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ORIGINAL ARTICLE

Clinical significance of miR-139-5p and FGF2 in ovarian cancer

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Summary

Purpose: *To estimate the expression and clinical significance of miR-139-5p and fibroblast growth factor 2 (FGF2) in ovarian cancer (OC).*

Methods: *Of the 198 female patients undergoing surgical treatment in our hospital, 101 patients with ovarian tumor resection were allocated in a study group and 97 with ovarian resection for benign lesions were allocated in a control group. MiR-139-5p and FGF2 expression was quantified, and associations between miR-139-5p and FGF2 and clinicopathological features of OC were analyzed, as well as their diagnostic performances (receiver operating characteristic (ROC) curve).*

Results: *The study group presented lower miR-139-5p level and higher FGF2 level (both p<0.05). Significant associations of miR-139-5p and FGF2 with tumor differentiation and clinical stage were noted in OC (p<0.05). MiR-139-5p was reversely associated with clinical stage and positively associated with tumor differentiation (p<0.05), FGF2 was positively correlated with clinical stage and negatively correlated with tumor differentiation (p<0.05). The overall survival in the study group was 70.41%. The survival in high miR-139-5p expression group and low FGF2 expression group improved remarkably (p<0.05). The area under the curve (AUC) of combined detection (0.91) was higher than that of single detection.*

Conclusion: *MiR-139-5p shows a decreased expression and FGF-2 shows an increased expression in OC, and they are associated with clinical stage and tumor differentiation. Combined detection of miR-139-5p and FGF-2 contributes to the diagnosis and treatment of OC, and is an available biomarker for the diagnosis and prognosis of patients.*

Key words: *miR-139-5p, FGF2, ovarian cancer, clinical significance*

Introduction

Cancer is a great threat to the life and safety of all humans. Although the overall cancer mortality continues to decline, the incidence in females has increased slightly [1]. The ovary is a vulnerable site for metastasis of various cancers, and ovarian tumors are metastatic lesions originating from non-ovarian organs [2]. Ovarian cancer (OC), coupled with its invasiveness [3], is one of the most frequent fatal malignancies of the female reproductive system [4]. With a median survival time after recurrence of 2.5-4 years, OC recurrence is responsible for most OC deaths [5]. Malignant ovarian tumors are heterogeneous and have different etiology and molecular biology, among which 90% of them are epithelial, with serous carcinoma being the most common type [6]. There were ~22,240 newly diagnosed OC cases and 14,070 deaths in the United States in 2018. Serous carcinomas were mostly diagnosed at stage III (51%) or stage IV (29%), and the 5-year cause-specific survival rates from 2007 to 2013 were 42% and 26%, respectively [7].

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MicroRNAs (miRs), a kind of non-coding RNAs with a length of ~22nt, exist widely in animals, plants and viruses, and affect fine-tuning gene expression regulation. MiR identification and annotation have been the key point in epigenomics research over the past decade [8]. Functioning as proto-oncogenes or tumor suppressors, miRs are involved in tumorigenesis [9] and have potential to be biomarkers for diagnosis, treatment and prognosis of diseases [10]. Downregulation of miR-139-5p partly saves the suppression of TTN-AS1 on OC cells [11]. Fibroblast growth factor 2 (FGF2) participates in OC progression, which may become a novel available target for its treatment [12].

The present study evaluated the levels and clinical significance of miR-139-5p and FGF2 in OC, hoping to provide theoretical basis for early diagnosis and treatment of OC.

Methods

General data

Of the 198 female patients undergoing surgical treatment in our hospital and enrolled, 101 with ovarian tumor resection were allocated in a study group (mean age 50.47±5.21 years) and another 97 with ovarian resection for benign lesions were allocated in a control group (mean age 51.24±5.62 years). Inclusion criteria: Patients accompanied by family members upon admission, as well as those with intact clinical and pathological data; patients in the study group pathologically diagnosed with OC and without chemoradiotherapy. Exclusion criteria: Patients with a history of mental illness, autoimmune system defect, severe organ diseases, or drug dependence, as well as those with a family history of mental illness or cancers; patients with communication disorders, aphasia, irritability, or unconsciousness. All participants were consented in advance and signed the informed consent form. The present study was performed after approval from the Ethics Committee.

