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SFRP1 inhibited the epithelial ovarian cancer through inhibiting Wnt/β-catenin signaling

Hao Zhang[1]*, Duohe Sun[2]*, Jianping Qiu[3]✉ and Liangqing Yao[1]✉

[1]Department of Gynecology, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, 200011, P.R. China; [2]Department of Obstetrics and Gynecology, Shanghai Jiaotong University, Affiliated Sixth People's Hospital South Campus, Shanghai, 201499, P.R. China; [3]Department of Obstetrics and Gynecology, Nanjing Medical University Hospital Affiliated Suzhou, Suzhou 215008, P.R. China

Background: Epithelial ovarian cancer is the most malignant gynecologic neoplasm accounting for 90% of the ovarian cancer patients. Objective: Researchers proved that epigenetic alterations could disrupt gene expression as often as genetic alterations. Secreted frizzed related protein (SFRP1), a Wnt antagonist, exerts a significant effect on ovarian cancer. The aim of this research was to investigate the effects and the mechanism of action of SFRP1 on epithelial ovarian cancer. Methods: Clinical specimens (including fallopian tubes epithelium from 60 epithelial ovarian cancer patients' and 20 healthy subjects who were undergoing surgical treatments), transgenic mice (overexpressing *SFRP1* gene), and 4 epithelial ovarian cancer cell lines (including OVCAR4, SKOV3, COV644, TOV21G) were used in this study. Overexpression of *SFRP1* in cells was carried out on OVCAR4 cells by transfection using Lipofectamine 2000. Gene transcription was analyzed by qRT-PCR. The methylation of *SFRP1* gene was quantified by methylation-specific PCR. The level of protein expression was measured by Western blot or immunohistochemistry analysis. Cell proliferation was analyzed by CCK8 methods. The ability of cell migration and invasion were measured by wound healing assay and transwell assay. Results: Abnormal expression level and hypermethylation status of SFRP1 were found in clinical epithelial ovarian cancer samples and cell lines. We observed that SFRP1 knockdown could promote proliferation, migration and invasion abilities of epithelial ovarian cancer cells. Additionally, we discovered a potential inhibitory effect of SFRP1 on Wnt/β-catenin signaling pathway in epithelial ovarian cancer cells. Furthermore, the anti-tumor effect of SFRP1 was tested in *SFRP1* transgenic mice. Conclusion: SFRP1 inhibited epithelial ovarian cancer through inhibiting Wnt/β-catenin pathway, suggesting that SFRP1 could be considered as a potential therapeutic target in epithelial ovarian cancer.

Key words: SFRP1, methylation, Wnt/β-catenin, epithelial ovarian cancer

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✉e-mail: JianpingQiuzxc@163.com (JQ); LiangqingYaozxc@163.com (LY)
*co-first authors

Abbreviations: DKK, Dickkopf-related protein; DMEM, dulbecco's modified eagle medium; IHC, immunohistochemistry analysis; MSP, methylation-specific PCR (MSP); PARP, poly ADP-ribose polymerase inhibitors; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SFRP1, secreted frizzed related protein

INTRODUCTION

Originating in the endometrium, epithelial ovarian cancer is thought to arise from the fallopian tube rather than from ovary or other pelvic organs (Cho & Shih, 2009; Lheureux *et al.*, 2019). Similarly to other types of epithelial neoplasms, epithelial ovarian cancer could metastasize by lymphatics, renal hilus or peritoneum (Lheureux *et al.*, 2019). Epithelial ovarian cancer is proposed as the most malignant gynecologic neoplasm, accounting for 90% ovarian cancer patients (Kurman & Shih, 2010). To date, the mainstream therapeutic method is chemotherapy, including single non–platinum-based agents, taxanes, and poly ADP-ribose polymerase inhibitors (PARP), surgery, including oophorectomy (Baylin & Herman, 2000). However, given many factors, including endocrine disorders and immune dysfunction, epithelial ovarian cancer patients often suffer from tumor recurrence (Morgan *et al.*, 2011). The molecular pathogenic mechanisms underlying epithelial ovarian cancers are complicated, including the gene mutations, endocrine etiology, etc. Interestingly, abnormalities of gene function or chromosome segments are considered as the most important risk factors (Kurman & Shih, 2011). Gene function in the context of cancer might be disrupted by epigenetic modifications or by genetic alterations (Baylin *et al.*, 2001), as the classic genetics theory alone cannot explain the diversity of cancer subtypes. It is well established that aberrant patterns of gene expression, epigenetic modification of DNA and chromatin conformation play an important role in the tumor. Among which, the DNA hypermethylation and hypomethylation are the research hotspots (Baylin *et al.*, 2001).

