Exosomal miR-143-3p Derived from PCOS Follicular Fluid Induces Granulosa Cell Apoptosis by Targeting BMPR1A and Suppression of Smad1/5/8 Signaling

Yuanyuan Zhao
the fourth hospital of Shijiazhuang

Shuhong Pan
the fourth hospital of Shijiazhuang

Yunying Li
the fourth hospital of Shijiazhuang

Xiaohua Wu (✉️ wuxiaohua1965@163.com)
the fourth hospital of Shijiazhuang

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**Abstract**

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorders disease in women of reproductive age. The anovulation caused by abnormal follicular development is still the main characteristic of infertile patients with PCOS. Granulosa cells (GCs), an important component of follicular microenvironment, affect follicular development through GCs dysfunction. Increasing evidence indicates that exosomal miRNAs derived from follicular fluid (FF) of patients play critical roles during PCOS. However, which and how follicular fluid derived exosomal miRNAs play a pivotal role in controlling granulosa cells function and consequently follicular development remain largely unknown. Herein, we showed that miR-143-3p is highly expressed in follicular fluid exosomes of PCOS patients and can be delivered into granulosa cells. Furthermore, the functional experiments showed that the translocated miR-143-3p promoted granulosa cell apoptosis, which are important in follicle development. In terms of mechanism, we demonstrated that BMPR1A was identified as a direct target of miR-143-3p. Overexpression of BMPR1A reversed the effects of exosomal miR-143-3p on GCs apoptosis and proliferation by activating Smad1/5/8 signaling pathway. These results demonstrate that miR-143-3p-containing exosomes derived from PCOS follicular fluid promoted granulosa cell apoptosis by targeting BMPR1A and blockading Smad1/5/8 signaling pathway. Our findings provide a novel mechanism underlying the roles of exosomal-miRNA in follicular fluid of PCOS and facilitate the development of therapeutic strategies for PCOS.

**Introduction**

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorders disease of reproductive age women, characterized by chronic anovulation, polycystic ovarian morphology and hyperandrogenism\(^1,2\). The etiology is complex and the pathogenesis remains unclear. However, previous studies revealed that PCOS patients exhibited abnormal follicular development, which is the main cause of anovulatory infertility\(^3\).

The follicular microenvironment is essential for follicular development, oocyte maturation and quality\(^4\). It has been reported that the oocyte and surrounding granulosa cells (GCs) display an interdependent relationship in the stage of follicle development via direct gap junctions. It was also found that there was nutrient exchange and biological signal transmission between GCs and oocytes in the follicular fluid microenvironment through paracrine or autocrine manners which regulated the growth, development and maturation of follicles\(^5,6\). Thus, the dysregulation of GCs affects the ovarian follicular microenvironment, which might impair folliculogenesis and finally lead to poor reproductive outcome in PCOS patients. Therefore, exploring the function of GCs can explain abnormal follicular development of PCOS patients. Previous studies have reported that the apoptosis of GCs affected follicles development, oocyte growth and maturation, and triggered follicular atresia during the early phase of follicular development\(^7\). Increasing evidence indicates that the apoptosis of GCs in PCOS patients was significantly higher than
those in healthy controls group\textsuperscript{8,9}. However, the underlying mechanism of abnormal apoptosis of GCs involved in the process of abnormal follicular development in PCOS remains unclear.

Follicular fluid is the critical microenvironment for follicular development and contains a wide variety of biologically active molecules, including exosomes that have a lipid bilayer membrane structure with diameter of 30-150 nm\textsuperscript{10}. The exosomes contain cell-specific proteins, lipids, and nucleic acids which act as a carrier passes biological information into target cells\textsuperscript{11}. MicroRNAs (miRNAs) are small single stranded non-coding RNA molecules with 21-24 nucleotides, which can regulate target gene expression by binding to the 3’ untranslated regions (3’ UTR) of target gene mRNAs to silence these genes at a post-transcriptional level\textsuperscript{12}. A growing number of studies report that follicular fluid and the exosomes derived from follicular fluid of PCOS patients (PCOS-FF exosomes) have many differentially expressed nucleic acids especially miRNAs\textsuperscript{13–15}. However, the effect of FF-exosomal miRNA on follicular development of PCOS patients remains unclear.

