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Article

Expression and Clinical Significance of miR-152 and CYFRA21-1 in Ovarian Cancer Tissues

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Abstract: To investigate the expression and clinical significance of miR-152 and CYFRA21-1 in ovarian cancer (OC) tissues. Seventy-four OC patients diagnosed in our hospital from March 2016 to April 2019 (research group, RG) and 30 patients with benign ovarian tumor at the same time (control group, CG) were collected as research objects in this experiment. qRT-PCR and ELISA were used to detect and observe the expression levels of miR-152 in patient tissues and CYFRA21-1 in serum. ROC curves were drawn to analyze the diagnostic value of miR-152 and CYFRA21-1 in OC. The clinicopathological correlation analysis was observed, Pearson was used to examine the correlation between the expression levels of miR-152 and CYFRA21-1 and carcinoembryonic antigen (CEA), and the 3-year survival rate of patients was observed according to the high and low expression of miR-152 and CYFRA21-1. qRT-PCR and ELISA showed that the miR-152 expression in the RG was dramatically lower than that in the CG, while CYFRA21-1 (μg/L) was remarkably higher than that in the CG ($p < 0.05$). ROC was drawn based on the miR-152 and CYFRA21-1 expression levels. The area under miR-152 curve was 0.724 ($p < 0.05$), and the area under CYFRA21-1 curve was 0.714 ($p < 0.05$). The expression levels of miR-152 and CYFRA21-1 were relevant to lymph metastasis, differentiation degree and pathological stage of OC patients ($p < 0.05$). Pearson test analysis identified that miR-152 and CYFRA21-1 were positively correlated with CEA ($p < 0.001$). The 3-year overall survival rate of miR-152 Low Expression Group (LEG) was 61.54%, that of high expression group (HEG) was 84.85%, that of CYFRA21-1 LEG was 83.75%, and that of HEG was 60.54%. miR-152 shows low expression in the tissues of patients and CYFRA21-1 shows high expression in serum, and both indexes have good diagnostic efficacy. The higher the miR-152 expression is, the higher the survival rate is, while the higher the CYFRA21-1 expression is, the lower the survival rate is.

Keywords: Ovarian cancer; miR-152; CYFRA21-1; expression

1 Introduction

Ovarian cancer (OC) is a very common malignancy in female reproductive organs, and is also one of the high incidence tumors in female body [1]. According to survey data, the number of new OC patients worldwide has exceeded 300,000 in 2016, an increase of about 6 times over 10 years ago [2,3]. And there are also data displaying that the morbidity is getting younger and younger. Currently, 25.63% of OC patients are under 40 years old [4]. It is estimated that OC will become the malignancies with the highest incidence among gynecological diseases by 2030 [5]. It not only has a very high morbidity, but also threaten women's life safety. According to statistics, it has a mortality of 72.8% within 5 years, which is the highest mortality among gynecological diseases [6]. Facing the huge threat of OC, it has been accepted as one of the key research diseases clinically. With the development of modern medical technology, if treated early, patients' 5-year

survival rate may be increased to more than 70% [7, 8]. Nevertheless, as the pathogenesis is indistinct, current diagnosis has great limitations and it still needs to be tested through cancer markers and imaging methods [9].

Recently, with the deepening of research, scholars at home and abroad have begun to focus on gene research. miRNA (microRNA) is a kind of small non-coding RNA, and it may be employed as endogenous RNA interference to control target genes and take part in regulating various physiological and pathological functions [10]. Bioinformatics data show that a single miRNA can bind to about hundreds of targets mRNA, thus playing a vital role in various biological processes. Currently, it has been proved to be tightly associated with the development and progression of various tumor diseases in human body [11,12]. Among them, miR-152 is a tumor suppressor gene proved to be down-regulated in breast cancer and gastric cancer tissues [13,14]. Al-Shagahin et al. [15] have pointed out that cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) is also relevant to tumor development and it is a tumor biomarker, but its role in OC is vague. Thus, we suspect that miR-152 and CYFRA21-1 also have abnormal expression in OC, and may be relevant to its development and progression. Through experimental analysis, we can verify our hypothesis, so as to supply reference and guidance for future diagnosis and therapy.