Detection methods

RT-PCR was performed for miR-139-5p and FGF2 quantification. Total RNAs extracted with a TRIzol kit (Shenyang Wanlei Biotechnology Co., Ltd., WLA088b) were treated with DNase I (RNA free) (Shanghai Hengfei Biotechnology Co., Ltd., K003399P) digestion to eliminate contaminating genomic DNA. The purity and concentration were determined by an ultraviolet spectrophotometer (Shanghai Hengfei Biotechnology Co., Ltd., UV-1100), and the integrity was measured by 1.5% agarose gel electrophoresis. cDNAs were obtained from RNA samples (500 ng/μL) with a reverse transcription kit (Shanghai Even Bridge Biotechnology Co., Ltd., 4368814). RT-PCR system (20 μL): 2x Ultra SYBR one step Qrt-PCR Buffer (10 μL), RNA template (2 μL), nuclear-free water (5.5 μL), upstream and downstream primers (1μL each), Super enzyme mix (0.5μL). RT-PCR conditions: 95℃ for 10 min, 40 cycles of 95℃ for 15 s, and 60℃ for 1 min. Designed by Primer Premier 5.0 (Premier, Palo Alto CA, USA), primers were produced by Tianjin Saier Biotechnology Co., Ltd. U6 and GAPDH served as internal control of miR-139-5p and FGF2, respectively for specific primer sequences (Table 1). $2^{-\Delta Ct}$ was adopted to calculate the relative expression of miR-139-5p and FGF2. Ct (cycle threshold): number of cycles corresponding to the inflection point where the fluorescence signal begins to enter the exponential growth phase from the background during PCR amplification).

Table 1. Primer sequence

Primer name	*Primer sequence 5'-3'*
miR-139-5p	5'-GCTCTACAGTGCACGTGTC-3'
U6	5'-GCGCTCGTAAGCGTTC -3'
FGF2	5'- TTCCTGCGCCTGATGTCC-3'
GAPDH	5'- AGTCTTTTGGGTAGCA -3'

Outcome measures

miR-139-5p and FGF2 expression in OC tissues; correlations between miR-139-5p and FGF2 and clinicopathological features in the study group; associations of miR-139-5p and FGF2 with clinical stage and tumor differentiation. At the 6th, 12th, 24th, 36th, 48th and 60th months after treatment, the patients in the study group were followed up by telephone or outpatient reexamination for 5 years. Patient survival was recorded and the associations with miR-139-5p and FGF2 were analyzed. Diagnostic value of single and combination of miR-139-5p and FGF2 for OC was estimated by the receiver operating characteristic (ROC) curve.

Statistics

SPSS20.0 software (IBM Corp, Armonk, NY, USA) and GraphPad Prism 7 (GraphPad Co., Ltd., San Diego, USA) were employed for data processing and graphing. Counting data were presented by [n(%)], and inter-group comparisons adopted chi-square test. Measurement data were presented by mean±standard deviation, and inter-group comparisons adopted *t*-test. Spearman correlation coefficient was used for correlation analyses, ROC for evaluations of sensitivity and specificity of single and combined diagnosis, and binary Logistic regression analysis for diagnostic value of combined detection for OC. Significance was set at $p<0.05$.

Results

Comparison of general data

No significant differences were revealed in general data of age, body mass index, history of smoking and alcohol drinking between the two groups ($p>0.05$) (Table 2).

Comparison of miR-139-5p and FGF2 expression

miR-139-5p expression in the study group decreased evidently compared with the control group, while FGF2 expression increased (both p<0.05) (Figure 1).

Correlations between miR-139-5p and FGF2 and pathological features of patients with OC

miR-139-5p and FGF2 had no significant correlations with age, pathological types and tumor size in OC tissues (p>0.05), but they were correlated with tumor differentiation and clinical stage (p<0.05) (Table 3).

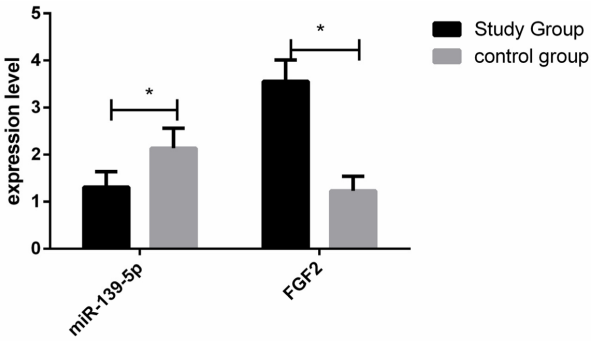

Figure 1. Comparison of miR-139-5p and FGF2 expression. miR-139-5p expression in study group is significantly lower than that in control group. FGF2 expression in study group is significantly higher than that in control group (*p<0.05).

