

ORIGINAL ARTICLE

MiR-877 suppresses tumor metastasis via regulating FOXM1 in ovarian cancer

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Summary

Purpose: Ovarian cancer (OC) is a serious threat to women's life. OC is insidious and lacks early diagnosis and effective treatment. Therefore, it is vital to look for new therapeutic targets and biomarkers.

Methods: MicroRNA-877 (miR-877) expression level in OC was accessed via quantitative real-time polymerase chain reaction (qRT-PCR). Transwell assay, Matrigel assay and wound healing assay were used to analyze the ability of miR-877 on cell migration and invasion. Luciferase reporter assay was employed for verification the target of miR-877. Western blotting was taken in for the determination of the expression level of FOXM1.

Results: MiR-877 had low expression level in OC tissues and cell lines. MiR-877 over-expression induced inhibition of cell migration and invasion. FOXM1 was a direct target of miR-877. MiR-877 restrained cell migration and invasion by negatively regulating FOXM1 expression in OC.

Conclusions: Our research elucidated that miR-877 played a role of tumor suppressor in OC by negatively regulating FOXM1 which may bring a novel insight into new molecular therapeutic targets and biomarkers for OC.

Key words: ovarian cancer, miR-877, migration, invasion, FOXM1

Introduction

Ovarian cancer (OC) is a serious threat to women's life with more than 22,280 new cases in the United States in one year [1,2]. OC is insidious and lacks early diagnosis and effective treatment. When diagnosed, 67% of OC are in advanced stage, with extensive abdominal or distant metastasis, and the 5-year survival rate is still low, hovering at 25-30%. Thus, it is vital to seek for novel therapeutic targets and biomarkers for OC.

MicroRNAs (miRs) are a newly discovered class of small non-coding RNAs with a length of 19 to 25 nucleotides that regulate gene expression in a variety of eukaryotes [3]. MiRs can regulate a variety of physiological and pathological processes in the body, but increasing evidence shows that they are closely related to the occurrence, development, invasion and metastasis of tumors [4]. MiR-25 is

highly expressed in OC tissues and OC cell lines, and up-regulation of miR-25 expression *in vitro* can inhibit the apoptosis of cancer cells and promote the proliferation of cancer cells [5]. By reducing the expression of miR-200 in OC cell lines or tissues, the expression of e-cadherin can be down-regulated, thus inducing epithelial-mesenchymal transition (EMT) to promote tumor metastasis [6]. Nevertheless, the role of miR-877 in OC has been rarely studied.

This research was conducted to examine the role of miR-877 in OC. Through using *in vitro* experiments, we found miR-877 suppressed cell migration and invasion. Its possible molecular mechanism is perhaps the regulation of its down-stream target FOXM1. All data provided evidence for novel OC treatment targets.

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Methods

Clinical samples

This study was approved by the Ethics Committee of Dongda Hospital. Signed written informed consents were obtained from all participants before the study entry. All the clinical samples were extracted from OC patients in Dongda Hospital during 2016-2017. All 46 paired of OC and normal tissues were included in this study.

Cell culture

Cell lines involved in this study including 6 ovarian cell lines (OVCAR3, PEO1, A2780, 3AO, CAOV3, SKOV3) and human ovarian surface epithelial cells (HOSEpiCs) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) and supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 ug/mL streptomycin at 37°C with 5% CO₂.

Cell transfection

MiR-877 over-expression was realized by using oligonucleotides (GenePharma, Shanghai, China). The transfection was performed according to the manufacturer's instruction. The lentivirus vector for over-expressing FOXM1 was generated by RiboBio (Shanghai, China).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of tissue specimens and cell lines were extracted through TRIzol reagent (Invitrogen, Carlsbad, CA, USA). miR-877 expression level was determined by SYBR Green real-time PCR. The primer sequences: miR-877:forward 5'-GTAGAGGAGATG-GCGCAGGG-3', reverse 5'-CAGTGCGTGTCTGGAGT-3';

U6: forward 5'-CTCGCTTCGGCAGCACA-3', reverse 5'-AACGCTTCACGAATTTGCGT-3'; FOXM1: forward 5'-GGACATCTACACTTGGATTGAGG-3', reverse 5'-TGTCATGGAGAGAAAGGTTGTG-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward 5'-CAAGGTCATCCATGACAACCTTTG-3', reverse 5'-GTCCACCACCCTGTTGCTGTAG-3'.

