

## Original Article

# MicroRNA-365 inhibits ovarian cancer progression by targeting Wnt5a

Yanli Wang<sup>1</sup>, Chunling Xu<sup>2</sup>, Yun Wang<sup>3</sup>, Xiaomeng Zhang<sup>2</sup>

<sup>1</sup>Department of Gynecology, The First Hospital of Jilin University, Changchun 130021, China; <sup>2</sup>Department of Ophthalmology, The Second Hospital of Jilin University, Changchun 130041, China; <sup>3</sup>The Affiliated Hospital, Changchun University of Chinese Medicine, 1478# Gongnong Road, Chaoyang District, Changchun 130021, China

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**Abstract:** MicroRNA-365 (miR-365) has been reported to play an important role in tumorigenesis in many types of cancers; however, the role of miR-365 in the carcinogenesis of ovarian cancer remains unknown. In this study, we focused on the roles and underlying mechanisms of miR-365 in ovarian cancer. Here, we found that miR-365 expression level was significantly decreased in ovarian cancer tissues and cell lines, and that low miR-365 expression was negatively significantly associated with advanced stages as defined by the International Federation of Gynecology and Obstetrics (FIGO), histological grading, and lymph node metastasis. Further functional assays showed that transfection with a miR-365 mimic significantly decreased ovarian cancer cell proliferation, colony formation, migration, and invasion. In addition, Wnt5a was identified as a target gene of miR-365 in ovarian cancer by bioinformatic analysis, luciferase reporter assay, qPCR, and western blot. Wnt5a expression levels were upregulated and inversely correlated with miR-365 expression in ovarian cancer tissues ( $r = -0.638$ ,  $P < 0.0001$ ). Overexpression of Wnt5a could effectively reverse the miR-365 overexpression-induced suppression of proliferation and invasion in ovarian cancer cells. Additionally, *in vivo* studies utilizing a xenograft model demonstrated that overexpression of miR-365 could reduce tumor growth by repressing Wnt5a. Taken together, these findings suggest that miR-365 may be a promising candidate for therapeutic application in ovarian cancer treatment.

**Keywords:** microRNAs, miR-365, ovarian cancer, Wnt5a

### Introduction

Ovarian cancer is the most common cause of death among gynecological malignancies [1, 2]. Although significant progress in the diagnosis and treatment of ovarian cancer has been made in the past decade, the 5-year survival rate for all stages is 35-38% [3, 4]. The mechanism underlying the tumorigenesis of ovarian cancer is not yet fully understood; hence, exploring the key molecular mechanisms of ovarian cancer initiation and development is urgently needed to develop novel approaches and agents to diagnose and treat this disease.

MicroRNAs (miRNAs) are small RNA molecules, approximately 22 nucleotides in length, that negatively regulate gene expression by binding to the 3' untranslated regions (UTRs) of target gene mRNA, thus inhibiting or destabilizing

translation of transcripts [5]. Increasing evidence suggests that miRNAs are involved in many diverse physiological and pathological processes, such as cell proliferation, cell cycle, differentiation, apoptosis, fat metabolism, oncogenesis, and drug resistance [6, 7]. miRNAs had been reported to play essential roles in tumor initiation, development, and progression, and function as tumor suppressors or oncogenes in various cancers [8, 9]. Previous investigations have identified a series of oncogenic and tumor suppressive miRNAs involved in human ovarian cancer tumorigenesis and progression [10, 11], which suggests that miRNAs could serve as diagnosis markers and therapeutics for ovarian cancer.

The newly discovery miRNA, miR-365, has recently been reported to serve as a potential tumor suppressor or oncogene in many types of

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**Table 1.** Correlation between clinicopathological features and miR-365 expression in ovarian cancer tissues

Variables	No. of cases	miR-365 expression		P value
		Low (n%)	High (n%)	
Age (years)				P > 0.05
< 60	22	11 (50.0)	11 (50.0)	
≥ 60	24	13 (54.2)	11 (45.8)	
Tumor size				P > 0.05
< 5	30	17 (56.7)	13 (43.3)	
≥ 5	16	7 (43.8)	9 (56.2)	
FIGO stage				P < 0.01
I-II	36	15 (41.7)	21 (58.3)	
III-IV	10	9 (90.0)	1 (10.0)	
Histological grading				P < 0.01
1/2	39	18 (46.1)	21 (53.9)	
3	7	6 (85.7)	1 (14.3)	
Lymph node metastasis				P < 0.01
No	38	16 (42.1)	22 (57.9)	
Yes	8	8 (100)	0 (0)	

cancers [12-20]. However, the detailed biological function and underlying molecular mechanism of miR-365 in ovarian cancer remains unclear. Therefore, the aims of this study were to investigate the expression of miR-365 in ovarian cancer tissues and cell lines, test its role in ovarian cancer growth and metastasis, and determine its target genes and underlying molecular mechanism in ovarian cancer.

### Materials and methods

#### Patients and tissue samples

A total of 46 ovarian cancer and adjacent normal ovary tissues were collected from The First Affiliated Hospital of Jilin University (Changchun, China) from January 2013 to December 2014. After surgery, all tissue samples were immediately frozen in liquid nitrogen, and stored at -80°C until use. The clinical characteristics of all the patients with ovarian cancer were recorded and were described in **Table 1**. Informed consent was obtained from all patients who participated in the study. This study was approved by the Research Ethics Committee of The First Affiliated Hospital of Jilin University.