An extracellular signaling molecule named secreted frizzed related protein (SFRP1), was reported to be a modulator of the important cell signaling pathway in which the Wnt ligand bounds to frizzled membrane receptors (Katoh & Katoh, 2006). Hypermethylation of *SFRP* gene was observed in ovarian cancer, colon cancer, acute myeloid leukemia (Ba *et al.*, 2017). Interestingly, SFRP is a Wnt antagonist that modulates Wnt signaling pathway (Holly *et al.*, 2014). Furthermore, the effects of SFRP on Wnt signaling pathway were reported under physiological or pathological conditions. Both bone mass and metabolism and cancer and systemic sclerosis could be regulated by SFRP/Wnt signaling (Dees *et al.*, 2014). Additionally, Wnt signaling participates in the development of epithelial neoplasms through increasing the expression of proliferation-associated proteins, such as c-Myc and cyclin D1 (Cowling *et al.*, 2007). However, the influence of epigenetic inactivation of *SFRP* gene on Wnt signaling pathway and effects on epithelial ovarian

cancer remain unknown. In this study, with the aim to clarify the effects of epigenetic alterations of *SRFP* on ovarian cancer, we reported abnormal expression levels and methylation status of *SFRP1* in patients. We further explored the effect of *SFRP1* overexpression or knockdown in cell models. Moreover, the regulation of SFRP1 was mediated by Wnt/β-catenin signaling pathway. Interestingly, *SFRP1* gene overexpression could inhibit the growth of epithelial ovarian cancer *in vivo*.

MATERIALS AND METHODS

Experimental subjects. Clinical specimens. A total of 60 epithelial ovarian cancer patients' fallopian tubes epithelium and 20 healthy subjects' fallopian tubes epithelium were obtained from the Chinese female subjects (Peking University People's Hospital, Beijing, China) who were undergoing surgical treatments. All the specimens were ethically justified. All fallopian tube epithelial cell samples were conserved in physiological saline solution after brushing and frozen immediately.

Reagents. The inhibitor of β-catenin named XAV-939 was purchased from Selleck Chemical (S1180).

Cell lines. Four epithelial ovarian cancer cell lines were used in this study, named OVCAR4, SKOV3, COV644, TOV21G. OVCAR4 and SKOV3 were purchased from R&D Systems (Minneapolis, MN). COV644 and TOV21G were bought from American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained at 37°C with 5% CO_2, and cultured in Roswell Park Memorial Institute-1640 medium (RPMI-1640) (Gibco, Grand Island, NY) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, Grand Island, NY), 100 IU penicillin and streptomycin. Human ovarian surface epithelial (HOSE) was chosen as the control cells. HOSE cells were established by immortalizing normal human ovarian surface cells with papilloma virus E6/E7, and cultured in DMEM supplemented with 10% FBS and 50 μg/mL gentamicin sulfate.

Animals. Transgenic mice overexpressing *SFRP1* gene were purchased from Cyagen Biosciences (Guangzhou, China) and were crossed more than 10 generations. The implantation experiment was performed in the 8-week-old transgenic mice. All the experimental procedures were approved by the Obstetrics and Gynecology Hospital Ethics Committee and the institutional animal care and use committee. The animal experiments were in full compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) or with the Guidelines laid down by the NIH in the US.

Establishment of epithelial ovarian cancer animal model. 8-week-old nude mice (Charles River Laboratories, Beijing, China) were utilized to generate the ovarian cancer model. Mice were irradiated with neutron source using a dose of 2.7 Gy. After 1–2 month, ovarian and subcutaneous tumor cells were extracted from the irradiated mice and then implanted into the *SFRP1* transgenic mice and wild-type mice. The size and volume of the tumor were assessed 1 month post implantation.

***SFRP1* plasmid construction and transfection**. The pCMV6 and pCMV6-SFRP1 plasmids were bought from ORIGENE (Rockville, MD). *SFRP1* overexpression was carried out on OVCAR4 cells by transfecting with empty pCMV6 vector as the negative control (NC) and pCMV6-*SFRP1* using Lipofectamine 2000 (ThermoFisher, Cheshire, UK) as in the previous work (Kim *et al.*, 2016). Cells were selected in medium supplemented with Geneticin G418 (ThermoFisher Cheshire, UK) for six weeks.

Knockdown of *SFRP1* was conducted on OVCAR4 cells by RNAi transfection. The oligonucleotide fragments of the negative control (siNC) and siRNA targeting *SFRP1* (siSFRP1) were purchased from GENECHEM (Shanghai, China). OVCAR4 cells were transiently transfected with siSFRP1 using FuGENE HD (Roche, Shanghai, China). Medium was replaced with fresh DMEM 8 hours post transfection.

Isolation of genomic DNA. The genomic DNA was isolated from fallopian tubes epithelium of epithelial ovarian cancer patients and healthy subjects as well as from the epithelial ovarian cancer cell lines. The procedure was performed using DNeasy Blood and Tissue kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's instructions. The isolated genomic DNA was processed for methylation-specific PCR.

