Journal of Ovarian Research

RESEARCH

Open Access

miR-34a-5p modulation of polycystic ovary syndrome via targeting the NOTCH signaling pathway

Kexin Zhang[1,2], Xiaomeng Wang[1], Fang Liu[3], Hong Lin[4], Yan Wang[5], Min Zhao[1], Xiaofei Wang[1], Yijing Chu[5] and Lin Xu[1*]

Abstract

Purpose Polycystic ovary syndrome (PCOS) is currently recognized as a condition that affects several systems in the body, including the reproductive, endocrine, and cardiovascular systems. Prevalent among teenagers and women of reproductive age. Prior research has demonstrated an elevation of miR-34a-5p within the follicular fluid (FF) of women of PCOS. Despite this, the precise mechanisms through which miR-34a-5p influences granulosa cells (GC) development and function remain poorly characterized.

Methods Therefore, this study investigates the involvement and pathogenic mechanisms of miR-34a-5p within GCs in the context of PCOS. The human granulosa-like tumor cell line (KGN) got transfected at a control, as well as a miR-34a-5p mimic and inhibitor, respectively. Monitor cellular proliferation in each experimental group. The experimental methods included RT-qPCR, CCK8, flow cytometry and western blotting. Also, the interaction between miR-34a-5p and the particular sequence of JAG1 has been verified using the dual luciferase assay. Further investigation of the connection involving miR-34a-5p and the Notch signaling pathway was conducted using bioinformatics analysis and experimental methods.

Results The results demonstrated that miR-34a-5p expression was significantly elevated in the serum($p<0.0001$)and FF ($p=0.0402$) of PCOS, whereas its expression in GCs ($p=0.5522$) showed no significant variation. Overexpressing miR-34a-5p caused a decrease in the rate at which KGN cells multiplied and an increase in programmed cell death. Conversely, inhibiting miR-34a-5p resulted in an increase in cell growth and a decrease in programmed cell death. Bioinformatics analysis and experimental results further demonstrated thatmiR-34a-5p interacts with the 3'UTR region of JAG1, leading to a negative regulation of the Jagged1-Notch signaling pathway.

Conclusion In summary, the miR-34a-5p molecule inhibits the growth of GCs as well as triggers programmed cell death by regulating the Jagged1-Notch signaling pathway. Silencing miR-34a-5p prevents dysfunction in GCs. Our analysis implies that miR-34a-5p is a new molecular site to treat PCOS.

Keywords Polycystic ovarian syndrome, miR-34a-5p, JAG1, Notch signaling pathway

*Correspondence:
Lin Xu
xulinqd@sina.com

Full list of author information is available at the end of the article

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent metabolic endocrine disorder that primarily affects adolescent and reproductive-aged women, with global prevalence estimates ranging from 6 to 21%. In China, the prevalence is approximately 5–6% [1–3]. Despite its widespread occurrence, the exact pathogenesis of PCOS remains poorly understood [4]. The clinical manifestations of PCOS are highly variable, with common symptoms including infertility, menstrual irregularities, obesity, and hyperandrogenism. Ovulatory dysfunction often leads to infertility, whereas hyperandrogenism is associated with symptoms such as hirsutism and acne [5]. Beyond its effects on the reproductive system, PCOS is closely linked to metabolic, endocrine, and cardiovascular abnormalities. Chronic elevation of estrogen levels in PCOS increases the risk of endometrial and breast cancers, while dyslipidemia and hyperinsulinemia exacerbate cardiovascular risks and may progress to type 2 diabetes mellitus [6–9]. These findings underscore the complexity of PCOS as a systemic disorder, with far-reaching implications for reproductive health, metabolic regulation, and overall cardiovascular risk. A deeper understanding of its pathogenesis and comprehensive management strategies is essential to mitigate its multifaceted impacts. At the microscopic level, patients with PCOS exhibit an increased number of preluminal follicles, which contributes to the formation of cystic follicles, thinning of the granulosa cell layer, and hyperplasia of the theca cell layer [10]. Excessive proliferation of GCs leads to granulosa cell tumours, as their proliferation and differentiation are closely linked to follicular maturation and ovulation. Conversely, apoptosis and degeneration of GCs often result in follicular atresia, contributing to the development of PCOS [11, 12].

microRNAs (miRNAs) are endogenous short-stranded RNAs with roughly 20–24 nucleotides. They are highly conserved and stable in eukaryotes [13]. Additionally, distinct miRNA profiles have been identified in serum/plasma, follicular fluid (FF), GCs, and thecae cells with PCOS patients when compared to those of healthy individuals [14]. miR-34a in pancreatic cancer has the ability to specifically target Notch1, hence facilitating the process of apoptosis and plays a role in several key pathways, such as MAPK, Notch, PI3K/AKT [15, 16]. miR-34a-5p is derived from the same precursor miRNA (pre-miR-34a) as miR-34a, is functionally similar to miR-34a, and is involved in the regulation of various cellular biological processes and has a fundamental impact on the onset and progression of obstetric and gynecological diseases.