2 Information and Methods

2.1 General Information

This experiment collected 74 OC patients diagnosed in our hospital (Longyan People's Hospital, Longyan, China) from March 2016 to April 2019 (research group, RG) and 30 patients with benign ovarian tumor at the same time (control group, CG) as research objects, aged 38-60 years old, with an average age of (48.24 ± 10.2). This experiment has been approved by the Medical Ethics Committee.

2.2 Inclusion and Exclusion Criteria

Inclusion criteria: patients met FIGO stage [16] issued in 2014, were diagnosed and treated in our hospital, and had integral case data; those consented to cooperate and take part in the investigation work; those had no adjuvant treatment before admission; they were 30-65 years old; there was no other serious organ diseases affecting the study; the informed consent form shall be signed by themselves or their immediate family members.

Exclusion criteria: patients died in the course of treatment; those were complicated with other tumors, cardiovascular and cerebrovascular diseases, the physically disabled; gravida; those were complicated with other autoimmune diseases, chronic diseases; those were transferred from one hospital to another; those had contraindications to surgery, mental diseases and language dysfunction, as well as diseases influencing this research.

2.3 Main Reagents

CYFRA21-1 kit was purchased from Smart-Lifesciences Co., Ltd., Changzhou, China. Art. No. H30100. Both TRIzol reagent and miRNA reverse transcriptase kit were bought from Invitrogen, USA. Both EasyPure miRNA Kit and TransScript Green miRNA Two-Step qRT-PCR SuperMix were bought from TransGen Biotech, Beijing, China. Art Nos. ER 601-01, AQ202-01. KH19A desktop high-speed high-performance centrifuge was bought from KAIDA, Foshan, Guangdong, China. The –80°C low temperature refrigerator was bought from Thermo Fisher Scientific, Waltham, MS, USA. The miR-152 primer sequence was devised and synthetised by Shanghai Sangon Bioengineering Co., Ltd., Shanghai, China (Tab. 1).

Table 1: Primer sequence

	Upstream sequence	Downstream sequence
U6	5′-CTCGCTTCGGCAGCACA-3′	5′-AACGCTTCACGAATTTGCGT-3′
miR-152	5'-CAAAGGTCCATAGCAAGGGT-3′	5'-CTCGCTTCGGCAGCACA-3′

2.4 qRT-PCR and ELISA Detection

Altogether 5 mL of fasting venous peripheral blood was collected before and early in the morning after the treatment of patients. After it stood 30 min at indoor temperature and was centrifuged for 10 min (3000 rpm/min), the upper layer serum was obtained. The upper layer serum was subpackaged with enzyme-free EP tubes. Some parts were taken for experiments, and the rest were preserved at –80°C. The total RNA was extracted by EasyPure miRNA Kit, and its purity, concentration and integrity were tested by ultraviolet spectrophotometer and agarose gel electrophoresis. The extracted total RNA was reverse transcribed via TransScript Green miRNA Two-Step qRT-PCR SuperMix (TransGen Biotech, AQ202-01). The steps were conducted in the light of the kit instructions and cDNA was collected for PCR amplification experiments. Each sample was supplied with 3 repeated wells, and the test was conducted 3 times. This study employed β-actin as internal reference and data were assessed via $2^{-\Delta ct}$. CYFRA21-1 was detected by ELISA (enzyme linked immunosorbent assay) and it maintains a perfect compliance of the kit instructions.

2.5 Follow-Up of Patients

Patients were followed up for 3 years, 3, 6, 9 and 12 months each year, and their survival conditions were collected through telephones and outpatient medical records.

2.6 Outcome Measures

Main outcome measures were as follows: miR-152 and CYFRA21-1 expression levels in ^{OC} patients; diagnostic value of miR-152 and CYFRA21-1 in OC.

Secondary outcome measures were as follows: correlation analysis of clinical pathology of OC; correlation between miR-152 and CYFRA21-1 expression levels and carcinoembryonic antigen (CEA). The 3-year survival rate was observed in view of high and low expression of miR-152 as well as CYFRA21-1.