In the present study, our study mainly focused on exosomal-miR-143-3p and explored the effect of PCOS-FF derived exosomal-miR-143-3p on the apoptosis and proliferation of ovarian GCs, and then analyzed its underlying molecular mechanisms involved in follicular dysplasia in PCOS. Our findings will hopefully afford perspectives to understand the progression of PCOS.

**Results**

**Comparison of clinical general information, laboratory data and apoptosis related indexes of granulosa cells between PCOS and normal controls**

A total of 146 patients were enrolled in this study, including 80 healthy controls and 66 PCOS patients. As shown in Table 1, there were no substantial differences of patients’ age, infertility years, the basal levels of E2, prolactin (PRL) and days of stimulation in two groups (p>0.05). Body mass index (BMI), the basal levels of LH, testosterone (T) and antral follicle count (AFC) in the PCOS group were significantly higher than these in the controls group (p<0.05). Meanwhile, the basal levels of FSH and the Gn dosage (IU) were significantly lower in PCOS group than that in the healthy control group (p<0.05). Laboratory data analysis indicated that the number of retrieved oocytes increased significantly, while MII oocytes rate, 2PN fertilization rate and high-quality embryos rate in the PCOS group decreased significantly compared with the healthy controls group (p<0.05). Consistently, we found that the pro-apoptotic gene Bax mRNA levels were significantly increased, and the anti-apoptotic gene Bcl-2 mRNA levels was significantly decreased in the PCOS group compared with the healthy controls group (Supplementary Fig.S1).
Table 1
Comparison of clinical characteristics between healthy control group and PCOS group

| variables                  | Healthy control group (n = 80) | PCOS group (n = 66) | P values |
|----------------------------|-------------------------------|--------------------|----------|
| Age (years)                | 30.08±3.42                    | 29.05±3.20         | 0.065    |
| Body mass index (kg/m²)    | 23.30±3.67                    | 26.12±4.21         | 0.000*   |
| Infertility years (years)  | 2.59±1.52                     | 2.86±1.74          | 0.317    |
| Basal FSH (IU/L)           | 5.28±1.60                     | 4.72±1.48          | 0.030*   |
| Basal LH (IU/L)            | 3.61±2.30                     | 6.08±5.52          | 0.001*   |
| Basal E2 (pg/mL)           | 47.82±44.99                   | 45.90±33.74        | 0.775    |
| Basal PRL (µg/L)           | 14.75±10.71                   | 13.76±7.18         | 0.519    |
| Basal T (nmol/L)           | 1.01±0.31                     | 1.44±0.48          | 0.000*   |
| Antral follicle count (n)  | 18.33±7.53                    | 28.94±13.34        | 0.000*   |
| Days of stimulation (days) | 12.31±2.30                    | 11.56±2.60         | 0.066    |
| Gn dosage (IU)             | 2613.96±675.51                | 2316.11±747.21     | 0.013*   |
| Number of retrieved oocytes (n) | 16.83±6.02                  | 19.18±6.07         | 0.020*   |
| MII oocytes rate (%)       | 92.51±8.04                    | 89.33±11.09        | 0.047*   |
| 2PN fertilization rate (%) | 74.39±13.49                   | 68.69±16.44        | 0.023*   |
| high-quality embryos rate (%) | 54.19±26.01                 | 41.37±19.64        | 0.001*   |

Note. FSH: follicle-stimulating hormone; E2: estradiol; LH: luteinizing hormone; PRL: prolactin; T: testosterone; MII: metaphase II oocytes