Researches indicate that miR-34a-5p is involved in various obstetric and gynecological diseases, playing a crucial role in the regulation of ovarian and uterine functions. For example, circulating serum miR-34a-5p may serve as a diagnostic marker for endometriosis [17]. The level of miR-34a-5p drops in both histiocytes and serum samples with those suffering from ovarian cancer. miR-34a-5p may regulate epithelial-mesenchymal transition by directly targeting TRIM 44 and TIRM 44 may serve as a diagnostic maker of ovarian cancer. Additionally, miR-34a-5p is implicated in regulating cisplatin chemotherapy resistance in ovarian cancer cells through the PD-L1 axis [18, 19]. Additionally, miR-34a-5p is related to certain pregnancy-related diseases, such as preeclampsia. Increased concentrations of miR-34a-5p reduce the capacity of trophoblast cells to grow and spread, which is associated with the development of early-onset preeclampsia [20]. Based on these findings, we propose that miR-34a-5p may play a critical role in the onset and progression of PCOS.

The primary objective of this article is to examine the differential expression of miR-34a-5p between patients with PCOS and normal individuals, and to investigate its role in modulating GCs growth through the Notch signaling pathway. Understanding this mechanism could contribute to the development of more effective clinical treatments for PCOS.

Methods and materials

Clinical samples

FF, serum, and GCs were collected from patients at the Reproductive Centre of the Affiliated Hospital of Qingdao University during the period of August 2023 to October 2023. The subjects' ages ranged from 25 to 42 years, and their body mass index (BMI) ranged from 18 to 32 kg/m^2, and basal follicle-stimulating hormone (FSH) < 10 IU/L on menstrual cycle days 2–3. These patients underwent in vitro fertilization and embryo transfer (IVF/ET) due to male factor or tubal factor infertility. The experimental group (PCOS group) include 19 patients, while the control group (NC group) consists of 20 patients. Prior to their enrolment in the trial, all participants were required to provide informed written permission. The study was granted approval by the ethical review board of Qingdao University (Ethics No. QYFY WZLL 28336).

PCOS group: according to the revised Rotterdam Conference criteria for the diagnosis of PCOS, which requires the presence of at least two of the following symptoms: (1) irregular ovulation or anovulation; (2) hyperandrogenism; and (3) polycystic ovarian alterations.

NC group: Fertile and healthy women with normal menstrual cycle, without polycystic changes in the ovaries, and without clinical signs of hyperandrogenemia (those who underwent assisted reproduction for male factor or tubal factor) were selected.

The specific exclusion criteria are as follows: patients with endometriosis, endocrine disorders, unexplained infertility, ovarian insufficiency, and other causes of

ovulatory dysfunction, as well as those with hyperandrogenism and amenorrhea caused by other factors, are excluded from the study.

Ovulation induction protocol

All patients underwent ovulation induction, with dosages according to follicular development. Once at least three antral follicles ≥ 17 mm or two follicles ≥ 18 mm were observed, 250 μg of recombinant Human chorionic gonadotrophin (hCG) was injected to promote ovulation. Oocyte retrieval took place 36 to 38 h following the hCG injection.

Collection of FF and isolation of GCs

Under ultrasound guidance, all follicles of appropriate size and maturity were punctured, and the FF was collected. The collected FF was centrifuged at 4 °C, and the clear, transparent supernatant was separated and stored at -80 °C for subsequent analysis. The cell pellet was treated with an appropriate amount of red blood cell lysis buffer to isolate GCs. The mixture was lysed on ice for 15 min and then centrifuged at low temperature to purify the GCs. The purified GCs were used for further experimental analyses.

