Clinical and Experimental Obstetrics & Gynecology

Original Research

SFRP1 suppresses granulosa cell proliferation and migration through inhibiting JNK pathway

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DOI:10.31083/j.ceog4805190

Submitted: 6 April 2021 Revised: 21 May 2021 Accepted: 15 June 2021 Published: 15 October 2021

Background: Secreted frizzled-related protein 1 (SFRP1) functions as a Wnt antagonist to repress the proliferation and migration of epithelial ovarian cancer cells. Recent research has shown that SFRP1 was reduced in the subcutaneous abdominal adipose stem cells isolated from patients with polycystic ovarian syndrome (PCOS). Regardless, the regulatory role and mechanism of SFRP1 in the proliferation and migration of granulosa cells during development of PCOS are scarce. *Methods*: SFRP1 expression was analyzed in plasma samples from patients with PCOS or immortalized human granulosa cells (KGN). Cell counting kit-8 (CCK-8) and colony formation assays were used to analyze the cell viability and proliferation of KGN, respectively. Cell apoptosis was analyzed by flow cytometry, and migration was detected by transwell. *Results*: SFRP1 expression was lower in plasma samples isolated from patients with PCOS than the healthy control. Immortalized human granulosa cells (KGN) also showed decreased SFRP1 expression compared to normal ovarian epithelial IOSE80 cells. pcDNA-mediated over-expression of SFRP1 reduced the cell viability and proliferation of KGN via cell counting kit-8 (CCK-8) and colony formation assays, respectively. Flow cytometry, analysis showed that the cell apoptosis of KGN was promoted by SFRP1. Ectopic expression of SFRP1 retarded cell migration with down-regulation of MMP2, MMP9, and vimentin. JNK phosphorylation was reduced in KGN with SFRP1 over-expression. *Conclusion*: SFRP1 contributed to the suppression of granulosa cell proliferation and migration through inhibition of JNK activation, providing a promising molecular target for PCOS.

Keywords

SFRP1; Granulosa cell; Proliferation; Migration; JNK; Polycystic ovarian syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) is a disease with the highest incidence of endocrine disorders in reproductive-age women, and is the most common cause of poor fertility [1]. The most common characteristics of PCOS are excessive androgen secretion, low ovulation, polycystic ovary, sterility and metabolic dysfunction, and so on [2]. Genetic basis and environmental factors are considered as the main etiology of PCOS [3]. However, the exact pathogenesis of PCOS has not been fully understood and remains to be clarified.

Granulosa cells that provide growth regulators and nutrients to the oocyte are the important cellular components of the ovary, and the normal proliferation of granulosa cells is mainly involved in the physiological process of transition from primitive follicles to mature follicles [4]. However, granulosa cells have a higher proliferative rate in the ovaries of PCOS patients than that of healthy persons [5], and the increased granulosa cell proliferation is associated with abnormal folliculogenesis and ovulation in PCOS [5]. Silence of lncRNA UCA1 suppressed human granulosa-like tumor cell proliferation, and ameliorated pathological characteristics, including ovary structural damage and granule cell layers, of mice with PCOS [6]. Therefore, the inhibition of excessive proliferation of granulosa cell is one of the therapeutic strategies for the treatment of PCOS.

Proliferation of mouse granulosa cells is regulated by Wnt2/beta-catenin [7]. The Wnts interact with Frizzled receptors to modulate ovarian steroidogenesis, luteogenesis and normal folliculogenesis [8]. Activation of Wnt pathway was related to the hallmarks of PCOS, including estrogen deficiency and insulin resistance [8]. Secreted frizzled-related protein 1 (SFRP1) belongs to SFRPs family, that function as the extracellular regulators of Wnt pathway, competes with the Frizzled receptors for Wnt binding and participates in tumorigenesis [9]. SFRP1 has been shown to promote colorectal cancer cell apoptosis and repress cell proliferation and metastasis [10]. SFRP1 suppressed Wnt/β-catenin signaling to inhibit the progression of epithelial ovarian cancer [11], and SFRP1 was reduced in the subcutaneous abdominal adipose stem cells isolated from patients with PCOS [12].

Therefore, SFRP1 was hypothesized to be closely associated with the pathogenesis of PCOS.

In this study, the expression of SFRP1 in plasma samples of patients with PCOS was firstly analyzed, which indicated the diagnostic or prognostic roles o SFRP1 in the progression of PCOS. The effects and mechanism of SFRP1 on KGN cell proliferation and migration were then assessed, which could provide a promising molecular target for PCOS.

2. Materials and methods

2.1 Clinical samples

The outpatients and inpatients with PCOS (N = 15) and qualified non-PCOS patients (N = 15) with written informed consents were recruited at endocrinology and gynecology department of Hwa Mei Hospital, University of Chinese Academy of Sciences between 2017 and 2020. The local research was approved by the Hwa Mei Hospital, University of Chinese Academy of Sciences, University of Chinese Academy of Sciences and in accordance with 1964 Helsinki Declaration. The blood samples were from volunteers, and then centrifuged at 1200 g for 10 minutes to collect the plasma samples.

