MicroRNA-32 promotes ovarian cancer cell proliferation and motility by targeting SMG1

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Abstract. Ovarian cancer (OC) is the most lethal gynecological malignancy and one of the leading causes of cancer-related deaths among women. Metastasis is the main cause of poor prognosis in OC. MicroRNA (miRNA/miR) has been shown to play an important role in tumorigenesis and metastasis in various cancer types by affecting the expression of its targets. In the present study, the role of miR-32 (miR-32-5p) in OC was explored. Reverse transcription-quantitative PCR results showed that miR-32 expression was significantly upregulated in both OC tissues and cell lines. Inhibition of miR-32 by transfection with miR-32 inhibitor in OC cells markedly suppressed cell proliferation, migration and invasion. In addition, a luciferase assay showed that suppressor of morphogenesis in genitalia 1 (SMG1) is a direct target of miR-32, and interference in SMG1 expression with transfection of SMG1 small hairpin RNA restored miR-32-mediated OC cell proliferation, migration and invasion. Taken together, these results indicate that miR-32 may promote OC cell growth and motility by targeting SMG1. The data of the present study suggest that miR-32 may serve as a potential therapeutic target for OC treatment in the future.

Introduction

Ovarian cancer (OC) is the most lethal gynecological malignancy and one of the leading causes of cancer-related deaths in women (1-3). The high mortality rate is due to the late diagnosis or advanced stage of at the time of diagnosis, with the majority of patients possessing stage III-IV cancer (2). The incidence of OC is still increasing. Currently, the most commonly used therapeutic strategies are surgery and chemotherapy, with radiotherapy occasionally being used (4-6). However, the 5-year survival rate is only ~50% due to the development of recurrent disease that is often resistant to chemotherapy (7). The treatment of recurrent OC is limited and recurrence of OC is still considered as incurable. Thus, the development of new and efficient therapeutic strategies is urgently needed.

MicroRNA (miRNA/miR) is a small non-coding RNA, 22 nt in length, that is able to regulate gene expression by binding to the complementary sequence at the 3'-untranslated region (3'-UTR) of its target mRNAs (8-12). Each miRNA can have multiple targets, inducing either upregulation or downregulation of the expression of each target (13). It has been reported that the dysregulation of miRNAs is related to a variety of human diseases, including cancer (14-16). miRNAs act as either tumor suppressors or oncogenes in different types of cancer and their role is dependent on their expression pattern and function (16,17). In OC, a number of miRNAs have been identified to have altered expression leading to tumorigenesis. Among these miRNAs, miR-16, miR-20a, miR-27a, miR-26, miR-182, miR-146, miR-221 and miR-508 have been reported to be upregulated in OC, while miR-145, miR-125b, miR-377, miR-210, miR-493 and miR-106b have been reported to be downregulated in OC (18-20). Investigating the role of these miRNAs in the development and progression of OC may provide new insights for the detection, diagnosis and treatment of OC.

miR-32 (miR-32-5p) has been reported to be overexpressed in several types of cancer, including breast, prostate, endometrial, colorectal and hepatocellular cancer, and has been shown to promote cancer cell proliferation and development (21-26). By contrast, miR-32 acts as a tumor suppressor in human oral squamous cell carcinoma (27). However, its expression and biological role in OC is still largely unknown. Therefore, the present study aimed to investigate the expression levels and functional roles of miR-32 in OC tissues and cell lines. The findings of the present study might highlight the potential of miR-32 as a therapeutic target for treatment of OC in the future.

Patients and methods

Patients and clinical specimens. A total of 38 paired malignant OC tissues and adjacent normal ovarian tissues were collected

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in Tianjin Medical University General Hospital (Tianjin, China) from female patients aged of 24-73 who underwent surgical resection between December 2015 and December 2016. Written informed consent was obtained from each patient, and the study was approved by The Ethics Committee of the Tianjin Medical University. All patient information is listed in Table I. The collected tissues were immediately frozen in liquid nitrogen and stored at -80˚C prior to RNA isolation.