Validation of GCs

The presence of follicle-stimulating hormone receptor (FSHR) was examined using immunofluorescence staining techniques [21]. Extracted primary GCs were uniformly seeded onto cover slips and, after 72 h, washed with PBS buffer three times for 3 min each. Cells were fixed in 4% paraformaldehyde for 15 min. Then samples permeabilized with Triton for 10 min before being blocked with BSA for 30 min. They were then incubated at 4 °C overnight with a rabbit polyclonal antibody against FSHR(1:500). Subsequently, cleanse using PBS. Fluorescence-labeled antibody secondary (1:200) was administered for one hour at room temperature in the dark, followed by another set of PBS washes (5 times, 5 min each). Finally, the slides were stained with DAPI and then sealed using an anti-fade mounting solution that also included DAPI. The expression of FSHR was subsequently visualized using a fluorescence microscope.

Cell culture and transfection

KGN, which display physiological characteristics similar to those of ovarian GCs, are commonly used to study their function and regulatory mechanisms. The KGN cell line derived from human ovarian GCs was grown using specified complete medium (DMEM/F12, Procell, Wuhan, China). Cells are cultivated in incubators.

Table 1 Sequences of primers used for RT-qPCR

Gene	Sequences(5′-3′)
miR-34a-5p mimic	UGGCAGUGUCUUAGCUGGUUGU
miR-34a-5p inhibitor	ACAACCAGCUAAGACACUGCCA

Transfection of small interfering RNAs (siRNAs), MiRNAs, and MiRNA inhibitors

The siRNAs as well as the miRNA mimics and inhibitors used in this study, were provided by Gemma (Shanghai, China). Cells were transfected with Lipo3000 according to the instruction manual. The sequences of the siRNAs, miRNA mimics and inhibitors are presented in the accompanying Table 1. Cells were collected 48–72 h after transfection for subsequent investigation.

RNA extraction and quantitative Real-Time PCR (RT-qPCR)

Serum, FF, and GCs samples were collected from participants. RNA extraction was performed on serum, FF, and GCs using Trizol reagent. The process of cDNA synthesis was carried out via the miRNA 1st Strand cDNA Synthesis Kit, followed by quantitative PCR (qPCR) employing the SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biology, China). The degree of miR-34a-5p and JAG1 expression were evaluated. Small nuclear U6 and GAPDH were used as internal controls for miRNA and mRNA quantification. All primers were synthesized by Biochem, and Table 2 lists the sequences. The $2^{\wedge-\blacktriangle\blacktriangle CT}$ approach allowed one to compute relative fold changes in gene expression.

CCK8 assay

CCK8 assay was used to evaluate cell growth. In a 96-well plate, plant 5000 KGN cells each well. One set of cells was genetically modified by introducing NC and miR-34a-5p mimic. Another group was transfected with a negative control (NC) and an inhibitor for miR-34a-5p. At time points of 24, 48, 72, and 96 h after transfection, 10 μL of CCK8 reagent was introduced to each well and kept at a temperature of 37° in an environment containing 5% CO^2 for a duration of 2 hours. Subsequently, the absorbance was then determined with microplate reader.

Table 2 Sequences of primers used for RT-qPCR

Gene	Forward primer	Reverse primer
miR-34a-5p	GGCAGTGTCTTAGCTGGTTGTAA	
U6	CTCGCTTCGGCAGCACATATAC	AACGCTTCACGAATTTGCGT
GAPDH	GAAGGTGAAGGTCGGGTGTG	GAAGATGGTGATGGGGATTTC
JAG1	TGCTGCCGTTGCAGAAGTAAG	AGTAGAAGGCCGTCACCAAGC

Flow cytometry

The cells were centrifuged after being digested with EDTA-free trypsin(MeilunBio, China). Discard the supernatant and rinse the precipitate with PBS three times, using centrifugal force at 1000 rpm for 5 min after each rinse. The remaining cell pellet was resuspended in binding buffer. Apoptosis was detected using the FITC-Annexin V/PI Apoptosis Detection Kit (UElandy, China). In accordance with the manufacturer's protocol, the cell suspension was treated with FITC-Annexin V and PI staining solution. The cells were gently mixed and incubated at 5 °C for 10–15 min in the dark. After the incubation period, the suspension of cells was moved to a tube specifically designed for flow cytometry, followed by the addition of 400 μL of 1× Annexin V binding buffer. Flow cytometric analysis was performed using bandpass filters for FITC (530 nm) and PI (617 nm) fluorescence, with excitation at 488 nm.