Isolation and characterization of follicular fluid-derived exosomes

Exosomes derived from FF of healthy controls group and PCOS patients were isolated and characterized according to the above method. TEM analysis revealed that exosomes derived from FF of PCOS patients were round membrane-bound vesicles with a diameter of 30-150 nm, which were consistent with exosomes from controls (Fig. 1A). Western blotting showed that exosome marker protein TSG101 and HSP70 were enriched in these exosomes as expected (Fig. 1B). To further confirm whether FF-exosomes can be taken up by primary GCs, we labeled the FF-exosomes with PKH67 fluorescent dye and then co-incubated with GCs for 12 h. Immunofluorescence staining revealed that the PKH67-labeled exosomes were efficiently absorbed by GCs and transferred to the cytoplasmic compartment (Fig. 1C). Overall, these results imply that FF-exosomes can be successfully isolated and can be internalized by GCs.
PCOS-FF derived exosomes promote granulosa cells apoptosis and inhibit proliferation in vitro

To determine the functional role of PCOS-FF derived exosomes on GCs apoptosis and proliferation, we cultured primary granulosa cells and KGN cells with normal-FF derived exosomes and PCOS-FF derived exosomes for 48 h, respectively. As shown in Fig. 2A and B, the mRNA and protein expression levels of pro-apoptosis gene Bax were upregulated in GCs exposed to PCOS-FF derived exosomes, while the mRNA and protein expression levels of anti-apoptosis gene Bcl-2 were downregulated in GCs exposed to PCOS-FF derived exosomes. As expected, the apoptotic rates of primary GCs and KGN cells were significantly enhanced in the PCOS-FF derived exosomes treated group as determined by PE Annexin V assay (Fig. 2C and D). We further evaluated the effects of PCOS-FF derived exosomes on granulosa cell proliferation in vitro via CCK8 assay, PCOS-FF derived exosomes treatment significantly inhibited growth capacity of primary GCs and KGN cells compared with PBS and normal-FF derived exosomes treatment (Fig. 2E and F). Taken together, our findings show that PCOS-FF derived exosomes promote apoptosis and inhibit proliferation of granulosa cells.

miR-143-3p is enriched in PCOS-FF derived exosomes rather than in normal-FF derived exosomes

Previous studies have shown that serum, follicular fluid as well as granulosa cell release large amounts of microvesicles containing both coding and non-coding RNAs, including miRNAs with multiple functional properties. Thus, we hypothesized that PCOS-FF-exosomes might enhance apoptosis of granulosa cells by transferring specific miRNAs. Hence, we screened the differentially expressed miRNAs in follicular fluid derived exosomes between normal population and PCOS patients according to the GRA001999 database of National Genomics Data Center, and found that miR-143-3p was enriched in exosomes of PCOS-FF, and the result was confirmed by the expression levels of miR-143-3p in normal-FF-exosomes and PCOS-FF-exosomes as detected by qRT-PCR analysis (Fig. 3A and B). In addition, the miR-143-3p levels was significantly increased in primary granulosa cells and KGN cells treated with PCOS-FF-exosomes compared with PBS and normal-FF-exosomes (Fig. 3C and D). Thus, our results suggest that miR-143-3p is enriched in PCOS-FF derived exosomes.

Exosomal-miR-143-3p promotes granulosa cells apoptosis and suppresses proliferation in vitro

To further clarify the biological roles of the miR-143-3p on granulosa cell apoptosis and proliferation, we successfully overexpressed or silenced miR-143-3p expression in KGN cells by using a miR-143 mimic or inhibitor, respectively (Fig. 4A). Western blotting results revealed that the protein levels of pro-apoptotic gene Bax was significantly increased by miR-143-3p mimic transfection and decreased by miR-143-3p inhibitor transfection. However, the protein levels of anti-apoptosis gene Bcl-2 was significantly decreased by miR-143-3p mimic transfection and increased by miR-143-3p inhibitor transfection. (Fig. 4B). Cell apoptosis assay showed that miR-143-3p overexpression significantly promoted cell apoptosis, whereas
miR-143-3p inhibition showed an anti-apoptotic phenotype (Fig. 4C and D). Meanwhile, CCK8 assay showed that miR-143-3p overexpression significantly enhanced cell proliferation, while miR-143-3p inhibition suppressed cell proliferation (Fig. 4E and F). These results reveal that PCOS-FF derived exosomal miR-143-3p plays a crucial role in enhancing granulosa cell apoptosis and reducing proliferation.