Cell culture and transfection. Three human OC cell lines: OVCAR3 (cat. no. HTB-161), SKOV3 (cat. no. HTB-77) and ES-2 (cat. no. CRL-1978) were purchased from the American Type Culture Collection and one human ovarian surface epithelial (HOSE) cell line (IOSE80; cat. no. CVCL_5546) was obtained from the Canadian Ovarian Tissue Bank (University of British Columbia). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin/streptomycin (Gibco; Thermo Fisher Scientific, Inc.), at 37˚C in a humidified chamber with 5% CO_2 atmosphere. Around 5-6x10^5 cells were seeded into 6-well plates at 24 h prior to transfection. 50 nM of miR-32 inhibitor, inhibitor negative control (NC), miR-32 mimic, mimic NC, SMG1 small hairpin (sh)RNAoligo and SMG1 NC were purchased from Shanghai GenePharma Co., Ltd., and transfected into ES-2 cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. A total of 50 nM RNA was used for each transfection. At 48 h after transfection, the functional experiments were performed. The inhibitor NC and the miR-32 mimic NC were synthesized with non-specific sequences of the same length as the miR-32 inhibitor and mimic, which could eliminate non-sequence-specific effects in the experiments. The sequences of genes mentioned above were listed as following. miR-32 inhibitor, 5'-UGCAACUUA GUAAUGUGCAAUA-3'; inhibitor NC, 5'-CAGUACUUU UGUGUAGUACAA-3'; miR-32 mimic, sense: 5'-UAUUGC ACAUUACUAAGUUGCA-3', antisense: 5'-CAACUUAGU AAUGUGCAAUAUU-3'; mimic NC, 5'-UUCUCCGAA CGUGUCACUGUU-3'; SMG1 (nonsense mediated mRNA decay associated PI3K related kinase) shRNA, 5'-GCCAUG ACUAACACUGAAAdTdT-3'.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the indicated cell lines, including OVCAR3, SKOV3, ES-2 and IOSE80, and the patient samples using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. cDNA was reverse transcribed from RNA using the PrimeScript RT reagent kit (Promega Corp.) (50-55˚C for 10 min, 80˚C for 10 min). U6 snRNA was used as normalization control gene for the detection of miR-32. RT-qPCR analyses for SMG1 and the normalization control gene GAPDH were performed using SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd.). The condition for qPCR was: 95˚C 30 sec; 95˚C 5 sec, 60˚C 30 sec for 40 cycles. The relative expression of each gene was calculated and normalized to U6 snRNA or GAPDH using the $2^{-\Delta\Delta Cq}$ method (28). The correlation between miR-32 and SMG1 mRNA expression in OC tissues was analyzed by the Spearman's correlation analysis. The following primers were used for RT-qPCR: miR-32, Forward: 5'-GCGGCGTATTGC ACATTACT-3', reverse: 5'-TCGTATCCAGTGCAGGGTC-3'; SMG1, forward: 5'-GTGCATTAGCCACCAAAGAC-3' and reverse: 5'-CTCAGAGAAGCACAGAGAAG-3'.

Cell proliferation assay. Transfected cells were placed into 96-well plates at a density of 2x10^3 cells/well and were cultured for 24, 48, 72 and 96 h. Next, 10 μl Cell Counting Kit-8 (CCK-8) reagent (Beyotime Institute of Biotechnology) was added into each well. The 96-well plates were placed in a 5% CO_2 incubator at 37˚C and the cells were incubated for 2 h. The absorbance was measured at 450 nm using a microplate reader.

Cell migration and invasion assays. Following transfection for 24 h, the cells were cultured in serum-free medium for another 12 h. The cells were then collected and their density was adjusted to 4-5x10^5/ml. A Transwell chamber with 8-μm pores (Corning, Inc.) was used for the migration assay. Complete DMEM (500 μl) (Hyclone; GE Healthcare Life Sciences), containing 10% FBS, was added in the lower layer, and 200 μl of the cell suspension in serum-free media was added in the upper chamber. After a 10-h incubation, the cells on the lower surface of the chamber were fixed with glacial acetic acid for 15-30 min at room temperature and stained with 0.2% crystal violet for 30 min at room temperature. A total of 10 fields from each chamber were selected randomly for counting. The cells (4-5x10^5/ml) were plated into the upper layer of the chamber covered with Matrigel (BD Biosciences), and the same culture method was used to perform cell invasion assays. After staining with 0.2% crystal violet, at least 10 fields from each chamber were selected and the invasive cells were counted and quantified under an inverted light microscope (Olympus) with x20 magnification.

Bioinformatics analysis. TargetScan version 6.2 (targetscan.org/vert_72/) was used to predict the potential targets of miR-32. Several potential targets, including ANP32E, ARRDC3, FXR1, SMG1, EVI5, GRAMD1B, KIF1B, BMP7 and SPHK2, were selected to analyze the target-miR-32 association and the role of miR-32 in the regulation of their expression. The primers of ANP32E, ARRDC3, FXR1, SMG1, EVI5, GRAMD1B, KIF1B, BMP7 and SPHK2 are listed in Table SI.

Dual-luciferase reporter assay. The 3'-UTR sequence of wild-type (WT) SMG1 and target-site mutant-type (MT) PCR products were cloned into a dual-luciferase reporter vector plasmid (Promega Corp.), and the products were termed as pGL3-SMG1-3'-UTR-WT (WT vector) and pGL3-SMG1-3'-UTR-MT (MT vector). Logarithmic growth-phase ES-2 cells were seeded into 96-well plates at a density of 1.5x10^4 cells/well prior to transfection. ES-2 cells were then co-transfected with the WT or MT vector and miR-32 mimic or mimic NC using the Attractene Transfection Reagent (Qiagen, Inc.), because miR-32 was most significantly expressed in ES-2 cells. After 48 h of transfection, the Firefly to *Renilla* luciferase activity ratio was detected using a dual-luciferase reporter system (Promega Corp.). Also, subsequent experiments were conducted in ES-2 cells.

Western blot analysis. Lysis buffer [150 M NaCl, 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM DTT, 1 mM PMSF

Table I. Associations between miR-32 expression and clinicopathological characteristics of 38 ovarian cancer patients.

Characteristics	Patients, n	miR-32 expression, n		P-value
		High (n=23)	Low (n=15)	
Age, years				0.646
<50	16	9	7	
≥50	22	14	8	
Clinical stage				0.021[a]
I-II	8	2	6	
III-IV	30	21	9	
Pathological grade				0.225
1-2	11	5	6	
3	27	18	9	
Histological type				0.475
Serous	28	16	12	
Non-serous	10	7	3	
Residual tumors after surgery, cm				0.254
<1	21	11	10	
≥1	17	12	5	

[a]P<0.05. miR, microRNA.