Wound healing assay

Cells were planted onto 6-well plates after transfection. All the linear scratches were made by pipette tips. The scratches were photographed at 0 h, 24 h to monitor the migration process. The experiments were performed in triplicate.

Transwell assay and Matrigel assay

Transwell assay was performed to figure out the invasion ability of transfected OC cells. Transwell chambers and 24-well plates were obtained from Corning (Corning, NY, USA). 1×10⁵ cells were suspended with serum-free medium (100 ul). In matrigel assay, cells were transferred to the Matrigel (BD Bio-sciences, Franklin Lakes, NJ, USA) coated upper chamber. After 36 h, the invasive cells were observed and counted.

Western blotting

Total protein was isolated by radioimmunoprecipitation assay (RIPA) buffer (Thermo, Waltham, MA, USA) and Phenylmethanesulfonyl fluoride (PMSF). Protein lysates were then transferred to polyvinylidene fluoride (PVDF) membrane. Then, the membrane was immunostained at 4°C by rabbit anti-FOXM1 (1:1000, CST, Danvers, MA, USA). Rabbit anti-GAPDH (1:5000, CST, Danvers, MA, USA) was taken as a loading control. Protein relative expression level was evaluated by Image J software (NIH, Bethesda, MD, USA).

Table 1. Association of miR-877 expression with clinicopathologic characteristics of ovarian cancer

Parameters	Number of cases	miR-877 expression		p value
		High (%)	Low (%)	
Age (years)	0.708			0.708
<60	17	9	8	
≥60	29	17	12	
Tumor size, cm				0.147
<4	24	16	8	
≥4	22	10	12	
T stage				0.959
T1-T2	28	16	12	
T3-T4	18	11	8	
Lymph node metastasis				0.012
No	30	21	9	
Yes	16	5	11	
Distance metastasis				0.022
No	33	23	11	
Yes	13	4	9	

Statistics

All experiments in this study was performed at least in triplicate. All data recorded were exhibited as mean \pm standard deviation (SD). Pearson's correlation method was performed for correlation analysis. Student's unpaired t-test was used to undergo statistics analysis. In this study, $p < 0.05$ was significant.

Results

miR-877 had low expression level in OC

qRT-PCR assay of Figure 1A found that miR-877 was down-expressed in tumor tissues. Similarly, miR-877 was also down-expressed in OC cells

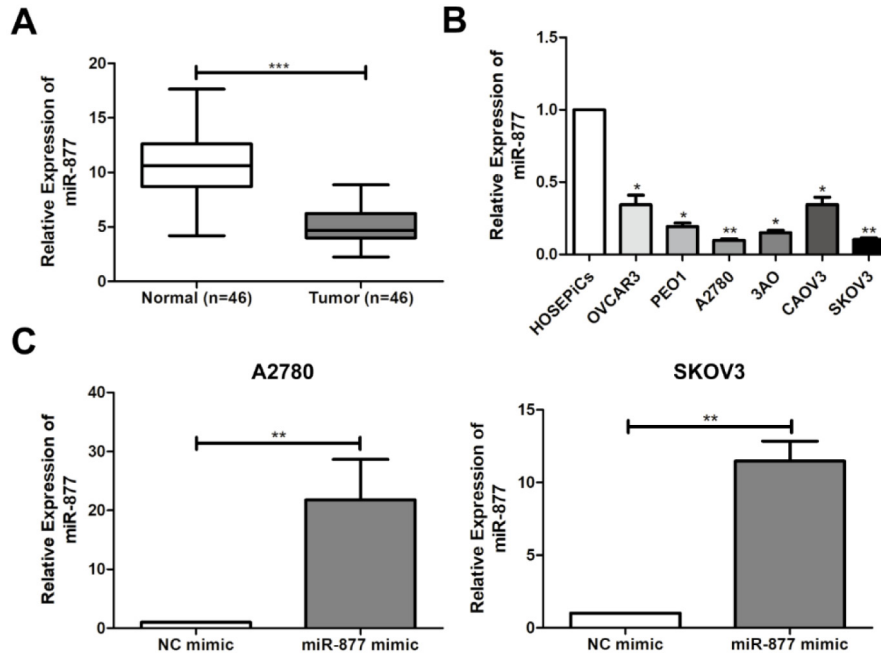


Figure 1. The expression level of miR-877 in OC tissues and cell lines. **A:** Relative expression level of miR-877 was detected in 46 paired OC tumor and normal tissues. **B:** qRT-PCR was used to verify the relative expression level of miR-877 in OC cell lines. **C:** Transfection efficiency was accessed by qRT-PCR. The data are expressed as the mean \pm SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to control group.