**BMPR1A is a direct target gene of miR-143-3p**

According to the online bioinformatics prediction based on Target Scan, a binding sequence of miR-143-3p was found in the 3’UTR of BMPR1A (Fig. 5A). Subsequently, dual-luciferase reporter gene assay was applied to verify their interaction. PGL-3-BMPR1A-wt plasmids and PGL-3-BMPR1A-mut plasmids were constructed and co-transfected with miR-143-3p mimic into the KGN cells. The results displayed that luciferase activity was reduced in KGN cells co-transfected with miR-143-3p mimic and wild-type BMPR1A 3’UTR reporter, while no luciferase activity change was found in KGN cells co-transfected with miR-143-3p mimic and mutant BMPR1A 3’UTR reporter (Fig. 5B).

To investigate the relationship between BMPR1A and miR-143-3p, we examined BMPR1A mRNA and protein expression in KGN cells transfected with the miR-143-3p mimic or inhibitor. As shown in Fig. 5C and D, the mRNA and protein expression of BMPR1A were decreased by miR-143-3p mimic transfection and increased by miR-143-3p inhibitor transfection. These data indicate that BMPR1A is a direct target of miR-143-3p.

**BMPR1A overexpression attenuates the effects of miR-143-3p on cell apoptosis and proliferation in KGN cells**

To explore whether BMPR1A was involved in the regulation of miR-143-3p on cell phenotype, we co-transfected miR-143-3p mimic and pcDNA-BMPR1A plasmid in KGN cells. Cell apoptosis and CCK8 assays indicated that the combined transfection of miR-143-3p mimic and BMPR1A expression plasmid showed the effect of inhibiting cell apoptosis and promoting cell proliferation ability compared with miR-143-3p mimic transfection alone (Fig. 6A and B). These data suggest that BMPR1A mediates the role of miR-143-3p in promoting cell apoptosis and inhibiting cell proliferation in KGN cells.

**Smad1/5/8 signaling pathway may be involved in the regulation of exosomal miR-143-3p on cell apoptosis and proliferation**

Previous studies have shown that Smad1/5/8 are BMPR1A downstream signaling molecules that regulate cell proliferation and apoptosis\(^{20}\). Next, we detected whether the down-regulation of BMPR1A by miR-143-3p affected downstream signals, including Smad1/5/8 and its phosphorylation. As shown in Fig. 6C, Overexpression of miR-143-3p significantly decreased the activity of phosphorylated Smad1/5/8, accompanied by the increase of pro-apoptotic protein Bax and the decrease of anti-apoptotic protein Bcl-
Nevertheless, co-treatment with miR-143-3p mimic and BMPR1A expression plasmid could rescue the expression of p-Smad1/5/8 and anti-apoptotic protein Bcl-2 compared to miR-143-3p mimic alone. Meanwhile, overexpression of BMPR1A inhibited the expression of pro-apoptotic protein Bax triggered by miR-143-3p. These results suggest that the BMPR1A/Smad1/5/8 signaling pathway mediates the role of miR-143-3p on cell apoptosis and proliferation in KGN cells.

**Discussion**

Follicular fluid provides an important microenvironment for follicular development and oocyte maturation. The major components of follicular fluid are proteins, hormones, amino acids and metabolites. It can reflect the secretory activity of the oocyte, granulosa and theca cells. The bi-directional communication between oocytes and the surrounding somatic cells in the follicular microenvironment is mainly mediated by either direct gap junctions or secretion of paracrine and autocrine molecules such as exosomes.

