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miR-532-5p is a prognostic marker and suppresses cells proliferation and invasion by targeting TWIST1 in epithelial ovarian cancer

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Abstract. – OBJECTIVE: Dysregulated miR-532-5p has been observed in epithelial ovarian cancer (EOC). However, the potential biological function and clinical significance have not been fully explained. The study aimed to investigate the prognostic value and potential role of miR-532-5p in EOC.

PATIENTS AND METHODS: MiR-532-5p and Twist homolog 1 (TWIST1) mRNA expression were examined using quantitative real-time PCR. The correlation of miR-532-5p expression with clinicopathological factors was statistically analyzed. Kaplan-Meier analysis and Cox proportional hazards regression models were explored to reveal the correlations of miR-532-5p expression with survival of patients. Cell Counting Kit-8, colony formation and transwell invasion assays were performed to evaluate the effects of miR-532-5p on cell proliferation and invasion, respectively. MiR-532-5p target genes were confirmed using luciferase activity, RT-PCR and Western blot assays.

RESULTS: We found that miR-532-5p was significantly decreased in EOC tissue and cell lines, and its expression levels were highly correlated with grade ($p = 0.011$), FIGO stage ($p = 0.004$) and distant metastasis ($p = 0.008$). In addition, overall patient survival for those with high miR-532-5p expression was significantly longer than those patients with low miR-532-5p expression ($p = 0.0058$). Multivariate regression analysis identified miR-532-5p down-regulation as an independent unfavorable prognostic factor in EOC patients. Function assays showed that overexpression of miR-532-5p inhibited proliferation, colony formation and invasion of EOC cells. Mechanistic investigations confirmed TWIST1 as a direct target of miR-532-5p. Further *in vitro* assay indicated that restored expression of TWIST1 dampened miR-532-5p-mediated suppression of tumor progression.

CONCLUSIONS: Our data suggested that miR-532-5p may act not only as a novel prognostic marker, but also as a potential target for molecular therapy of EOC.

Key Words:
MiR-532-5p, Epithelial ovarian cancer, Tumor suppressor, Prognosis.

Introduction

Ovarian cancer is the sixth common cancer in women around the world and is the leading cause of morbidity among malignant gynecologic diseases[1]. Epithelial ovarian carcinoma (EOC) is the most common form of ovarian cancer[2]. In China, more than 50000 new cases of this disease are diagnosed each year[3]. Despite advances in surgery and chemotherapy over the last few decades, 80% of patients diagnosed with advanced EOC relapse and only 30% of patients survive for 5 years after diagnosis[4]. The 5-year survival of the EOC patients diagnosed at FIGO I stage was around 90 %. Unfortunately, most EOC patients are diagnosed with advanced disease[5]. Therefore, major efforts have focused on the identification of the diagnostic and prognostic potential of specific and sensitive biomarkers in EOC.

MicroRNAs are small (18-25nt), single stranded, noncoding RNAs that play important regulatory roles in the post-transcriptional level of gene expression by binding to the 3'UTR sequence of their target mRNA, thus leading to mRNA degradation or translational repression[6]. More and more studies have demonstrated that miRNAs are

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involved in various cellular activities, including proliferation, apoptosis, metastasis and differentiation[7]. Recently, aberrant miRNAs expressions are frequently observed in cancers that serve as oncogenes or tumor suppressors[8,9]. In addition, several studies reported that aberrant miRNAs expression might be of potential use as a diagnostic and prognostic biomarker for human cancer, including EOC[10,11]. MiR-532-5p, located at human chromosome Xp11.23, has been reported to be dysregulated in several tumors, including EOC[12-14]. However, the clinical significance of miR-532-5p and its related molecular mechanisms involved in the progression of EOC remain poorly investigated.

In this work, we detected the expression levels of miR-532-5p in both EOC tissues and cell lines. Then, the association of miR-532-5p with clinicopathological factors or EOC patient prognosis was also statistically analyzed. Moreover, we performed gain-function assay to explore the role of miR-532-5p in EOC cells proliferation and invasion. In addition, we performed several cells experiments to explore the potential mechanism by which miR-532-5p influenced the cells behavior of EOC. Our findings suggest miR-532-5p as potential therapeutic candidates and prognostic biomarker for EOC.