2.2 Cell culture

Human granulosa-like tumor cell line (KGN) and normal ovarian epithelial IOSE80 cells were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). KGN was incubated in DMEM/F-12 medium with 10% fetal bovine serum, 0.1 mg/mL streptomycin sulfate and 100 U/mL penicillin G (Hyclone, South Logan, UT, USA) at a 37 °C incubator. IOSE80 cells were also maintained in DMEM (Hyclone) in a 37 °C incubator.

2.3 qRT-PCR

RNAs were isolated from blood samples, KGN and IOSE80 cells via Trizol (TaKaRa, Shiga, Japan). RNA was reverse-transcribed into cDNA, and qRT-PCR analysis was assessed by Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), and GAPDH was used as the endogenous control. The conditions were shown as: 94 °C for 15 minutes, 40 cycles of 94 °C for 30 seconds; 59 °C for 1 minute. The fold change of SFRP1 was calculated by the $2^{-\Delta\Delta Ct}$ method with the following primers (Table 1).

Table 1. Primers used for qRT-PCR.

ID	Sequences (5′-3′)
GAPDH F	AGGTCGGTGTGAACGGATTTG
GAPDH R	TGTAGACCATGTAGTTGAGGTC
SFRP1 F	GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT
SFRP1 R	CTCAACCTACAATCAAAAACAACACAAACA

2.4 Cell transfection

The pcDNA3.1-OSR1 was constructed by RiboBio (Guangzhou, China). KGN cells were plated in the 96-well plates, and transfected with pcDNA3.1-SFRP1 or pcDNA vector (300 μg) via Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Cells without transfection and treated with Lipofectamine 2000 were used as the control group.

2.5 Western blot

The plasma samples, KGN and IOSE80 cells were lysed with RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific). The protein concentration of lysates was calculated by acid protein kit (Thermo Fisher Scientific). The lysates (30 μg) were separated by SDS-PAGE, and electro-transferred onto PVDF membrane (Thermo Fisher Scientific). Membranes were blocked with 5% bovine serum albumin, and probed with primary antibodies: anti-SFRP1 and anti-Bax (1 : 2000, Cell Signaling, Beverly, MA, USA), anti-Bcl-2 and anti-cleaved caspase-3 (1 : 2500, Cell Signaling), anti-MMP2 and anti-MMP9 (1 : 3000, Cell Signaling), anti-JNK and anti-p-JNK (1 : 3500, Cell Signaling), anti-vimentin and anti-β-actin (1 : 4000, Cell Signaling). Following incubation with the corresponding horseradish peroxidase-labeled secondary antibody (1 : 5000; Cell Signaling), the immunoreactivities of bands in the membranes were detected by enhanced chemiluminescence (KeyGen, Nanjin, China).

2.6 Cell viability and colony formation

KGN cells were plated in the 96-well plates for 24, 48, 72 or 96 hours, and then incubated with 10 μL of CCK8 solution (Dojindo, Kumamoto, Japan) for 2 hours. Absorbance at 450 nm of each well was measured by ELISA reader (BioTek, Winooski, VT, USA). KGN cells were plated in the 6-well plates, and cultured for two weeks to assess the cell colony formation. The formaldehyde-fixed cells were stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA), and counted under light microscope (Olympus Corp. Tokyo, Japan).

2.7 Cell migration

KGN cells in 100 μL serum-free DMEM/F-12 medium was plated in the upper chamber of transwell chamber (Biosciences, San Jose, CA, USA). The lower chamber was filled with 600 μL DMEM/F-12 medium with 20% fetal bovine serum. Two days later, cells in the lower chamber were fixed in 10% formaldehyde and then stained with 0.1% crystal violet (Sigma-Aldrich) before measurement under microscope (Olympus Corp).

2.8 Cell apoptosis

KGN cells were harvested following centrifugation at 1000 g for 5 minutes. Cells were then resuspended in 100 μL Annexin-binding buffer with 5 μL Annexin V-FITC plus 1 μL propidium iodide (Sigma-Aldrich). The apoptotic cells were analyzed under flow cytometry (Becton Dickson Immunocytometry-Systems, San Jose, CA, USA).

2.9 Statistical analysis

Data were expressed as mean $\pm$ SEM, and performed with one-way analysis of variance or student's *t* test under GraphPad Prism 5.0 (GraphPad Software, SanDiego, CA, USA). The p value < 0.05 was considered as statistically significant.

