Abstract. Expression of miR-34c and miR-141 in serum of colon cancer patients and their association with clinicopathological features and diagnostic value for colon cancer were investigated. A total of 64 patients with colon cancer admitted to Hubei Cancer Hospital from January 2016 to March 2018 were included in the experimental group, and 64 healthy subjects undergoing physical examination during the same period were the control group. The expression of miR-34c and miR-141 in serum of patients in the two groups were detected by RT-qPCR, and the association of miR-34c and miR-141 with the clinicopathological characteristics of colon cancer patients was analyzed. The receiver operating characteristic (ROC) curve was used to assess the diagnostic efficiency of miR-34c and miR-141 in colon cancer. The expression of miR-141 in serum of patients in the experimental group was significantly higher than that in the control group (P<0.05). Expression of miR-34c in serum of patients in the experimental group was significantly lower than that in the control group (P<0.05) and the expression of miR-34c and miR-141 in serum of the experimental group were associated with tumor diameter, clinical stage, degree of differentiation and lymph node metastasis (P<0.05). AUC of serum miR-34c in the diagnosis of colon cancer was 0.857 (95% CI: 0.795-0.919), with the cut-off value of 0.800, the diagnostic sensitivity of 84.38%, and the specificity of 68.75%. In conclusion, miR-34c and miR-141 might be involved in the occurrence and progression of colon cancer and could be used as biological indicators for early diagnosis of colon cancer.

Introduction

Colon cancer is one of the most common malignant tumors in the clinical practice. Its morbidity and mortality worldwide are gradually increasing, posing a great threat to human life (1). There is lack of specific early stage symptoms and the digestive system signs appear in the middle and late stages, which can cause serious adverse effects (2,3). At present, the main treatment for clinical colon cancer is still surgical resection, but due to the lack of sensitive diagnostic indicators, most patients have already developed lymph nodes or distant metastases at the time of diagnosis, and the surgical resection rate and prognosis are not ideal (4,5). Therefore, it is very important to find a biomarker for early diagnosis of colon cancer.

It has been found that miRNAs are differentially expressed in colon cancer cells (6). They are closely related to the biological and clinical characteristics of colon cancer, and play an important role in the occurrence and progression of colon cancer (7). As studies have shown, miRNAs are thought to have a regulator role in tumor suppression and tumorigenesis. For example, miR-185 can inhibit the proliferation and invasion of colon cancer cells by targeting Wnt1, and regulating the level of miR-185 may have a therapeutic effect on colon cancer patients (8). miR-223-3p can promote the progression of colon cancer by negatively regulating PRDM1 (9). Based on these results, miRNAs are attracting attention as a potential target for the treatment and diagnosis of colon cancer. Recently, the role of miR-141 in tumor growth has been described. Expression level of miR-141 in patients with colon cancer at stage IV has increased, which can easily distinguish patients with distant metastasis, other stages and healthy control, showing that plasma miR-141 is a potential prognostic factor for predicting poor survival of colon cancer patients (10). miR-34C is a tumor suppressor regulator, which is down-regulated in most forms of cancer, and can inhibit the growth of malignant tumors by inhibiting genes related to proliferation, anti-apoptosis.
and migration (11). A study has shown that the expression of miR-34C is down-regulated in colon cancer, and the loss of expression is consistent with the data of colon cancer cell lines (12).

There are few previous studies on the diagnosis of colon cancer with serum miR-34c and miR-141 (13-15), and the study on the role of miRNA expression in colon cancer provided new diagnostic methods and thinking for the diagnosis and treatment of colon cancer. In this study, by observing the expression of miR-34c and miR-141 in the serum of colon cancer patients, the diagnostic value of miR-34c and miR-141 in colon cancer and their relationship with clinicopathological features were investigated.

Patients and methods

General data. A total of 64 patients diagnosed with colon cancer and treated in Hubei Cancer Hospital (Wuhan, China) were selected as the experimental group, including 44 males and 20 females. The patients were aged 25-65 years, with an average age of (46.5±8.4) years. According to TNM staging system, there were 22 cases of stage I+II, and 42 cases of stage III. There were 19 cases of lymph node metastasis, and 15 cases of poor differentiation, 49 cases of high and medium differentiation. Sixty-four healthy subjects were included in the control group, including 38 males and 26 females. The controls were aged 26-57 years, with an average age of (44.5±7.9) years. The study was approved by the Ethics Committee of the Hubei Cancer Hospital, and the subjects and/or their families were informed and signed an informed consent.

