ISSN 2706-6819 Vol.6, Issue 10: 39-43, DOI: 10.25236/IJFM.2024.061006

# Expression of serum miRNA34a gene in patients with colorectal cancer

**Haiyan Zhu***

*Department of Oncology, Weifang People's Hospital, Weifang, 261041, China*
**Corresponding author: Hyzwfrmyy@163.com*

***Abstract:*** *80 patients who were hospitalized in the General Surgery Department of Weifang People's Hospital from March 2022 to October 2023 were selected. The research subjects were divided into three groups: Group A: 40 patients with colorectal adenocarcinoma confirmed by histopathological biopsy; Group B: 40 patients with colon adenomatous polyps confirmed by histopathology; Group C: 40 healthy individuals matched with the first two groups in age and gender as controls. We completed medical history data, laboratory and examination results from all research subjects were collected. The laboratory results included serum cancer markers CA19-9 and CEA, and the gene expression level of miRNA34a. This study explores the potential significance of miRNA34a gene expression as a biomarker for colorectal cancer.*

***Keywords:*** *MiRNA34a gene; Colorectal cancer; CA19-9; CEA*

## 1. Introduction

Colorectal cancer ranks third among malignant tumors worldwide and fourth among causes of cancer death. Fecal occult blood test, serum CA19-9 and CEA biomarker detection have certain clinical predictive significance for the diagnosis of colon cancer, but their sensitivity and specificity are not satisfactory. MiRNAs are non coding RNAs with 21-25 nucleotides, which may be related to cell proliferation, differentiation, and apoptosis. The miRNA34 family consists of miRNA34a, miRNA34b, and miRNA34c, which exhibit tumor suppressive properties due to their involvement in cell cycle and apoptosis. MiRNA34a affects Notch signaling and protein levels by binding to the 3'UTR of the messenger RNA sequence of the Notch receptor, inhibiting the Noth-1 and Notch-2 pathways and thus affecting the occurrence and development of colon cancer. This study explores the potential significance of miRNA34a gene expression as a biomarker for colorectal cancer.

## 2. Object and method

### *2.1 Research object*

Select 80 patients who were hospitalized in the General Surgery Department of Weifang People's Hospital from March 2022 to October 2023, and include 40 healthy volunteers during the same period. The research subjects were divided into three groups: Group A: 40 patients with colorectal adenocarcinoma confirmed by histopathological biopsy; Group B: 40 patients with colon adenomatous polyps confirmed by histopathology; Group C: 40 healthy individuals matched with the first two groups in age and gender as controls. Patients who have received preoperative radiotherapy and/or chemotherapy, as well as inflammatory bowel disease and hereditary non polypic colorectal cancer, were excluded from this study. All research subjects in this study voluntarily participated and signed informed consent forms, and the research plan was approved by the Ethics Committee of our hospital.

### *2.2 Teaching methods*

Collect complete medical history data, laboratory and examination results from all research subjects. The laboratory results include blood routine, liver and kidney function, serum cancer markers CA19-9 and CEA, and the gene expression level of miRNA34a was detected through real-time PCR.

ISSN 2706-6819 Vol.6, Issue 10: 39-43, DOI: 10.25236/IJFM.2024.061006

### *2.3 Detection method for 3 miRNA34*

#### *2.3.1 Instruments and reagents:*

The miRNA extraction kit was purchased from Beijing Biotech, while the mi TNA fluorescence quantitative PCR kit and miRNAc DNA first strand synthesis kit were purchased from Beijing Kangwei Century. Taq DNAPolymerase, Oligo (d T), 10 mmol/L d NTP, and reverse transcriptase were all purchased from Promega. The primers used in fluorescence quantitative PCR were synthesized by Beijing Kangwei Century Company, and the primer sequence was:

MinRNA34a F: 5 '- TGGCAGTGTCTTAGCTG-GTTTG-3',

R: 5 '- GCGAGACACAGATATATACGAC-3'

U6 F: 5 '- CTCGCTTTCGGCAGCACA-3',

R: 5 '- AACGTTCACGATTTGCGT-3'.