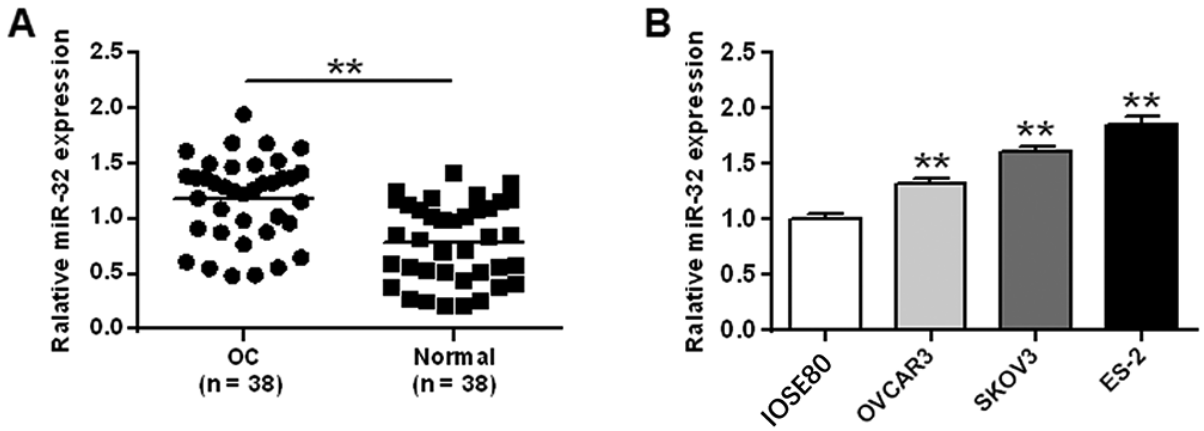

Figure 1. miR-32 is upregulated in OC tissues and cell lines. (A) RT-qPCR was performed to determine the relative expression of miR-32 in 38 paired human OC and normal ovarian tissues. (B) RT-qPCR was conducted to detect the relative expression of miR-32 in three OC cell lines (OVCAR3, SKOV3, and ES-2) and IOSE80 cells. U6 served as an internal control. The experiments were repeated at least three times and similar results were obtained. **P<0.01. OC, ovarian cancer; RT-qPCR, reverse transcription PCR.

and 10% glycerol)] was used to digest the sample tissues and cells. The protein concentration of each sample was measured using a BCA protein assay kit (cat. no. 23225; Thermo Fisher Scientific, Inc.). Total proteins (30 μg) from each sample were separated by polyacrylamide gel electrophoresis with 10% SDS and then transferred to polyvinylidene fluoride membranes at 100 V for 1.5 h. The membranes were blocked with 5% skimmed milk in TBST (1 ml/l Tween-20, 100 mM Tris-Cl, 9 g/l NaCl, pH 7.5) for 1 h at room temperature, and were incubated with primary antibodies (anti-SMG1; ab30916; 1:500; Abcam,) at 4˚C overnight. After washing, secondary antibodies (horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G; 1;1,000; cat. no. ab6721; Abcam) were added and the membranes were incubated at room temperature for 2 h. Protein bands were visualized using enhanced chemiluminescence (ECL) reagents (EMD Millipore). ImageJ version 1.46 software (National Institutes of Health) was used to quantify the protein expression levels. GAPDH (cat. no. ab181602; 1:1,000; Abcam) was used as the loading control.

Statistical analysis. Statistical analysis was performed using the SPSS 17.0 software (SPSS, Inc.). Data are expressed as the mean ± SD. The independent-samples or paired t-test was used for comparisons between two groups.