Inclusion and exclusion criteria. Inclusion criteria were: Patients met NCCN colon cancer tumor clinical practice guidelines (16). CT, color Doppler ultrasound and MRI were performed to rule out distant metastasis. According to TNM staging system there were Stage I, II and III. Patients did not receive previous chemotherapy, or radiotherapy. Patients were diagnosed for the first time, with detailed clinicopathological data. Exclusion criteria were: Patients without other malignant tumors, hematological diseases. Patients with severe complications and immune system diseases. Patients with poor treatment compliance caused by severe mental illness, and patients unwilling to participate in the present study.

Main instruments and reagents. ABI PRISM 7500 quantitative PCR instrument (Beijing Image Trading Co., Ltd.; cat. no. 100005). M-MLV reverse transcription kit (Beijing Shengkeboyuan Biotechnology Co., Ltd.; cat. no. RTP50). TRIzol extraction kit (Shanghai Xinfan Biological Technology Co., Ltd.; cat. no. XFR1030). UV-visible spectrophotometer (Biotek corporation; cat. no. ND5000). microRNA PCR premix kit (AcebioX; cat. no. PAMI1000). The primers of miR-34c, miR-141 and U6 were synthesized by Beijing Lvyanbode Biotechnology Co., Ltd. (Table I).

RT-qPCR detection. Elbow venous blood (5 ml) of the subjects were taken, after 10-15 min, the blood was centrifuged at 1,500 x g and 4˚C for 10 min, and then stored at -70˚C. The total RNA in the serum was extracted using TRIzol kit (Takara), and the absorbance values of RNA at 260 and 280 nm were measured by ultraviolet-visible spectrophotometer. The RNA concentration and purity were analyzed. Then, 2 µl of total RNA was taken to reversely transcribe the first strand cDNA, according to the reverse transcription kit. Reverse transcription reaction conditions: 42˚C for 30 min, 95˚C for 5 min. The synthesized cDNA sample was stored at -80˚C. U6 was used as an internal reference. The total volume of 20 µl includes 10 µl of PCR Premix, 2 µl of upstream primer, 2 µl of downstream primer and dd water (Rnase and Dnase free). PCR amplification cycle conditions were: 92˚C for 5 min, 95˚C for 5 sec, 65˚C for 30 sec, 72˚C for 5 sec, a total of 45 cycles. 2-∆△Ct method was used to analyze the relative expression of the target gene.

Results

General data. There was no difference between the experimental group and the control group in gender, age, body mass index (BMI), smoking history, drinking history, residence, educational level or other general clinical data (P>0.05) (Table II).

Expression levels of miR-34c and miR-141 in colon cancer. The relative expression of miR-34c and miR-141 in the serum of subjects in the two groups were detected, and it was found that the serum level of miR-34c in the experimental group was significantly lower than that in the control group (P<0.05), and the relative expression of miR-141 in serum of patients in the experimental group was significantly higher than that in the control group (P<0.05) (Table III and Fig. 1).

Association of the expression of miR-34c and miR-141 with clinicopathologic features of patients with colon cancer. The expression of miR-34c and miR-141 in serum of colon cancer was not associated with the clinicopathological parameters such as age, gender, local tumor invasion, vascular invasion, degree of differentiation, and neural invasion (P>0.05), but was associated with tumor diameter, lymph node metastasis, carcinoembryonic antigen, and TNM staging (P<0.05) (Table IV).

The relative expression of serum miR-34c and miR-141 in the diagnosis efficiency of colon cancer. ROC curve of relative expression of serum miR-34c and miR-141 for the diagnosis of colon cancer was drawn. AUC of serum miR-34c for the diagnosis of colon cancer was 0.857 (95% CI: 0.795-0.919), its cut-off value was 0.800; diagnostic sensitivity was 84.38%, and specificity was 68.75%. AUC of serum miR-141 for
diagnosis of colon cancer was 0.876 (95% CI: 0.810-0.941), its cut-off value was 0.282; diagnostic sensitivity was 70.31%, and specificity was 96.88%. ROC curve for the diagnosis of colon cancer combined with serum miR-34c and miR-141 was further drawn. The combined AUC of serum miR-34c and miR-141 for the diagnosis of colon cancer was 0.929 (95% CI: 0.884-0.974), the cut-off value was 0.566; diagnosis sensitivity was 84.38% and the specificity was 93.75%. More details are shown in Table V and Fig. 2.