Table 2. Comparison of general clinical data (x±sd)/[n (%)]

	Study group (n=101)	*Control group (n=97)*	t/x^2	*p*
Age (years)	50.47±5.21	51.24±5.62	1.00	0.32
Body mass index (kg/m^2)	22.43±2.14	22.39±2.23	0.13	0.90
Smoking, n (%)			0.00	0..98
Yes	21 (20.79)	20 (20.62)	-	-
No	80 (79.21)	77 (79.38)	-	-
Alcohol drinking, n (%)			0.00	0.96
Yes	18 (17.82)	17 (17.53)	-	-
No	83 (82.18)	80 (82.47)	-	-
Residence, n (%)			0.01	0.94
Urban	62 (61.39)	59 (60.82)	-	-
Rural	39 (38.61)	38 (39.18)	-	-
Pathological classification, n (%)			-	
Serous	55 (54.46)	0 (0.00)	-	-
Other	46 (45.54)	0 (0.00)	-	-

Table 3. Correlations between miR-139-5p and FGF2 and clinicopathological features (x±sd)

	n	*miR-139-5p*	*t*	*p*	*FGF2*	*t*	*p*
Age (years)			0.32	0.75		0.34	0.74
<50	51	1.30±0.34			3.55±0.47		
≥50	50	1.32±0.29			3.58±0.42		
Pathological classification			0.58	0.56		0.34	0.74
Serous	55	1.29±0.36			3.57±0.41		
Other	46	1.33±0.32			3.54±0.48		
Tumor size (cm)			0.90	0.37		0.65	0.52
<4	39	1.28±0.35			3.53±0.49		
≥4	62	1.34±0.31			3.59±0.43		
Tumor differentiation			5.93	0.00		15.17	0.00
Moderately and highly differentiated	76	1.36±0.29			2.96±0.31		
Poorly differentiated	25	0.97±0.27			4.02±0.28		
Clinical stage			8.16	0.00		8.93	0.00
I-II	45	1.40±0.31			3.15±0.38		
III-IV	56	0.92±0.28			3.86±0.41		

Associations of miR-139-5p and FGF2 with clinical stage and tumor differentiation

miR-139-5p was reversely associated with clinical stage (r=-0.69, p<0.05), that is, miR-139-5p decreased with the aggravation of OC. There was a significant positive association between FGF2 and clinical stage (r=0.79, p<0.05), that is, FGF2 increased with the aggravation of OC (Figure 2).

Associations of miR-139-5p and FGF2 with tumor differentiation

miR-139-5p was positively associated with differentiation of OC cells (r=0.62, p<0.05), that is, the higher the tumor differentiation, the higher the miR-139-5p level while FGF2 was negatively correlated with OC cell differentiation (r=-0.75, p<0.05) (Figure 3).

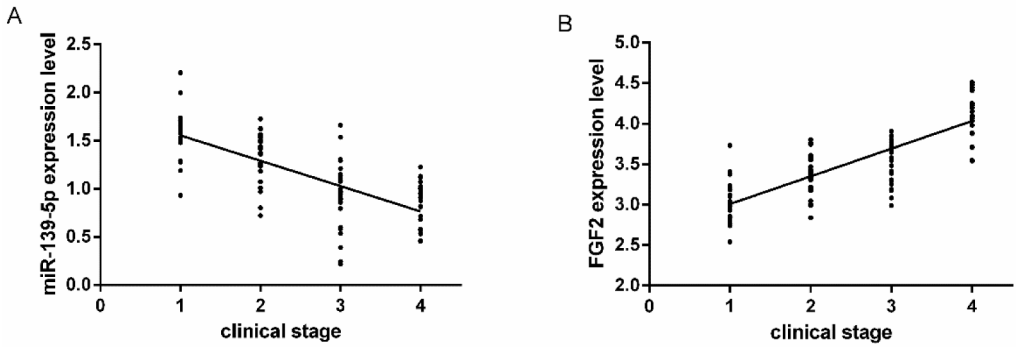

Figure 2. Associations of miR-139-5p and FGF2 with clinical stage. **A:** miR-139-5p is reversely associated with clinical stage. **B:** FGF2 is positively associated with clinical stage. 1 indicates stage I, 2 indicates stage II, 3 clinical stage III, and 4 indicates stage IV.