Patients and Methods

Patient Specimens

Paired tissue specimens (tumor and adjacent normal tissues) from 145 patients with EOC were obtained and histologically confirmed by a pathologist at Linyi People's Hospital from January 2011 to December 2013. None of the patients had received chemotherapy, radiotherapy or adjuvant hormonal therapy prior to surgery. Specimens were preserved in liquid nitrogen immediately and stored at -80°C until RNA extraction. All patients from cohort 2 were taken on 5-year follow-up. Overall survival was defined as the date of surgery until the date of death. The clinical information is summarized in Table I. Tissue sample use was approved by the Ethics Committees of our hospital and written informed consent was obtained from all study participants.

Cell Culture

Human EOC (OVCAR3, SKOV3, ES-2 and CAOV-3) cell lines were primary purchased from Shanghai Institute of Biochemistry and Cell Biology (Xuhui, Shanghai, China). Human normal ovarian epithelial NOEC cell lines were purchased from

Table I. Relationship between miR-532-5p expression and clinicopathological variables in EOC patients.

Variable	Number	miR-532-5p expression		*p*-value
		High	Low	
Age (y)				0.574
<50	76	35	41	
≥50	69	35	34	
Tumor size (cm)				0.202
≤ 10	68	29	39	
> 10	77	41	36	
CA125 level (U/ml)				0.216
< 600	92	48	44	
≥ 600	53	22	31	
Ascites				0.671
< 100	74	37	37	
≥ 100	71	33	38	
FIGO stage				0.004
I + II	84	49	35	
III + IV	61	21	40	
Grade				0.011
G1	88	50	38	
G2 + G3	57	20	37	
Distant metastasis				0.008
Yes	65	23	42	
No	82	47	35	

Table II. The primers for PCR.

Primer name	Sequences (From 5′ to 3′)
miR-532-5p Fwd	CGGCCATGCCTTGAGTGTA
miR-532-5p Rev	GCAGGGTCCGAGGTATTC
TWIST1 Fwd	CCAGGTACATCGACTTCCTCTA
TWIST1 Rev	CCATCCTCCAGACCGAGAA
GAPDH Fwd	CCAGGTGGTCTCCTCTGA
GAPDH Rev	GCTGTAGCCAAATCGTTGT

the American Type Culture Collection (Manassas, VA, USA). All cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco, Jianglin Technology, Pudong, Shanghai, China), 100 U/ml penicillin (Meilunbio®, Dalian, Niaoning, China) and 100 μg/ml streptomycin (Haoran Technology, Yuzhong, Chongqing, China) at 37°C in a humidified atmosphere containing 5% CO_2.

Plasmid Construction And Cell Transfection

MiR-532-5p mimics and its negative control were purchased from RiboBio (Guandong, Guangzhou, China). For TWIST1 over-expression, the open reading frame of TWIST1 was inserted into the pcDNA3.1 vector to generate TWIST1 overexpression vectors. Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) were used to transfect the cells once they reached 60% confluency. Transfection efficiencies were detected by qRT-PCR after 48 h of transfection.

RNA Isolation And Quantitative Real-Time Reverse Transcription-PCR (qRT-PCR)

Total RNA from tissues and cell lines was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Complimentary DNA (cDNA) was synthesized using the PrimeScript II First Strand cDNA Synthesis kit (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. The qPCR was performed by using the miRNA-specific TaqMan MiRNA Assay Kit (Biosystems, Foster City, CA, USA) under 7900 Real-Time PCR System (Biosystems, Foster City, CA, USA). The reaction was performed in triplicate for 30 min at 16°C, 30min at 42°C, and 5 min at 85°C. TWIST1 mRNA expression was measured by RT of total RNA into complementary DNA using M-MLV Reverse Transcriptase (Promega Corporation, Madison, WI, USA). For both TWIST1 mRNA and miR-532-5p detection, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The relative expression of TWIST1 mRNA and miR-532-5p was calculated by the $2^{-\Delta\Delta T}$ method. The primer sequences used in this study were shown in Table II.