Methylation-specific PCR (MSP). The genomic DNA was treated with bisulfite for the bisulfite modification of DNA. EpiTect Fast DNA Bisulfite Kit (Qiagen, Düsseldorf, Germany) was used to convert cytosine to uracil in the unmethylated genomic DNA. We used MethPrimers software to design 5'-TGTAGTTTTCGGAGTTAGTGTCGCGC-3' and 5'-CCTACGATCGAAAACGACGCGAACG-3' (amplicon length: 126 bp; annealing temperature: 60°C) primers for the methylated sequence of *SFRP1*, and 5'-GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT-3' and 5'-CTCAACCTACAATCAAAAACAACACAAACA-3' (amplicon length 135 bp; annealing temperature: 60°C) primers for the unmethylated sequence of *SFRP1*.

qRT-PCR (quantitative reverse transcription-polymerase chain reaction). Total RNA from HOSE, OVCAR4, SKOV3, COV644, TOV21G cells and fallopian tubes epithelium was extracted using Trizol Reagent (Invitrogen, Cheshire, UK). RNA was reverse-transcribed into cDNA using Super Script reverse transcriptase (ThermoFisher, Cheshire, UK). To assess the mRNA level and the transfection efficiency, qRT-PCR analysis was performed with the following primers: *SFRP1* forward, 5'-GATGCTTAAGTGTGACAAGTTCC-3' and *SFRP1* reverse, 5'-TCAGATTTCAACTCGTTGTCACAG-3' (amplicon length: 130 bp; annealing temperature: 62°C); glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) forward, 5'-AGGTGAAGGTCGGAGTCAACG -3' and *GAPDH* reverse, 5'-AGGGGTCATTGATGGCAACA-3' (amplicon length: 130 bp; annealing temperature: 62°C). The transfection efficiency of SFRP1 siRNA was ~70%.

Western blot. Proteins were extracted from OVCAR4 cells post-transfection using lysis solution containing Tris-HCl (20 mM, pH=7.5), Triton X-100 (1%), PMSF (1%). After total protein estimation BCA Protein Assay Kit (ThermoFisher, Cheshire, UK), protein samples were separated by 10% SDS-PAGE, then transferred onto the PVDF membranes. Membranes were incubated with rabbit anti-SFRP1 antibody (Abcam, Cambridge, MA) and goat anti-c-Myc antibody, goat anti-Cyclin D1 antibody, goat anti-β-catenin antibody, goat anti-GAPDH antibody (Abcam, Cambridge, MA) for 16 h at 4°C, and subsequently with secondary anti-goat HRP-conjugated antibody for 1 h at 22°C.

Immunohistochemistry analysis (IHC). Immunohistochemistry analysis was used to test the cell proliferation and invasion and SFRP1 protein expression in the epithelial ovarian cancer tissue. The anti-SFRP1 antibody for IHC was bought from Millipore (Bedford, MA). Pa-

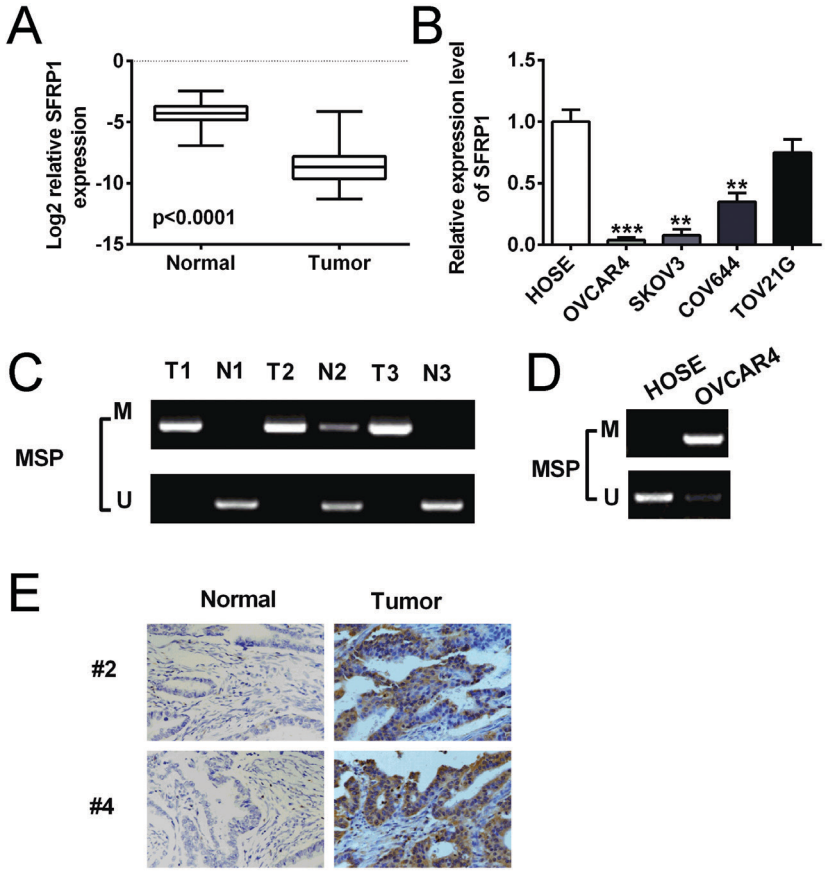

Figure 1. Aberrant expression level and methylation status of *SFRP1*.
(**A**) Log_2 relative mRNA level of SFRP1. (**B**) Relative SFRP1 mRNA levels in five cell lines. (**C**) DNA methylation status of *SFRP1* in clinical samples. (**D**) DNA methylation status of *SFRP1* in OVCAR4 cells. (**E**) IHC analysis showing SFRP1 protein expression in the fallopian tube. (n=10; One-way ANOVA analysis; *p<0.05, **p<0.01, ***p<0.001).

tients' tissues were embedded in paraffin after the conventional treatment, then subjected to IHC analysis.