#### Cell culture and transfection

Human ovarian carcinoma cell lines (SKOV3, A2780, OVCAR, and HO-8910) and a human

ovarian surface epithelial cell line (HOSEpiC) were purchased from the Tumor Cell Bank of the Chinese Academy of Medical Science (Beijing, China). All cell lines were maintained in RPMI-1640 medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA) and 1% penicillin/streptomycin in a humidified chamber with 5% CO<sub>2</sub> at 37°C. miR-365 mimic or corresponding negative control (miR-Ctrl) were purchased from GenePharma (Shanghai, China). The Wnt5a overexpression plasmid (pCDNA3.1-Wnt5a) was provided by Dr. Dongxu Zhao (China-Japan Union Hospital of Jilin University, Changchun, China). Transfection was performed using Lipofectamine™ 3000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

#### RNA extraction, reverse transcription PCR and quantitative real time-PCR

RNA was isolated from harvested cells or tissues with TRIzol® reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. To measure the expression levels of miR-365, quantitative real time-PCR was performed on the ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) as previously described [13]. Expression of U6 was used as an endogenous control. To determine the mRNA levels of Wnt5a, total RNA was reverse-transcribed using the PrimeScript RT Reagent Kit with oligo dT primer (Takara, Dalian, China). The cDNAs were amplified using SYBR Premix DimerEraser (Takara) on a 7900HT system (Applied Biosystems). GAPDH was used as the internal control. Primers used to amplify Wnt5a and GAPDH have been described previously [21]. The relative expression of miR-365 and Wnt5a was analyzed by the 2<sup>-ΔΔCt</sup> method.

#### Cell proliferation and colony formation assays

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan). Briefly, the transfected cells were seeded into 96-well plates (2 × 10<sup>3</sup> cells/well) and cultured for 24-72 h. After transfection, 10 μl CCK-8 solution was added to each well at the indicated time points (24 h, 48 h and 72 h), and incubated at 37°C for 4 h. The optical density

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value (OD) of each well was measured at 450 nm using a microplate spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT, USA).

For colony formation assay, 1000 transfected cells were seeded in 6-well plates and incubated for 14 days. Then, the medium was refreshed with medium containing 10% FBS once every 3 days at 37°C under 5% CO<sub>2</sub>. After washing 3 times with PBS, the colonies were fixed with 75% ethanol, dried, and stained with 0.1% crystal violet solution (Sigma, St. Louis, MO, USA) for 10 min. Finally, the colony formation was observed and counted using a light microscope (Olympus, Tokyo, Japan).

### *Cell migration and invasion assays*

Wound-healing assay was performed to measure cell migration. Briefly, transfected cells were plated into 6-well plates at a density of  $2 \times 10^5$  cells per well. A sterile plastic tip was used to scratch the cell layer when the cells reached 90% confluency. After wounding, the medium was changed to fresh serum-free RPMI-1640 medium to remove cellular debris. Photographic images were taken at different time points (0 h and 24 h) with a light microscope (Olympus, Tokyo, Japan).

A 24-well Boyden chamber with 8 µm pore size polycarbonate membrane (Corning, Corning, NY, USA) was used to evaluate cell invasion. Transfected cells ( $1 \times 10^5$ ) were seeded on transwell chambers with Matrigel in 200 µl serum-free medium. An aliquot of 600 µl medium with 10% FBS was added to the lower chamber as a chemoattractant. After 24 h of incubation at 37°C with 5% CO<sub>2</sub>, cells on the upper surface of the membrane were removed by cotton swabs, whereas cells on the lower surface of the inserts were stained with 0.1% crystal violet. The number of cells was counted at five randomly selected fields under a light microscope (Olympus).

### *Luciferase reporter assay*

Wnt5a was identified as a miR-365 target in TargetScan 7.1 software ([http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)). Wild-type (Wt) human Wnt5a 3'-UTR and mutated (Mut) Wnt5a 3'-UTR (with mutant sequence on the miR-365 binding site) were amplified by PCR from a human ovarian cDNA library and inserted into the pGL3-

control vector (Ambion, Austin, TX, USA) at the *NheI* and *XhoI* restriction sites. For luciferase assays,  $1 \times 10^5$  cells were plated in 24-well plates and cultured for 24 h. Cells were then co-transfected with 100 nM of miR-365 mimic or miR-Ctrl and 100 ng wild-type or mutant-type Wnt5a-3'UTR reporter plasmid using Lipofectamine™ 3000 Reagent (Invitrogen) according to the manufacturer's protocol. The relative luciferase activity was calculated after 48 h by normalizing Firefly luminescence to that of *Renilla* using the Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA).

### *Western blot analysis*

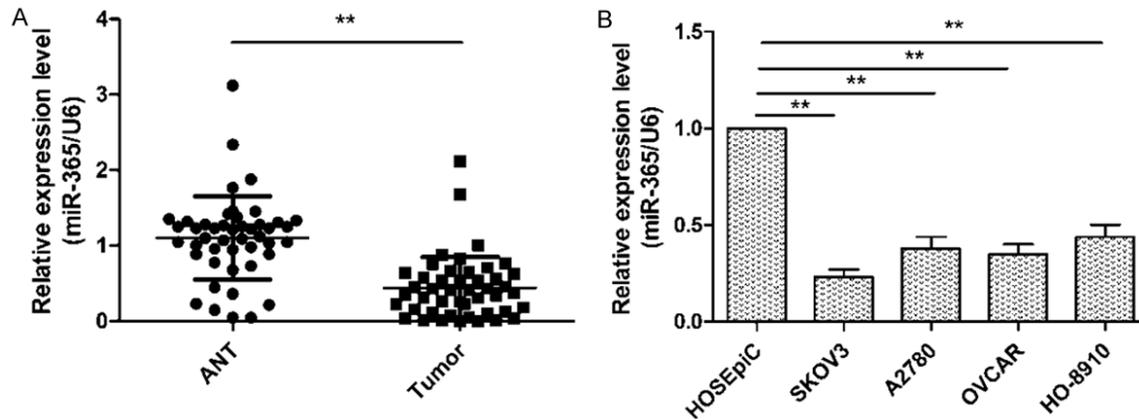
Tissues or cells were lysed using ice-cold lysis buffer (Beyotime, Shanghai, China), and were centrifuged at 4°C. Subsequently, proteins in the supernatants were quantified by the bicinchoninic acid (BCA) kit (Pierce, Rockford, IL, USA). For western blot analysis, 20 µg of protein was fractionated by 10% SDS-PAGE, and the proteins were blotted onto nitrocellulose membranes (Amersham BioSciences, Buckinghamshire, UK). The membranes were blocked with 10% nonfat milk in PBS for 1 h and immunoblotted with antibodies against Wnt5a (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and GAPDH (Santa Cruz Biotechnology) in PBS overnight at 4°C. Then, the membranes were immunoblotted by HRP-linked secondary antibodies (Santa Cruz Biotechnology) at room temperature for 1 h. The protein bands were detected by the SuperSignal West Pico Chemiluminescent Substrate kit (Pierce, Rockford, IL, USA).