Cell Proliferation Assay

The *in vitro* cell proliferation of EOC cells was measured using the Cell Counting Kit-8 (CCK8) assay. In brief, OVCAR3 and ES-2 cells were seeded into 96-well plates (3×10^4/well) and incubated in RPMI 1640 at 37°C and 5% CO_2 atmosphere for 96 hours. The cell viability assay was performed using Cell Counting Kit-8 (CCK8; Dojindo, Pudong, Shanghai, China) according to the manufacturer's protocol. Absorbance levels were measured at the wavelength of 490 nm using an automatic microplate reader (Gene, Haidian, Beijing, China). All experiments were performed at least three times in triplicate.

Colony Formation Assay

Approximately 3×10^4 cells were seeded per well for six-well plates and were grown for 10 days with normal medium. The cells were then fixed and staining by crystal violet. All experiments were performed at least three times in triplicate.

Invasion Assay

1×10^5 cells in serum-free medium were placed into the upper chamber of an insert coated with Matrigel (Sigma-Aldrich, St. Louis, MO, USA). The chambers were then incubated in culture medium supplemented with 10% FBS (Gibco, Jianglin Technology, Pudong, Shanghai, China) in the bottom chambers for 24 h before examination. Following incubation, cells on the upper surface of the membrane were scraped off and the invasive cells to the bottom of the membrane were fixed and stained. Then, the number of invaded cells was quantified using ImageJ (National Institutes of Health, Bethesda, MD, USA)

Luciferase Reporter Assays

A fragment of the TWIST1 3'-UTR and a mutated 3'-UTR of TWIST1 that contained the putative miR-532-5p binding sites were prepared to construct reporter plasmids containing the 3'-UTR regions of TWIST1. DNA fragments were cloned into subcloned into the pmirGLO-control. OVCAR3 and ES-2 cells were seeded at a density of 4000 cells in a 96-well plate. The next day, OVCAR3 and ES-2 cells were co-transfected with

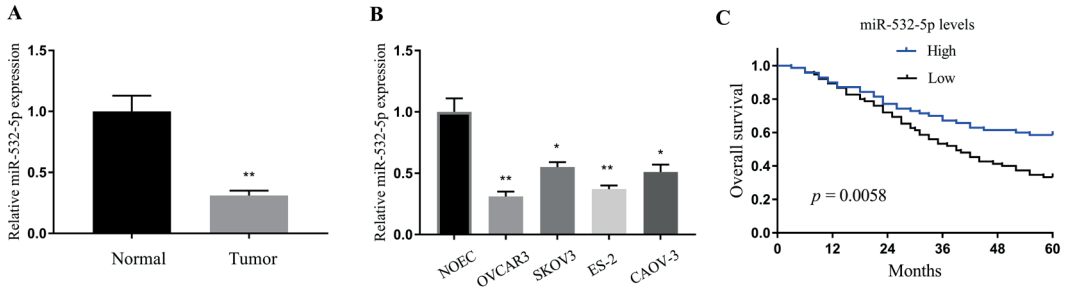

Figure 1. Relative miR-532-5p expression in EOC tissues and its clinical significance. (***A)*** Tumor tissues and adjacent normal tissues were extracted from EOC patients. The expression of miR-532-5p was compared by qRT-PCR. ***(B)*** miR-532-5p expression was downregulated in four EOC cell lines (OVCAR3, SKOV3, ES-2 and CAOV-3) compared to human normal ovarian epithelial NOEC cell line. ***(C)*** Kaplan-Meier overall survival curves according to miR-532-5p expression level. The overall survival of the Low-miR-532-5p group was significantly lower than that of high- miR-532-5p group ($p = 0.0058$). $^{**}p < 0.01$.

wild-type (mutant) reporter plasmid and miR-532-5p mimics or Ctrl using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). At 48 h post-transfection, the relative luciferase activities were evaluated using Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Western Blot Assay