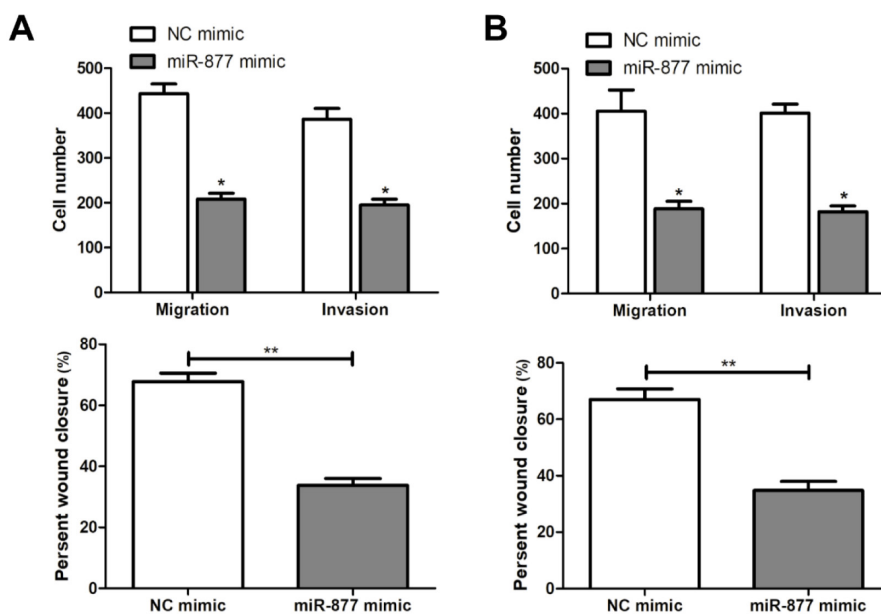


Figure 2. miR-877 suppressed cell migration and invasion *in vitro*. **A:** Transwell assay and Matrigel assay was used to detect the ability of cell migration and invasion. (magnification: 400 \times) **B:** Wound healing assay was performed in transfected cells. (magnification: 10 \times) The data expressed as the mean \pm SD. * $p < 0.05$, ** $p < 0.01$, compared with control group.

(Figure 1B). For further examination, we selected A2780 and SKOV3 cell lines. The transfection efficiency was determined by qRT-PCR assay (Figure 1C). Besides, we investigated the clinical statistics and the results indicated that the expression level of miR-877 was correlated with tumor metastasis (Table 1). Our results revealed out that miR-877 was down-expressed in OC.

miR-877 over-expression lowered cell migration and invasion in vitro

In order to examine the influence of miR-877 on cell migration and invasion, we used transwell assay and Matrigel assay. In transwell assay and Matrigel assay, miR-877 over-expressed group had less cell counts when compared with control group

which indicated that over-expression of miR-877 inhibited cell migration and invasion (Figure 2A). Besides, wound healing assay was also performed and showed that high expression of miR-877 impaired the ability of cell migration (Figure 2B).

FOXM1 was a down-stream target of miR-877

miRDB, microRNA.org and miRTarBase were used to get the down-stream target of miR-877. FOXM1 was taken as a potential target of miR-877. For validation, the dual luciferase reporter assay was involved. The results indicated that miR-877 had binding sites with FOXM1 3'UTR region (Figure 3A). In miR-877 over-expression group, we determined that the messenger (m) RNA and protein expression level of FOXM1