Post transfection, OVCAR4 cells were seeded onto a 6-well plate. Cells were incubated with DAPI (Abcam, Cambridge, MA) for 30 min. After the Transwell assay, the membranes were fetched and incubated with DAPI for 30 min.

Cellular proliferation assay. 1.5×10^6 OVCAR4 cells were seeded onto 12-well plates in DMEM medium. 36 h post-seeding cells were washed with 1× PBS, and incubated with DAPI for 30 min, observed and counted under a microscope.

The CCK-8 assay was also conducted to test cell proliferation. 1×10^5 cells were plated in 96-well plates and incubated with cell counting kit-8 solutions (R&D Systems, Minneapolis, MN) for 1.5 h at 37°C.

Migration and invasion assay. Wound healing assay was performed to evaluate the cell migration of OVCAR4 cells upon *SFRP1* overexpression or knockdown. Briefly, 2×10^6 OVCAR4 cells were plated in a 6-well plate with RPMI-1640 medium. When the cell confluence reached ~80%, a line in the middle plates was scratched using sterile pipette tip, and washed three times. IncuCyte system (ESSEN, American) was used to observe the wound width.

Transwell assay was conducted to study the cell invasion of OVCAR4 cells upon *SFRP1* overexpression or knockdown. Transwell chambers were inserted into the 6-well plate. The pore diameter of the membrane was 3.0 μm, and the membrane was coated with Matrigel. 36 h post-seeding, membranes were incubated with DAPI for 30 min, observed and quantified under a microscope.

Statistical analysis. All the values in this paper were presented as mean ± standard deviation (S.D.), and analyzed by SPSS 18.0. One-way ANOVA and Dunnett's post hoc test were used to compare several groups to the control. p<0.05 was considered statistically significant in this study (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001).

RESULTS

Expression level and methylation status of *SFRP1* in epithelial ovarian cancer patients and cell lines

Clinical fallopian tubes epithelium samples and the four epithelial ovarian cancer cell lines were collected or purchased as described above. Total RNA was extracted for qRT-PCR analysis. The methylation of *SFRP1* promoter was analyzed in 4 ovarian cancer cell lines and in fallopian tubes epithelium of epithelial ovarian cancer patients by MSP. Results showed that SFRP1 mRNA level in fallopian tube epithelium of epithelial ovarian cancer patients was lower than in healthy subjects (****p<0.0001) (Fig. 1A). We also tested the SFRP1 mRNA levels in four epithelial ovarian cancer cell lines (OVCAR4, SKOV3, COV644, TOV21G) and a control cell line named HOSE, and we observed lower SFRP1 mRNA levels in the four epithelial ovarian cancer cell lines in comparison to HOSE. It is worth mentioning that SFRP1 mRNA level in OVCAR4

