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Original Article

Expression of miR-92a in colon cancer tissues and its correlation with clinicopathologic features and prognosis

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Abstract: Objective: This study analyzed the expression of miR-92a in colon tumor tissues and its correlation with disease clinicopathologic features and prognosis. Methods: 83 cases of colorectal cancer tissues and paracancerous normal tissues acquired from colon cancer resection surgery during January 2015-January 2017 were collected. We detected the expression of miR-92a in cancer tissues and paracancerous tissues by qRT-PCR, and analyzed the correlation between the relative expression of miR-92 in colon cancer tissues and clinicopathologic characteristics, progression-free survival (PFS), and overall survival (OS) of the patients accordingly. Results: The relative expression level of miR-92a in colon cancer tissues was higher than of paracancerous tissues ($P<0.05$). Relative expression of miR-92a in cancer was correlated with the degree of differentiation, TNM stage, and lymph node metastasis ($P<0.05$), while uncorrelated with gender, age, tumor diameter, or invasion depth ($P>0.05$). Patients with low expression of miR-92a had superior PFS to the control group ($P>0.05$) and better OS ($P<0.05$). Conclusion: Abnormally high expression of miR-92a occurs in colon tumor tissues. Its expression is related to the occurrence, progression, and prognosis of patients with colon cancer. It may be a marker for diagnosis, treatment, and prognosis of disease.

Keywords: miR-92a, colon cancer tissues, clinicopathologic features, prognosis of survival

Introduction

Colon cancer is among the common malignant tumors in the human digestive system. In recent years, owing to environmental factors such as poor dietary habits and food pollution along with genetic factors, the incidence of the disease has been increasing. The prognosis, in addition, is quite poor [1]. Although large studies have been made on the occurrence and influencing factors of colon cancer, its specific mechanism has yet to be completely elucidated [2]. In addition, as colon cancer patients often lack specific symptoms and are delayed in screening, most are already in the middle and late stages of disease when diagnosed. Therefore, patients with colon tumor have poor treatment effect and a high mortality rate [3, 4]. Exploring the molecular markers related to the occurrence and progression of colon cancer is of great significance for improving the early diagnosis rate, therapeutic effect, and the survival of patients. Micro RNA (miRNA) is a type of small non-coding RNA that has regulatory functions. Scholars have discovered that miRNAs are involved in the occurrence and progression of a variety of neoplastic diseases [5]. According to studies in vitro [6], antagonizing miR-92a can cause growth inhibition and apoptosis of colon cancer cells. Aiming to further analyze the function of miR-92a in colon cancer patients, this study explored and analyzed the miR-92a expression in colon tumor tissues and its correlation with clinicopathologic features and prognosis.

Case and data

Clinical data

83 cases of colorectal tumor tissues and paracancerous tissues acquired from colon cancer resection surgery during January 2015-January 2017 were collected in this research. The spe-

Table 1. Primer sequences

Primer	Sequence
miR-92a	Forward primer: 5'-ACAGGCCGGGACAAGTGCAATA-3'
	Reverse primer: 5'-GCTGTCAACGATACGCTACGTAACG-3'
U6	Forward primer: 5'-CTCGCTTCGGCAGCACA-3'
	Reverse primer: 5'-AACGC TTCACGAATTTGCGT-3'

cimens included 45 males and 38 females aged from 34 to 79 years, with average age of (56.40±9.27) years old. The TNM staging of patients included 19 cases of stage I, 34 cases of stage II, 26 cases of stage III, and 4 cases of stage IV. There were 43 subjects with lymph node metastasis. The work was done under the approval of the Ethics Committee of our hospital.

Inclusion and exclusion criteria

Inclusion criteria: (1) The patients met diagnostic criteria of colorectal cancer in 8th edition of Surgery [7]; (2) The diagnosis was confirmed by pathologic examination after surgery; (3) The patients had not experienced chemoradiotherapy before surgery; (4) The distance between the normal tissues and the lesion was over 3 cm; and (5) The patients or their family members were informed to provide informed consent.

Exclusion criteria: (1) Those with metastatic colorectal cancer; or (2) Patients with occurrence of other malignancies.