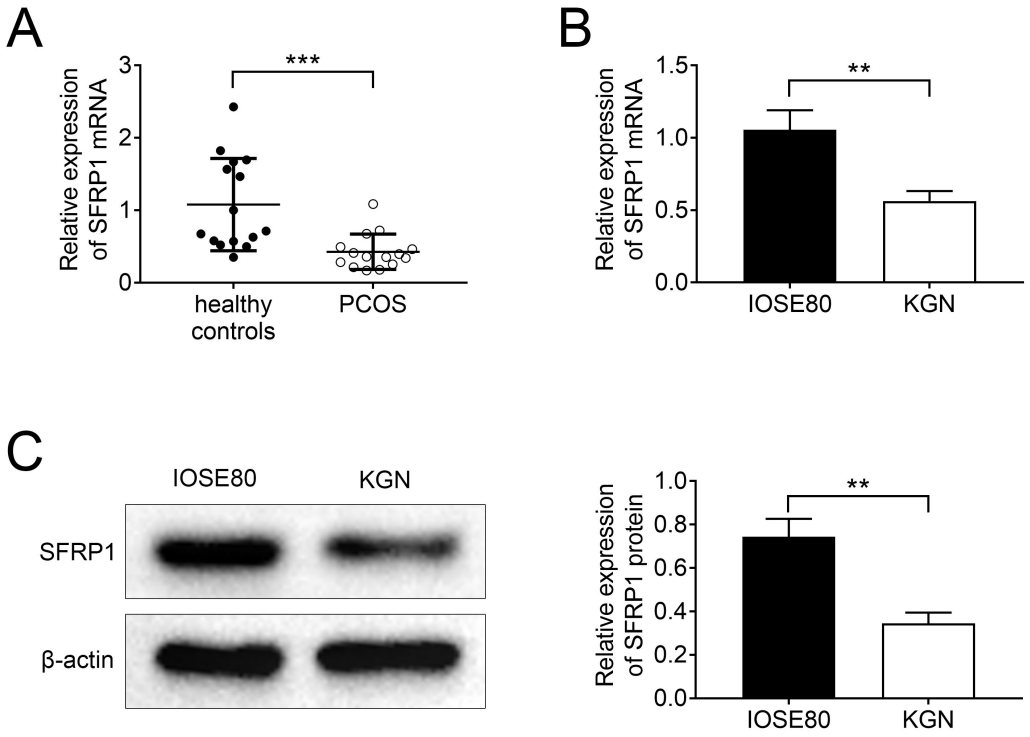

Fig. 1. Downregulation of SFRP1 in patients with PCOS. (A) SFRP1 was downregulated in the plasma samples from PCOS patients compared to qualified non-PCOS patients (healthy controls) by qRT-PCR. (B) The mRNA expression of SFRP1 was downregulated in KGN cells compared to that in IOSE80 cells by qRT-PCR. (C) The protein expression of SFRP1 was downregulated in KGN cells compared to that in IOSE80 cells by western blot. ** $p < 0.01$ *vs.* healthy controls or IOSE80 group.

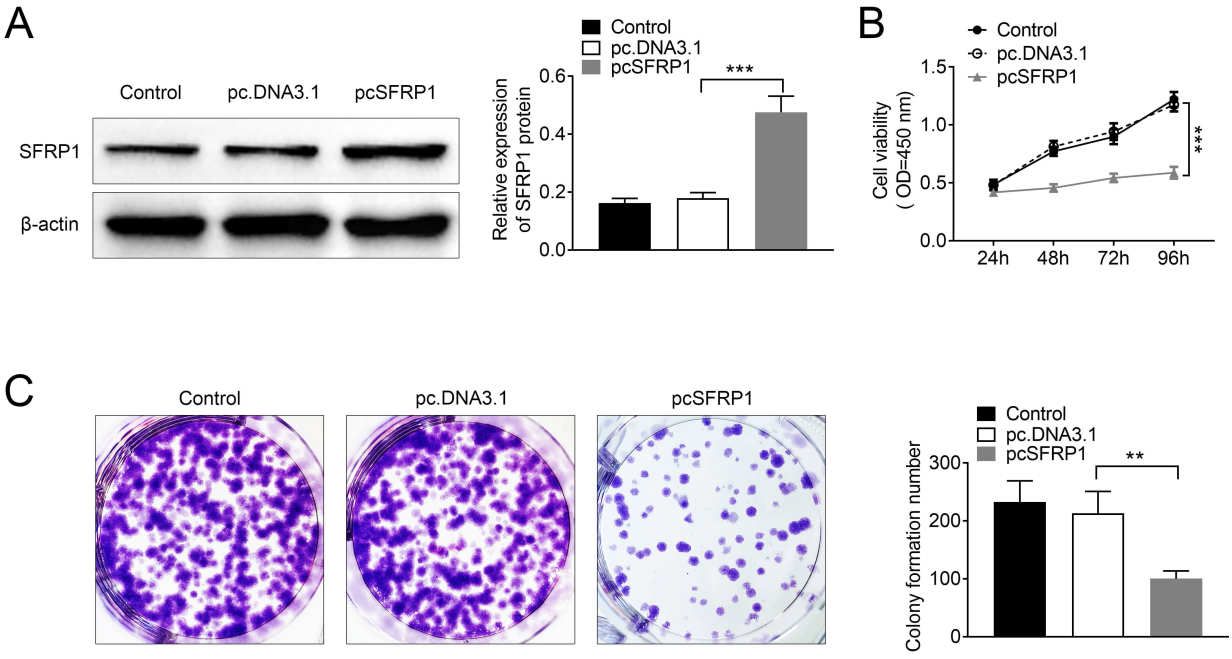

Fig. 2. SFRP1 decreased KGN cell proliferation and colony formation. (A) Protein expression of SFRP1 was increased in KGN cells that transfected with pcDNA-SFRP1 by western blot. (B) Ectopic expression of SFRP1 reduced KGN cell viability by CCK-8. (C) Ectopic expression of SFRP1 decreased KGN cell proliferation by colony formation assay. ** $p < 0.01$ and *** $p < 0.001$ *vs.* pcDNA3.1 group.