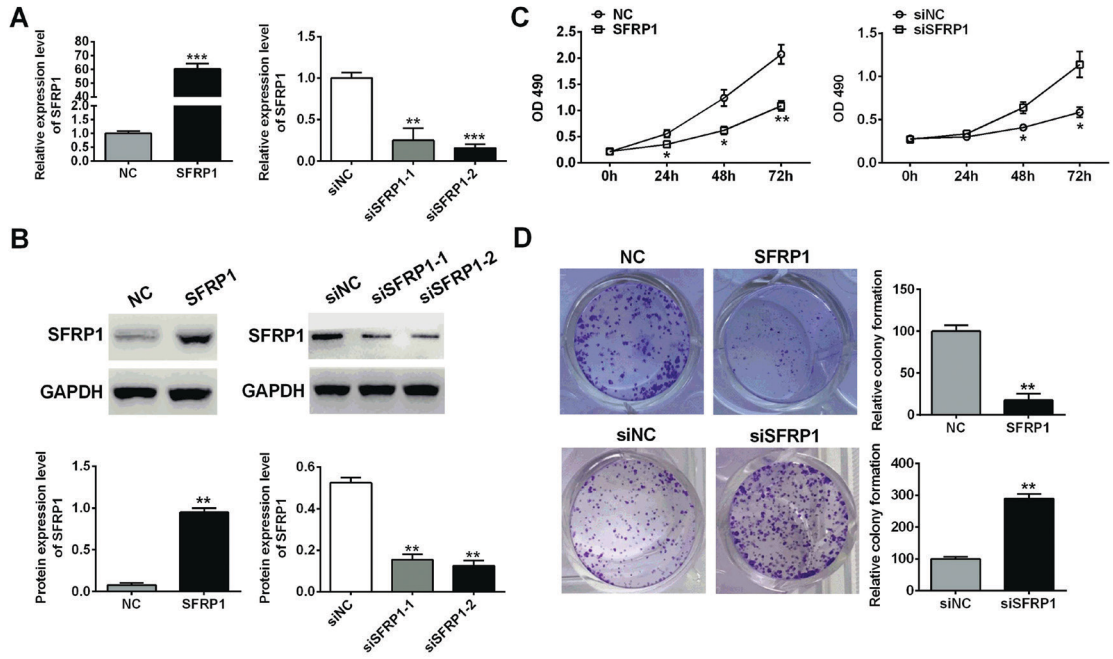

Figure 2. Effects of SFRP1 on cells proliferation.
(**A**) SFRP1 mRNA level upon *SFRP1* overexpression and knockdown. (**B**) SFRP1 protein level upon *SFRP1* overexpression and knockdown. (**C**) OVCAR4 cells activity upon *SFRP1* overexpression and knockdown. (**D**) OVCAR4 cells number upon *SFRP1* overexpression and knockdown. (n=7; One-way ANOVA analysis; **p*<0.05, ***p*<0.01, ****p*<0.001).

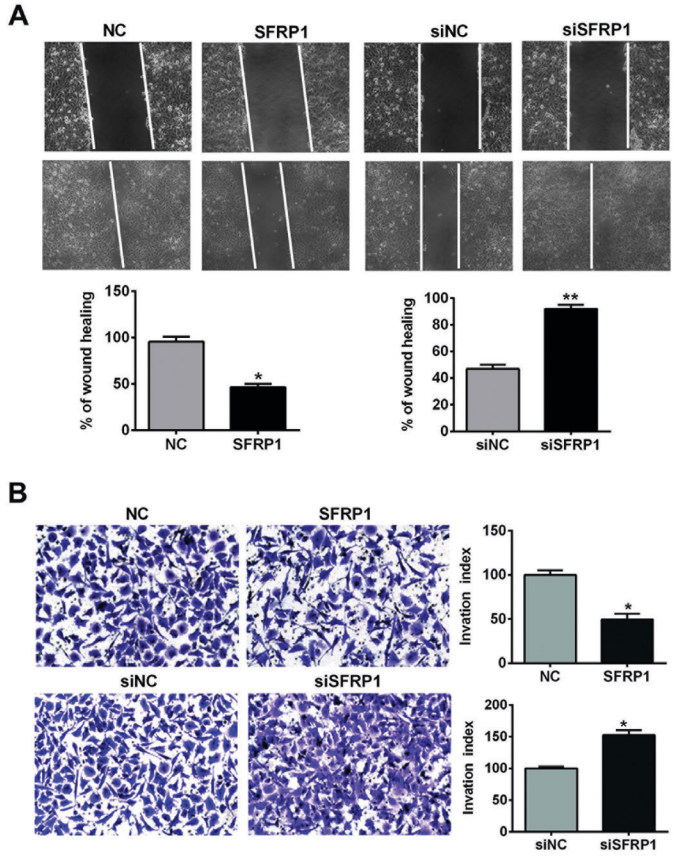

Figure 3. Effects of SFRP1 on cell migration and invasion.
(**A**) The migration rate of OVCAR4 cells upon *SFRP1* overexpression and knockdown. (**B**) Invasion ability of OVCAR4 cells upon *SFRP1* overexpression and knockdown. (n=7; One-way ANOVA analysis; **p*<0.05, ***p*<0.01, ****p*<0.001).

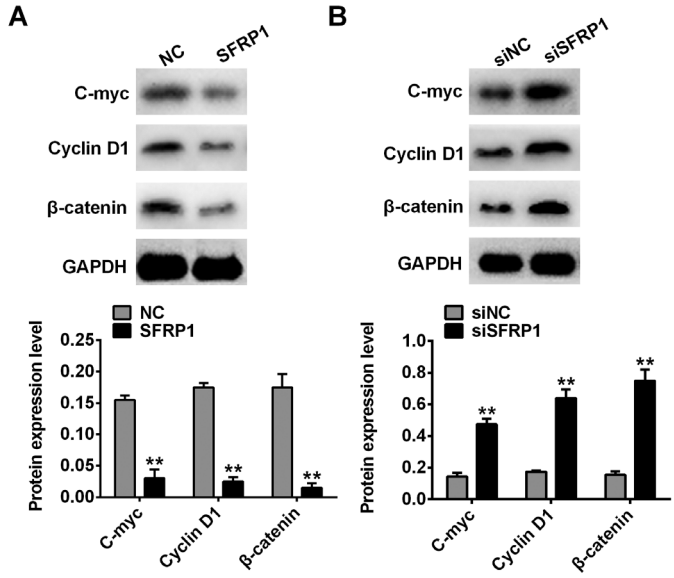

Figure 4. SFRP1 modulated Wnt signaling pathway in epithelial ovarian cancer cells.
(**A**) Effect of *SFRP1* overexpression on Wnt signaling pathway proteins: β-catenin, C-myc, Cyclin D1. (**B**) Effect of *SFRP1* knockdown on Wnt signaling pathway proteins: β-catenin, C-myc, Cyclin D1. (n=7; One-way ANOVA analysis; *p<0.05, **p<0.01, ***p<0.001).