2.7 Statistical Analysis

The collected data were statistically analyzed via SPSS20.0 and required pictures were drawn via GraphPad 7. The distribution of dose data was assessed via K-S test, where normal distribution data were represented via Meas ± SD. The inter-group comparison was analyzed through independent-samples *t* test, intra-group comparison was assessed via paired *t* test, and multi-group comparison was analyzed through one-way analysis of variance and expressed by F. The usage rate (%) of counting data was assessed by chi-square test and expressed by χ^2. The survival rate was counted via Kaplan-Meier method and analyzed through Log-rank test, and there were statistical differences when $p < 0.05$.

3 Results

3.1 Clinical Data

There was no remarkable difference between the RG and the CG in clinical data such as age, BMI, smoking history, drinking history, place of residence, marital status, exercise habits, disease course and other data, proving comparability ($p > 0.05$) (Tab. 2).

Table 2: Clinical basic data [n(%)]

	Research group (n = 74)	Control group (n = 30)	χ^2 or t	p
Age (years)	49.4 ± 9.6	48.7 ± 10.2	0.741	0.331
BMI	21.23 ± 4.86	21.16 ± 5.02	0.066	0.948
Smoking history				
Yes	48 (64.86)	18 (60.00)	0.218	0.641
No	26 (35.14)	12 (40.00)		
Drinking history				

Yes	19 (25.68)	9 (30.00)	0.203	0.652
No	55 (74.32)	21 (70.00)		
Place of residence				
Cities and towns	54 (72.97)	23 (76.67)	0.152	0.697
Countryside	20 (72.03)	7 (23.33)		
Marital status				
Married	69 (93.24)	27 (90.00)	0.316	0.574
Unmarried	5 (6.76)	3 (10.00)		
Exercise habits				
Yes	31 (7.32)	11 (0.00)	0.242	0.623
No	43 (92.68)	19 (100.00)		
Course of disease	3.74 ± 1.04	3.92 ± 0.86	0.838	0.404

3.2 Expression of miR-152 and CYFRA21-1 in OC Patients

qRT-PCR and ELISA detection showed that the miR-152 expression in the RG was obviously lower than that in the CG, while CYFRA21-1 (μg/L) was dramatically higher than that in the CG ($p < 0.05$) (Fig. 1).

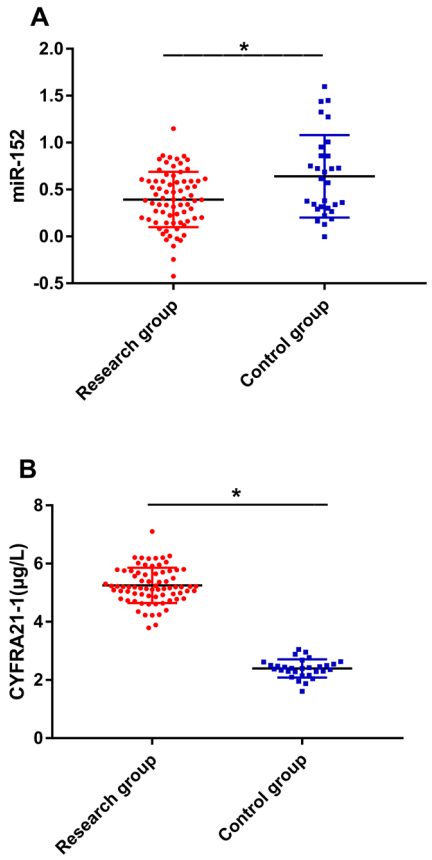

Figure 1: Expression of miR-152 and CYFRA21-1 in ovarian cancer patients. A: miR-152 is significantly lower than that of the control group; B: CYFRA21-1 (μg/L) is significantly higher than that of the control group. Note: * indicates the difference between the two groups ($p < 0.05$)

3.3 Diagnostic Value of miR-152 and CYFRA21-1 in OC

ROC was drawn according to the miR-152 and CYFRA21-1 expression levels. The area under miR-152 curve (AUC) was 0.724 ($p < 0.05$), and CYFRA21-1's AUC was 0.714 ($p < 0.05$) (Tab. 3, Fig. 2).