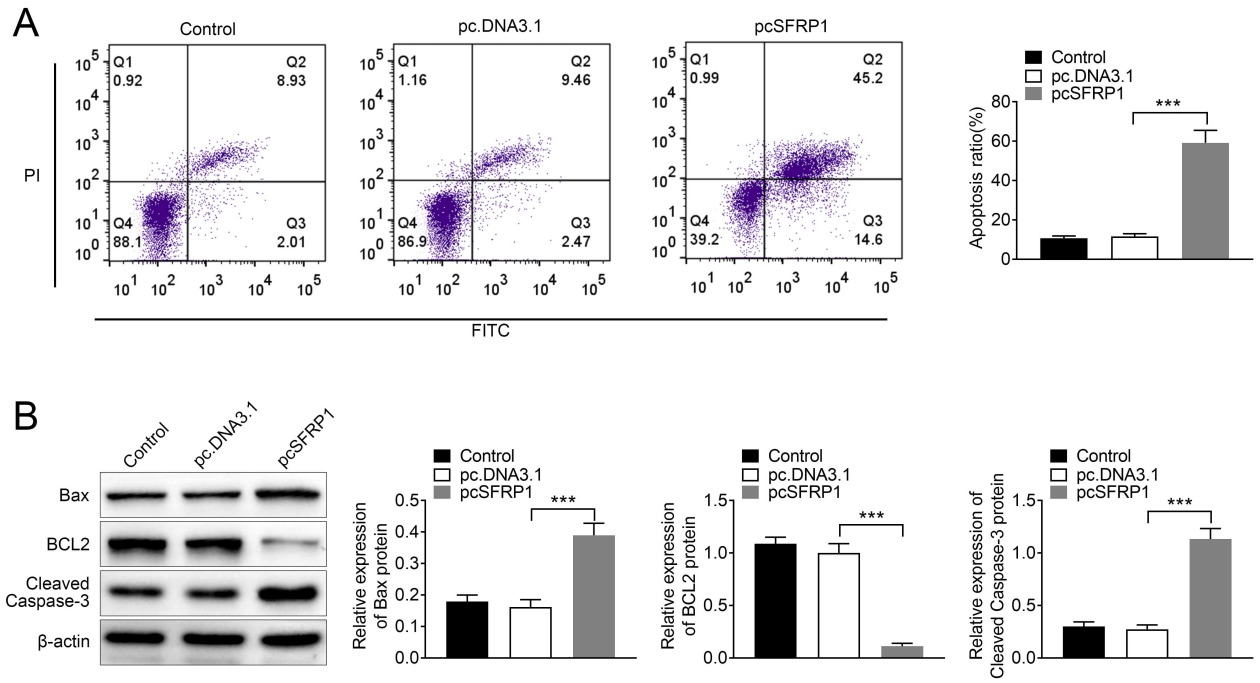

Fig. 3. SFRP1 promoted KGN cell apoptosis. (A) Ectopic expression of SFRP1 promoted KGN cell apoptosis by flow cytometry. (B) Ectopic expression of SFRP1 down-regulated the protein expression levels of Bcl-2, and up-regulated Bax and cleaved caspase-3 in KGN cells by western blot. *** $p < 0.001$ *vs.* pcDNA3.1 group.

3. Results

3.1 Downregulation of SFRP1 in patients with PCOS

To determine the expression level of SFRP1 in patients with PCOS, the plasma samples collected from PCOS patients and non-PCOS patients (healthy controls) were used for the measurement of SFRP1 expression. The mRNA expression of SFRP1 was downregulated in PCOS patients as compared to that in healthy controls (Fig. 1A). In KGN cell, the lower expression of SFRP1 was showed as compared to that in IOSE80 cells (Fig. 1B,C), suggesting the potentially regulatory role of SFRP1 in the progression of PCOS.

3.2 SFRP1 decreased KGN cell proliferation and colony formation

To further explore the mechanism underlying the role of SFRP1 in the progression of PCOS, KGN cells was transfected with pcDNA vector for the over-expression of SFRP1. The protein expression of SFRP1 was higher in KGN cells transfected with pcDNA-SFRP1 than the control or pcDNA vector, indicating the successful transfection efficiency (Fig. 2A). The functional results showed that the ectopic expression of SFRP1 reduced KGN cell viability (Fig. 2B), and decreased cell colony formation (Fig. 2C), demonstrating the anti-proliferative effect of SFRP1 on the granulosa cells.

3.3 SFRP1 promoted KGN cell apoptosis

The ectopic expression of SFRP1 significantly promoted KGN cell apoptosis compared to the control vector group (Fig. 3A). Besides, compared to the control vector group, transfection with pcDNA-SFRP1 down-regulated the protein expression of Bcl-2 (Fig. 3B), and up-regulated Bax and cleaved caspase-3 in KGN cells (Fig. 3B). These results indicated the pro-apoptotic effect of SFRP1 on the granulosa cells.