Luciferase reporter gene assay

The gene JAG1, which is the aim of miR-34a-5p, was inserted at the 3' end of the firefly luciferase gene. Construct plasmid vectors JAG1-WT and JAG1-MUT for perihelion assembly (GenePharma, Shanghai). The luciferase reporter plasmids were co-transfected into 293T cells with alternatively miR-34a-5p inhibitor or control using Lipofectamine 3000. Furthermore, JAG1-WT or JAG1-MUT was jointly transfected with miR-34a-5p in the control, overexpression, or inhibitor groups, respectively. Luciferase activity was measured 48 h post-transfection using the Firefly & Renilla-Light Luciferase Reporter Assay Kit (MeilunBio, China), with Renilla luciferase activity serving as the normalized internal standard.

Western blot

Western blot was performed using the same method as prior [22]. The subsequent principal antibodies were utilised in the analyses: JAG1 (CST, USA), NICD (CST, USA), Notch (ZEN-BIOSCIENCE, China), Bcl-2 (Elabscience, China), Bax (Elabscience, China), caspase-3 (Elabscience, China), caspase-9 (Elabscience, China), Hes1 (ZEN-BIOSCIENCE, China), and Hey1 (Affinity, China).

Data analysis

All statistical analyses were performed using GraphPad Prism (GraphPad; La Jolla, USA). For comparisons between two groups, independent t-tests were employed. Data are presented as the mean ± standard deviation (SD). A p-value of less than 0.05 was considered statistically significant.

Results

Immunofluorescence identification of GCs

FSHR is specifically expressed in ovarian GCs, where it mediates the effects FSH to promote GCs proliferation and follicular maturation [23]. In this study, immunofluorescence staining was performed to detect FSHR expression in GCs. The results demonstrated positive FSHR expression in the cytoplasm of all GCs, with green fluorescence observed in the cytoplasm and blue fluorescence in the nuclei (Fig. 1).

Measurement of miR-34a-5p levels and diagnostic accuracy

The levels of miR-34a-5p in serum, FF, together with GCs of PCOS patients were measured by RT-qPCR. In comparison to the NC, the quantity of miR-34a-5p were markedly higher within both the serum (Fig. 2A) and FF (Fig. 2B) of the PCOS group, with statistically significant differences noted ($p < 0.05$). In our study, no significant difference in miR-34a-5p expression was observed in GCs between PCOS and NC groups(Fig. 2C). ROC curves were employed to evaluate the diagnostic precision of miR-34a-5p from various sample sources in detecting PCOS. The area under the curve (AUC) for miR-34a-5p in FF and serum were0. 5360 and 0. 6819. The 95% confidence intervals were 0. 4318-0. 6422 for FF and 0. 5850-0. 7783 for serum, with corresponding p-values of 0.4787