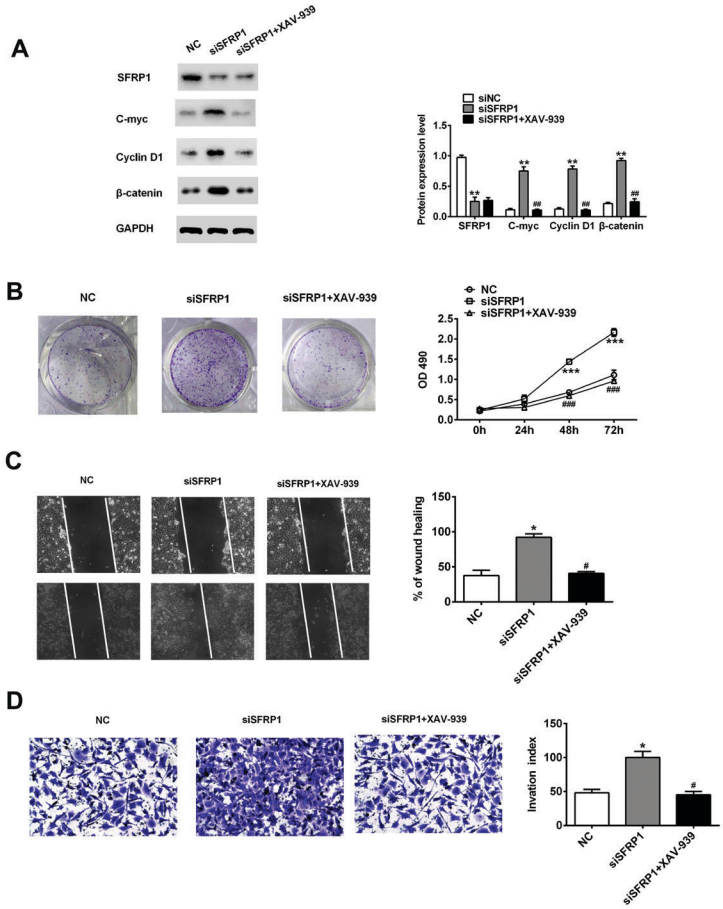

Figure 5. SFRP1 influenced cells proliferation, migration and invasion through modulating Wnt signaling pathway.
(**A**) Effect of β-catenin inhibitor, XAV-939 and *SFRP1* knockdown on Wnt signaling pathway proteins: β-catenin, C-myc, Cyclin D1. (**B**) Effect of β-catenin inhibitor, XAV-939 and *SFRP1* knockdown on the proliferation of OVCAR4 cells. (**C**) Effect of β-catenin inhibitor, XAV-939 and *SFRP1* knockdown on the migration of OVCAR4 cells. (**D**) Effect of β-catenin inhibitor, XAV-939 and *SFRP1* knockdown on the invasion of OVCAR4 cells. (n=7; One-way ANOVA analysis; *p<0.05, **p<0.01, ***p<0.001).

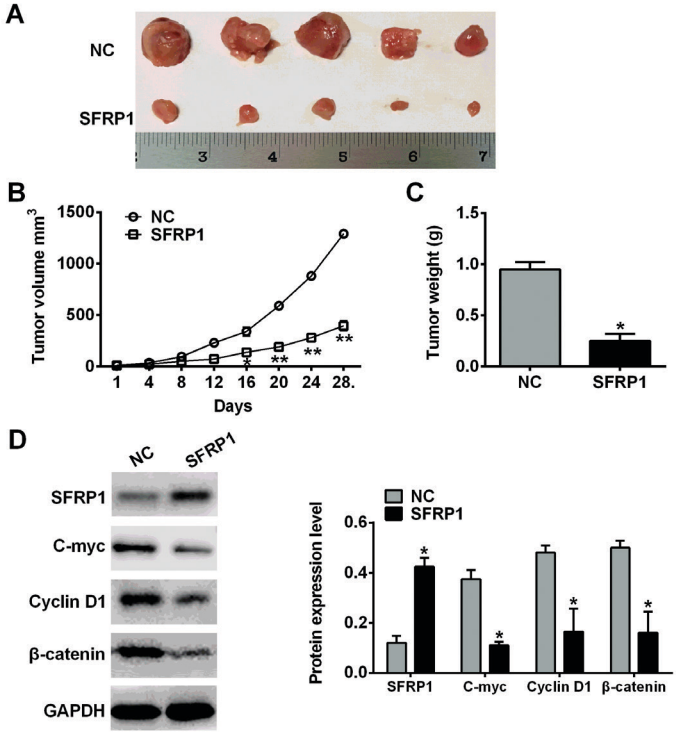

Figure 6. SFRP1 suppressed tumor growth and the expression of key proteins involved in Wnt signaling.
(**A**) Effect of *SFRP1* overexpression on tumor size. (**B**) Effect of *SFRP1* overexpression on tumor volume. (**C**) Effect of *SFRP1* overexpression on tumor weight. (**D**) Effect of *SFRP1* overexpression on the level of Wnt signaling pathway proteins: β-catenin, C-myc, Cyclin D1 in the tumor. (n=10; One-way ANOVA analysis; *p<0.05, **p<0.01, ***p<0.001).