3.4 SFRP1 suppressed KGN cell migration

In addition to the anti-proliferative and pro-apoptotic effects, the ectopic expression of SFRP1 also suppressed KGN cell migration (Fig. 4A). The protein expression of MMP2, MMP9 and Vimentin were decreased by pcDNA-SFRP1 compared to the control vector group (Fig. 4B), suggesting the anti-migratory effects of SFRP1 on the granulosa cells.

3.5 SFRP1 repressed the activation of p-JNK in KGN cells

The protein expression of JNK was not significantly affected by SFRP1 over-expression compared to the control vector group (Fig. 5). However, transfection with pcDNA-SFRP1 in KGN cells decreased the protein expression of JNK phosphorylation (p-JNK) than the control vector group (Fig. 5), revealing that SFRP1 repressed the activation of JNK pathway to inhibit granulosa cell proliferation and migration.

4. Discussion

SFRPs directly bind to Frizzled receptors or Wnt ligands to inhibit Wnt signaling, and participate in tumor progression, including endometrial cancer and ovarian cancer [13]. Since Wnt activation was found to be related to the hallmarks of PCOS [8], SFRPs might be involved in the development of PCOS. Indeed, SFRP4 was significantly increased in the apoptotic granulosa cells, and was implicated in the pre-

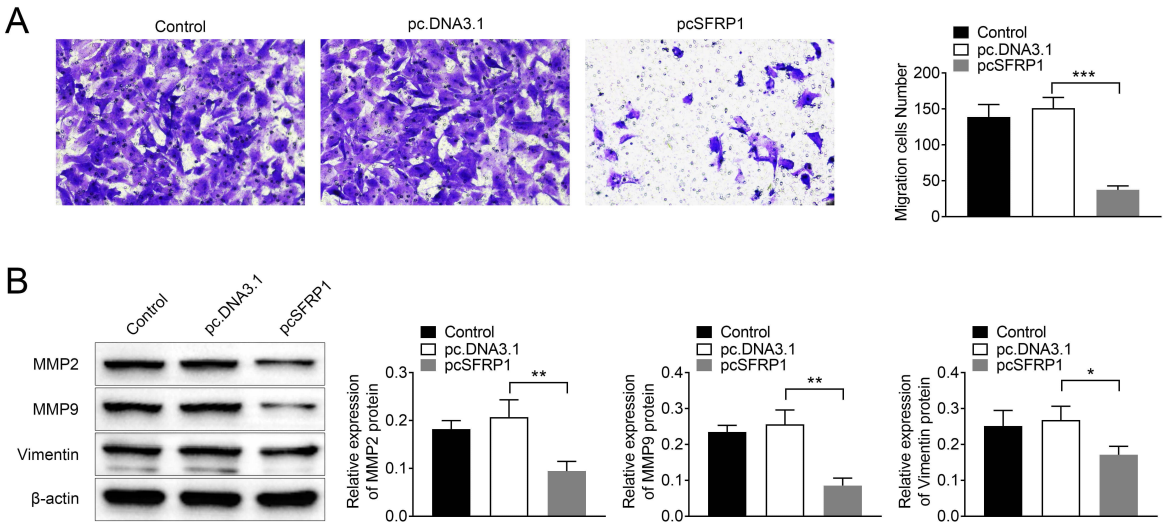

Fig. 4. SFRP1 suppressed KGN cell migration. (A) Ectopic expression of SFRP1 suppressed KGN cell migration by transwell assay. (B) Ectopic expression of SFRP1 reduced the protein expression levels of MMP2, MMP9 and Vimentin in KGN by western blot. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ *vs.* pcDNA3.1 group.

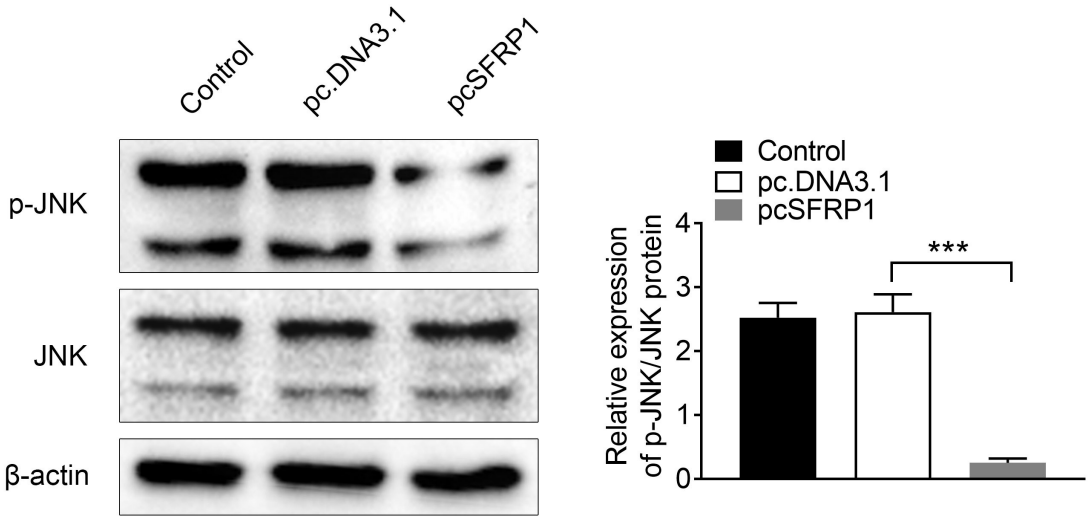

Fig. 5. SFRP1 repressed the activation of p-JNK in KGN cells. Ectopic expression of SFRP1 reduced the protein expression of p-JNK in KGN cells by western blot. *** $p < 0.001$ *vs.* pcDNA3.1 group.