Exosomes, as a new transmission medium in follicular fluid microenvironment, are able to carry genetic information involved in follicular growth and oocyte maturation. However, the mechanism by which FF-exosomes affect granulosa cells apoptosis and follicular development in PCOS is unclear. In this study, FF-derived exosomes were isolated and characterized from PCOS patients and healthy controls group. Meanwhile, FF-derived exosomes could be taken up by GCs. In addition, PCOS-FF-derived exosomes promoted apoptosis and inhibited proliferation of granulosa cells. Previous studies have found that aberrant expression of exosomal-miRNAs in follicular fluid from PCOS patients. Thus, we hypothesized that PCOS-FF exosomes might affect apoptosis of granulosa cells by transferring specific miRNA. Hereby, in this study, we identified the miRNA expression profiles in the exosomes derived from PCOS and healthy control patient’s FF. Small RNA sequencing analysis showed that 20 miRNAs differentially expressed between PCOS FF-exosomes and control group according to GRA001999 database of National Genomics Data Center, among which miR-143-3p was enriched in PCOS-FF exosomes. Meanwhile, some studies have shown that miR-143-3p is involved in various biological processes, such as cell proliferation, apoptosis. In this study, over-expression of miR-143-3p significantly enhanced the apoptosis rate of GCs, accompanied by the inhibition of cell proliferation. Therefore, it is suggested that exosomal miR-143-3p in PCOS follicular fluid may play an important role in PCOS follicular dysplasia by affecting the biological functions of GCs.

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor β (TGFβ) superfamily. Their biological effects are mediated by BMP-specific type I (BMPR1A and BMPR1B) and type II (BMPR2) serine/threonine kinase receptors as well as SMAD-related proteins. BMPs have been demonstrated required for normal folliculogenesis in the ovary by regulating several key biological processes including cell proliferation, differentiation, apoptosis, and steroidogenesis. Thus, it is suggested that disruption of BMPs system may be involved in folliculogenesis disorders.
BMPR1A, is a bone morphogenetic protein receptor activin-like kinase and a key signaling molecule in the BMP signaling pathway. In this study, the binding sites between miR-143-3p and BMPR1A were predicted by bioinformatics analysis, and luciferase reporter assays further verified the direct binding between miR-143-3p and BMPR1A in KGN cells.

Our results verified that treatment of KGN cells with the miR-143-3p promoted cell apoptosis and suppressed proliferation via directly targeting BMPR1A. Moreover, over-expression of BMPR1A protein partially conferred protection against miR-143-3p induced cell apoptosis and reversed the inhibitory effect of miR-143-3p on granulosa cell proliferation. In this study, we also found that miR-143-3p mediated cell apoptosis and proliferation in KGN cells were related to the phosphorylation of Smad1/5/8.

In conclusion, our research revealed for the first time that miR-143-3p-containing exosomes derived from PCOS follicular fluid promote granulosa cell apoptosis by targeting BMPR1A and blockading Smad1/5/8 signaling pathway. Therefore, these findings provide novel insights into GCs dysfunction in PCOS and suggest that exosomal-miR-143-3p derived from follicular fluid of PCOS patients may be a promising molecular target for the treatment of PCOS.

Methods

Clinical samples, ovarian stimulation

The clinical samples were obtained from 66 PCOS patients and 80 healthy controls undergoing in vitro fertilization (IVF) at the Department of Reproductive Medicine Center, the Fourth of Shijiazhuang Maternity Hospital between April 2020 and September 2021. PCOS was diagnosed in strict accordance with the 2003 Rotterdam criteria, excluding Cushing’s syndrome, androgen secreting tumors, thyroid dysfunction, endometriosis, hyperprolactinemia and other endocrine diseases. All analysis performed in studies involving human samples were approved by the Clinical Ethics Review Board of the Obstetrics and Gynecology Hospital of Shijiazhuang (approval number: 20210080). All methods were performed in accordance with the relevant guidelines and regulatory methods section. All participants signed informed consent. Meanwhile, this study was conducted in accordance with the 1964 Declaration of Helsinki.

Ovarian hormonal stimulation was performed according to a short-acting gonadotropin-releasing hormone agonist (GnRH-a) long protocol.