Total protein was collected using RIPA buffer (ThermoFisher Scientific, Waltham, MA, USA). Then the protein concentration was determined using the BCA Protein Assay kit (Thermo Fisher Scientific, Inc. Waltham, MA, USA). 20 mg of protein were separated by dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline and Tween 20 (TBST) for 1 h, and then incubated with anti-TWIST1 (1:500, Sigma-Aldrich, Xicheng, Beijing, China) or anti-GAPDH (1:1000, Sigma-Aldrich, Xicheng, Beijing, China) primary antibody overnight at 4°C before subsequent incubation with horseradish peroxidase(HRP)-conjugated secondary antibodies (Sigma-Aldrich, Xicheng, Beijing, China) for 1 h at 37°C. Protein signals were developed using the Amersham ECL Western Blotting Detection Reagent (GE Healthcare Life Sciences, Pittsburgh, PA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 16.0 software (SPSS Inc., Chicago, IL, USA) was applied in statistical analysis. The Student t-test was employed for comparison between two groups. The multi-group comparison was performed using one-way analysis of variance, followed by Least-Significant Difference (LSD) test. The x^2-test was used to examine the associations between miR-532-5p expression and the clinicopathological characters. Overall, survival curves were plotted according to the Kaplan-Meier method, with the log-rank test applied for comparison. Univariate and multivariate analyses were conducted to explore the independent risk factors for EOC using the Cox proportional hazard model. A p-value < 0.05 was considered statistically significant.

Results

MiR-532-5p Expression Level Was Decreased in EOC Tissues and Cell Lines

To determine the expression of miR-532-5p in EOC, 145 paired human EOC and normal ovarian tissues were subjected to qRT-PCR. As shown in Figure 1A, we found that the expression levels of miR-532-5p were significantly lower in EOC tissues compared to adjacent noncancerous tissues ($p < 0.01$). Subsequently, we further measured miR-532-5p expression in four EOC lines and one human normal ovarian epithelial NOEC cell line. As shown in Figure 1B, our results showed that miR-532-5p was downregulated in the four EOC cell lines compared with its expression in NOEC cells ($p < 0.01$). Our data from PCR revealed that miR-532-5p may be involved in the development of EOC. As downregulation was more evident in the OVCAR3 and ES-2 cell lines, we chose OVCAR3 and ES-2 for subsequent experiments.

Association Between miR-532-5p Expression and the Clinicopathological Features of EOC

In order to further the biological significance of miR-532-5p, we categorized miR-532-5p expression levels as high and low according to the mean value. The association between clinicopathological characteristics and miR-532-5p expression is summarized in Table II. We found that low miR-532-5p expression was closely related to advanced grade ($p = 0.011$), higher FIGO stage ($p = 0.004$) and positive distant metastasis ($p = 0.008$). However, no significant difference was observed between miR-532-5p expression and patients' age, tumor size, CA125 level and ascites ($p > 0.05$). Our results indicated miR-532-5p as a potential tumor suppressor.

Relationship of miR-532-5p to Overall Survival of EOC Patients

Then, Kaplan-Meier analysis was applied to examine the prognostic value of miR-532-5p expression to overall survival of patients with EOC. As shown in Figure 1C, we found that patients with lower miR-532-5p expression had a significantly shorter overall survival than patients with high miR-532-5p expression ($p = 0.0058$). Furthermore, univariate analysis revealed that FIGO stage ($p = 0.006$), grade ($p = 0.028$), distant metastasis ($p = 0.004$) and miR-532-5p expression ($p = 0.006$) were prognostic factors for patient's overall survival (Table III). More importantly, the results of multivariate analysis confirmed that miR-532-5p expression (HR = 3.774, 95% CI = 1.253-4.779, p = 0.006) was an independent predictor of overall survival in EOC. Overall, these findings suggested that miR-532-5p expression could be used as a powerful independent prognostic factor in EOC patients.

Effects of miR-532-5p on EOC Cell Proliferation and Invasion

To investigate the biological role of miR-532-5p in EOC progression, the OVCAR3 and ES-2 cell line was transfected with miR-532-5p or Ctrl. The transfection efficiency was confirmed by qRT-PCR ($p < 0.05$, Figure 2A). Then, we performed CCK-8 assay, finding that overexpressed miR-532-5p decreased the proliferation rate of OVCAR3 and ES-2 cells (Figure 2B and 2C). Similarly, the colony formation assay revealed that clonogenic survival was significantly suppressed in miR-532-5p -overexpressed OVCAR3 and ES-2 cell lines (Figure 2D). In addition, transwell invasion assays revealed that ectopic expression of miR-532-5p drastically suppressed OVCAR3 and ES-2 cells invasion (Figure 2E). Overall, our data suggested that miR-532-5p suppressed proliferation and invasion in EOC cells.