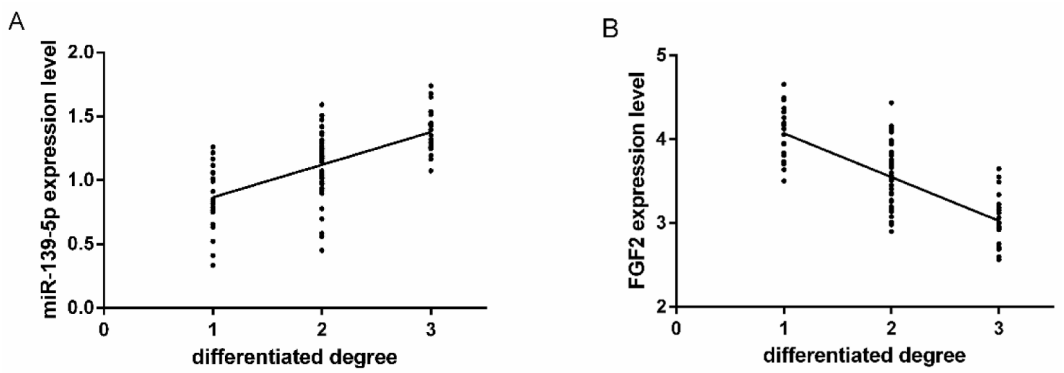

Figure 3. Associations of miR-139-5p and FGF2 with tumor differentiation. **A:** mIR-139-5p is positively associated with tumor differentiation. **B:** FGF2 is reversely associated with tumor differentiation. 1 indicates poorly differentiated, 2 indicates moderately differentiated, and 3 indicates highly differentiated.

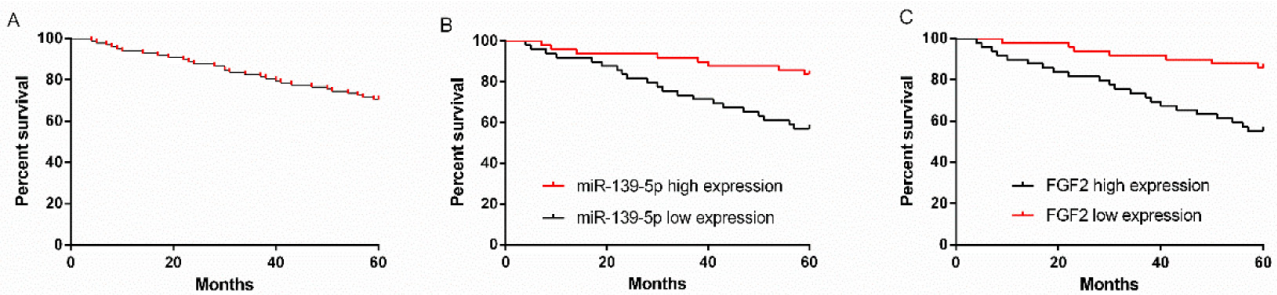

Figure 4. 5-year overall survival of patients in study group and subgroups **A:** Overall survival of patients in the study group is 70.41%. **B:** Survival in high miR-139-5p expression group is significantly higher than that in low miR-139-5p expression group (p<0.05). **C:** Survival of low FGF2 expression group is significantly higher than that in high FGF2 expression group (p<0.05).

Table 4. Diagnostic performances of single and combined detection of miR-139-5p and FGF2

	Specificity	*Sensitivity*	*Optimal cut-off value*	*You-den index*	*AUC*	*p*	*95% confidence interval*	
							Lower limit	*Upper limit*
miR-139-5p	93.07%	64.95%	>1.61	0.03	0.84	0.00	0.78	0.89
FGF2	75.25%	87.63%	<2.28	0.03	0.83	0.00	0.77	0.90
Combined detection	72.78%	92.78%	>0.32	0.02	0.91	0.00	0.88	0.95

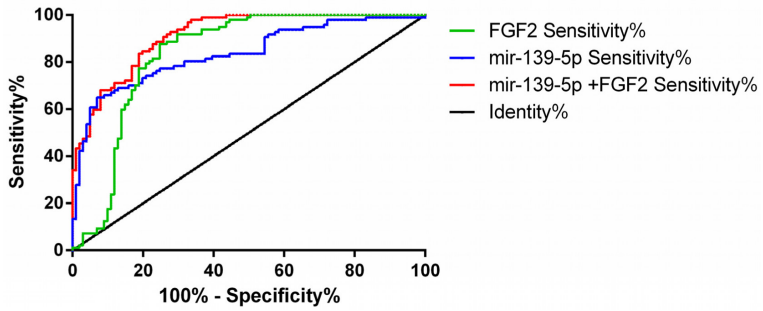

Figure 5. ROC curve for single and combined detection of miR-139-5p and FGF2 AUC reaches the highest in combined detection of miR-139-5p and FGF2.