### *Animal model*

Six-week-old female nude BALB/c mice (18-25 g) were purchased from the Laboratory Animal Center of Jilin University. All mice were housed in specific pathogen-free conditions (SPF) in accordance with the guidelines set forth by the Ethics Committee of Jilin University. All protocols were approved by the Animal Care and Use Committee and by the local ethics committee of Jilin University.

To produce experimental tumor formation, SKOV3 cells ( $5 \times 10^6$ ) transfected with miR-365 mimic or miR-Ctrl were harvested, washed and resuspended in serum-free RPMI-1640 medium, and then injected subcutaneously into the left flanks of nude mice. Tumor growth was

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**Figure 1.** miR-365 expression was downregulated in ovarian cancer tissues and cell lines. A. The expression levels of miR-365 were detected in 46 ovarian cancer samples and adjacent normal tissues (ANT) by qPCR. B. The expression of miR-365 was determined in four ovarian cancer cell lines (SKOV3, A2780, OVCAR, and HO-8910) and human ovarian surface epithelial cell line (HOSEpiC) by qPCR. \* $P < 0.05$ , \*\* $P < 0.01$ .

monitored by measuring the tumor width and length every 7 days using a Vernier caliper, using the formula: Volume ( $\text{mm}^3$ ) =  $1/2 \times \text{width}^2 \times \text{length}$ . Five weeks after inoculation, mice were euthanized with subcutaneous injection of sodium pentobarbital (50 mg/kg), and the tumor tissues were removed and weighted. Tumor tissues were snap-frozen and stored in liquid nitrogen for detection of miR-365 and Wnt5a expression.

### Statistical analysis

All data are presented as the mean  $\pm$  standard deviation (SD) from at least 3 separate experiments. Student's t-test was used to calculate the differences when comparing only two groups. A one-way analysis of variance (ANOVA) was used when comparing more than two groups. Association of miR-365 expression and Wnt5a expression was estimated using Pearson's correlation analysis. SPSS 19.0 (SPSS Inc., USA) was used to analyze all statistics. In all cases, a  $P < 0.05$  was considered statistically significant.

### Results

#### *miR-365 is downregulated in ovarian cancer tissues and cell lines*

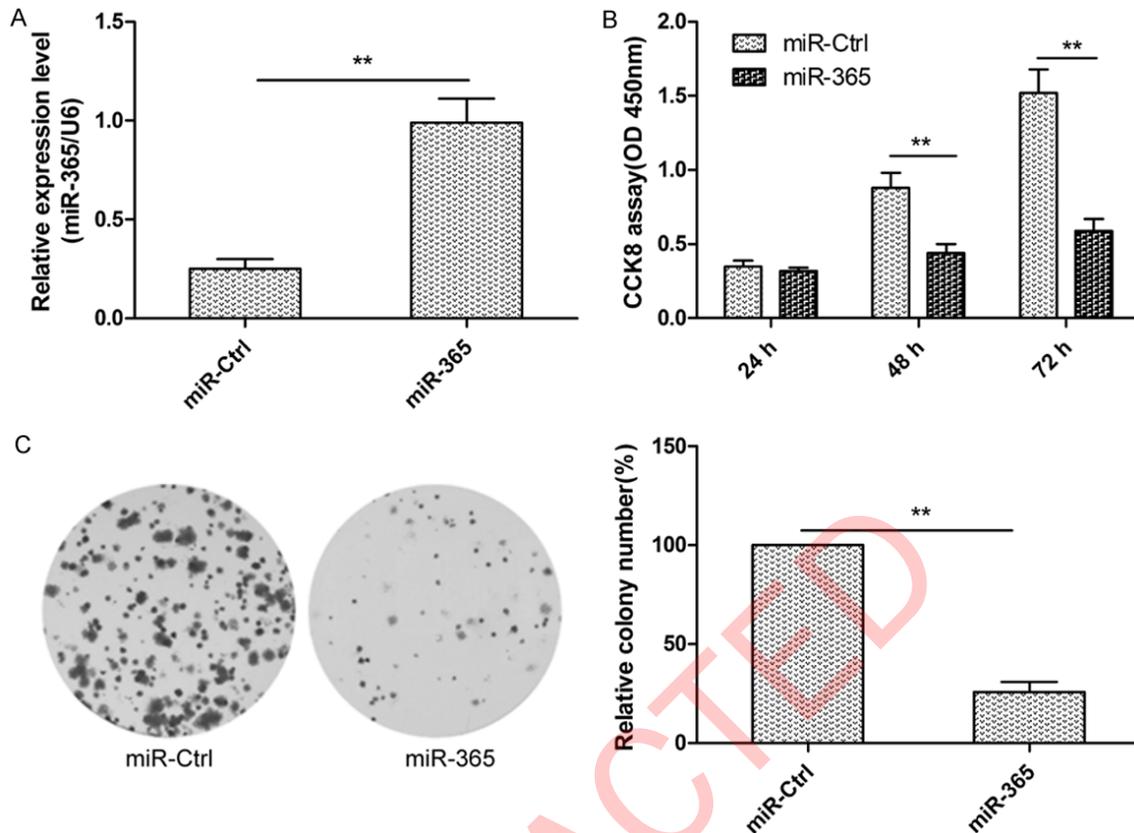
Expression levels of miR-365 were determined in 46 pairs of ovarian cancer tissue and paired adjacent non-tumor tissues (ANT) by qPCR. As presented in **Figure 1A**, expression of miR-365