TWIST1 is a Direct Target of miR-532-5p

To explore the underlying molecular mechanism by which miR-532-5p suppressed EOC cell proliferation and invasion, we explored its target genes through two bioinformatics tools. As shown in Figure 3A, the results of TargetScan and miRanda showed that TWIST1 might be the target for miR-532-5p (Figure 2B). To validate the specific regulation of miR-532-5p on TWIST1, luciferase reporter assays were performed. As shown in Figure 3B, we observed that forced miR-532-5p expression decreased the luciferase activity of wild type TWIST1 3' UTR construct, whereas the luciferase activity was not decreased in the mutated type in both OVCAR3 and ES-2 cell lines. Moreover, qRT-PCR and Western blot analyses showed that overexpression of miR-532-5p significantly downregulated the expression of TWIST1 at the mRNA and protein levels in both OVCAR3 and ES-2 cell lines (Fi-

Table III. Univariate and multivariate analyses of overall survivor rates in 147 ovarian cancer patients by Cox regression analysis.

Variable	Univariate Analysis HR (95% CI)	*p*-value	Multivariate Analysis HR (95% CI)	*p*-value
Age	1.223(0.732-1.780)	0.562	-	-
Tumor size	1.562(0.894-2.321)	0.473	-	-
CA125 level	0.932(0.573-1.774)	0.217	-	-
Ascites	1.266(0.426-2.218)	0.135	-	-
FIGO stage	3.652(1.263-5.263)	0.002	3.253(1.093-4.263)	0.006
Grade	3.251(1.445-4.273)	0.008	2.778(1.239-3.882)	0.028
Distant metastasis	3.792(1.377-5.298)	0.001	3.253(1.193-4.776)	0.004
miR-532-5p expression	4.263(1.557-6.372)	0.001	3.774(1.253-4.779)	0.006