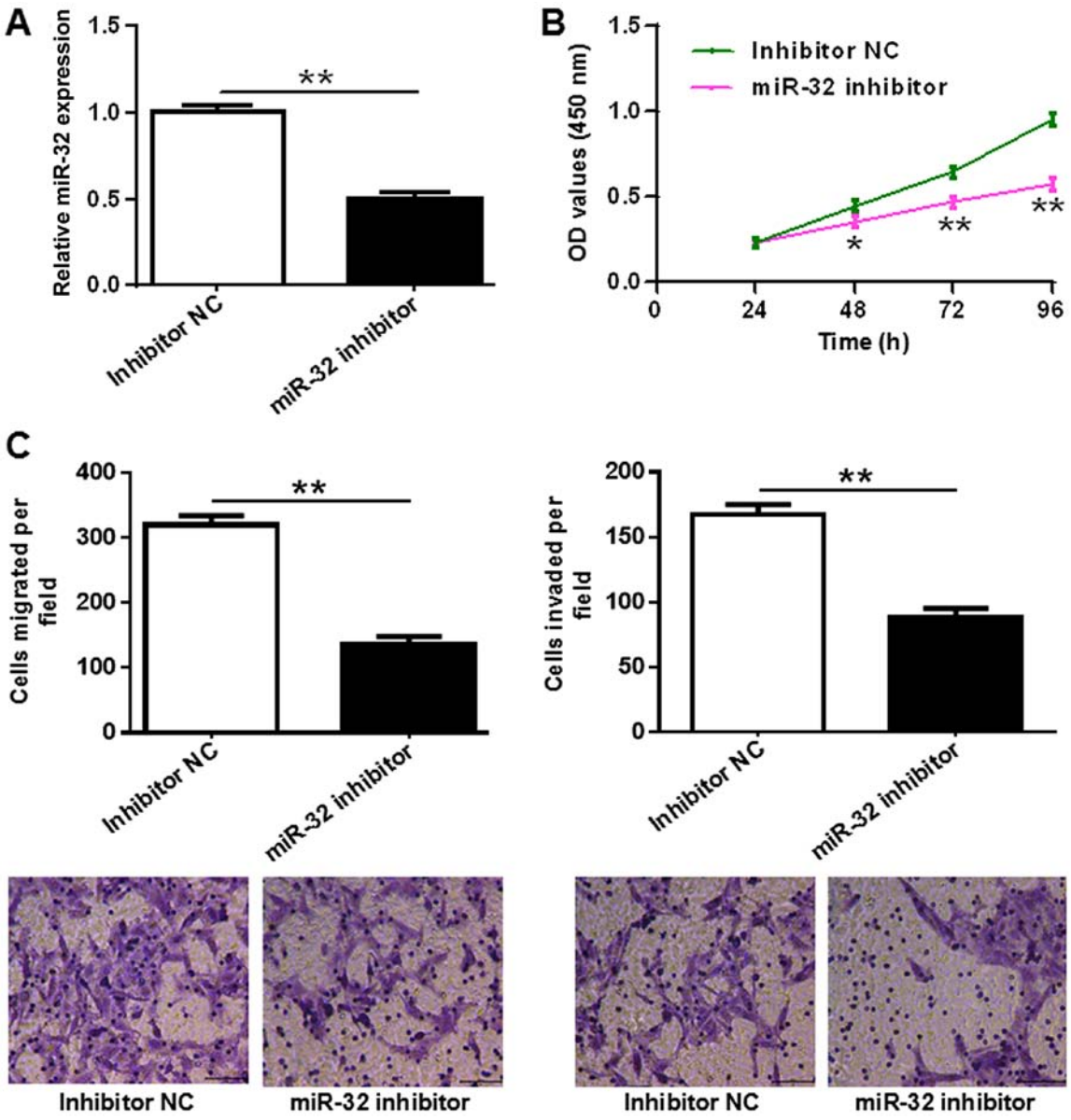

Figure 2. Inhibition of miR-32 suppresses OC cell proliferation and motility. (A) ES-2 cells were transfected with miR-32 inhibitor or inhibitor NC, and the relative expression levels of miR-32 were detected by reverse transcription-quantitative PCR. (B) Cell proliferation of pre-treated ES-2 cells examined by CCK-8 assay. (C) Cell motility of pre-treated ES-2 cells determined by Transwell migration and invasion assays by staining of the cells with 0.2% crystal violet. The experiments were repeated at least three times and similar results were obtained. *P<0.05, **P<0.01. OC, ovarian cancer; NC, negative control; CCK-8, Cell Counting Kit-8; miR, microRNA; OD, optical density. Scale bar, 200 μm.

One-way ANOVA, followed by Bonferroni's post-hoc test, was performed to analyze the differences among more than two groups. The correlation between miR-32 and SMG1 mRNA expression in OC tissues was analyzed by Spearman's correlation analysis. The Pearson's χ^2 test was used to analyze the association between miR-32 expression and clinicopathological parameters. Each experiment was repeated at least three times with triplicates in each experiment. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-32 is upregulated in OC tissues and cell lines. To determine the expression profile of miR-32 in OC, RT-qPCR was performed to detect the mRNA level of miR-32 in 38 paired human OC tissues and normal ovarian tissues. The expression of miR-32 was elevated in OC tissues compared with that in the normal ovarian tissues (P<0.01; Fig. 1A). Furthermore, the expression of miR-32 was significantly higher in the three OC cell lines (OVCAR3, SKOV3, ES-2) compared with that in the HOSE cell line (IOSE80) (P<0.01; Fig. 1B). These results indicate that the expression of miR-32 is upregulated in OC.

Inhibition of miR-32 suppresses OC cell proliferation and motility. To investigate the effect of miR-32 on OC cell proliferation and motility, ES-2 cells were transfected with miR-32 inhibitor or inhibitor NC. Transfection efficiency of miR-32 expression in ES-2 cells was confirmed by RT-qPCR (P<0.01; Fig. 2A). A CCK-8 assay was performed to detect the prolifera-