Isolation and identification of follicular fluid exosomes

Exosomes were isolated from healthy controls patients (female tubal factors) and PCOS patients’ follicular fluids using the exoEasy Maxi Kit (Qiagen, Germany) according to the manufacturer’s instructions. Finally, the pellets containing exosomes were resuspended in XE buffer and stored at -80°C for subsequent experiments.

Characterization of FF-exosomes was confirmed by transmission electron microscope (TEM): 5-10 µL exosomes were dropped onto the carbon-coated copper grids at room temperature for 3-5 min, and then
absorb the excess liquid with absorbent paper. Then 10 µL 2% phosphotungstic acid was pipetted on the grids for staining for 2-3 min, the excess fluid was removed, and the grid was dried at room temperature. Finally, the copper grid was detected under TEM at 80KV (HITACHI). Exosome-specific marker TSG101 and EV-associated protein marker HSP70 were analyzed by western blotting.

**Isolation and culture of granulosa cells from follicular fluids**

On the day of oocyte retrieval, the first tube of serum-free follicular fluid was collected, and then centrifuged at 2000 g for 10 min, the supernatant was stored at -80°C for further experiments. The cell pellets were resuspended in phosphate-buffered saline (PBS) and 1:1 added onto ficoll solution, then centrifuged for 15 min at 1500 g. Cells at the interface were removed and washed twice with PBS, the cell pellets were resuspended in DMEM/F-12 (Hyclone, Logan, UT, USA) medium containing 10% Fetal Bovine Serum (FBS) (Gibco, Carlsbad, CA, USA), supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in 5% CO₂ cell culture incubator.

**Exosome uptake assay**

The purified exosomes were labeled with PKH67 green Fluorescent Cell Liner Kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer’s instructions. Briefly, 20 µg exosome-XE was added to 0.5mL Dilution C, 1µL PKH67 was added to 0.5mL Dilution C, then fully mixed and incubated with each other at room temperature for 3 min. 1mL 1% bovine serum albumin was then added to neutralize the excess dye. Then exosome-PKH67 was extracted with exoEasy Maxi Kit following the manufacturer’s instructions (Qiagen, Germany). The labeled exosomes were then co-incubated with KGN cells for 24 h. Then the cells were fixed with 4% paraformaldehyde at room temperature for 10min, washed twice with PBS, the nuclei were stained with DAPI. Finally, the signal was observed under confocal microscope.

**RNA extraction, reverse transcription and RT-PCR analysis**

Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, USA), and then 1µg of RNA was reverse-transcribed into cDNA. QRT-PCR was performed using the SYBR Premix Ex Taq kit in accordance with the manufacturer’s protocol. The exosomal RNA was extracted using exoRNeasy Serum/Plasma Starter Kit (Qiagen, Germany). miRNA was then reverse-transcribed into cDNA by Bulge-Loop™ miRNA RT Primer (RiboBio Co., Ltd Guangzhou, China). U6 small nuclear RNA was used as the internal reference of miRNAs. Ct values were indicated by using 2^-ΔΔCt method.

To quantify apoptosis related gene Bax and Bcl-2 mRNA expression, we used oligo d(T) 18 primers to reverse transcribe total RNA into cDNA. Then, qRT-PCR was performed by using SYBR Green dye and specific primers for Bax, Bcl-2 and β-actin. The primer sequences we used are listed in Table S1.

**Western blotting**

SDS lysis buffer freshly mixed with a protease and phosphatase inhibitor cocktail (Thermo Scientific, Rockford, Cambridge, MA) was used to isolate proteins from cells. Western blotting assay was performed as described previously. All the antibodies we used are listed in Table S2.
Apoptosis and proliferation assay

Cell apoptosis assay was conducted as described PE AnnexinV Apoptosis Detection Kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer’s instructions. The cells were pretreated with Exosomes or miR-143-3p mimic or inhibitor (RiboBio Co., Ltd Guangzhou, China), and then 48 h after treatment, cells were collected and diluted into the density of $1 \times 10^5$ which resuspended in binding buffer, then 5 µL PE AnnexinV and 7-AAD were introduced. The cells were incubated at room temperature in darkness for 15 min. Apoptosis cells were observed by flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA).