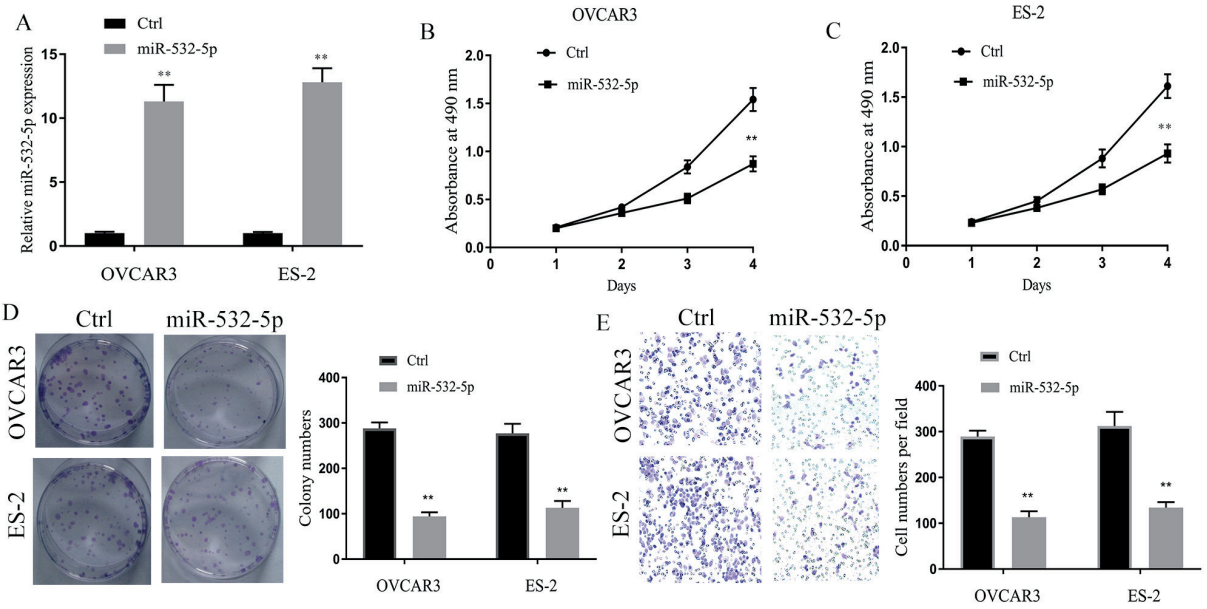

Figure 2. Overexpression of miR-532-5p could inhibit cell proliferation, colony formation and invasion in EOC cell lines. ***(A)*** Upregulation of miR-532-5p in miR-532-5p -stably-overexpressing OVCAR3 and ES-2 cells. ***(B-C)*** Cell viability was validated by MTT assay in OVCAR3 and ES-2 cells. ***(D)*** Relative colony formation rates of OVCAR3 and ES-2 cells with indicated treatment were determined by colony formation assays. ***(E)*** Transwell matrigel assay was used to determine OVCAR3 and ES-2 cells invasive capability. $^{**}p < 0.01$.

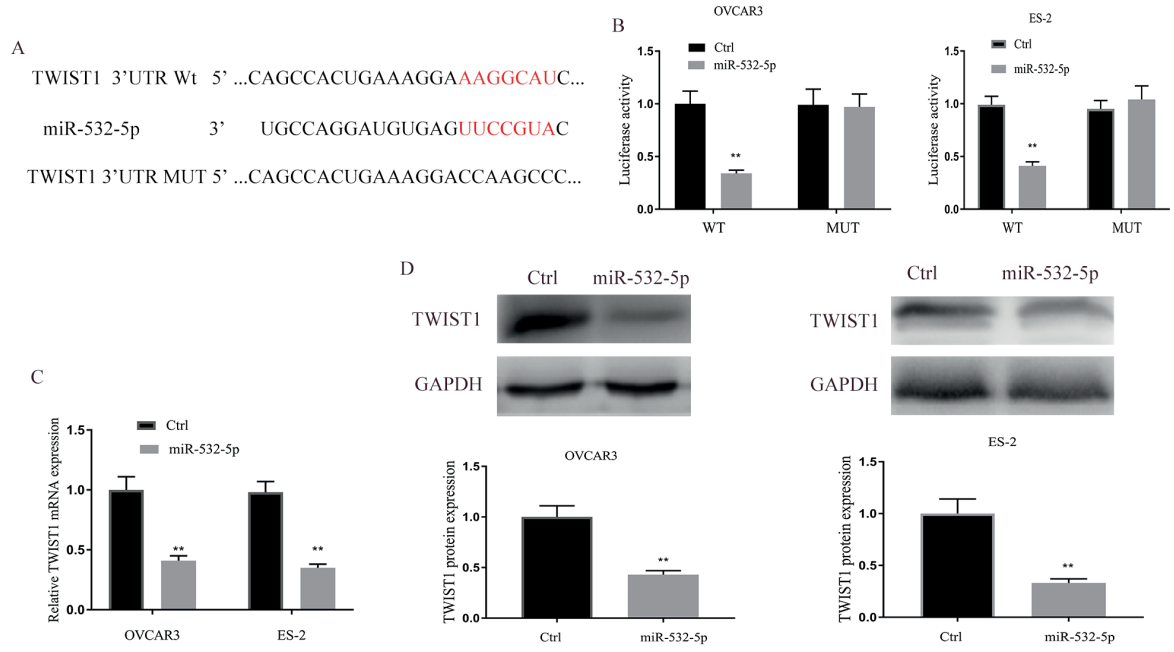

Figure 3. TWIST1 was a direct target of miR-532-5p in EOC cells. ***(A)*** The predicted binding sites for miR-532-5p in the 3'UTR of TWIST1 and the mutations in the binding sites are shown. ***(B)*** Luciferase assay was used to confirm the direct regulation of miR-532-5p on 3'UTR of TWIST1 in both OVCAR3 and ES-2 cells. ***(C)*** miR-532-5p decreased TWIST1 mRNA expression in OVCAR3 and ES-2 cells. ***(D)*** miR-532-5p decreased TWIST1 proteins expression in OVCAR3 and ES-2 cells. $^{**}p < 0.01$.