#### *2.3.2 qRT PCR*

Implementation steps: Collect 2ml of extracted peripheral venous blood into a tube containing EDTA, extract total RNA using the Trizol method, reverse transcribe it into cDNA, and use the product for qRT PCR amplification. qRT PCR reaction system: miRNA34a: F/R (10 Umol)/ μl) 0.5 eachμl. TaqPlus DNA Polymer 2.0μl. 10 x Buffer (15 mmol/L Mg $Cl_2$) 0.5μl. 10 mmol/L d NTP 0.5μl. Betaime3.0μl. RNA free water 12.5μl. The total volume of the reaction is 30μl. Reaction conditions: 94 ℃ for 3 minutes, 94 ℃ for 45 seconds, 58 ℃ for 45 seconds, 72 ℃ for 45 seconds, repeat for 40 cycles. After the reaction is completed, the software automatically generates a dynamic curve of fluorescence quantitative values, and analyzes the displayed results and cycle threshold (Ct). The control is the U6 gene. Using $F=2^{-\Delta\Delta Ct}$ method was used to analyze and compare the expression of target genes in the peripheral blood of patients with colorectal adenocarcinoma, colon polyps, and healthy individuals. ΔΔ Ct=(CtmiRNA34a -Ct U6) tuber - (CtmiRNA34a -Ct U6) normal, set the control group as $\Delta\Delta Ct=0.2^{-\Delta\Delta Ct}=1$.

### *2.4 Statistical methods*

SPSS 19.0 statistical software was used for data analysis. The measurement data are expressed by ($\bar{x}\pm s$) and compared by t test; The counting data is expressed in (%) and used for comparison χ 2 test, $P < 0.05$ means the difference is statistically significant.

## 3. Results

### *3.1 Statistical analysis of basic information of each group*

As shown in Table 1, There was no statistically significant difference in age between Group A, Group B, and Group C.($P < 0.05$)

*Table 1: Basic information of research subjects (n=40)*

| Group | Number | Age | Average age |
|---|---|---|---|
| A Group | 40 | 21-77 | 36.3±22.3 |
| B Group | 40 | 38-68[a] | 45.1±10.8 |
| C Group | 40 | 40-80 | 60.0±8.37 |

### *3.2 CEA, CA19-9, and MiRNA34a levels in each group*

*Table 2: Comparison of CEA, CA19-9, and MiRNA34a levels among different groups( $\bar{x}\pm s$)*

| Group | Number | CEA( mg/dl) | CA19-9( U /ml) | MiRNA34a |
|---|---|---|---|---|
| A Group | 40 | 23.12±3.21 | 30.21±1.89 | 2.36±1.89 |
| B Group | 40 | 10.35±4.28 [a] | 13.56±4.67 [a] | 7.24±3.20 [a] |
| C Group | 40 | 5.01±1.29 [ab] | 532±2.32 [ab] | 15.36±5.64 [ab] |

a is P<0.05, compared with Group A; b is P<0.05, compared with Group B.

Compared with Group A, the serum levels of CEA and CA19-9 in Group B and Group C increased;

ISSN 2706-6819 Vol.6, Issue 10: 39-43, DOI: 10.25236/IJFM.2024.061006

Compared with Group A, the serum levels of MiRNA34a in Group B and Group C decreased (P<0.05). Compared with Group B, the serum levels of CEA and CA19-9 in Group C increased; Compared with Group B, the serum levels of MiRNA34a in Group C decreased (P<0.05). See Table 2.