The cell proliferation assay was conducted according to manufacturer’s instructions. KGN cells were plated in 96-well plates at a concentration of 5000 cells/well. After 24 h, 48 h and 72 h incubation, cells were incubated with 10µL Cell Counting Kit-8 (CCK8) (Yeasen biotech Co., Ltd. Shanghai, China) for another 3 h at 37°C in 5% CO$_2$ cell culture incubator. Finally, the absorbance at 450 nm was measured by Microplate reader.

Vector construction and dual-luciferase reporter assay

MiRNAs that target BMPR1A were predicted by online software programs, TargetScan (TargetScan Human 7.1). The full-length of 3'UTR BMPR1A containing the predicted wild-type or mut-type miR-143-3p binding sites were amplified by PCR and then cloned into the PGL3-Basic reporter vector. PGL-3-BMPR1A-wt plasmids and PGL-3-BMPR1A-mut plasmids co-transfected with miR-143-3p mimic into the KGN cells along with pRL-TK vector using lipo2000 reagent (Invitrogen, Carlsbad, CA, USA). After 24 h of transfection, luciferase activity was determined using dual-luciferase reporter assay system (Promega, Madison, WI, USA) according to the manufacturer’s protocol, the Renilla luciferase activity was regarded as normalization.

Statistical analysis

Clinical data was analyzed by SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). The measured data was presented as average and standard deviation of three independent experiments. Quantitative data analysis was performed using Graph Pad Prism software version 7.0. The differences of statistics significance between different treatments were evaluated by one-way ANOVA or Student t-test. $P$ values lower than 0.05 was considered statistically significant.

Abbreviations

PCOS: Polycystic ovary syndrome; FF: Follicular fluid; GCs: Granulosa cells; exos: exosomes; TEM: transmission electron microscope; IVF: in vitro fertilization; FSH: follicle-stimulating hormone; E2: estradiol; LH: luteinizing hormone; PRL: prolactin; T: testosterone; MII: metaphase II oocytes

Declarations
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Author information

Affiliations

Center for Reproductive Medicine, the Fourth Hospital of Shijiazhuang (Obstetrics and Gynecology Hospital Affiliated to Hebei Medical University), Shijiazhuang 050011, Hebei, China;

Yuanyuan Zhao, Shuhong Pan, Yunying Li, Xiaohua Wu

The institute of Reproductive Health and Infertility, Shijiazhuang 050011, Hebei, China;

Yuanyuan Zhao, Shuhong Pan, Yunying Li, Xiaohua Wu

Contributions

Y.Z. designed the study, wrote the manuscript; Y. Z. and S.P. performed the research; Y.Z. and Y.L. collected data and statistical analysis. X.W. guided the experiments and revised the manuscript. All authors reviewed the manuscript.

Corresponding author

Correspondence to Xiaohua Wu.

Competing interests

The author(s) declare no competing interests.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics declarations

The study was approved by the Clinical Ethics Review Board of the Obstetrics and Gynecology Hospital of Shijiazhuang (Hebei, China), the ethical approval number: 20210080.

Supplementary Information

Supplementary Information
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**Figures**

**Figure 1**

*Isolation and identification of exosomes derived from human follicular fluids.* (A) Transmission electron microscopy (TEM) analysis of the ultrastructure of exosomes from FF of normal population (normal-FF-exos) and the exosomes from FF of PCOS patients (PCOS-FF-exos). Scale bars, 200 nm. (B) Western blotting analysis of exosome markers HSP70 and TSG101. (C) Detection of FF-exosomes uptake by KGN cells in vitro. Granulosa cells were incubated with PKH67-labeled exosomes for 24 h (PKH67 was shown in green, nuclei were stained with DAPI).