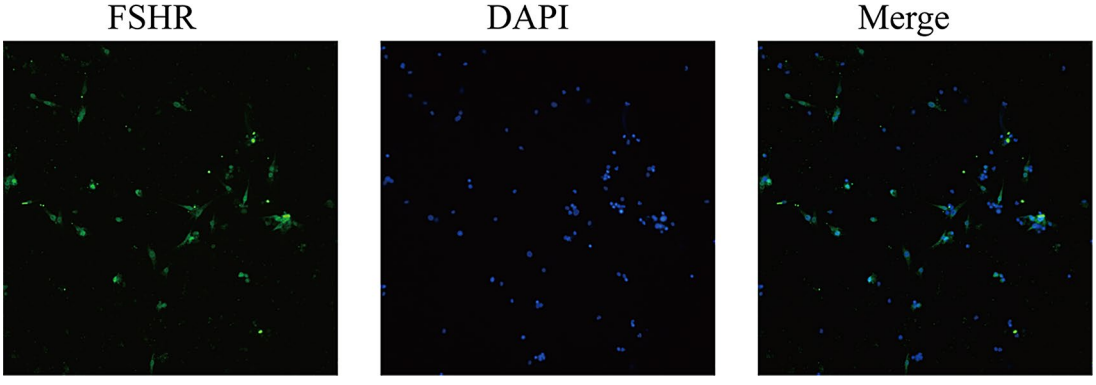

Fig. 1 Identification results of primary GCs

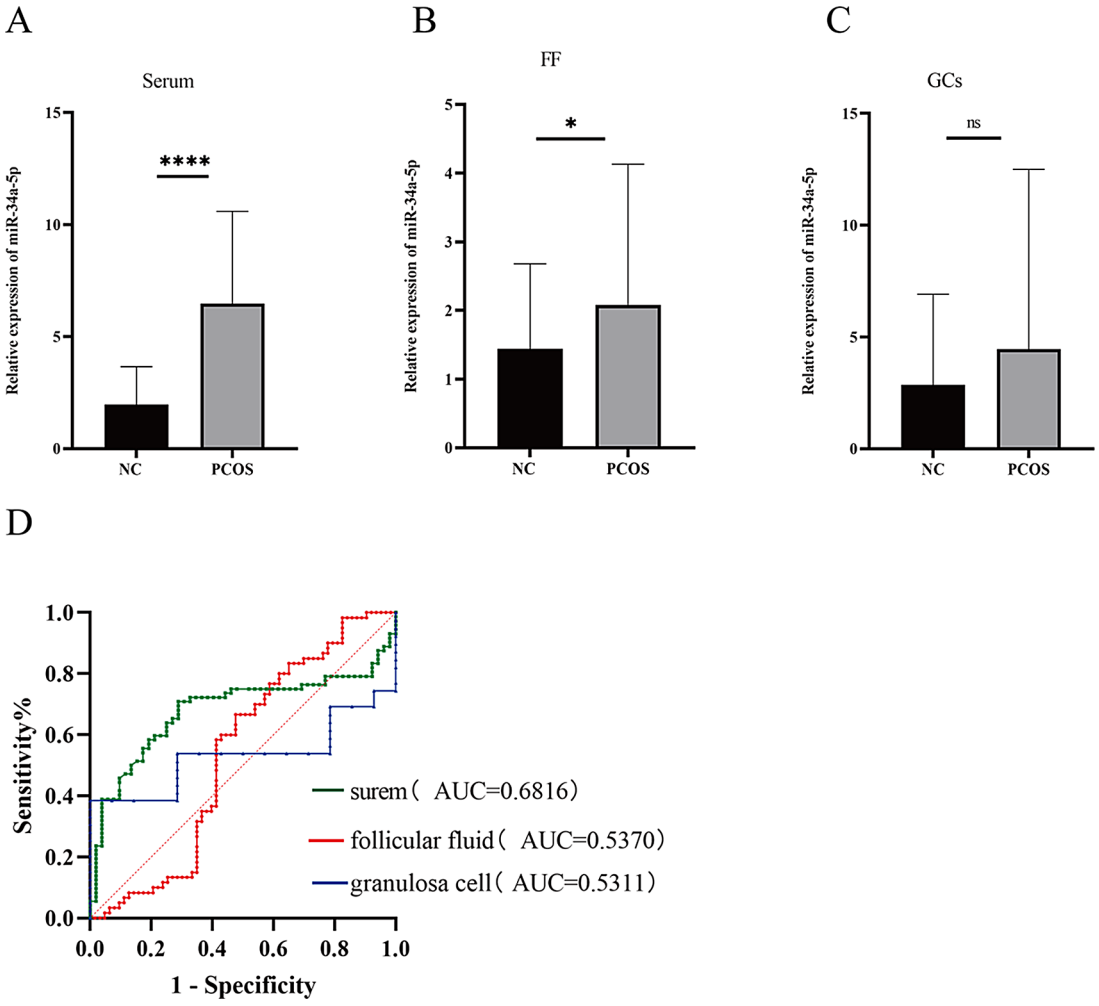

Fig. 2 A Relative expression of miR-34a-5p in serum, FF, and GCs of control women and women with PCOS. Data are expressed as means ± SEM of $^{2-\blacktriangle\blacktriangle CT}$ which indicates the miRNA expression in each individual considering the mean expression in the group control women as reference. B ROC curves based on miR-34a-5p in serum, FF, and GCs of 20 NC and 19 PCOS. *$p < 0.05$, ****$p < 0.0001$ vs. NC

and 0.0006 (Fig. 2D). The clinical features of people with PCOS and individuals without the condition (healthy controls) are displayed in Table 3.

Suppression of KGN cell proliferation through miR-34a-5p overexpression

We generated KGN cells with enhanced miR-34a-5p expression through transfection with miR-34a-5p mimics. Also we achieved miR-34a-5p downregulation by introducing specific inhibitors. As shown in Fig. 3A, miR-34a-5p mimics and inhibitors were efficiently introduced via transfection into KGN cells ($p < 0.001$).