in ovarian cancer tissues was significantly lower than that in adjacent non-tumor tissues ( $P < 0.01$ ). To investigate the potential associations between miR-365 expression and patients' clinicopathological variables, we divided the patients with ovarian cancer into two groups based on mean value (0.441) of miR-365 expression: high expression group ( $> 0.441$ ;  $n = 22$ ), and low expression group ( $< 0.441$ ,  $n = 24$ ). Statistical analysis showed that low miR-365 expression significantly correlated with FIGO stage, histological grading, and lymph node metastasis, whereas no statistical difference was found in the correlation of miR-365 expression with age and tumor size (**Table 1**). Furthermore, expression levels of miR-365 were determined in four ovarian cancer cell lines (SKOV3, A2780, OVCAR and HO-8910), with the human ovarian surface epithelial cell line (HOSEpiC) serving as a control. Expression levels of miR-365 were significantly less in all the ovarian cancer cell lines compared to HOSEpiC (**Figure 1B**), especially SKOV3, which showed the lowest miR-365 levels (**Figure 1B**), and was therefore selected for subsequent studies.

#### *miR-365 inhibits ovarian cancer cell proliferation and colony formation*

To examine the effect of miR-365 on ovarian cancer growth, SKOV3 cells were transfected with either a miR-365 mimic or miR-Ctrl. Increased expression of miR-365 in the SKOV3 cells transfected with the miR-365 mimic was

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**Figure 2.** miR-365 inhibits ovarian cancer cell proliferation and colony formation. A. The expression of miR-365 was detected in SKOV3 cells transfected with miR-365 mimic and miR-Ctrl. B. Cell proliferation was determined in SKOV3 cells transfected with miR-365 mimic and miR-Ctrl by CCK8 assay. C. Cell colony formation was determined in SKOV3 cells transfected with miR-365 mimic and miR-Ctrl by colony formation assay. \* $P < 0.05$ , \*\* $P < 0.01$ .

confirmed by qPCR (Figure 2A). The CCK8 assay was then performed on cells transfected with miR-365 or miR-Ctrl, and cells transfected with the miR-365 mimic demonstrated significantly reduced cell proliferation compared to cells transfected with the miR-Ctrl ( $P < 0.01$ ) (Figure 2B). The colony formation assay was also performed to assess proliferative capacity. Again, SKOV3 cells transfected with the miR-365 mimic exhibited significantly lower rates of colony formation than those of cells transfected with the miR-Ctrl (Figure 2C). These results suggest that miR-365 could suppress ovarian cancer cell growth *in vitro*.

### *miR-365 inhibits ovarian cancer cell migration and invasion*

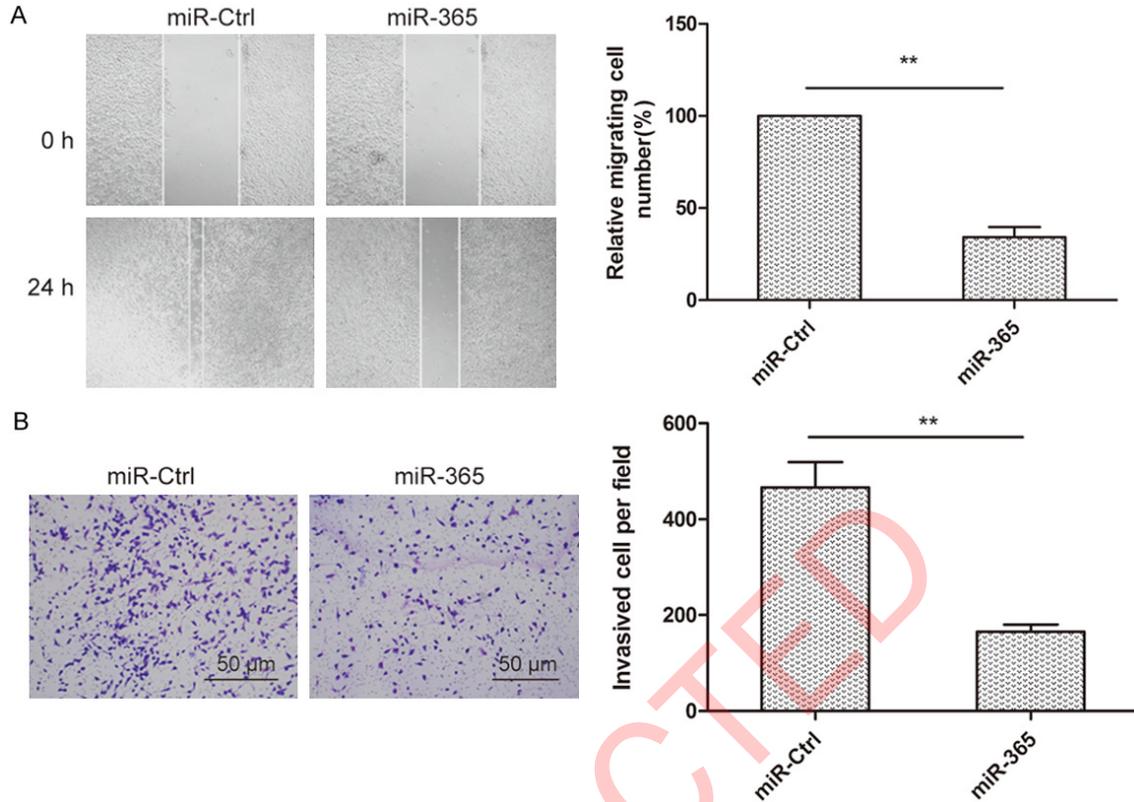
Cancer metastasis is the primary cause of cancer-associated death and is directly related to its migratory and invasive capacity; therefore, we investigated the effects of miR-365 on the

migration and invasion abilities of ovarian cancer cells by wound healing and transwell invasion assays, respectively. We found that SKOV3 cells that were transfected with the miR-365 mimic exhibited significantly reduced rates of migratory and invasive capacity compared to cells transfected with miR-Ctrl (Figure 3A and 3B).