## 4. Discussion

### *4.1 Advantages of Internet + PBL teaching under COVID-19*

Colorectal cancer (CRC) is a type of malignant tumor with a high incidence rate worldwide. Related statistics indicate that the number of cases diagnosed with colorectal cancer in clinical practice in China is becoming younger and younger, which is closely related to the rapid development of the domestic economy in the past decade [1]. People pursue more food intake for the quality of life after they meet food and clothing requirements, resulting in the body absorbing too much fat and protein every day, making the incidence rate of colorectal cancer younger and younger. Its incidence rate accounts for 1/5 of the total number of people under 40 years old, and has a trend of increasing year by year. In the world, China has entered the ranks of high incidence rate of colorectal cancer. In addition, colorectal cancer is difficult to detect and treat in a timely manner, resulting in often being in the middle and late stages after detection, which poses many difficulties for the prognosis and treatment of patients in the future. The increase in the incidence rate of colorectal cancer, as well as the high mortality rate caused by the increase in the difficulty of its detection, has seriously threatened the health of our people. With the advancement of technology, the development of clinical treatment, and the further maturity of targeted cancer therapy, this has to some extent improved the treatment difficulty and prognosis of colorectal cancer patients. However, in patients with advanced and metastatic colorectal cancer, the prognosis remains poor. In order to increase the survival rate of colorectal cancer patients, medical workers have been striving to explore the pathogenesis of CRC and explore new and more effective treatment strategies [2-3].

### *4.2 Improvement ideas of Internet + PBL teaching mode under COVID-19*

In this study, a total of 120 subjects were included in the study, divided into Group A (confirmed cases of colon adenocarcinoma), Group B (cases of colon adenomatous polyps), and Group C (healthy individuals matched in age and gender during the same period), with 40 cases in each group. The demographic data of three groups of patients, such as gender ratio, age, and body mass index, showed no statistically significant differences (P>0.05), indicating good homogeneity. This study also found that serum tumor markers CEA and CA19-9 were significantly elevated in colon cancer patients compared to the other two groups, which has certain diagnostic value. This is inconsistent with the research reports of Polat et al., who found that there was no statistically significant difference in the levels of serum CA19-9 between the control group and the colon cancer group (P>0.05), but the levels of serum CEA in colon cancer patients were significantly higher than those in the control group. These two research findings may be due to the small sample size in this study or individual differences between foreign and Chinese populations.

MiRNAs (micro RNAs) are defined as a class of non coding RNAs with short length (total length of fragments is about 18-22nt) that can regulate the expression of related genes after transcription. There are studies indicating that there are changes in the expression levels of multiple miRNAs and their associated genes in colorectal cancer cells. MiRNA-34a is a type of miRNA that plays an important role in cell development and apoptosis in living organisms. Its functions mainly include cell cycle arrest, affecting cell aging, and preventing cell migration. In the treatment of some malignant tumor diseases, reactivated miRNA-34a can prevent the migration and infiltration of tumor cells. Abnormal expression of miRNA-34a regulates the expression of related proteins by targeting different target miRNAs, leading to abnormal host cell function and tissue lesions, which can even lead to the occurrence of tumors [4-5]. Jin Fangling et al. [6] constructed a lentiviral vector overexpressing miRNA-34a and stably transfected colon cancer HT29 cells. Real time quantitative polymerase chain reaction confirmed that successful transfection significantly upregulated the expression of miRNA-34a, revealing that overexpression of miRNA-34a increased the sensitivity of colon cancer HT29 cells to X-ray irradiation, manifested in a decrease in cell proliferation ability and survival fraction, while clone formation ability was inhibited by miRNA-34a. The role of miRNA-34a in the sensitivity of colon cancer to radiotherapy was explained, and its regulatory mechanism was tested to promote the development of miRNA target gene action patterns in colon cancer radiotherapy resistance, providing valuable experimental evidence for the development of radiotherapy technology and protocols. Zhuang