Table 3: ROC curve

	miR-152	CYFRA21-1 (μg/L)
AUC	0.724	0.714
Std.Error	0.058	0.052
95%5CI	0.611–0.838	0.612–0.816
P	0.001	0.001
Cut-off	0.719	5.148
Sensitivity (%)	56.67	90.00
Specificity (%)	86.49	55.41

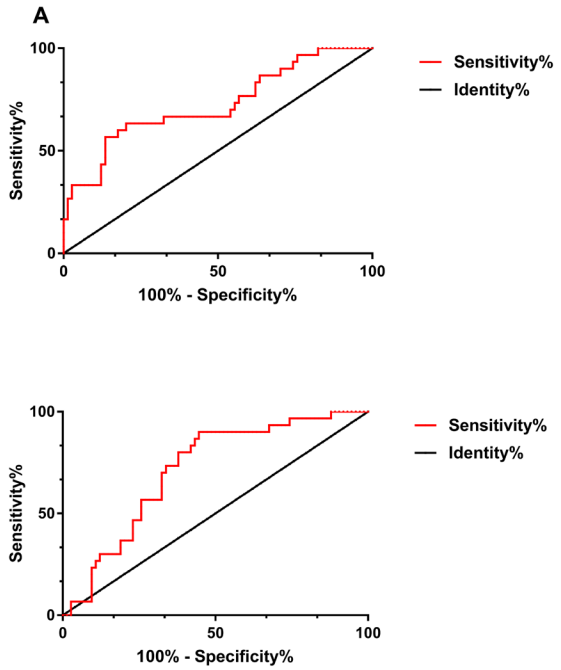

Figure 2: Diagnostic value of miR-152 and CYFRA21-1 for ovarian cancer. A: When the cut-off value is 0.719, the sensitivity and specificity of miR-152 are 56.67% and 85.49%, respectively; B: When the cut-off value is 5.148, CYFRA21-1 has a sensitivity of 90.00% and specificity of 55.41%

3.4 Clinicopathological Correlation Analysis of OC Patients

The miR-152 and CYFRA21-1 expression levels had no marked correlation with the age, tumor type, exercise and smoking habits of OC patients ($p > 0.05$), but was relevant to their lymph metastasis, differentiation degree and pathological stage ($p < 0.05$) (Tabs. 4, 5).

Table 4: Correlation analysis of miR-152 expression level and clinicopathology of patients in research group

	n	miR-152	t or F	*p*
Age				
≤49	45	0.24 ± 0.14	0.667	0.507
>49	29	0.26 ± 0.10		
Smoking				
Yes	48	0.26 ± 0.09	0.878	0.383
No	26	0.24 ± 0.10		
Lymphatic metastasis				
Yes	27	0.12 ± 0.06	10.862	0.001
No	47	0.35 ± 0.10		
Tumor type				
Serosity	49	0.27 ± 0.07	0.527	0.600
Non-serous	25	0.26 ± 0.009		
Pathological staging				
Ⅰ – Ⅱ	32	0.35 ± 0.04	14.453	0.001
Ⅲ–Ⅳ	42	0.15 ± 0.07		
Degree of differentiation				
Highly differentiated	19	0.38 ± 0.10	35.942	0.001
Moderately differentiated	24	0.22 ± 0.08		
Poorly differentiated	31	0.10 ± 0.14		
Exercise habits				
Yes	19	0.25 ± 0.08	0.517	0.607
No	55	0.24 ± 0.07		

Table 5: Correlation analysis between CYFRA21-1 expression level and clinical pathology of patients in research group

	n	CYFRA21-1 (μg/L)	t or F	*p*
Age				
≤49	45	5.22 ± 1.79	0.093	0.926
>49	29	5.18 ± 1.83		
Smoking				
Yes	48	5.23 ± 1.79	0.185	0.854
No	26	5.15 ± 1.74		
Lymphatic metastasis				
Yes	27	5.99 ± 1.99	2.138	0.037
No	47	5.11 ± 1.52		
Tumor type				
Serosity	49	5.15 ± 1.69	0.048	0.962
Non-serous	25	5.17 ± 1.71		
Pathological staging				
Ⅰ – Ⅱ	32	5.11 ± 1.61	2.209	0.030
Ⅲ–Ⅳ	42	6.03 ± 1.89		