Relationship between survival of OC patients and miR-139-5p and FGF2

Of 101 patients included in the study group, 98 were successfully followed up and 3 were lost to follow-up. The total survival rate in the study group was 70.41%. According to the median miR-139-5p and FGF2 expression, patients in the study group were assigned into high and low expression groups of miR-139-5p and FGF2. It turned out that the survival in the high miR-139-5p expression group and low FGF2 expression improved evidently (both $p<0.05$) (Figure 4).

Diagnostic performances of single and combination of miR-139-5p and FGF2

We compared the sensitivity, specificity, and area under the curve (AUC) of single and combination of miR-139-5p and FGF2 to evaluate their diagnostic performances in OC. The sensitivity was the highest (92.78%) in the combined detection, the specificity (93.07%) was the highest in the single miR-139-5p detection, and the AUC reached the highest (0.91) in the combined detection (Table 4 and Figure 5).

Discussion

OC is the fifth most prevalent cause of cancer-related death among women and the second most prevalent female malignancy in the world [13]. Approximately 200,000 new cases and 100,000 deaths of patients with OC are reported worldwide per year. Epithelial OC is the most common type, accounting for 4% of female cancer-related mortality globally [14]. Patients with advanced OC suffer from adverse prognosis, with a 5-year survival rate of less than 25% [15,16]. Therefore, it is necessary to seek ideal molecular targets for early diagnosis and treatment of this disease.

MiRs, that are frequently abnormally expressed in OC [17,18], play an anti-tumor or carcinogenic role and participate in the pathological process of OC [19,20]. FGF2 is a member of FGF family [21] that affects various physiological and pathological processes [22]. It has been reported to have abnormal expression in gastric cancer [23] and breast cancer [24]. Therefore, we estimated the expression and clinical significance of miR-139-5p and FGF2 in OC.

MiR-139-5p in OC tissues was remarkably lower and FGF2 was higher than that in normal controls, and the same conclusions were drawn by Jiang et al [25] and Feng et al [26]. An increase in miR-139-5p enhances the sensitivity of cisplatin-resistant ovarian cancer cells [25], and FGF2 exerts a carcinogenic effect in epithelial OC [27]. Therefore, miR-139-5p acts as a tumor suppressor and FGF2 acts as an oncogene in OC. In addition, close correlations of miR-139-5p and FGF2 with tumor differentiation and clinical stage indicate that both miR-139-5p and FGF2 are essential in OC. Inactivation of MAPK signaling pathway and miR-139-5p overexpression reverse cisplatin resistance in OC [27].

Besides, miR-936 inhibits the invasion of epithelial OC cells *in vitro* and *in vivo* by targeting FGF2-mediated PI3K-Akt pathway [28]. In the present study, it was concluded that miR-139-5p was reversely associated with clinical stage, and was positively associated with tumor differentiation, but FGF2 was quite the opposite, suggesting that both miR-139-5p and FGF2 may be promising targets for OC treatment. Of 101 patients in the study group, 98 were followed up for 5 years, and 3 were lost to follow-up, with a return visit rate of 97.03%. It was found that the overall 5-year survival rate was 70.41%, and patients with high miR-139-5p level or low FGF2 level showed improved survival. Downregulation of miR-139-5p and its correlations with clinical stage, lymphatic metastasis and overall survival have been reported in a previous study [29]. Huang et al revealed that FGF2 increases evidently in OC, and is negatively correlated with overall survival of patients [30]. All of these findings suggest that either a low miR-139-5p level or a high FGF2 level portends poor prognosis of patients with OC, which enlightens us a new direction for improving the treatment, prognosis, and survival of patients. We compared the sensitivity, specificity, and AUC of single and combined detection of miR-139-5p and FGF2 to evaluate their diagnostic performances in OC. It turned out that the AUC of joint detection was remarkably higher than that of single detection. Therefore, both miR-139-5p and FGF2 may be available targets for OC treatment, and the combined detection can be used as an indicator for its early diagnosis.

The clinical significance of miR-139-5p and FGF2 in OC was discussed to provide theoretical basis for early diagnosis and treatment of this disease. However, there are still several limitations. It is not clear how the differential expression of miR-139-5p and FGF2 is induced. Besides, the specific effects of miR-139-5p and FGF2 on OC cells remain to be identified. We will continuously supplement our conclusions in the future research to inspire clinical diagnosis and treatment of OC.

To sum up, miR-139-5p shows a decreased expression and FGF-2 shows an increased expression in OC, and they both are associated with clinical stage and tumor differentiation. Combined detection of miR-139-5p and FGF-2 contributes to the diagnosis and treatment of OC, and is an available biomarker for the diagnosis and prognosis of patients.

Conflict of interests

The authors declare no conflict of interests.

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