### *Wnt5a is a direct target of miR-365 in ovarian cancer cells*

To explore the molecular mechanisms by which miR-365 executes its biological function, we used bioinformatic prediction software (TargetScan) to determine the potential target of miR-365. Wnt5a, an oncogene associated with several cancers, was identified as a direct and functional target of miR-365, as shown in Figure 4A. To further confirm targeting of Wnt5a by miR-365, luciferase activity assay was performed. Our results demonstrate that miR-365

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**Figure 3.** miR-365 inhibits ovarian cancer cell migration and invasion. A. Cell migration activity was measured by wound healing assay in SKOV3 cells transfected with miR-365 mimic and miR-Ctrl. B. Cell invasion activity was measured by transwell assay in SKOV3 cells transfected with miR-365 mimic and miR-Ctrl. \* $P < 0.05$ , \*\* $P < 0.01$ .

dramatically inhibited the luciferase activity of the wild-type (Wt) 3'UTR but not that of the mutant-type (Mut) 3'UTR of Wnt5a (**Figure 4B**). Subsequently, we examined the Wnt5a expression level in SKOV3 cells transfected with miR-365 or miR-Ctrl, and found that Wnt5a was downregulated, at both the mRNA and protein level, in SKOV3 cells transfected with the miR-365 as compared to cells transfected with the miR-Ctrl (**Figure 4C and 4D**). These results suggest that Wnt5a might be a target of miR-365 in ovarian cancer cells.

*Wnt5a was upregulated and negatively correlated with miR-365 in ovarian cancer tissues*

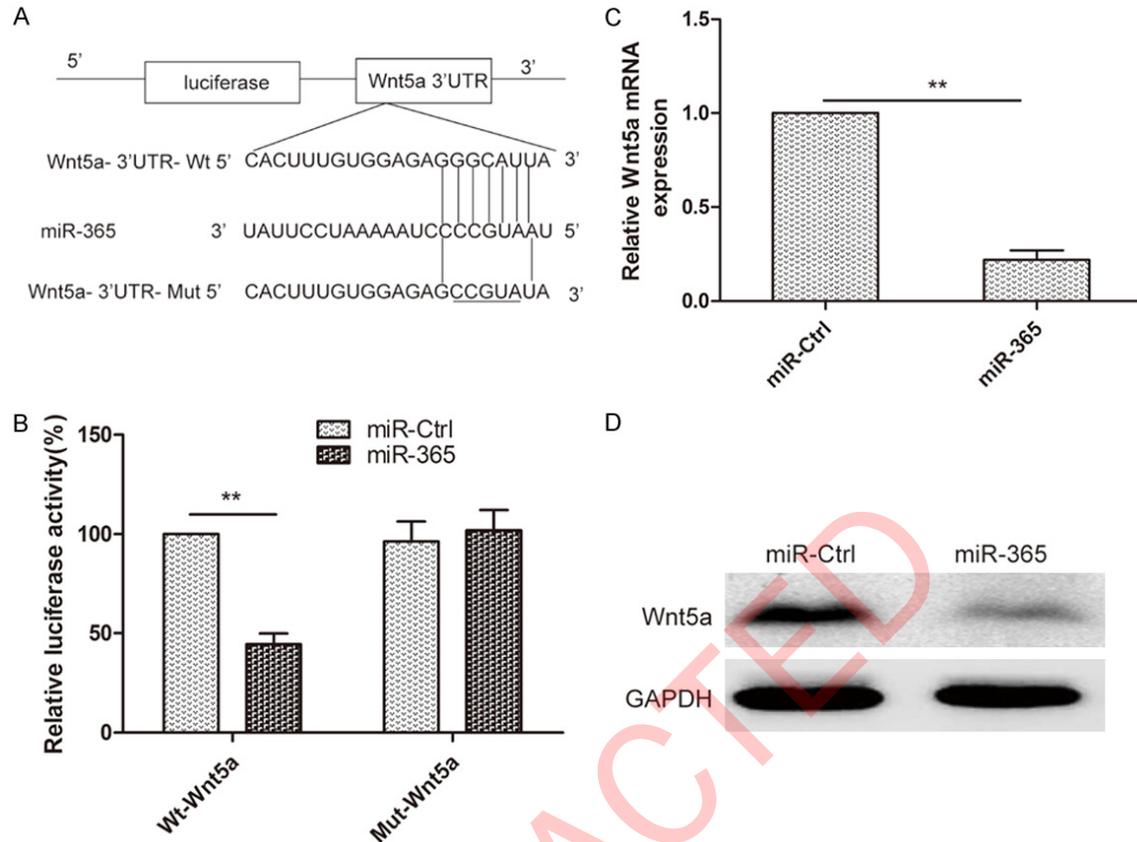
To further explore the relationship between miR-365 and Wnt5a, we examined the expression Wnt5a mRNA in ovarian cancer tissues and adjacent ovarian cancer tissues by qRT-PCR. As shown in **Figure 5A**, ovarian cancer tissues had significantly higher levels of Wnt5a mRNA than those of adjacent non-tumor tissues. Furthermore, Pearson correlation analy-

sis demonstrated an inverse relationship between Wnt5a mRNA levels and miR-365 levels in ovarian cancer tissues ( $r = -0.638$ ,  $P < 0.0001$ ) (**Figure 5B**). We also found that both Wnt5a mRNA and protein levels were significantly upregulated in four ovarian cancer cell lines compared to normal ovary cells (**Figure 5C and 5D**).