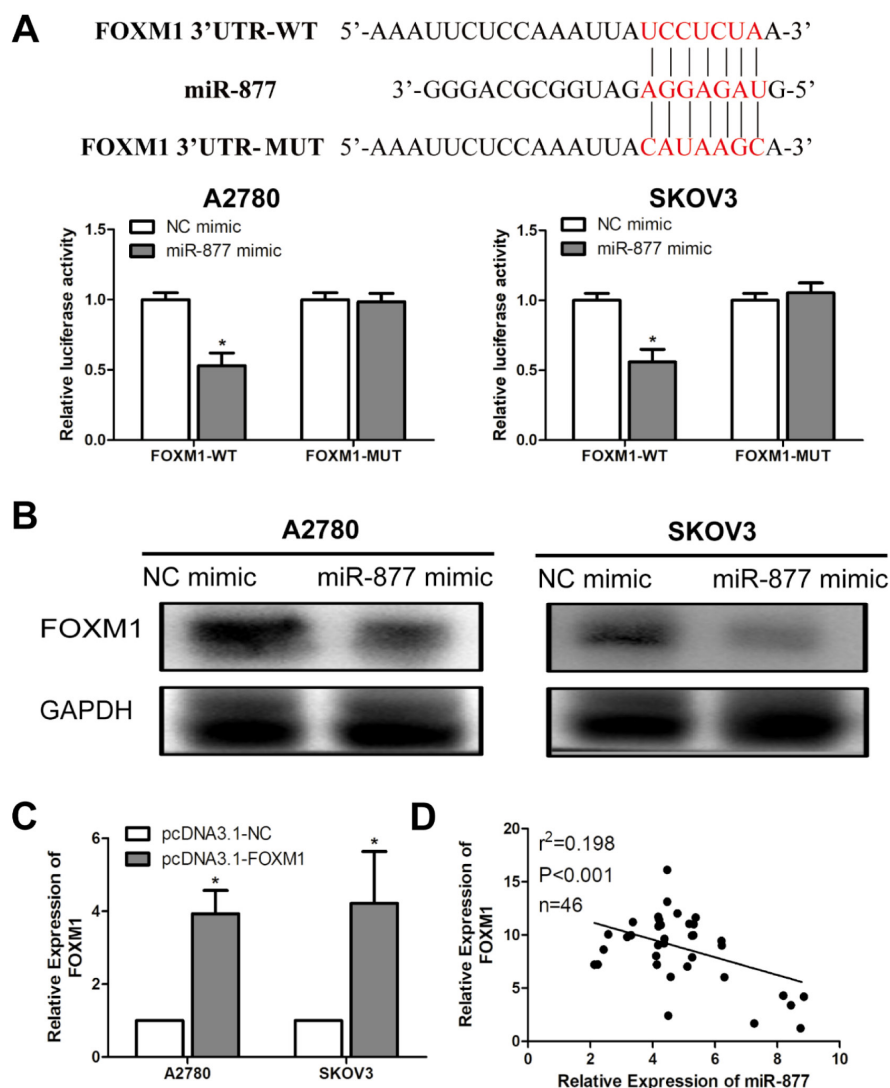


Figure 3. FOXM1 was a down-stream target of miR-877. **A:** Luciferase assay was constructed to prove that miR-877 directly bond to the 3'-UTR regions of FOXM1. **B:** The expression level of FOXM1 was detected by Western blotting in transfected cells. **C:** Transfection efficiency was examined by qRT-PCR. **D:** Pearson's correlation analysis was used to examine the correlation between miR-877 and FOXM1 ($p<0.001$). The data are expressed as the mean \pm SD. * $p<0.05$, compared with control group.