Table I. Primer sequences of miR-34c, miR-141 and U6.

| Gene   | Forward primers                        | Reverse primers                        |
|--------|----------------------------------------|----------------------------------------|
| miR-34c | 5’-CGCGGATCCCTCTATTTGCCATCGTCTA-3'    | 5’-CTGAAGCTTCAGGCAGCTTATTTGGAC-3'     |
| miR-141| 5’-GCGAAGCATTTGCAAGAA-3'              | 5’-CAATCACAGACCTGTTATTGCA-3'         |
| U6     | 5’-CTGCTTCGGCAGCACA-3'                | 5’-AACGCTTCAGAATTGGCT-3'             |

Table II. General data in the experimental group and control group [n (%), mean ± SD].

| Class                  | Experimental group (n=64) | Control group (n=64) | t/χ² | P-value |
|------------------------|---------------------------|----------------------|------|---------|
| Sex                    |                           |                      | 0.131| 0.717   |
| Male                   | 44 (68.75)                | 38 (59.38)           |      |         |
| Female                 | 20 (31.25)                | 26 (40.62)           |      |         |
| Age (years)            | 46.5±8.4                  | 44.5±7.9             | 1.388| 0.168   |
| BMI (kg/m²)            | 22.94±4.06                | 22.49±3.72           | 0.654| 0.514   |
| Smoking history        |                           |                      | 0.283| 0.595   |
| Yes                    | 36 (56.25)                | 33 (51.56)           |      |         |
| No                     | 28 (43.75)                | 31 (48.44)           |      |         |
| Drinking history       |                           |                      | 1.647| 0.199   |
| Yes                    | 44 (68.75)                | 37 (57.81)           |      |         |
| No                     | 20 (31.25)                | 27 (42.19)           |      |         |
| Place of residence     |                           |                      | 0.142| 0.707   |
| City                   | 20 (31.25)                | 22 (34.38)           |      |         |
| Country                | 44 (68.75)                | 42 (65.62)           |      |         |
| Education level        |                           |                      | 2.050| 0.152   |
| >Senior high school    | 33 (51.56)                | 41 (64.06)           |      |         |
| ≤Senior high school    | 31 (48.44)                | 23 (35.94)           |      |         |

Figure 1. Expression of miR-34c and miR-141 in the serum of colon cancer. (A) Serum level of miR-34c in the experimental group was significantly lower than that in the control group (P<0.05). (B) The relative expression of miR-141 in serum of patients in the experimental group was significantly higher than that of the control group (P<0.05). *P<0.05, compared with the normal control group.

Table III. Expression levels of miR-34c and miR-141 in serum of colon cancer (mean ± SD).

| Groups        | n   | miR-34c     | miR-141     |
|---------------|-----|-------------|-------------|
| Experimental  | 64  | 0.69±0.15   | 0.31±0.07   |
| Control       | 64  | 0.84±0.18   | 0.22±0.03   |
| t             | -   | 5.121       | 9.454       |
| P-value       | -   | <0.001      | <0.001      |
Colon cancer is a malignant lesion in mucosal epithelium caused by a variety of carcinogenic factors. It is mainly related to a high-fat, high-protein and low-fiber diet, and is one of the common malignant tumors (17). The onset of colon cancer is insidious and most of its onset is slow. In the early stage, there is no obvious symptoms, patients are often diagnosed in the middle or late stage, thus losing the best time for treatment (18). Therefore, how to improve the diagnostic rate of colon cancer and overall survival is a major problem for clinicians. Surgery is an early treatment method for colon cancer. In recent years, the treatment of colon cancer has improved to a certain extent, but the high postoperative complication rate of colon cancer has not changed (19). Hence, actively looking for indicators with high sensitivity is
miR-34c and miR-141 are implicated in colon cancer. Overexpression of miR-34c is observed in various malignancies, and it acts as a suppressor gene in colon cancer (26), while miR-141 might act as an oncogene (31). These microRNAs can form specific inclusions and are suitable for detection in body fluids, including serum and feces (23), making them potential biomarkers for colon cancer.

Serum miR-34c and miR-141 levels were measured using real-time PCR. The ROC curve analysis revealed that the AUC for serum miR-34c was 0.857 (95% CI: 0.795-0.919), with a diagnostic sensitivity of 84.38% and specificity of 68.75%. For serum miR-141, the AUC was 0.876 (95% CI: 0.810-0.941), and the diagnostic sensitivity was 70.31% with a specificity of 96.88%.

Combining the results of miR-34c and miR-141, the AUC was 0.929 (95% CI: 0.930-0.940), and the cut-off value was 0.282. This combination improved diagnostic sensitivity to 84.38% and specificity to 68.75%, indicating that miR-34c and miR-141 might be involved in the diagnosis of colon cancer.

In conclusion, miR-34c and miR-141 may be involved in the diagnosis of colon cancer, as they show good sensitivity and specificity. The combined detection of these microRNAs could improve the diagnostic accuracy of colon cancer. Further studies are needed to validate these findings and to explore the potential of miR-34c and miR-141 as biomarkers in colon cancer diagnosis.
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Availability of data and materials

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

Authors' contributions

HW and HY conceived and designed the study, acquired, analyzed and interpreted the experiment data, drafted the manuscript, and revised the manuscript critically for important intellectual content. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Hubei Cancer Hospital (Wuhan, China). Signed informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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