CCK8 assay was used to evaluate miR-34a-5p overexpression and downregulation in KGN cell growth. The findings demonstrated that the upregulation of miR-34a-5p significantly suppressed cellular growth($p < 0.05$, Fig. 3B). And the reduction of miR-34a-5p greatly amplified cellular replication ($p < 0.001$, Fig. 3B).

To analyze the mechanisms underlying miR-34a-5p-induced apoptosis, we performed flow cytometry analysis. Apoptosis in KGN cells was enhanced by the overexpression of miR-34a-5p. ($p = 0.0369$, Fig. 3E). Conversely, downregulation of miR-34a-5p expression led to a substantial reduction in apoptosis ($p = 0.0366$, Fig. 3E).

To further understand the molecular mechanisms driving these findings, we examined the expression levels of apoptosis-related proteins using qPCR following transfection. In the group with miR-34a-5p upregulation, there was a significant upregulation of protein levels for Caspase-3, Caspase-9, and Bax, accompanied by a downregulation of Bcl-2 levels (Fig. 3F). KGN cells harboring miR-34a-5p inhibitors exhibited the opposite pattern.

Bioinformatics predictions and validation of miR-34a-5p targeting JAG1

A conserved binding site for miR-34a-5p was discovered in the 3' untranslated region (3'UTR) of JAG1

Table 3 Anthropometric and metabolic parameters of all participants

Characteristics	NC ($n=20$)	PCOS ($n=19$)	*P* value
Age	33. 75 ± 3. 007	33. 95 ± 4. 428	0. 8709
BMI (kg/m^2)	20. 80 ± 1. 737	24. 36 ± 3. 443	0. 0002 ***
WHR	0. 8269 ± 0. 04297	0. 8732 ± 0. 05774	0. 0062 **
SBP (mmHg)	117. 2 ± 7. 63	121. 1 ± 11. 49	0. 2229
DBP (mmHg)	70. 65 ± 5. 55	78. 32 ± 11. 50	0. 0111 *
FPG (mmol/L)	4. 67 ± 0. 27	4. 85 ± 0. 30	0. 0451 *
FINS (mIU/L)	7. 71 ± 1. 75	10. 85 ± 4. 60	0. 0071 **
HOMA-IR	0. 1655 ± 50. 04006	0. 2337 ± 0. 09702	0. 0063 **
TC(mmol/L)	4. 643 ± 0. 579	4. 856 ± 1. 048	0. 4201
TG(mmol/L)	0. 8000 ± 0. 2547	1. 452 ± 1. 185	0. 0214 *
HDL(mmol/L)	1. 607 ± 0. 2247	1. 433 ± 0. 2849	0. 0401 *
LDL(mmol/L)	2. 751 ± 0. 4479	2. 626 ± 0. 8709	0. 5760
FSH (mIU/mL)	7. 410 ± 1. 163	5. 636 ± 0. 9047	< 0. 0001 *****
LH (mIU/mL)	5. 608 ± 1. 875	7. 632 ± 3. 216	0. 0207 *
E2(PG/mL)	223. 3 ± 159. 9	191. 9 ± 190. 9	0. 5805
P (nmol/L)	1. 613 ± 3. 862	2. 075 ± 5. 464	0. 7633
T (nmol/L)	0. 6195 ± 0. 1855	1. 222 ± 0. 4499	< 0. 0001 ****
LH/FSH	0. 7945 ± 0. 3530	1. 375 ± 0. 5618	0. 0004 ***
AFC	15. 75 ± 8. 039	34. 11 ± 12. 83	< 0. 0001 ****

Data are presented as mean ± SD

BMI, body mass index; WHR, waist-to-hipratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FINS, fasting plasma insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglyceride; HDL, high-density plasma insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglyceride; high-density lipoprotein; LDL, low-density lipoprotein; FSH, follicular stimulating hormone; LH, luteinising hormone; E2, estradiol; P, progesterone; T, testosterone; AFC, antral follicle count

using bioinformatics research applying TargetScan, miRTarBase, and mirDIP. (Table 4). To experimentally validate this interaction, the 293T cell was utilised as a tool cell. Then cells were subjected to transfection with miR-34a-5p mimics or inhibitors, along with luciferase reporter constructs containing either the unaltered (WT) or modified (MUT) JAG1 3' untranslated region (3'UTR). In cells transfected with the WT JAG1 3'UTR construct, the introduction of miR-34a-5p mimics brought about a substantial reduction in luciferase activity. Conversely, introducing miR-34a-5p inhibitors resulted in a marked increase in luciferase activity (Fig. 4C). However, there were no significant alterations in luciferase enzyme activity observed in cells that were incorporated with the MUT JAG1 3'UTR construct, regardless of whether they were administered with the miR-34a-5p mimic or inhibitor.