*Expression of Wnt5a could partially reverse the inhibition effects induced by miR-365*

Having confirmed that miR-365 acts to reduce Wnt5a levels in ovarian cancer cells, we then questioned whether this action of miR-365 is responsible for its tumorigenic effects. We therefore restored Wnt5a expression in SKOV3 cells that overexpressed miR-365 by transfection with a Wnt5a overexpression plasmid (**Figure 6A and 6B**). We found that restoration of the expression of Wnt5a could effectively reverse the inhibition effect on cell proliferation, colony formation, migration, and invasion induced by miR-365 overexpression in SKOV3

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**Figure 4.** Wnt5a is a direct target of miR-365 in ovarian cancer. A. The putative miR-365-binding sites and mutant (Mut) 3'-UTR Wnt5a sites are shown. The replaced site is underlined. Wt: wide-type; Mut: mutant-type. B. Relative luciferase activity was measured in SKOV3 cells cotransfected with wide-type-Wnt5a-3'UTR or mutant-type-Wnt5a-3'UTR reporter plasmid and miR-365 mimic or miR-Ctrl. C, D. Wnt5a mRNA and protein expression was measured in SKOV3 cells transfected with miR-365 mimic and miR-Ctrl. GAPDH was used as an internal control.

cells (**Figure 6C-F**). These findings suggest that Wnt5a is responsible for the tumorigenic effects in ovarian cancer cells, and reduction of Wnt5a via miR-365 inhibits these effects.

### miR-365 suppressed tumor growth *in vivo*

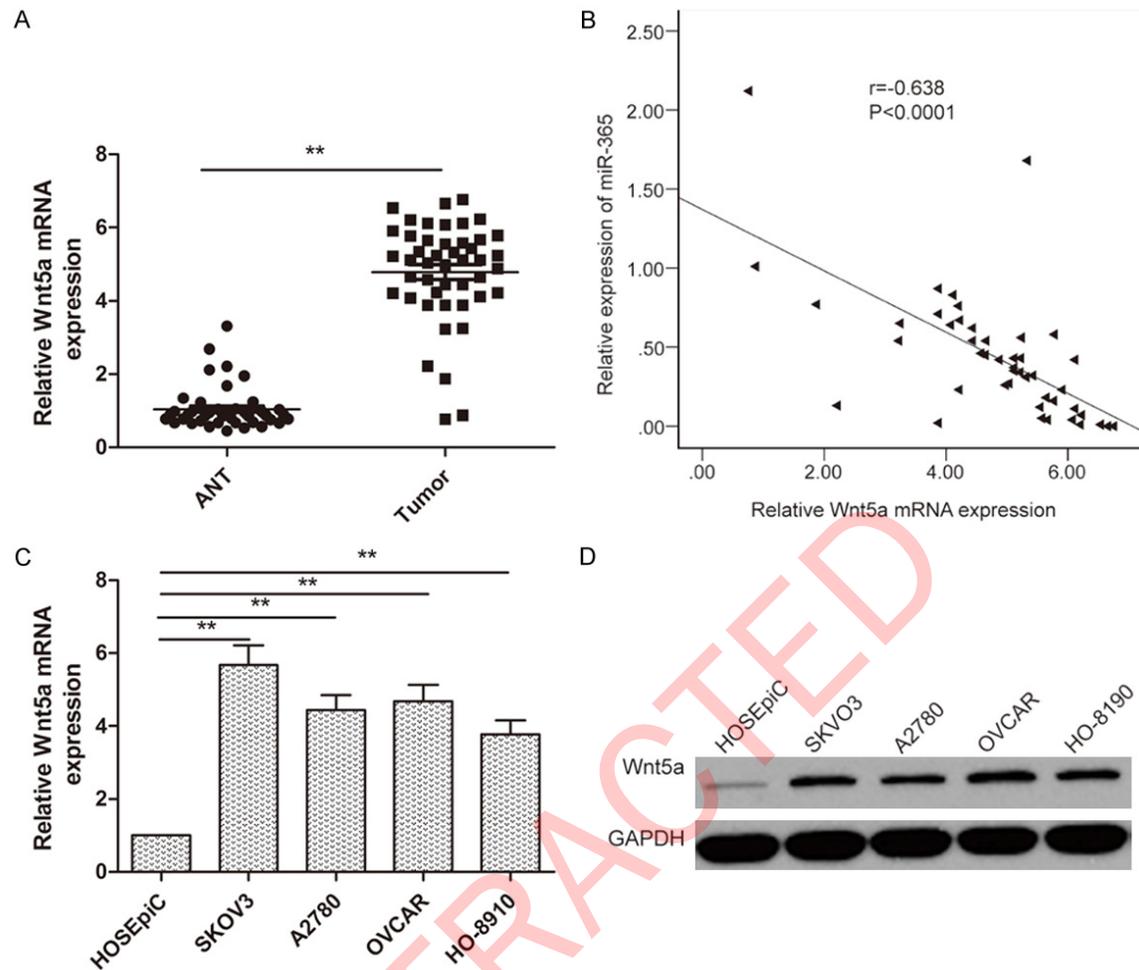
To determine the effects of miR-365 on tumorigenicity *in vivo*, the SKOV3 cells overexpressing either miR-365 or miR-Ctrl were subcutaneously injected into the flanks of nude mice. It was found that tumor growth was slower in the SKOV3/miR-365 group than that of the SKOV3/miR-Ctrl group (**Figure 7A**). Consistent with the tumor growth curve, the size and weight of tumors from the SKOV3/miR-365 group were significantly decreased compared to tumors from the SKOV3/miR-Ctrl group (**Figure 7B and 7C**). In addition, we detected miR-365 and Wnt5a expression in tumor tissues, and found that the miR-365 expression level was upregu-

lated (**Figure 7D**), while Wnt5a expression was downregulated in SKOV3/miR-365 compared to SKOV3/miR-Ctrl (**Figure 7E and 7F**). These data suggest that miR-365 suppresses ovarian cancer growth *in vivo* by repressing Wnt5a.