mature differentiation of follicles during the development of PCOS [14]. SFRP5 level was related to the insulin and inflammatory markers in patients with PCOS [15]. However, to our best knowledge, the effects of SFRPs on PCOS have not been reported yet. Considering the fact that SFRP1 was reduced in the subcutaneous abdominal adipose stem cells isolated from patients with polycystic ovarian syndrome [12], this study is the first evidence demonstrating that SFRP1 suppressed granulosa cell proliferation and migration to attenuate PCOS.

A dramatically down-regulation of SFRP1 was identified in both of plasma samples from PCOS patients and KGN cells. Epigenetic alterations, such as DNA methylation of transcription factors, have been shown to be associated with the follicular development of granulosa cells, and involved in the development of PCOS [16]. SFRPs were often down-regulated in tumor tissues through hypermethylation of the promoters, and epigenetic modifying agents that repressed the methylation reversed the expression of SFRPs and further antagonized Wnt-driven tumorigenesis [13]. Hypermethylation of SFRP1 lead to impaired transcription and reactivation of SFRP1 attenuated pulmonary fibrosis in mice [17]. Therefore, the dynamic methylation of SFRP1 in PCOS should be investigated in the further research to provide po-

tential therapeutic strategy for clinical implication of SFRP1 in PCOS. Drug-resistant cells, such as cancer stem cells, are responsible for the high rate of recurrence in epithelial ovarian cancer (Cytogenetic analysis of epithelial ovarian cancer's stem cells: an overview on new diagnostic and therapeutic perspectives), and methylation of SFRP1 promoter is related to the primary cytogenetic resistance of chronic myeloid leukemia to imatinib mesylate (sFRP1 promoter methylation is associated with persistent Philadelphia chromosome in chronic myeloid leukemia). Therefore, SFRP1 might be a potential therapeutic target for the treatment of PCOS, and drug-resistant ovarian cancer.

Androgens are converted into estrogens in the granulosa cells, thus participating in steroidogenesis [18]. Disturbance of steroidogenesis results in hormonal abnormality, and is implicated in the pathogenesis of PCOS [19]. Regulation of granulosa cells is linked to the steroidogenic property of PCOS [20]. Functional analysis in this study showed that ectopic expression of PCOS reduced KGN cell viability and proliferation, while promoted cell apoptosis. Previous study has shown that patients with PCOS demonstrated higher proliferative rate and lower apoptotic rate in the granulosa cells than that in the normal control [5]. The dysregulated granulosa cell proliferation resulted in the prenatal folliculogenesis of PCOS patients [21]. Suppression of granulosa cell proliferation has been regarded as a potential strategy for ovulation and folliculogenesis in PCOS patients [22]. The antiproliferative effect of SFRP1 on KGN cells suggested that SFRP1 might be a potential therapeutic target for the treatment of PCOS. Granulosa cell migration has been reported to essential for the follicle development [23], and the aberrant migration of granulosa cells regulated maturation of the oocyte and contributed to PCOS development [24]. Suppression of granulosa cell migration could repress PCOS progression [25]. In this study, over-expression of SFRP1 reduced the protein expression of MMP2, MMP9 and Vimentin to suppress the KGN cell migration. Therefore, SFRP1 exerted anti-migratory effect on granulosa cell to suppress PCOS progression. Accumulating evidence has suggested that insulin-resistance is one of the most important mechanism of PCOS pathogenesis [26], and insulin-sensitizer, such as inositol isoforms, has been widely studied in the treatment of PCOS due to the safety profile and effectiveness [27]. Since the mRNA expression of SFRP1 was found to be negatively related to insulin resistance [28], and mice with Sfrp1$^{-/-}$ showed systemically insulin resistant [29]. Therefore, SFRP1 might regulate insulin resistance and is thus involved in PCOS.

JNK is involved in the non-canonical Wnt signaling pathway and associated with SFRPs-mediated tumor progression [13]. JNK pathway was activated in PCOS rats with elevated JNK phosphorylation level [30]. Inhibition of JNK reduced ovary fibrosis and suppressed inflammation to attenuate PCOS progression [31]. Moreover, JNK inactivation was also implicated in the suppression of granulosa cell proliferation and migration [32]. Phosphorylated JNK was enhanced in SFRP1 delete mice [33], and forced SFRP1 decreased JNK phosphorylation to protect cardiac myoblasts against doxorubicin-induced apoptosis [34]. This study indicated that ectopic expression of SFRP1 decreased JNK phosphorylation in KGN cells, revealing that JNK pathway was involved in SFRP1-mediated PCOS progression.