cells was the lowest (Fig. 1B). Therefore, we chose OVCAR4 cells as our model cells.

Extensive research reported the aberration of *SFRP1* gene expression in cancer due to abnormal methylation status (Sugiyama *et al.*, 2013). To further investigate the relationship between SFRP1 mRNA level and methylation, we performed MSP analysis to test the methylation level in clinical fallopian tubes epithelium samples and OVCAR4 cells. Results showed that methylation levels of *SFRP1* gene in fallopian tubes epithelium of epithelial ovarian cancer patients and OVCAR4 cells were higher than in negative control (Fig. 1C–D). Histological staining indicated that SFRP1 protein was decreased in the fallopian tubes epithelium of epithelial ovarian cancer patients (Fig. 1E). Collectively, our data suggest that low expression level of SFRP1 is elevated in epithelial ovarian cancer due to the abnormal methylation of the *SFRP1* gene.

Effects of *SFRP1* on cells proliferation

To further investigate the effects of SFRP1 on epithelial ovarian cancer, we performed overexpression and knockdown of *SFRP1* in OVCAR4 cells. We tested the SFRP1 mRNA and protein level in the OVCAR4 cells, showing that transfection using pCMV6-FoxQ1 plasmid successfully changed SFRP1 mRNA level and SFRP1 protein level (Fig. 2A, B). Moreover, CCK-8 assay was performed to test the cell viability and proliferation. Consistently, *SFRP1* overexpression inhibited, while *SFRP1* knockdown promoted the viability and proliferation of OVCAR4 cells (Fig. 2C, D).

Effects of *SFRP1* on cell migration and invasion

To further explore the influence of *SFRP1* on the migration and invasion, we performed wound healing assay and trans-well assay. Wound healing assay results suggested that *SFRP1* overexpression significantly inhibited the migration ability of OVCAR4 cells, while *SFRP1* knockdown significantly promoted the migration ability (Fig. 3A). Transwell assay demonstrated that *SFRP1* overexpression significantly decreased the invasion rate of OVCAR4 cells, while *SFRP1* knockdown significantly elevated the invasion rate (Fig. 3B).

Effects of SFRP1 on Wnt signaling

According to the previous studies, SFRP1 is considered an antagonist of Wnt signaling, and the aberrant methylation of *SFRP1* gene caused hyperactivation of Wnt pathway (Yamaguchi *et al.*, 2015). In our experiments, we investigated the effect of *SFRP1* on Wnt signaling pathway protein β-catenin and proliferation-associated proteins c-Myc and cyclin D1 by western blot. Results showed that SFRP1 overexpression decreased β-catenin level, which was accompanied by the reduced expression of proliferation-associated proteins c-Myc and cyclin D1 (Fig. 4A). However, *SFRP1* knockdown elevated β-catenin protein level, which was accompanied by the increased expression of c-Myc and cyclin D1 (Fig. 4B).

To further confirm the effect of SFRP1 on Wnt signaling, we next investigated Wnt signaling pathway-related proteins level upon *SFRP1* knockdown. According to the previous results, the proliferation, migration and invasion ability of OVCAR4 cells were increased upon *SFRP1* knockdown. We used the inhibitor of β-catenin, XAV-939 to study the effect of *SFRP1* knockdown without β-catenin activity. Interestingly, results indicated that *SFRP1* knockdown promoted the proliferation, migration and invasion of OVCAR4 cells (Fig. 5B, C, D),

but XAV-939 could reverse such SFRP1-mediated cellular events, which was followed by the decreased c-Myc and cyclin D1 protein expression (Fig. 5A).

Effects of SFRP1 on tumor growth

Given the inhibitory effect of SFRP1 on epithelial ovarian cancer cells, we hypothesized that SFRP1 could also inhibit the tumor growth *in vivo*. With the aim to study the effect of SFRP1 *in vivo*, we subcutaneously injected the epithelial ovarian cancer cells to establish an epithelial ovarian cancer model in *SFRP1* transgenic mice and wild mice. Intriguingly, the size, volume and weight of tumor were decreased in *SFRP1* transgenic mice (Fig. 6A, B, C). Moreover, we further discovered that β-catenin protein level was also lowered in *SFRP1* transgenic mice, which was accompanied by the reduced c-Myc and cyclin D1 levels (Fig. 6D).