Detection of miR-92a expression

We extracted total RNA from tissues by Trizol method (purchased from Chagan Corporation, USA) and tested the purity and concentration of RNA. Subsequently, we reverse-transcribed the RNA into cDNA by a reverse transcription kit (purchased from Takara, Japan). The qRT-PCR reaction system (20 μl) consisted of 2 μl forward primer, 2 μl reverse primer, 2 μl cDNA, 10 μl SYBR® Primix Ex Taq™, 0.4 μl ROX and 3.6 μl ddH_2O. The reaction proceeded under 95°C for 8 min, 95°C for 25 s, 62°C for 38 s and then 67°C for 25 s, with a total of 45 reaction cycles was used, and each sample experiment was repeated three times. Using U6 as an internal reference gene, the relative expression of miR-92a was calculated by $2^{-\Delta\Delta Ct}$ method. The gene primers were synthesized by Shanghai Shenggong Biological Engineering Technology Co., Ltd., and the sequences are shown in **Table 1**.

Postoperative follow-up

We recorded the progression free survival (PFS) and overall survival (OS) after operation, with follow-up ending by December 1, 2020. PFS refers to the period of time between the 1st day after operation and the day observed with disease progression or death due to any cause. OS refers to the period from 1 day after surgery to death for any reason.

Statistical analysis

Data process and analysis were conducted by SPSS 23.0. The comparison of measurement data and enumeration data were by *t*-test and χ^2 test respectively. Survival conditions were plotted by Kaplan-Meier survival curve, and the comparison of survival conditions was performed by Log-rank test. *P<0.05* was considered significant. The graphic software was by Graphpad prism 9.

Results

Comparison of miR-92a expression between colon cancer and paracancerous tissues

The relative expression level of miR-92a in colon cancer tissues was higher than that of paracancerous tissues [(1.897±0.331) vs. (1.072±0.210)] (*P<0.05*) (**Table 2** and **Figure 1**).

Relationship between relative expression level of miR-92a and clinicopathologic characteristics of colon cancer

The relative expression level of miR-92a in colon cancer tissues was correlated with the degree of differentiation, TNM stage, and lymph node metastasis of patients (*P<0.05*), and not correlated with gender, age, tumor diameter, or invasion depth (*P>0.05*) (**Table 3**).

Relationship between miR-92a expression in colon tumor tissues and PFS of subjects

Taking the relative expression level of miR-92a in colon tumor tissues 1.897 as the cut-off value, we divided the patients into a miR-92a high expression group (>1.897) and a miR-92a

Table 2. Comparison of miR-92a expression between colon cancer and paracancerous normal tissues ($\bar{x} \pm s$)

Group	n	Expression level of miR-92a	t	P
Cancer tissues	83	1.897±0.331	19.174	<0.001
Paracancerous normal tissues	83	1.072±0.210		

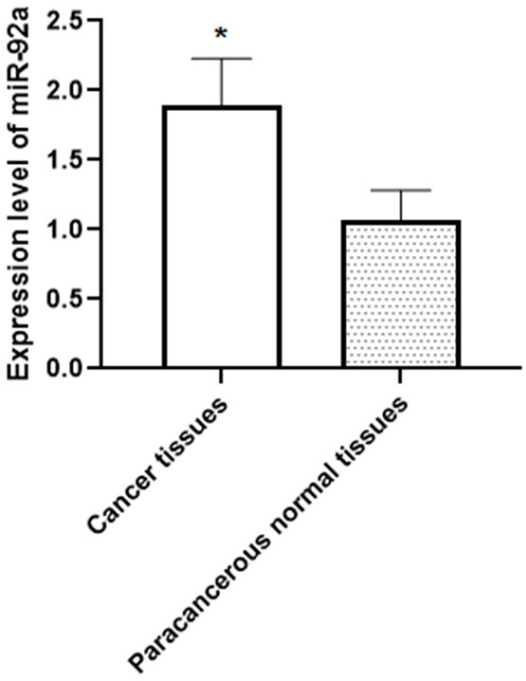

Figure 1. Comparison of miR-92a expression between colon cancer and paracancerous normal tissues. Note: Compared with paracancerous normal tissues, *P<0.05.

low expression group (≤1.897). The median PFS of patients with low miR-92a expression was 48.25 months, and that of patients with high miR-92a expression was 31.65 months. Patients with low expression of miR-92a had superior PFS to the control group (*P>0.05*) (**Figure 2A**).