### Discussion

A number of miRNAs have been suggested to be involved in ovarian cancer progression and development through regulation of cell proliferation, migration, apoptosis, and invasion [10, 11]. For example, Li *et al.* reported that miR-143 inhibited ovarian cancer cell proliferation, migration, and invasion by suppressing CTGF expression [22]. Wen *et al.* demonstrated that miR-338-3p significantly inhibited ovarian cancer cell proliferation, colony formation, migration and invasion, and induced cell apoptosis and enhanced caspase-3, -8, and -9 activities, as well as tumor growth *in vivo* by targeting

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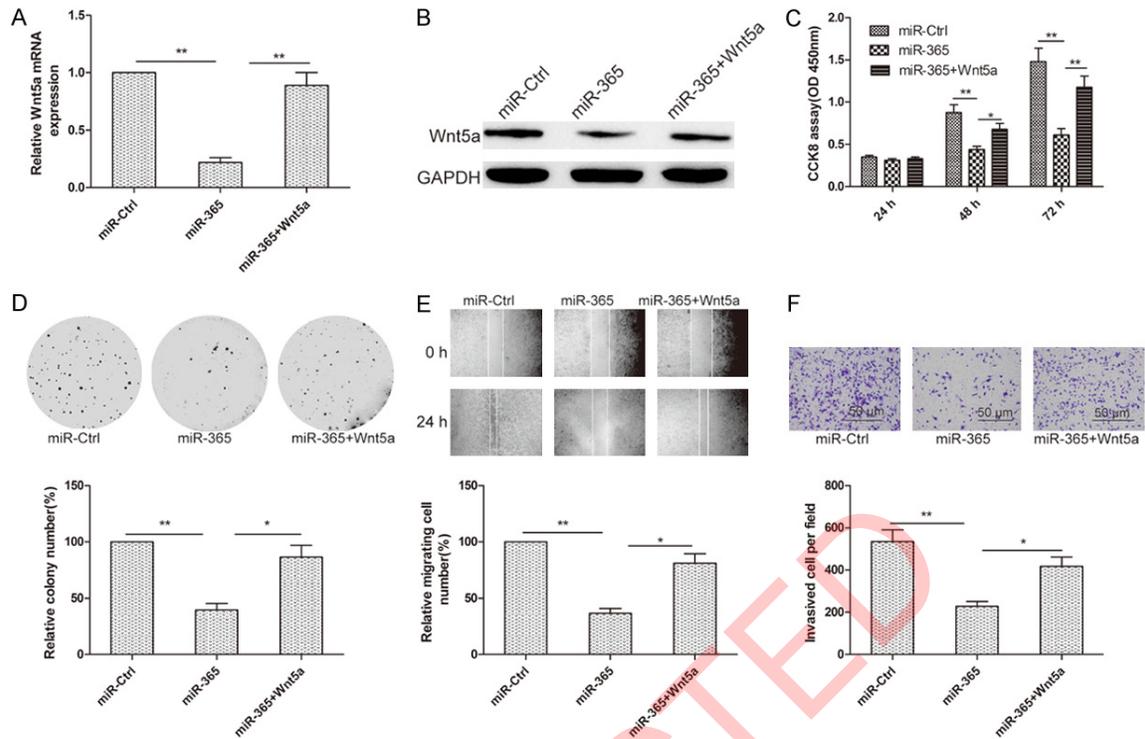


**Figure 5.** Wnt5a was upregulated and inversely correlated with miR-365 expression in ovarian cancer tissues. A. The Wnt5a mRNA levels in 46 ovarian cancer samples and adjacent normal tissues (ANT) were determined by qPCR. GAPDH was used as an internal control. B. The correlation of the expression levels of Wnt5a and miR-365 was analyzed in ovarian cancer tissues by Pearson's correlation assay ( $n = 36$ ). C, D. Wnt5a mRNA and protein expression was determined in four ovarian cancer cell lines (SKOV3, A2780, OVCAR, and HO-8910) and human ovarian surface epithelial cell line (HOSEpiC) by qPCR. \* $P < 0.05$ , \*\* $P < 0.01$ .

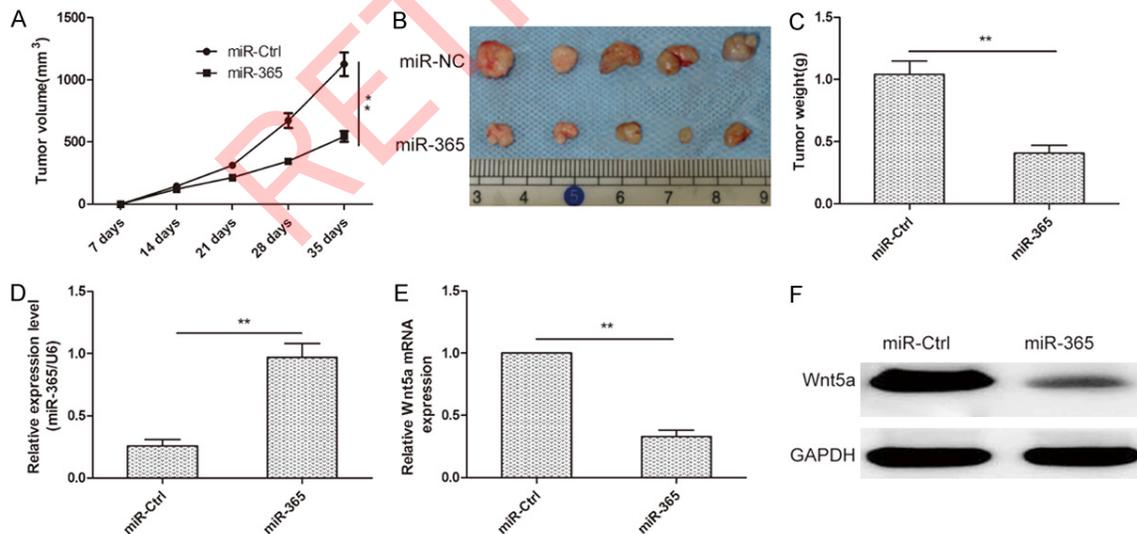
Runx2 [23]. Li *et al.* found that miR-494 could remarkably inhibit ovarian cancer cell proliferation, colony formation, migration, and invasion, as well as induce cell apoptosis and G0/G1 phase arrest, and suppress tumor growth in a nude mouse xenograft model system by targeting IGF1R [24]. In the present study, we found that miR-365 was significantly decreased in ovarian cancer cell lines and tissues, and its expression was negatively correlated with advanced FIGO stage, histological grading, and lymph node metastasis. Further studies revealed that miR-365 suppressed ovarian cancer growth *in vitro* and *in vivo* by targeting Wnt5a. These data suggest that miR-365 might serve as a novel biomarker or therapeutic target for ovarian cancer.