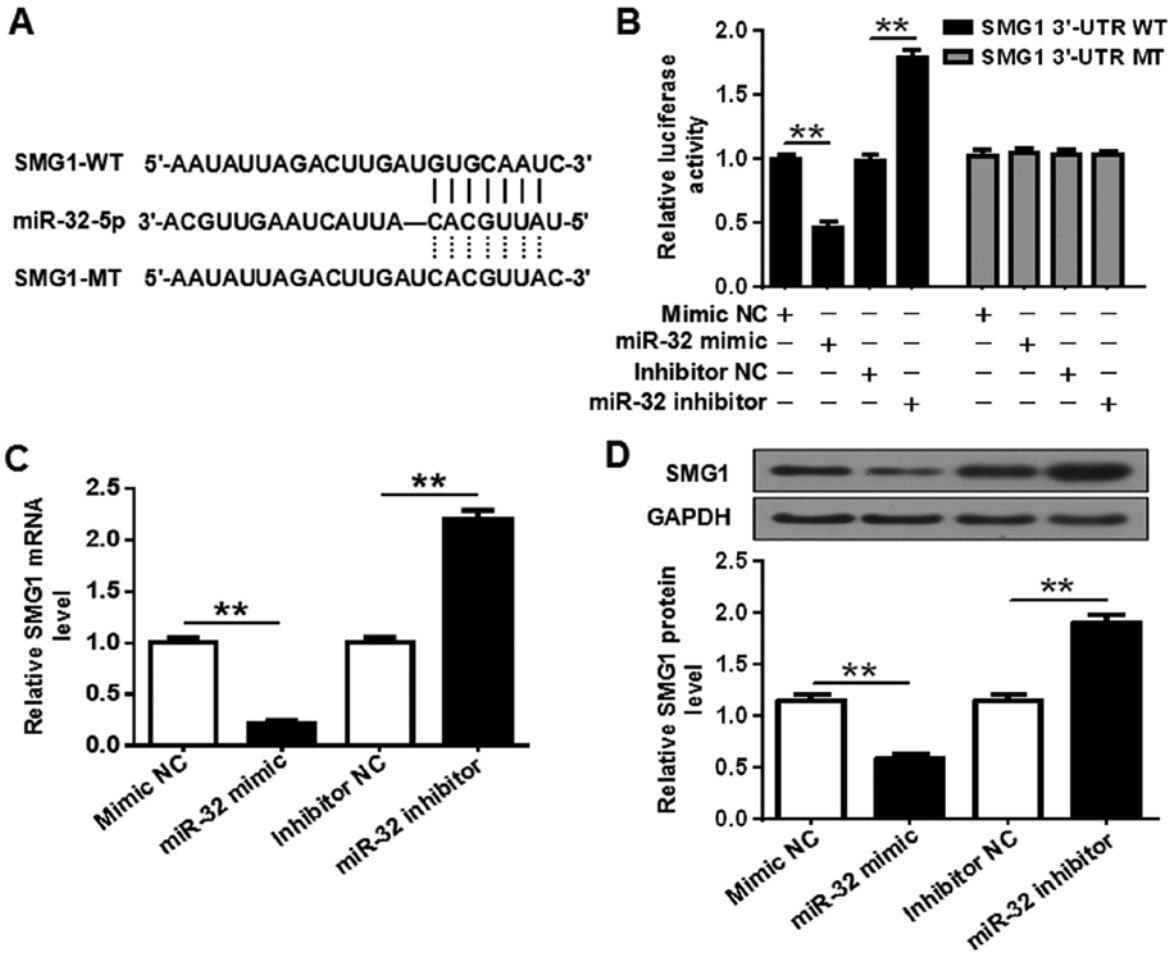

Figure 3. SMG1 is a direct target of miR-32. (A) The predicted miR-32-5p target sequence in SMG1 3'-UTR is shown. (B) Luciferase activity was measured after co-transfection with luciferase reporter plasmids (SMG1 3'-UTR WT/MT), and miR-32 mimic/mimic NC or miR-32 inhibitor/inhibitor NC in ES-2 cells. The sequences of WT and MT plasmids are shown in (A). (C) Reverse transcription-quantitative PCR analysis of SMG1 mRNA expression in ES-2 cells after transfection. (D) Western blot analysis of SMG1 protein expression in ES-2 cells after transfection. The experiments were repeated at least three times and similar results were obtained. **P<0.01. SMG1, suppressor of morphogenesis in genitalia 1; 3'-UTR, 3'-untranslated region; WT, wide-type; MT, mutant-type; NC, negative control; miR, microRNA.

tion of the transfected ES-2 cells. The results showed that miR-32 inhibitor significantly suppressed OC cell proliferation compared with the inhibitor NC (P<0.05 at 48 h, and P<0.01 at 72 and 96 h; Fig. 2B). Next, cell motility was measured by Transwell migration and Matrigel invasion assays, and it was shown that the inhibition of miR-32 effectively suppressed OC cell migration and invasion (both P<0.01; Fig. 2C). These results reveal that the inhibition of miR-32 suppresses OC cell proliferation and motility.

SMG1 is a direct target of miR-32. TargetScan 6.2 was used to explore the potential targets of miR-32 in OC. SMG1, a tumor suppressor in human tumorigenesis, was predicted and selected as the target of miR-32 in the present study (Fig. 3A). To confirm this, the cells were co-transfected with WT or MT SMG1 3'-UTR vectors, with miR-32 mimic or mimic NC (Fig. S1), and a luciferase activity assay was conducted. The results showed that the overexpression of miR-32 significantly suppressed the WT, but not the MT 3'-UTR of SMG1, while inhibition of miR-32 significantly promoted the WT, but not the MT 3'-UTR of SMG1 (P<0.01; Fig. 3B). In addition, RT-qPCR and western blot analysis revealed that the inhibition of miR-32 significantly increased the mRNA and protein levels of SMG1, while overexpression of miR-32 significantly decreased the mRNA and protein levels of SMG1 (both P<0.01; Fig. 3C and D). The decrease in SMG1 protein expression induced by miR-32 mimic is lower than the decrease in SMG1 mRNA expression, because the post-transcriptional, translational and degradation regulation determines the concentration of the protein, thus, the protein and mRNA levels may not be well correlated. Taken together, these results demonstrate that SMG1 is a direct target of miR-32.