Relationship between miR-92a expression in colon cancer tissues and OS

The median survival time of patients with low miR-92a expression was 62.93 months, and that of patients with high miR-92a expression was 43.21 months. Those with low miR-92a expression did better than the control group with respect to OS (χ^2=6.581, *P<0.05*) (**Figure 2B**).

Discussion

With continuous changes in modern living standards and in people's dietary habits, the incidence and mortality of colon cancer are on the rise year by year, according to related surveys and studies [8]. As the occurrence and progression of carcinogenesis is a multi-gene and multi-step complex process, an in-depth study into the specific pathogenesis of colon cancer is of great importance for the early diagnosis, treatment guidance, and prognosis [9, 10]. miRNA is a type of endogenously expressed non-coding small RNA, with 17-25 nucleotides in length. miRNA can inhibit the translation of target gene mRNA through incomplete pairing with the non-coding region at 3' end of the target gene, and participate in the regulation of cell ontogeny, apoptosis, proliferation, and differentiation. It is closely connected to the occurrence, metastasis, drug resistance, and other pathologic processes of tumor [11, 12]. It has been demonstrated [13] that the expression of miRNA in tissues and cells has significant tumor relevance, tissue specificity, and expression stability. Studies have confirmed that the overexpression or silencing of specific miRNAs in colon cancer is associated with the progression of colon cancer, tumor cell metastasis, and drug resistance [14].

miR-92a, which is categorized as miR-17-92 gene cluster family, has been confirmed as an oncogenic miRNA. The primary mechanism of miR-92a is to induce the proliferation and differentiation of cancer cells through the PI3K/AKT pathway mediated by the tissue oncogene PTEN, thus accelerating the growth of tumor, and further leading to the progression of disease [15]. In addition, studies were also suggesting that miR-92a exerted an impact on tumor cells by down-regulating expression of apoptosis protein (BIM) [16]. Colon cancer patients show critically high expression of miR-92 in plasma and tissues, and decreased expression level of miR-92a in plasma after surgery. It is considered that the detection of plasma miR-92a level is conductive for judging the therapeutic effect and recurrence in patients [17]. Since high expression of miR-92a can also be detected in stool of patients,

Table 3. Correlation between relative expression level of miR-92a and clinicopathologic characteristics of patients with colon cancer

Clinicopathologic feature	Number of cases	Expression level of miR-92a	t	P
Gender				
Male	45	1.921±0.365	0.756	0.452
Female	38	1.863±0.327		
Age (y)				
<60	48	1.844±0.302	1.154	0.252
≥60	35	1.942±0.471		
Tumor diameter				
<5 cm	43	1.831±0.334	1.253	0.214
≥5 cm	40	1.939±0.447		
Differentiation degree				
Poorly differentiated	21	2.428±0.510	7.903	<0.001
Medium to high differentiation	62	1.674±0.323		
TNM staging				
Phase I-II	53	1.695±0.384	7.610	<0.001
Phase III-IV	30	2.391±0.428		
Lymph node metastasis				
Yes	43	2.218±0.339	6.467	<0.001
No	40	1.701±0.321		
Depth of infiltration				
T_1-T_2	23	1.885±0.332	0.536	0.594
T_3-T_4	60	1.938±0.427		

this detection can help to distinguish advanced adenocarcinoma and colon cancer from healthy individuals [18]. Antagonism against miR-92a can induce apoptosis of colon cancer cells, and it is proven that miR-92a exerts an important influence in the occurrence and progression of colon cancer [19].