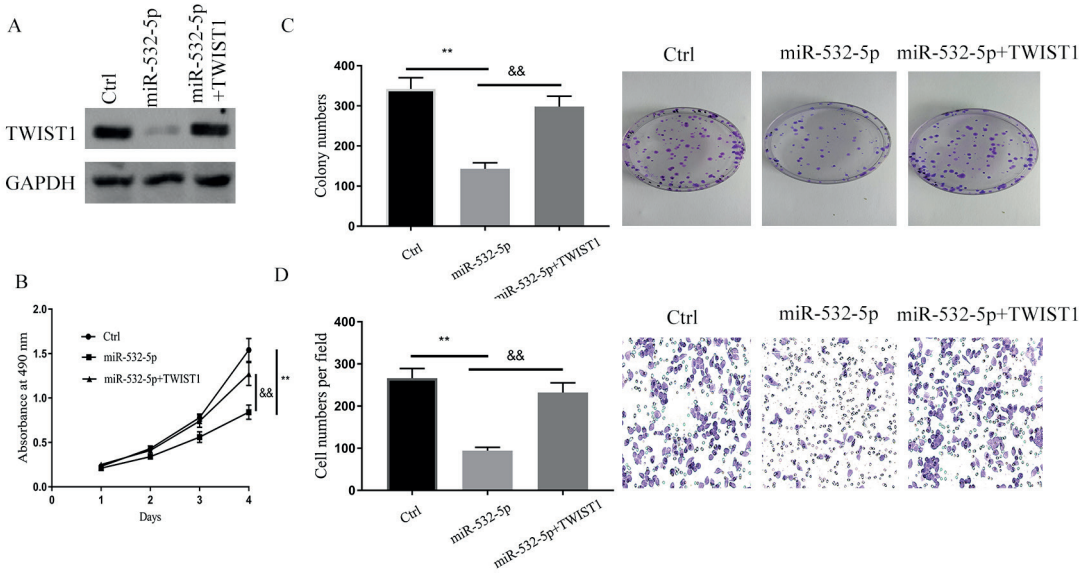

Figure 4. miR-532-5p suppressed EOC progression mainly by down-regulating TWIST1. ***(A)*** Western blotting showed TWIST1 protein expression in OVCAR3 cells transfected with Ctrl, miR-532-5p and miR-532-5p + TWIST1 plasmid, respectively. ***(B)*** CCK-8 assay detected the effect of miR-532-5p overexpression on miR-532-5p-induced cell growth arrest in OVCAR3 cells. ***(C)*** Colony formation assay was used to evaluate the cell viability in OVCAR3 cells. ***(D)*** The invasion of OVCAR3 cells was assessed using a transwell assay. $^{**}p < 0.01$ vs. Ctrl group; $^{\&\&}p < 0.01$ vs. miR-532-5p group.

gure 3C and 3D). Overall, these results confirm that TWIST1 is a direct target of miR-532-5p in EOC cells.

Overexpression of TWIST1 Reversed the Effect of miR-532-5p

In order to further explore whether TWIST1 is involved in the miR-532-5p-induced suppression of EOC cell proliferation and invasion, we performed a rescue experiment in OVCAR3 cells. Western blotting analysis indicated that pcDNA3.1-TWIST1 transfection effectively replenished miR-532-5p miRNA mimics-induced TWIST1 loss (Figure 4A). In addition, transfected with pcDNA3.1-TWIST1 decreased the suppressive effects of miR-532-5p on cell viability (Figure 4B; $p < 0.01$), colony formation (Figure 4C; $p < 0.01$) and invasion (Figure 4D; $p < 0.01$) of OVCAR3 cells. In summary, the data indicated that miR-532-5p could inhibit cell viability and invasion by down-regulation of TWIST1.

