| I                         | 26           | 7    | 19   | .014       |
| II                        | 36           | 14   | 22   |            |
| III                       | 35           | 22   | 13   |            |
| IV                        | 29           | 21   | 8    |            |

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Figure 1. Expression level of miR-663 in CRC patients, cases with benign colorectal diseases and healthy controls.
MiR-663 is a member of the miRNA family and also expressed in CRC, such as 11, 12, 13a, 196, 20, 21, 22, 23, 24, 26, 29a, 25. Reports have shown that miR-663 promotes the proliferation and cell cycle progression of nasopharyngeal carcinoma cells through directly targeting CDKN2A, suggesting that miR-663 may be an effective target in treating cancer [29]. Therefore, miR-663 might be an important regulator during the development and progression of human cancers and may be a candidate biomarker for human cancers.

To the best of our knowledge, there has been no research on the expression level of miR-663 in CRC patients. Since miR-663 acts as a tumour promoter or suppressor in an organ-specific fashion [13,14,30], its effects on the malignant progression of tumours are controversial [31]. In the present study, the level of miR-663 was higher in CRC patients than in colorectal adenomas cases and healthy controls. The result indicated that miR-663 acted as a tumour promoter in CRC. Further exploration through ROC curve suggested that miR-663 held fine diagnostic value in CRC. It is worth mentioning that this was the first study showing the clinical value of the miR-663 expression in CRC.

In conclusion, our findings provided convincing evidence for the first time demonstrating the upregulation of miR-663, which might serve as a novel molecular marker for the diagnosis of CRC, and its expression level was influenced by clinical stages, infiltration degree and distant metastasis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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