5. Conclusions

In conclusion, this study provided the first evidence showing that over-expression of SFRP1 suppressed granulosa cell proliferation and migration through inhibition of JNK pathway. Therefore, SFRP1 might be a novel therapeutic target for PCOS treatment. However, the *in vivo* effect of SFRP1 on PCOS should be further investigated for its clinical application.

Abbreviations

SFRP1, Secreted frizzled-related protein 1; KGN, Immortalized human granulosa cells; CCK8, cell counting kit-8; PCOS, polycystic ovarian syndrome; DMEM, Dulbecco's Modified Eagle Medium; qRT-PCR, Quantitative Reverse Transcription PCR; PVDF, Polyvinylidene Fluoride; SDS-PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Author contributions

SZ designed the study, supervised the data collection, LX analyzed the data, interpreted the data, LYH prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of Hwa Mei Hospital, University of Chinese Academy of Sciences (Approval No. PJ-NBEY-KY-2020-182-01). Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Acknowledgment

Thanks to all the peer reviewers for their opinions and suggestions.

Funding

This work was supported by the Hwa Mei Research Fund of Hwa Mei Research Fund of Hwa Mei Hospital, University of Chinese Academy of Sciences (Grant No. 2019HMKY32).

Conflict of interest

The authors declare no conflict of interest.

References

[1] Azziz R. Polycystic Ovary Syndrome. Obstetrics & Gynecology. 2018; 132: 321–336.