SMG1 expression is decreased in both OC tumor tissues and tumor cells, and negatively correlated with the expression of miR-32. Next, the expression of SMG1 in both OC tumors and tumor cell lines was analyzed. RT-qPCR results demonstrated that the expression of SMG1,compared with other potential targets, including ANP32E, ARRDC3, FXR1, SMG1, EVI5, GRAMD1B, KIF1B, BMP7 and SPHK2, was significantly decreased in both OC tumor tissues and tumor cell lines compared with that in normal adjacent tissues and the HOSE cell line (IOSE80), respectively (all P<0.01; Figs. 4A, B and S1). Hence, SMG1 was the most relevant target to be used in this study. Moreover, the correlation between the SMG1 and miR-32 expression levels was also analyzed.

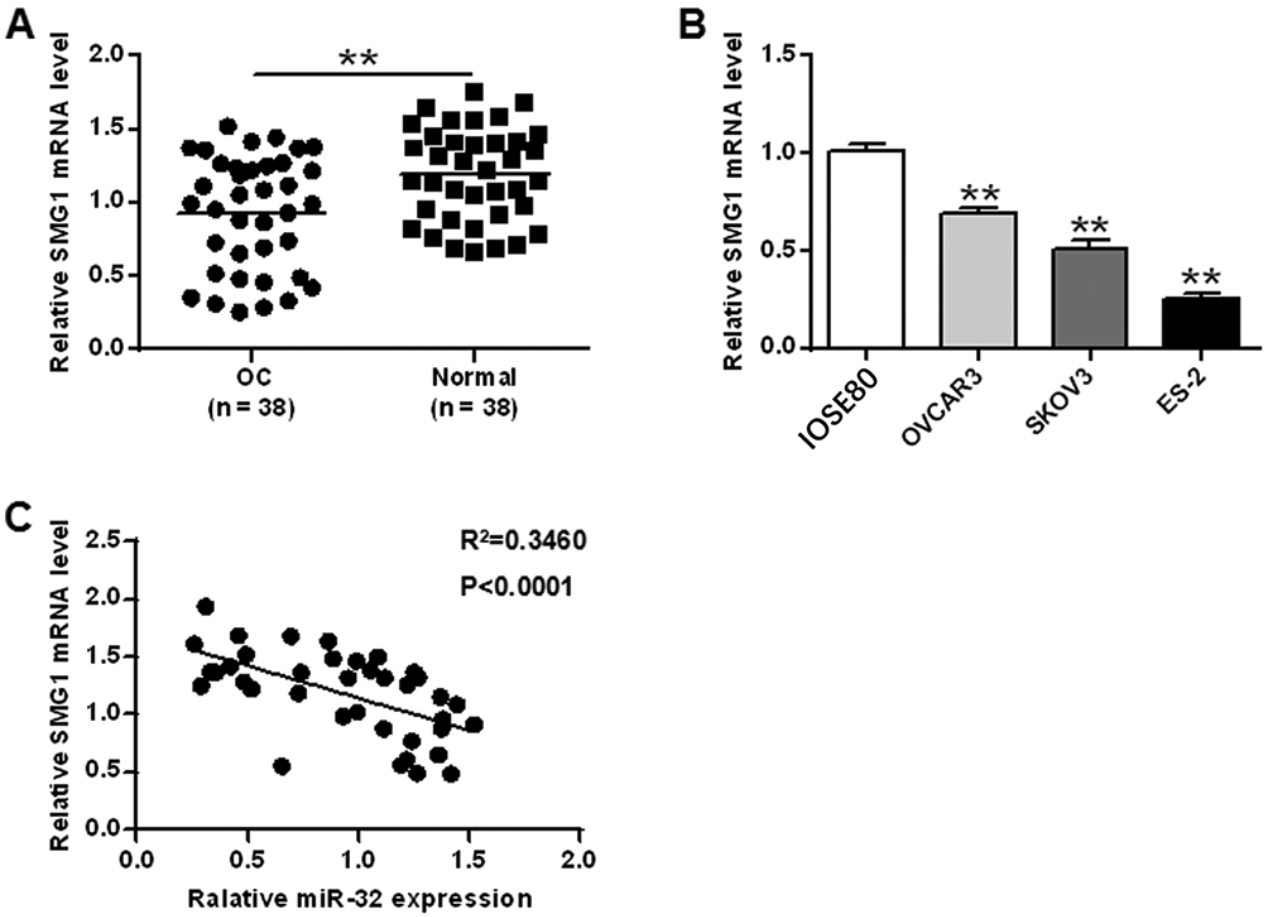

Figure 4. Expression of SMG1 in OC tissues and tumor cells, and correlation between SMG1 and miR-32 expression levels. (A) The expression of SMG1 was determined by RT-qPCR in both OC and normal tissues. (B) The expression of SMG1 in OC cell lines OVCAR3, SKOV3 and ES-2, as well as the human ovarian surface epithelial cell line (IOSE80) was detected by RT-qPCR. (C) The correlation between the expression of SMG1 and miR-32 was analyzed. The experiments were repeated at least three times and similar results were obtained. **P<0.01. SMG1, suppressor of morphogenesis in genitalia 1; OC, ovarian cancer; RT-qPCR, reverse transcription-quantitative PCR.

The results revealed that SMG1 was negatively correlated with miR-32 (r^2=0.3460, P<0.0001; Fig. 4C). Taken together, these results suggest that SMG1 is downregulated in OC and negatively correlated with miR-32.