**Figure 2**

*PCOS-FF exosomes accelerate granulosa cell apoptosis and reduce cell proliferation compared with normal-FF exosomes.*

(A) Quantitative PCR analysis of expression levels of apoptosis related genes in primary granulosa cells and KGN cell lines treated with normal-FF-exos or PCOS-FF-exos for indicated time. (B) Western blotting analysis of the expression levels of apoptosis related protein in primary granulosa cells and KGN cell lines treated with normal-FF-exos or PCOS-FF-exos for indicated time. (C-D) Flow cytometry detection of apoptosis after primary granulosa cells and KGN cell incubation with normal-FF-exos or PCOS-FF-exos for 48 h. (E-F) CCK8 analysis of cell viabilities after primary granulosa cells and KGN cell incubation with normal-FF-exos or PCOS-FF-exos for four days, respectively. All data were presented as mean ± SD. Student’s t-test was used for statistical analysis. ns: no significance, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 for comparison with control.

**Figure 3**

*miR-143-3p is enriched in PCOS-FF derived exosomes rather than in normal-FF derived exosomes*
(A) GRA001999 database analysis of miR-143-3p expression in PCOS-FF-exos. (B) Quantitative PCR analysis of the expression levels of miR-143-3p in normal-FF-exos and PCOS-FF-exos (n=5). (C-D) Quantitative PCR analysis of the expression levels of miR-143-3p in primary granulosa cells and KGN cells after normal-FF-exos or PCOS-FF-exos treatment for 48 h. All data were presented as mean ± SD. Student’s t-test was used for statistical analysis. ns: no significance, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Figure 4

**Exosomal-miR-143-3p promotes apoptosis and inhibits growth in KGN cells.**

(A) Quantitative PCR analysis of the expression levels of miR-143-3p in KGN cells transfected with miR-143-3p mimic or miR-143-3p inhibitor for 24 h. (B) Western blotting analysis of the expression levels of apoptosis related protein in KGN cells treated with miR-143-3p mimic or miR-143-3p inhibitor for 48 h. (C-D) Flow cytometry detection of apoptosis after KGN cells incubation with miR-143-3p mimic or inhibitor for 48 h. (E-F) CCK8 analysis of cell viabilities after primary granulosa cells and KGN cell incubation with miR-143-3p mimic or inhibitor for four days, respectively. All data were presented as mean ± SD. Student’s t-test was used for statistical analysis. ns: no significance, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Figure 5

**BMPR1A is a direct target gene of miR-143-3p.**

(A) The binding site of miR-143-3p on the BMPR1A gene was predicted by Target Scan. The wild-type BMPR1A-3’UTR (BMPR1A-3’UTR-wt) and mutant BMPR1A-3’UTR (BMPR1A-3’UTR-mut) were used to construct recombinant plasmids for dual luciferase reporter assays. wt, wild type; mut, mutant; UTR: untranslated region; (B) The relative luciferase activity was measured following co-transfection of miR-143-3p mimic with the plasmids encoding the BMPR1A-3’UTR-wt or BMPR1A-3’UTR-mut into the KGN cells. The renilla luciferase activities were normalized by firefly luciferase activities. (C) Quantitative PCR analysis of BMPR1A mRNA expression in KGN cells transfection with miR-143-3p mimic or inhibitor. (D) Western blotting analysis of the expression of BMPR1A protein in KGN cells transfection with miR-143-3p mimic or inhibitor. ns: no significance, *P<0.05, **P<0.01, ***P<0.001, All results were presented as mean ± SD.

Figure 6
miR-143-3p promotes cell apoptosis and suppresses cell proliferation by inhibiting of BMPR1A/Smad1/5/8 signaling pathway.

(A) Flow cytometry detection of apoptosis after KGN cells co-treatment of BMPR1A-expressing plasmid with miR-143-3p mimic. (B) CCK8 analysis of cell viabilities after KGN cells co-treatment of BMPR1A-expressing plasmid with miR-143-3p mimic. (C) Western blotting analysis showing the expression of BMPR1A, Smad1/5/8, p-Smad1/5/8 and apoptosis related protein in KGN cells treated with miR-143-3p mimic or/and BMPR1A-expressing plasmid. ns: no significance, *P < 0.05, **P < 0.01, ***P < 0.001, All results were presented as mean ± SD.

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