In order to provide a clearer understanding of how miR-34a-5p regulates the regulation of JAG1 expression, we evaluated the mRNA and protein of JAG1. Consistent with expectations, compared to the negative control group, miR-34a-5p overexpression markedly reduced JAG1 mRNA along with protein levels. (Fig. 4A, B). These data collectively show that miR-34a-5p directly controls JAG1 by attaching to its 3'UTR.

miR-34a-5p inhibits cell growth by suppressing JAG1 expression

To function the role of JAG1 in cell growth, KGN cells were transfected with JAG1 siRNA to knock down JAG1 expression. As illustrated in Fig. 5A and B, in the JAG1 siRNA group, JAG1 mRNA and protein levels were considerably reduced, thereby confirming the successful silencing of JAG1 ($p<0.0001$) (Fig. 5A and B). The findings further indicate that reducing the expression of JAG1 impacts the proliferation of cells (Fig. 5C, D and E). Collectively, these findings suggest that silencing the JAG1 gene inhibits KGN cell proliferation and induces apoptosis.

miR-34a-5p suppresses the notch pathway

Bioinformatic analysis was conducted using three online databases—mirDIP, miRTarBase, and TargetScan—to identify predicted and experimentally validated miR-34a-5p target genes (Fig. 6A). Intersection of the predicted targets from the three datasets identified 20 common miR-34a-5p targets. The pathway enrichment analysis showed a significant abundance of genes related to the Notch signalling system. (Fig. 6B, C). The study analyzes how upregulating miR-34a-5p in KGN cells affects Notch signaling pathway. Subsequently, the expression levels of crucial constituents of the Notch pathway, such as JAG1, Notch1, NICD, Hes1, and Hey1, were assessed. Following miR-34a-5p overexpression, a marked downregulation of JAG1, Notch1, NICD, Hes1, and Hey1 was observed (Fig. 6D), indicating that miR-34a-5p directly downregulates the Notch signaling pathway by affecting both transcriptional and post-transcriptional processes. This evidence points that miR-34a-5p functions as a suppressor of the Notch pathway, contributing to its regulatory role in cellular proliferation and apoptosis.

miR-34a-5p suppresses cell proliferation via suppressing JAG1 expression

We performed a simultaneous transfection of KGN cells with miR-34a-5p mimics and JAG1 agonists. The outcomes confirmed that JAG1 expression considerably decreased miR-34a-5p's cell growth inhibitory effect. ($p<0.0001$, Fig. 7A). Moreover, JAG1 overexpression reversed the pro-apoptotic effect induced by miR-34a-5p ($p<0.01$, Fig. 7B, C). According to the findings, miR-34a-5p is primarily responsible for inhibiting cell proliferation by suppressing the expression of JAG1.

Discussion

Previous studies have demonstrated that miR-34a-5p is significantly upregulated in the FF exosome of patients with PCOS, where it may regulate metabolic pathways involved in follicular development and oocyte