miR-365 has been reported to be involved in multiple biological processes such as proliferation, invasion, apoptosis, differentiation, and cell cycle distribution [12-20, 25]. miR-365 expression was upregulated in several types of cancers such as gastric cancer [16], cutaneous squamous cell carcinoma [14, 15], and pancreatic cancer [17], suggesting it acts as an oncogene in these cancers. In contrast, in lung cancer [12, 13], liver cancer [26], melanoma [19], breast cancer [18], and colon cancer [20], miR-365 expression was downregulated and functioned as a tumor suppressor. These findings suggest that the functional role of miR-365 in different cancers may be paradoxical. However, the exact roles and mechanisms of miR-365 remain unknown in human ovarian cancer. This

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**Figure 6.** Expression of Wnt5a partially reverses the inhibition effects induced by miR-365 in ovarian cancer cells. A, B. Wnt5a mRNA and protein expression was determined in SKOV3 cells transfected with miR-365 or miR-Ctrl and with or without Wnt5a overexpression plasmid by qPCR and western blot, respectively. GAPDH was used as an internal control. C-F. Cell proliferation, colony formation, migration, and invasion were determined in SKOV3 cells transfected with miR-365 or miR-Ctrl and with or without Wnt5a overexpression plasmid. \* $P < 0.05$ , \*\* $P < 0.01$ .



**Figure 7.** miR-365 retards the tumor growth of xenografts in nude mice. A. The curve of tumor growth. B. Photographs of xenograft tumors. C. Average tumor weight. D. The expression levels of miR-365 were detected in tumor tissues by qPCR. E, F. Wnt5a mRNA and protein expression was measured in tumor tissues by qPCR and western blot, respectively. GAPDH was used as an internal control. \* $P < 0.05$ , \*\* $P < 0.01$ .

study is the first report that miR-365 expression level was downregulated in ovarian cancer tissues and cell lines compared to adjacent non-tumor tissues and normal ovarian cells, and that miR-365 overexpression inhibited ovarian cancer cell proliferation, colony formation, migration, and invasion *in vitro*, as well as suppressing tumor growth *in vivo*. These results imply that miR-365 functions as tumor suppressor in ovarian cancer.

Wnt5a, a member of the embryonic signaling pathway Wingless (Wnt), has been reported to play critical roles in postnatal cellular functions, as well as development through Wnt signal pathways [27]. Growing evidence suggests that Wnt5a is involved in diverse pathogenesis, including cancers, metabolic diseases, and inflammatory diseases, through multiple Wnt signaling receptors [27-29]. Altered Wnt5a expression has been linked to multiple cancers, including breast, gastric, colorectal, lung, and prostate cancer [30]. In ovarian cancer, Wnt5a expression was found to be upregulated in all major subtypes (serous, endometrioid, clear cell, and mucinous) of ovarian cancer compared to borderline tumors and benign controls [31]. In addition, Wnt5a could enhance the vasculogenic capacity, motility, and invasiveness of ovarian cancer cells by regulating the epithelial-mesenchymal transition (EMT) [32]. These results suggest that Wnt5a served as oncogene in ovarian cancer cells. Here, luciferase reporter assay analysis revealed that miR-365 downregulated Wnt5a expression by directly binding to the 3'-UTR of Wnt5a mRNA. Further studies showed that overexpression of miR-365 decreased both mRNA and protein Wnt5a expression in ovarian cancer cells. These results suggest that Wnt5a was a direct target of miR-365 in ovarian cancer. In addition, our results show that the expression level of Wnt5a was significantly upregulated in ovarian cancer tissues, and its expression was inversely correlated with miR-365 expression in ovarian cancer tissues. Overexpression of Wnt5a partially abrogated the suppression effect on cell proliferation, migration, and invasion induced by miR-365 in ovarian cancer cells. *In vivo* studies also revealed that miR-365 inhibited tumor growth of nude mice by repressing Wnt5a. These data suggest that miR-365 exerted its suppressive role in ovarian cancer by targeting Wnt5a.

In summary, the results presented here are the first to demonstrate that miR-365 expression level was decreased in ovarian cancer tissues and cell lines, and that decreased miR-365 expression was negatively correlated with advanced FIGO stage, histological grading, and lymph node metastasis. Our findings also suggest that miR-365 impaired ovarian cancer cell proliferation, colony formation, migration, and invasion *in vitro*, as well as retarding tumor growth *in vivo* by directly targeting Wnt5a. These results suggest that miR-365 might be developed as a new therapeutic target in ovarian cancer.

### Acknowledgements

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Xiaomeng Zhang, Department of Ophthalmology, The Second Hospital of Jilin University, Changchun 130041, China. E-mail: zhangxm15106@sina.com

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