DISCUSSION

In this study, we unraveled a link between SFRP1, Wnt signaling and epithelial ovarian cancer. We observed aberrant expression level and methylation status of *SFRP1* in both epithelial ovarian cancer tissues cell models, which were consistent with previous studies. The aberrant expression and methylation of *SFRP1* in cancer were reported extensively: Vedran Kardum *et al.* reported the loss of SFRP1 protein expression observed in low-grade serous ovarian carcinomas (Kardum *et al.*, 2017). The CpG island hypermethylation of *SFRP1* is a common event observed in many kinds of cancers (Atschekzei *et al.*, 2012), which might be one of the key reasons for the lower expression in epithelial ovarian cancer. Jasenka Partl and others (Partl *et al.,* 2014) reported that loss of SFRP1 and SFRP3 expression was involved in the development of human trophoblastic tumors. We analyzed the clinical epithelial ovarian cancer tissue samples and cell models and confirmed the lower expression of *SFRP1* in epithelial ovarian tumor. Furthermore, we verified the relationship between *SFRP1* and epithelial ovarian cancer and proved that SFRP1 protein could inhibit epithelial ovarian cells *in vitro*.

The effect was observed in other cancers such as colon cancer and pancreatic cancer (Bu *et al.*, 2008a, Wang *et al.*, 2018), SFRP1 was considered as a factor inhibiting the tumor growth through Wnt signaling modulation. Moreover, epigenetic inactivation of SFRP1 *via* hypermethylation could be a shared event. Other anti-tumor factors like Dickkopf-related protein 1 (DKK1) could also be methylated in CpG sequences of the promoter (Revet *et al.*, 2010). Briefly, epigenetics alterations should not be neglected in cancer, especially the DNA methylation (Esteller, 2008). DNA methylation is the best known epigenetic marker, which plays an indispensable role in genetic expression. For example, the certain tissue-specific genes: *MASPIN* (a member of the serum protease inhibitor family) and *MAGE* (germ-line genes) are silent in almost all tissues (Berardi *et al.*, 2013). However, hypomethylation and expression of *MASPIN* and *MAGE* genes were detected in cancer cells (Tellez *et al.*, 2009). The hypomethylation of DNA was one of the first epigenetic alterations to be found in human cancer, whose the major reason is CpG islands hypermethylation of tumor-suppressor genes (Tellez *et al.*, 2009). In addition, histone modifications such as acetylation and methylation play an essential role in cancer (Esteller, 2007). Hence, we hypothesized that epigenetic regulations might promote cancer. Nevertheless, the DNA methylation degree varies with each individual and cell line. In our paper, we chose six subjects to perform the MSP analysis, and the results showed that N2 (Fig. 1C) was different from the others. Additionally, cell lines displayed diversity in DNA methylation status and protein level, as OVCAR4 cells were characterized by the highest DNA methylation degree and lowest SFRP1 mRNA level. However, it was not clear whether the cause of the difference was an individual variation or the status of the cells. Considerably, the cell cycle might be controlled in the phase of pre-mRNA or protein synthesis. And the different grades of tumor might be the reason for the different DNA methylation degree, similarly as glutathione S-transferase gene which has different methylation status at the early and late stages of prostate cancer (Lee *et al.*, 1994, Jeronimo *et al.*, 2001).

We also discovered that SFRP1 inhibited tumor growth through regulating Wnt signaling, including β-catenin, cyclin D1, and C-myc. SFRP1 could competitively bind to β-catenin receptor, Fz or directly influence the Wnt-protein functions (Satoh *et al.*, 2010). Cyclin D1 is a nuclear protein required for cell cycle progression in G1 phase, while the anti-cyclin D1 antibody could prevent cells from entering S phase. Cyclin D1 gene is a target of β-catenin (Tetsu & Mccormick, 1999). C-myc, another downstream target of β-catenin, inhibits epithelialization and wound healing (Stojadinovic *et al.*, 2005). Hence, inhibition of the β-catenin expression by SFRP1 was accompanied by the decreased cyclin D1 and C-myc levels, which might partially explain SFRP1-mediated anti-tumor effects. Similarly to SFRP1, other SFRPs family and DKKs family members also function as Wnt antagonists in different tissues and in the context of various diseases (Revet *et al.*, 2010). *SFRP1* gene overexpression could prominently improve the epithelial ovarian cancer in the animal model, indicating that SFRP1 could be a key therapeutic target in epithelial ovarian cancer.

CONCLUSION

We demonstrated an aberrant expression level and methylation status of SFRP1 in both epithelial ovarian cancer tissues and cell models. We reported that SFRP1 influenced the proliferation, migration and invasion via inhibiting β-catenin signaling, which was accompanied by the decreased expression levels of cyclin D1 and C-myc. Importantly, SFRP1 could inhibit epithelial ovarian cancer growth *in vivo*. Collectively, this study shed light on SFRP1 as a potential therapeutic target of epithelial ovarian cancer treatment from bench to clinic.

Conflict of interest

The authors declare no conflict of interests.

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