Interference of SMG1 restores miR-32-mediated OC cell proliferation and motility. Since SMG1 is a direct target of miR-32, downregulation of SMG1 was performed to determine its effect on the inhibition of miR-32-induced cell proliferation and motility regression. To this end, the SMG1-targeting shRNA oligo was employed to deplete endogenous SMG1 in OC cells. The downregulation effect was confirmed by western blot analysis (Fig. 5A). miR-32 inhibitor or inhibitor NC and SMG1 shRNA oligo or SMG1 NC were co-transfected into ES-2 cells. According to the results, SMG1 shRNA attenuated miR-32 inhibitor-triggered SMG1 protein elevation in ES-2 cells (P<0.01; Fig. 5A). In addition, downregulation of SMG1 by shRNA also attenuated the miR-32 inhibitor-induced OC cell proliferation regression (*P<0.05 and **P<0.01 vs. control group; Fig. 5B). Moreover, downregulation of SMG1 by shRNA also attenuated miR-32 inhibitor-induced ES-2 cell migration and invasion regression (P<0.01; Fig. 5C). These findings suggest that miR-32 promotes OC cell proliferation and motility by the regulation of SMG1.

Discussion

miRNAs have been studied for decades, and the dysregulation of miRNAs has been reported in tumor tissues and serums (29,30). miRNAs act as tumor suppressors or oncogenes in the development and progression of different types of cancer, depending on their proliferation, biological function and targets. Therefore, the investigation of specific miRNAs, their role in cancer and their targets would be valuable for cancer diagnosis and therapy. miR-32, located at chromosome band Xq26.2, has been reported to serve as an oncogene in several types of cancer, including breast, prostate, endometrial, colorectal and hepatocellular cancer (21-26), while acting as a tumor suppressor in human oral squamous cell carcinoma (27). However, its expression profile and biological function in OC is still under investigation. In this study, the expression and biological function of miR-32 in OC was explored. There are two types of miR-32, miR-32-5p and its complementary strand miR-32-3p, derived from the miR-32-5p/-3p duplex, which is processed from intron 14 of the C9orf5 gene. Since miR-32-5p has been widely explored and identified as an important regulator in tumorigenesis in different types of cancer, its role in OC was explored in the present study to characterize its target genes and physiological functions.