[2] Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nature Reviews. Endocrinology. 2018; 14: 270–284.
[3] McCartney CR, Marshall JC. Polycystic Ovary Syndrome. New England Journal of Medicine. 2016; 375: 54–64.
[4] Tu J, Cheung AH, Chan CL, Chan WY. The Role of microRNAs in Ovarian Granulosa Cells in Health and Disease. Frontiers in Endocrinology. 2019; 10: 174–174.
[5] Das M, Djahanbakhch O, Hacihanefioglu B, Saridogan E, Ikram M, Ghali L, *et al.* Granulosa Cell Survival and Proliferation are Altered in Polycystic Ovary Syndrome. Journal of Clinical Endocrinology & Metabolism. 2008; 93: 881–887.
[6] Yang D, Wang Y, Zheng Y, Dai F, Liu S, Yuan M, *et al.* Silencing of lncRNA UCA1 inhibited the pathological progression in PCOS mice through the regulation of PI3K/AKT signaling pathway. Journal of Ovarian Research. 2021; 14: 48.
[7] Wang H, Li T, Kidder GM. WNT2 Regulates Proliferation of Mouse Granulosa Cells through Beta-Catenin. Biology of Reproduction. 2009; 81: 376–376.
[8] Qiao G, Dong B, Zhu C, Yan C, Chen B. Deregulation of WNT2/FZD3/β-catenin pathway compromises the estrogen synthesis in cumulus cells from patients with polycystic ovary syndrome. Biochemical and Biophysical Research Communications. 2017; 493: 847–854.
[9] Caldwell GM, Jones C, Gensberg K, Jan S, Hardy RG, Byrd P, *et al.* The Wnt Antagonist sFRP1 in Colorectal Tumorigenesis. Cancer Research. 2004; 64: 883–888.
[10] Wang Z, Li R, He Y, Huang S. Effects of secreted frizzled-related protein 1 on proliferation, migration, invasion, and apoptosis of colorectal cancer cells. Cancer Cell International. 2018; 18: 48.
[11] Zhang H, Sun D, Qiu J, Yao L. SFRP1 inhibited the epithelial ovarian cancer through inhibiting Wnt/β-catenin signaling. Acta Biochimica Polonica. 2019.
[12] Leung KL, Sanchita S, Pham CT, Davis BA, Okhovat M, Ding X, *et al.* Dynamic changes in chromatin accessibility, altered adipogenic gene expression, and total versus de novo fatty acid synthesis in subcutaneous adipose stem cells of normal-weight polycystic ovary syndrome (PCOS) women during adipogenesis: evidence of cellular programming. Clinical Epigenetics. 2020; 12: 181.
[13] Surana R, Sikka S, Cai W, Shin EM, Warrier SR, Tan HJG, *et al.* Secreted frizzled related proteins: Implications in cancers. Biochimica et Biophysica Acta. 2014; 1845: 53–65.
[14] Bicer M, Alarslan P, Guler A, Demir I, Aslanipour B, Calan M. Elevated circulating levels of secreted frizzled-related protein 4 in relation to insulin resistance and androgens in women with polycystic ovary syndrome. Journal of Endocrinological Investigation. 2020; 43: 305–313.
[15] Inal ZO, Inal HA, Erdem S. The effect of serum and follicular fluid secreted frizzle-related protein-5 on in vitro fertilization outcomes in patients with polycystic ovary syndrome. Molecular Biology Reports. 2018; 45: 2037–2044.
[16] Qu F, Wang F, Yin R, Ding G, El-Prince M, Gao Q, *et al.* A molecular mechanism underlying ovarian dysfunction of polycystic ovary syndrome: hyperandrogenism induces epigenetic alterations in the granulosa cells. Journal of Molecular Medicine. 2012; 90: 911–923.
[17] Zhou J, Yi Z, Fu Q. Dynamic decreased expression and hypermethylation of secreted frizzled-related protein 1 and 4 over the course of pulmonary fibrosis in mice. Life Sciences. 2019; 218: 241–252.
[18] Hertelendy F, Asem EK. Steroidogenesis in granulosa cells during follicular maturation: evidence for desensitization-resensitization during the ovulation cycle. Journal of Experimental Zoology. 1984; 232: 513–520.
[19] Bakhshalizadeh S, Amidi F, Shirazi R, Shabani Nashtaei M. Vitamin D3 regulates steroidogenesis in granulosa cells through AMP-activated protein kinase (AMPK) activation in a mouse model of polycystic ovary syndrome. Cell Biochemistry and Function. 2018; 36: 183–193.
[20] Hasegawa T, Kamada Y, Hosoya T, Fujita S, Nishiyama Y, Iwata N, *et al.* A regulatory role of androgen in ovarian steroidogenesis by rat granulosa cells. Journal of Steroid Biochemistry and Molecular Biology. 2017; 172: 160–165.
[21] Stubbs SA, Stark J, Dilworth SM, Franks S, Hardy K. Abnormal preantral folliculogenesis in polycystic ovaries is associated with increased granulosa cell division. Journal of Clinical Endocrinology and Metabolism. 2007; 92: 4418–4426.
[22] Cai G, Ma X, Chen B, Huang Y, Liu S, Yang H, *et al.* MicroRNA-145 Negatively Regulates Cell Proliferation through Targeting IRS1 in Isolated Ovarian Granulosa Cells from Patients with Polycystic Ovary Syndrome. Reproductive Sciences. 2017; 24: 902–910.
[23] Buensuceso AV, Deroo BJ. The Ephrin Signaling Pathway Regulates Morphology and Adhesion of Mouse Granulosa Cells in Vitro1. Biology of Reproduction. 2013; 88: 25.
[24] Wang D, Di X, Wang J, Li M, Zhang D, Hou Y, *et al.* Increased Formation of Follicular Antrum in Aquaporin-8-Deficient Mice is Due to Defective Proliferation and Migration, and not Steroidogenesis of Granulosa Cells. Frontiers in Physiology. 2018; 9: 1193.
[25] Jia C, Wang S, Yin C, Liu L, Zhou L, Ma Y. Loss of hsa_circ_0118530 inhibits human granulosa-like tumor cell line KGN cell injury by sponging miR-136. Gene. 2020; 744: 144591.
[26] Laganà AS, Rossetti P, Sapia F, Chiofalo B, Buscema M, Valenti G, *et al.* Evidence-Based and Patient-Oriented Inositol Treatment in Polycystic Ovary Syndrome: Changing the Perspective of the Disease. International Journal of Endocrinology and Metabolism. 2017; 15: e43695.
[27] Facchinetti F, Unfer V, Dewailly D, Kamenov ZA, Diamanti-Kandarakis E, Laganà AS, *et al.* Inositols in Polycystic Ovary Syndrome: an Overview on the Advances. Trends in Endocrinology & Metabolism. 2020; 31: 435–447.
[28] Ehrlund A, Mejhert N, Lorente-Cebrián S, Aström G, Dahlman I, Laurencikiene J, *et al.* Characterization of the Wnt inhibitors secreted frizzled-related proteins (SFRPs) in human adipose tissue. Journal of Clinical Endocrinology and Metabolism. 2013; 98: E503–E508.
[29] Gauger KJ, Bassa LM, Henchey EM, Wyman J, Bentley B, Brown M, *et al.* Mice deficient in Sfrp1 exhibit increased adiposity, dysregulated glucose metabolism, and enhanced macrophage infiltration. PLoS ONE. 2013; 8: e78320.
[30] Wu Y, Li P, Zhang D, Sun Y. Metformin and pioglitazone combination therapy ameliorate polycystic ovary syndrome through AMPK/PI3K/JNK pathway. Experimental and Therapeutic Medicine. 2018; 15: 2120–2127.
[31] Bulut G, Kurdoglu Z, Dönmez YB, Kurdoglu M, Erten R. Effects of jnk inhibitor on inflammation and fibrosis in the ovary tissue of a rat model of polycystic ovary syndrome. International Journal of Clinical and Experimental Pathology. 2015; 8: 8774–8785.
[32] Xia H, Zhao Y. MiR-155 is high-expressed in polycystic ovarian syndrome and promotes cell proliferation and migration through targeting PDCD4 in KGN cells. Artificial Cells, Nanomedicine, and Biotechnology. 2020; 48: 197–205.
[33] Matsuyama M, Nomori A, Nakakuni K, Shimono A, Fukushima M. Secreted Frizzled-related protein 1 (Sfrp1) regulates the progression of renal fibrosis in a mouse model of obstructive nephropathy. Journal of Biological Chemistry. 2014; 289: 31526–31533.
[34] Tu M, He L, You Y, Li J, Yao N, Qu C, *et al.* EFTUD2 maintains the survival of tumor cells and promotes hepatocellular carcinoma progression via the activation of STAT3. Cell Death & Disease. 2020; 11: 830.