Degree of differentiation				
Highly differentiated	19	5.12 ± 1.69	3.404	0.039
Moderately differentiated	24	6.31 ± 1.94		
Poorly differentiated	31	6.51 ± 1.98		
Exercise habits				
Yes	19	5.15 ± 1.77	0.086	0.932
No	55	5.11 ± 1.75		

3.5 Correlation between miR-152, CYFRA21-1 Expression Level and CEA

Pearson test analysis identified that miR-152 was positively associated with CEA ($r = 0.655$, $p < 0.001$) and CYFRA21-1 was positively associated with it, too ($r = 0.567$, $p < 0.001$) (Tab. 6, Fig. 3).

Table 6: Correlation between miR-152, CYFRA21-1 expression level and CEA

	miR-152	CYFRA21-1
r	0.655	0.567
95%CI	0.543–0.745	0.426–0.681
p	0.001	0.001

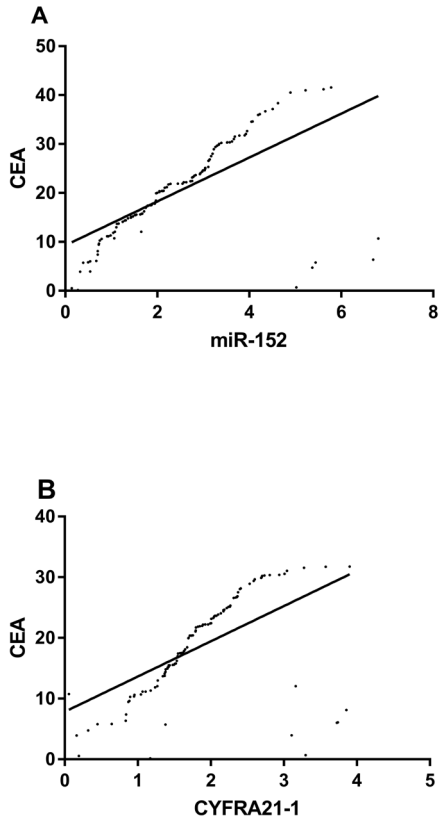

Figure 3: Correlation between miR-152, CYFRA21-1 expression level and CEA. A: correlation analysis between miR-152 and CEA; B: correlation analysis between CYFRA21-1 and CEA

3.6 Prognosis and Survival

Of the 74 patients, 72 cases in the RG were successfully followed up, about 97.30%. Based on the miR-152 and CYFRA21-1 median expression levels, patients were divided into two groups: 36 cases in high expression group (HEG) and 36 cases in low expression group (LEG). Prognostic follow-up results revealed that the 3-year overall survival rate of miR-152 LEG was 61.54%, and that of miR-152 HEG was 84.85%; the rate of CYFRA21-1 LEG was 83.75%, and that of CYFRA21-1 HEG was 60.54% (Fig. 4).

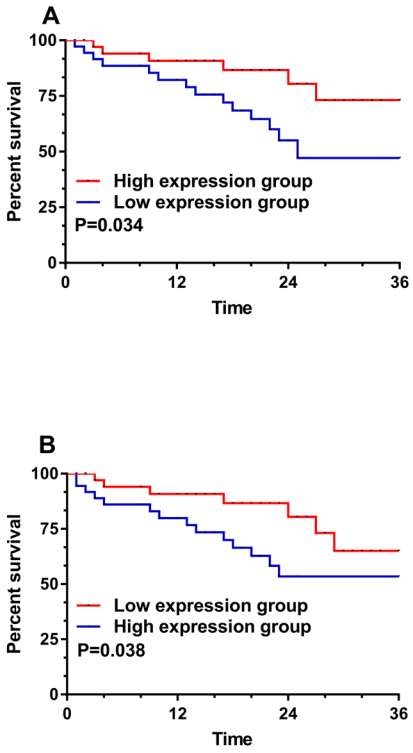

Figure 4: Prognosis and survival. A: The 3-year overall survival rate of miR-152 low expression group is 61.54%, and that of high expression group is 84.85% ($p = 0.034$); B: The 3-year survival rate of CYFRA21-1 low expression group is 83.75%, and that of high expression group is 60.54% ($p = 0.038$)