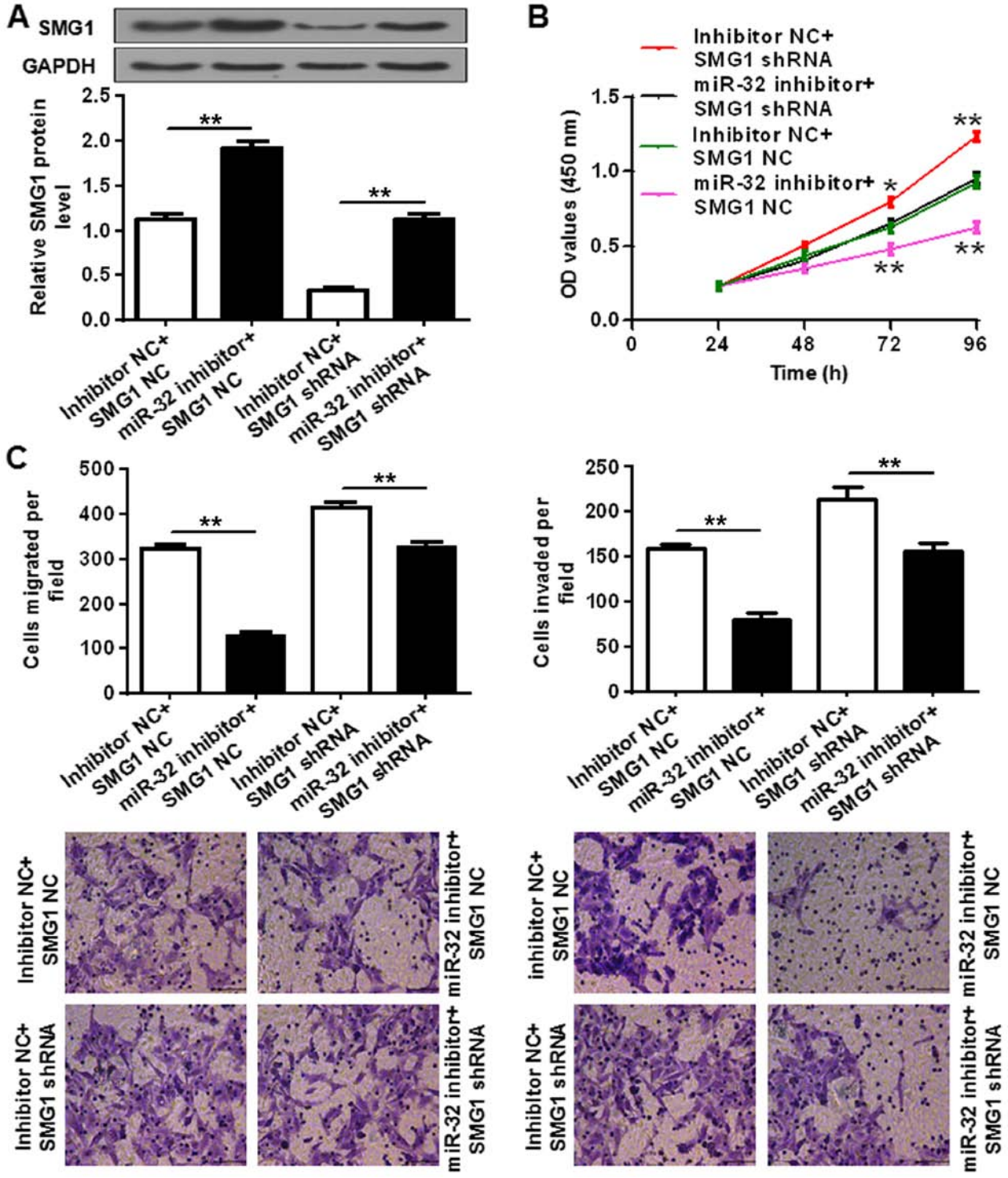

Figure 5. Effect of SMG1 depletion on miR-32-mediated OC cell proliferation and motility. (A) SMG1 protein expression was determined in ES-2 cells co-transfected with miR-32 inhibitor, inhibitor NC, SMG1 shRNA oligo or SMG1 NC. (B) Cell proliferation and (C) motility (migration and invasion) were determined in ES-2 cells co-transfected with miR-32 inhibitor, inhibitor NC, SMG1 shRNA oligo or SMG1 NC by Transwell assay and staining with 0.2% crystal violet respectively. The experiments were repeated at least three times and similar results were obtained. *P<0.05 and **P<0.01 vs. control group. SMG1, suppressor of morphogenesis in genitalia 1; OC, ovarian cancer; NC, negative control; OD, optical density; miR, microRNA. Scale bar, 200 μm.

In the present study, different types of OC patients were enrolled and the expression of miR-32 was analyzed in tumor tissues and paired adjacent normal tissues. The data indicated that miR-32 was significantly upregulated in both OC tumor tissues and cell lines, when compared with normal adjacent tissues and normal ovarian cells, respectively. These results suggest that miR-32 may play an oncogenic role in OC development and progression. However, there are still limitations to our understanding of the expression profile of miR-32 due to the lack of a large number of participants. In future studies, more OC patients will be included to confirm the oncogenic role in the development and progression of OC. Furthermore, a CCK8 assay showed that downregulation of miR-32 markedly inhibited OC cell proliferation, and a Transwell assay proved

that the downregulation of miR-32 profoundly inhibited cell motility by decreasing cell migration and invasion. Together, these findings confirm the oncogenic role of miR-32 in OC cells.

Each miRNA can have multiple targets and can regulate its targets to either promote or inhibit tumor cell proliferation, growth and motility. In breast cancer, miR-32 was reported to promote cell proliferation and motility, and suppress apoptosis by targeting FBXW7 (22). In hepatocellular carcinoma, miR-32 was proven to induce cell proliferation and motility by targeting PTEN (24). Also, in human squamous cell carcinoma, miR-32 was shown to act as tumor suppressor and directly target EZH2 (27). Thus, exploring the targets of miR-32 could advance our understanding of the mechanism of miR-32 in OC development and progression. In the present study, several potential targets, including ANP32E, ARRDC3, FXR1, SMG1, EVI5, GRAMD1B, KIF1B, BMP7 and SPHK2, were detected and RT-qPCR was performed to check the mRNA expression level of these targets in both OC cell lines and related normal cells (Figs. 4B and S2). Among these potential targets, the expression decrease of SMG1 was most significant. Hence, SMG1 was selected for further research. SMG1 is an enzyme encoded by the SMG1 gene that belongs to the phosphatidylinositol 3-kinase-related kinase protein family, which is involved in nonsense-mediated mRNA decay (31,32). Recent studies have shown that SMG1 is a potential tumor suppressor gene in acute myeloid leukemia and in planarians (33,34). In the present study, SMG1 was predicted and proven to be a direct target of miR-32. Downregulation of SMG1 restored miR-32-mediated OC cell proliferation, migration and invasion. Therefore, miR-32 may promote OC cell growth and motility by targeting SMG1. Thus, inhibition of miR-32 by its inhibitor has the potential to be considered as a therapeutic strategy for the treatment of OC.

In conclusion, the present results revealed that miR-32 was upregulated in OC tissue samples and cells, and that downregulation of miR-32 inhibited OC cell proliferation and motility. To the best of our knowledge, this is the first time that the oncogenic role of miR-32 in the development and progression of OC has been demonstrated. Inhibition of miR-32 may be a therapeutic strategy in the treatment of OC. Furthermore, SMG1 was shown to be a direct target of miR-32. Downregulation of SMG1 was found to attenuate the inhibition of miR-32-triggered OC cell proliferation and motility. Hence, miR-32 promotes OC cell proliferation and motility via regulation of SMG1. Together, these results uncover the mechanism through which miR-32 serves as an oncogene in OC to promote cancer development and progression, and miR-32 can be explored as a therapeutic target for the clinical treatment of OC. However, there are still limitations to this study. Since ES-2 cells have the highest expression of miR-32, only ES-2 was selected to be extensively studied. In the future, more OC cell lines will be used to confirm the role of miR-32 in OC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

SZ and FX designed and performed the study. SL, JF and JG participated in conducting the experiments and in the statistical analysis. SZ and FX wrote the manuscript. FX revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by The Ethics Committee of Tianjin Medical University (Tianjin, China). Written informed consent was obtained from each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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