ISSN 2706-6819 Vol.6, Issue 10: 39-43, DOI: 10.25236/IJFM.2024.061006

Biao et al. [7] confirmed that overexpression of miRNA-34a can inhibit the proliferation, invasion, and metastasis of human colon cancer HCT116 cells. This effect may be related to regulating the expression of Bcl-2/BAX, MMP-2, and MMP-9 proteins, preliminarily revealing the potential value of miRNA-34a in the treatment and diagnosis of colon cancer. Fu Yujuan et al. [8] found in their study on the mechanism of XIST/miRNAA-34a signaling axis regulating the radiotherapy sensitivity of colorectal cancer cells SW480 that the expression level of XIST increased in colorectal cancer tissues and cell lines. Downregulating the expression of XIST can increase the radiotherapy sensitivity of colorectal cancer SW480 cells, and inhibiting the expression of miRNA-34a can reverse this effect. Li Jun et al. found that miRNA-34a may inhibit colon cancer cell proliferation and promote cancer cell apoptosis by downregulating the expression level of SIRT1. It is speculated that miRNA-34a may become a new target for biological therapy of colon cancer. MiRNA-34a also plays a significant role in the diagnosis of malignant tumors. Ma et al. [9] investigated the differential expression of miRNA-34a in benign and malignant colorectal lesions. We detect the expression of miRNA-34a by conventional real-time polymerase chain reaction method. In addition, colon cancer cells were cultured with the demethylating agent 5-azacytidine to screen for differentially expressed miRNA-34a. After drug treatment, the expression of miRNA-34a in colorectal cancer cell lines was enhanced. Real time polymerase chain reaction results showed that the expression levels of miRNA-34a in both cell lines and colorectal cancer tissues were lower compared to adjacent tissues (P<0. 05). The expression of polyp tissue was significantly higher than that of adjacent cancerous tissue and colorectal cancer tissue (P<0 05). MiRNA-34a-5p may play a role as a tumor suppressor gene in colorectal cancer and is associated with DNA methylation. This study showed that compared with Group A, the serum levels of CEA and CA19-9 in Group B and Group C increased, while the levels of MiRNA34a decreased (P<0.05) Compared with Group B, the serum levels of CEA and CA19-9 in Group C increased, while the levels of MiRNA34a decreased (P<0.05).

## 5. Conclusion

In short, the miRNA34a gene can serve as a potential biomarker for colorectal cancer, and its specific mechanism needs further research.

## Acknowledgements

This paper was funded by Shandong province medical and health science and technology development plan (202102080115)

## References

*[1] Yang Chaomei, Feng Jie, Lang Lei, Yan Guangtao The clinical value of combined detection of CTC, CEA, and CA125 in colorectal cancer [J]. Laboratory Medicine, 2021, 36 (09): 901-905*

*[2] Li Yujiang, Zhang Dongli, Qian Yun, Fu Yiwei, Yang Bin, Liu Cuixia. The relationship between the expression of PEA15 and MSI-2 in colorectal cancer tissue and liver metastasis and prognosis [J]. Journal of Difficult Diseases, 2021-20 (09): 924-928+947*

*[3] Wu Xiuqin, Du Lihua, Li Huihui, Chen Xiaoli. Analysis of influencing factors on coping strategies of postoperative chemotherapy patients with colorectal cancer [J]. Hebei Medical Journal, 2021-43 (17): 2695-2698*

*[4] Ji Yangsong, Rao Cheng, Long Yilin, Xie Tianhong, Wang Shifei. The effects and mechanisms of miRNA-34a and SIRT6 on apoptosis of auditory cortical neurons in aged mice [J]. Journal of Zunyi Medical University, 2020, 43 (05): 589-592+598*

*[5] MicroRNA-34a promotes apoptosis of retinal vascular endothelial cells by targeting SIRT1 in rates with diabetic retinopathy Cell cycle (Georgetown, Tex.) 19.21 (2020): doi: 10.1080/15384101. 2020.1827509*

*[6] Jin Fangling, Cai Zhongfang, Ge Yangli, etc. MiRNA-34a targeted inhibition of Rb/E2F1 pathway activation enhances radiotherapy sensitivity in colon cancer [J]. Chinese Medical Journal, 2015; 12 (32): 13-6, 29*

*[7] Zhuang Biao, Min Zhijun, Wang Tingfeng, etc. The effect of miRNA-34a on the proliferation, invasion, and metastasis of human colon cancer cell line HCT116 [J]. Journal of Practical Cancer, 2016; 31 (2): 179-82186*

*[8] Fu Yujuan, Yin Tao, Ni Meng, etc. Mechanism of XIST/miRNA-34a signaling axis regulation of*

ISSN 2706-6819 Vol.6, Issue 10: 39-43, DOI: 10.25236/IJFM.2024.061006

*radiotherapy sensitivity in colorectal cancer cells SW480 [J]. Cancer Progress, 2019; 17 (6): 661-5*
*[9] Ma ZB, Kong XL, Cui G, et al. Expression and clinical significance of miRNA-34a in colorectal cancer [J] Asian Pac J Cancer Prev, 2014; 15 (21): 9265-70*