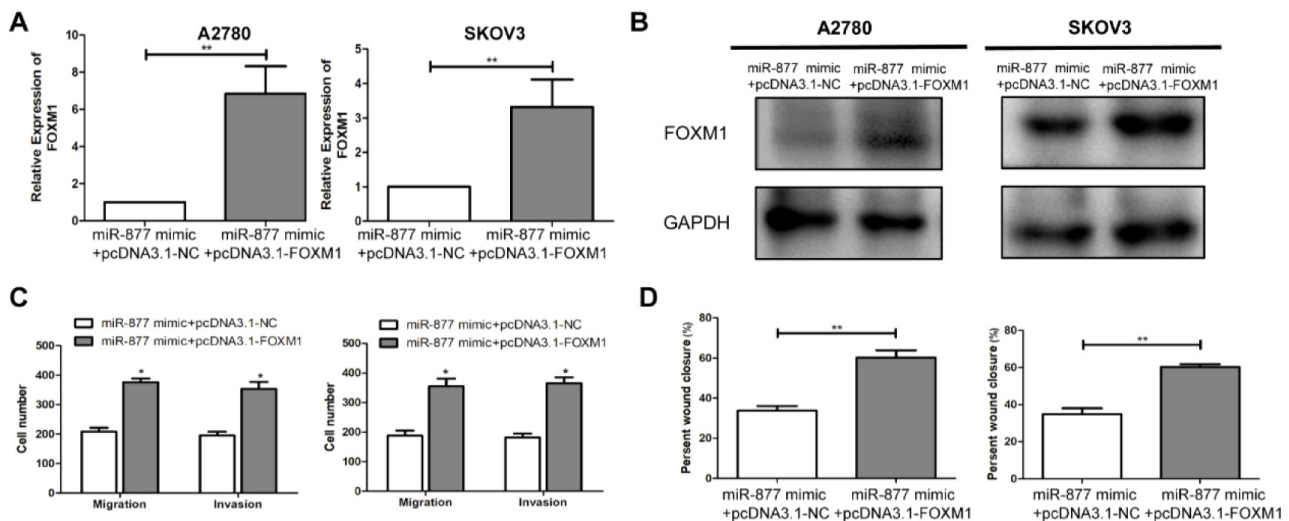


Figure 4. miR-877 negatively regulated the expression of FOXM1 in OC. **A:** qRT-PCR was used to examine the mRNA of FOXM1 in co-transfected cells. **B:** The expression of FOXM1 in co-transfected cell lines via Western blotting. **C:** Cell migration or cell invasion was determined by Transwell assay or Matrigel assay. (magnification: 400×) **D:** Wound healing assay was employed to examine the migration ability of co-transfected cells. (magnification: 10×) The data expressed as the mean \pm SD. * $p < 0.05$, ** $p < 0.01$, compared to control group.

were both decreased when compared with control group (Figure 3B, 3C). Pearson's correlation analysis showed that miR-877 was negatively correlated with FOXM1 (Figure 3D). Our findings verified that FOXM1 was a directly down-stream target of miR-877 in OC.

miR-877 suppressed OC progression through regulating FOXM1 in vitro

The rescue assay was performed in our study for further validation. We co-transfected with FOXM1 over-expression plasmid in transfected cell lines and the transfection efficiency was examined by qRT-PCR assay and Western blotting assay (Figure 4A, 4B). FOXM1 over-expression cancelled the inhibition effect on cell migration and invasion induced by up-regulated miR-877 (Figure 4C). In wound healing assay, over-expressed FOXM1 regained the ability of cell migration which was impaired by miR-877 over-expression (Figure 4D). Taken together, all data showed that miR-877 functioned as a tumor suppressor in OC by regulating FOXM1.

Discussion

OC is a common malignant tumor of the female reproductive system. It is characterized of insidious onset, rapid progression, high mortality and low 5-year survival [7]. At present, an over increasing attention has been paid to the pathogenesis, diagnosis and treatment of OC [8].

In recent years, it has been found that miRs may play either the role of tumor suppressors or

of oncogenes. Many studies have shown that there are up-regulations or down-regulations of miRs in a variety of tumor cells, which is closely related to the occurrence and development of tumors [9]. MiR-877 has been reported to function as a tumor suppressor in diverse tumor types including hepatocellular carcinoma, cervical cancer, colorectal cancer and non-small cell lung cancer [10-13]. Herein, our study was designed to figure out the role of miR-877 in OC progression. Our data verified that over-expression of miR-877 inhibited cell migration and invasion *in vitro*.

MiR sequences are highly conserved in evolution, and each miR may have multiple mRNA targets. It has a certain regulatory function on genes, and miRs can be incomplete or even completely paired and complementary with the corresponding mRNA, ultimately leading to mRNA translation modification or degradation, thus further controlling gene expression at the transcription level [14]. Through several public databases, we found that perhaps FOXM1 serve as a downstream target of miR-877. Subsequently the luciferase reporter assay determined that FOXM1 was a downstream target of miR-877. FOXM1 was reported to inhibit cell growth, migration and invasion in bladder cancer [14]. It is also reported that FOXM1 promoted cell growth and metastasis of colorectal cancer [15]. In our research, we found that FOXM1 was a target of miR-877. Through the rescue assay, our data determined that miR-877 exerted its function via regulating FOXM1 expression in OC.

Conclusions

In conclusion, this study found that miR-877 can inhibit the migration and invasion of cancer cells in OC by down-regulating the expression level of FOXM1, which provides a reliable molecular target

for the clinical treatment of OC and also provides new therapeutic schemes and ideas for the treatment of OC.

Conflict of interests

The authors declare no conflict of interests.

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