Discussion

At present, human EOC is still a complex malignancy, which has multiple histological subtypes[15]. The 5-year overall survival of EOC patients diagnosed at an advanced stage is relatively low. Therefore, it is of great importance to identify novel genetic or protein biomarkers for accurate diagnosis and prediction of prognosis. In this study, we demonstrated that miR-532-5p expression was decreased in human EOC tissues and cell lines compared with matching adjacent non-tumoral tissue and normal cell lines, in accordance with previous studies[16]. Then, we firstly analyzed its clinicopathologic and prognostic significance. Our data demonstrated that low miR-532-5p expression was closely related to advanced grade, higher FIGO stage and positively distant metastasis, indicating that miR-532-5p may act as a negative regulator in clinical progression of EOC patients. Moreover, we performed Kaplan-Meier analysis with five-year following-up, finding that the patients with lower levels of miR-532-5p expression had significantly shorter survival time. This supports the hypothesis that miR-532-5p plays a potential role in EOC progression. More importantly, further univariate and multivariate survival analysis showed that miR-532-5 could be used as an independent potential prognostic biomarker for EOC patients. Taken together, these findings, together with previous, highlighted the clinical significance of miR-532-5p in EOC patients and implied potentially im-

portant role for miR-532-5p in predicting the prognosis of EOC patients. Previous studies have shown that miR-532-5p was involved in biological processes in cancers. For instance, Hu et al[17] reported that miR-532 expression was significantly up-regulated in gastric cancer and its overexpression promote gastric cancer cells migration and invasion by targeting NKD1. Xu et al[18] further showed that miRNA-532-5p exerted its oncogenic role by directly targeting RUNX3. Of note, Song et al[19] reported that miR-532-5p was significantly lowly expressed in hepatocellular carcinoma and its knockdown promoted cell proliferation and metastasis by influencing CXCL2 expression, indicating miR-532-5p as a tumor suppressor in hepatocellular carcinoma. Song et al[20] found that miR-532-5p downregulation in colorectal cancer tissues and its association with aggressive clinicopathological characteristics. *In vitro* and *in vivo* revealed that up-regulation of miR-532 suppressed colorectal cancer cells proliferation, migration and invasion by directly targeting IGF-1R and regulating the PI3K/Akt signaling pathway, supporting miR-532-5p as a tumor suppressor in colorectal cancer. The above results revealed that miR-532-5p may serve as a tumor suppressor or a tumor promoter according the types of tumors. Importantly, previously Bai et al[16] reported that the expression levels of miR-532 were significantly down-regulated in ovarian cancer tissues and cell lines. They also observed that miR-532 served as a tumor suppressor by inhibiting ovarian cancer cell proliferation and invasion via regulating hTERT. However, the evidence about the expression pattern and biological role of miR-532-5p is limited. In this study, our *in vitro* experiments also confirmed that overexpression of miR-532-5p significantly suppressed EOC cells proliferation and invasion. However, the potential mechanism by which miR-532-5p exerted its tumor-suppressive role in EOC progression remains largely unknown. Identification of miR-532-5p target genes is critical for understanding the role of miR-532-5p in tumorigenesis, and is important for exploring novel therapeutic targets. Then, we screened TWIST1 (Twist homolog 1) as a potential target of miR-532-5p according to microRNA target databases and published studies. As a highly conserved basic helix-loop-helix transcription factor, TWIST1 plays an essential role in many biological processes[21]. Increasing evidence confirms that TWIST1 function as a tumor promoter in various tumor progression, such as breast cancer, lung cancer and EOC[22-24]. Current findings showed that TWIST1 was a key regulator of cancer-associated fibroblasts[25]. In addition, several studies reported TWIST1 was modulated by several miRNAs, such as miR-186, miR-548c and miR-320[26-28]. In this work, we suggested that TWIST1 is one of miR-532-5p target genes. According to the results of a luciferase reporter assay, TWIST1 was identified as a target gene of miR-532-5p. In order to further confirm the association between miR-532-5p and TWIST1, we performed rescue experiments, finding that restoration of TWIST1 reversed the effects of miR-532-5p overexpression in EOC cells. Taken together, miR-532-5p/TWIST1 based targeted therapy could be new therapeutic strategies for EOC patients.

Conclusions

We found that miR-532-5p was downregulated in EOC tissues, and low miR-532-5p expression was intimately associated with poor prognosis, which is considered as an independent prognostic indicator for overall survival. Moreover, we for the first time suggested that miR-532-5p suppressed the proliferation and invasion of EOC cells by targeting TWIST1. Therefore, the miR-532-5p/TWIST1 axis could serve as a potential target for the treatment of EOC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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