This study manifested that the relative expression of miR-92a in colon tumor tissues was critically higher than that in paracancerous normal tissues. The relative expression level of miR-92a in colon cancer tissues was correlated with the degree of differentiation, TNM stage, and lymph node metastasis, and uncorrelated with gender, age, tumor diameter, or invasion depth. miR-92a is notably highly expressed in colon tumor tissues, and its high expression is related to tumor progression, differentiation, and lymph node metastasis. These conclusions are in line with the results reported by others [20, 21], suggesting that miR-92a is connected with the tumorigenesis and progression of colon cancer. In addition, the PFS and OS in objects with low miR-92a expression were notably superior to those of the control group. This indicates that miR-92a is closely related to the survival and prognosis of colon cancer, and can be employed as a biologic indicator for the prediction of survival and prognosis in patients to better guide clinical treatment and prolong survival.

The specific biological mechanism of miR-92a has yet been completely clarified. miR-92a gene is located on 13q13 [22]. Studies have shown that miR-17-92 clusters can promote tumor cell proliferation, inhibit apoptosis, promote tumor angiogenesis and accelerate tumor progression [23]. It has been reported that miR-92a can promote apoptosis of cancer cells in colon cancer tissue by targeting the Bcl-2 family [24]. There are also reports stating that miR-92a promotes the proliferation, invasion and metastasis of colon cancer cell line SW480 by targeting PTEN [25]. A single miRNA may have multiple target genes and act in different tumor cells through different signaling pathways. It is also possible that several miRNAs work combinatively on exerting or inhibiting the oncogene. However, its specific molecular biologic mechanisms still await further study [26-28].

To conclude, abnormally high-expression of miR-92a exists in colon tumor tissues. Its expression is related to the occurrence, progression, and survival prognosis of patients with colon cancer, and is likely to be a biologic indicator for diagnosis, treatment and prognostic prediction of colon cancer.

Disclosure of conflict of interest

None.

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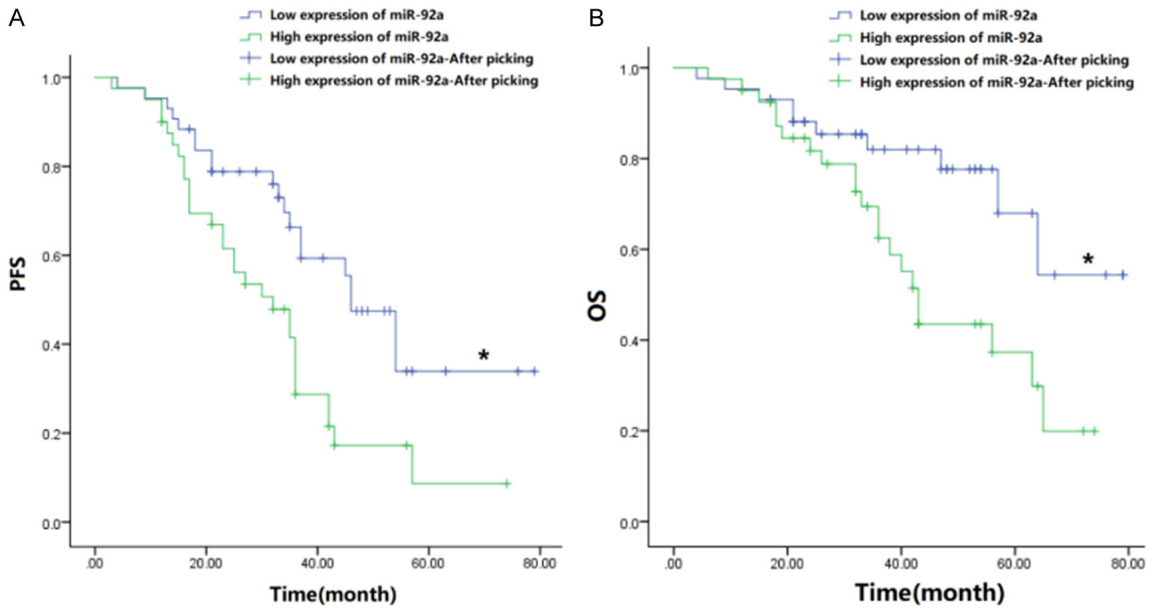

Figure 2. Relationship between miR-92a expression and PFS and OS in colon cancer. A: Relationship between miR-92a expression in colon cancer tissues and PFS of patients; B: Relationship between miR-92a expression in colon cancer tissues and OS of patients. Note: Compared with high expression of miR-92a.

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