4 Discussion

OC is currently the malignancy with the highest mortality among female patients. There are no significant special symptoms in the early stage, and it is easy to be neglected or mistreated. Most patients have reached the middle and late stages of tumor diagnosis. At this time, most tumors have metastasis and invasion, the treatment difficulty greatly increases, and the prognosis is even less upbeat [17,18]. At the moment, its pathogenesis is vague, and there is not an exact and active indicator for reference in the clinical diagnosis of early OC [19]. Active biological indicators in diagnosing and treating OC are currently a major research focus clinically. With the continuous deepening of research, micro RNA supplies a new direction for targeted research of modern tumors [20]. miRNA is a kind of endogenous expression non-coding small molecule RNA which occupies only 1%–3% of human genome sequence, only 17–25 nucleotides long [21]. Fragmentary pairing of the 3'UTR end non-coding region of the target gene leads to blocking of target gene mRNA translation and life activities like development and progression with cells involved in regulation, which are constantly proved to be effective in various tumor diseases as cancer promotion or tumor suppressor genes [22,23].

miR-152 is made up of four highly conserved small molecule non-coding RNA with similar structures and sequences. It located on the 4th intron of chromosome 2 polar shadow CTDSP1 and is a member of miR-152 family. It inhibits HCC cells' proliferation and invasion through regulating the EphA2 and c-Myc expression levels. It has been proved that there is abnormal expression in various tumors [24–26], CYFRA21-1 is a cytokeratin released into the blood under the condition of tumor cell necrosis or lysis. Its sensitivity and concentration increase with the progress of cancer cells [27]. However, its current part in OC is vague. Thus, this experiment uses qRT-PCR and ELISA techniques to detect the miR-152 expression level in OC tissue and CYFRA21-1 in serum, and discusses the abnormal expression of miR-152 and CYFRA21-1 and their relationship with the pathological conditions of patients.

In this experiment, we first used qRT-PCR and ELISA to test the miR-152 and CYFRA21-1 expression levels in patients. And we found that the miR-152 expression level in the RG was dramatically lower than that in the CG, while the CYFRA21-1 expression level was higher than that in the CG, suggesting that the two were relevant to OC's development and progression and might be used as protooncogenes to participate in its pathological process, which was also coincided with the research results of Liu et al. [28], and could support the results of this experiment. We further drew ROC curve and discovered that miR-152's AUC was 0.724, and CYFRA21-1's AUC was 0.714, which had high diagnostic value, suggesting that the two were expected to be effective targets for diagnosing OC in the future. We analyzed the differences between miR-152 and CYFRA21-1 in the clinical pathology of OC patients, and found that their expression levels were not significantly correlated with age, tumor type, exercise and smoking habits, but were closely correlated with pathological stage, tumor differentiation degree and lymph node metastasis, suggesting that miR-26b was tightly correlated with cells' differentiation and proliferation, and the disease deterioration degree of patients could be judged through the miR-152 and CYFRA21-1 expression levels. Pearson correlation analysis identified that the miR-152 expression levels in patient tissues and CYFRA21-1 in serum were positively correlated with CEA. In the end, we compared the 3-year survival of the miR-152 and CYFRA21-1 HEG and LEG and found that the miR-152 LEG was dramatically worse than the miR-152 HEG, while the CYFRA21-1 LEG was markedly better than the CYFRA21-1 HEG, indicating that miR-152 and CYFRA21-1 were relevant to prognosis of patients and could be used as an active biological marker.

We have initially proved miR-152 and CYFRA21-1's clinical value, but we still have certain limitations. First of all, the basic cell experiment has not been carried out in this study. Secondly, the sample base of our experiment is small and the population is relatively single. Hence, we hope to supplement our research results by conducting basic cell experiments and expanding the sample size of the research subjects in future studies.

5 Conclusion

To summarize, miR-152 and CYFRA21-1 are low expressed in ovary, which are closely related to pathological stage, tumor differentiation degree and lymph node metastasis of OC, suggesting that the two take part in its development and progression, and are expected to become active indicators in future diagnosis and treatment and prognosis of patients.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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