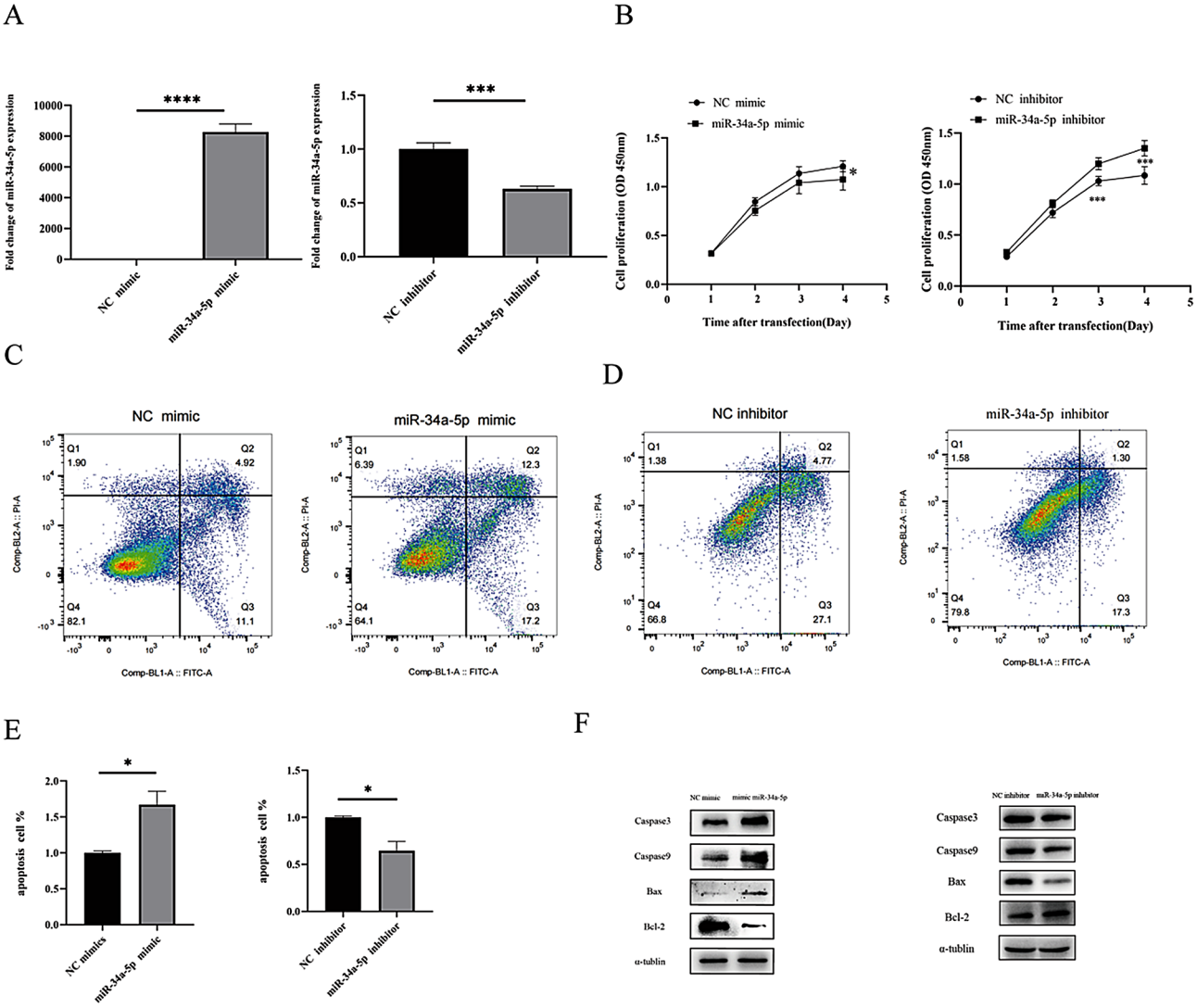

Fig. 3 miR-34a-5p inhibition of KGN cell viability affects apoptosis. A miR-34a-5p mimic, miR-34a-5p inhibitor, NC mimic, NC inhibitor were transfected into KGN cells using RT-qPCR. B Cell proliferation was detected by CCK8. C, D, E Flow cytometry was used for the apoptosis was detected. F Effects of apoptotic proteins were observed using western blotting $*p < 0.05$, $**p < 0.001$

Table 4 Potential binding sequences of miR-34a-5p to JAG1 were predicted using TargetScan, MiRTarBase, and MirDIP website

JAG1 3'UTR WT 5'-AUUUUGCCAUAGAGUACACUGCCUGCCU-3'
hsa-miR-34a-5p 3'-UGUUGGGUCGAUUCU GUGACGGU-5'
JAG1 3'UTR MUT 5'-AUUUUGCCAUAGAGUAGUGACGGUGCCU-3'

maturation, ultimately contributing to the pathophysiology of PCOS [24]. Our study extends these findings by showing that miR-34a-5p is upregulated in both serum and FF in PCOS patients. However, we did not observe significant changes in GCs expression, which could be attributed to the small sample size. We also found that serum miR-34a-5p had greater diagnostic value for PCOS compared to FF. We hypothesize that the differential expression of miR-34a-5p in PCOS patients may be associated with localized ovarian changes. Furthermore, the abnormal expression of miR-34a-5p may provide new insights into the molecular mechanisms underlying PCOS pathogenesis.

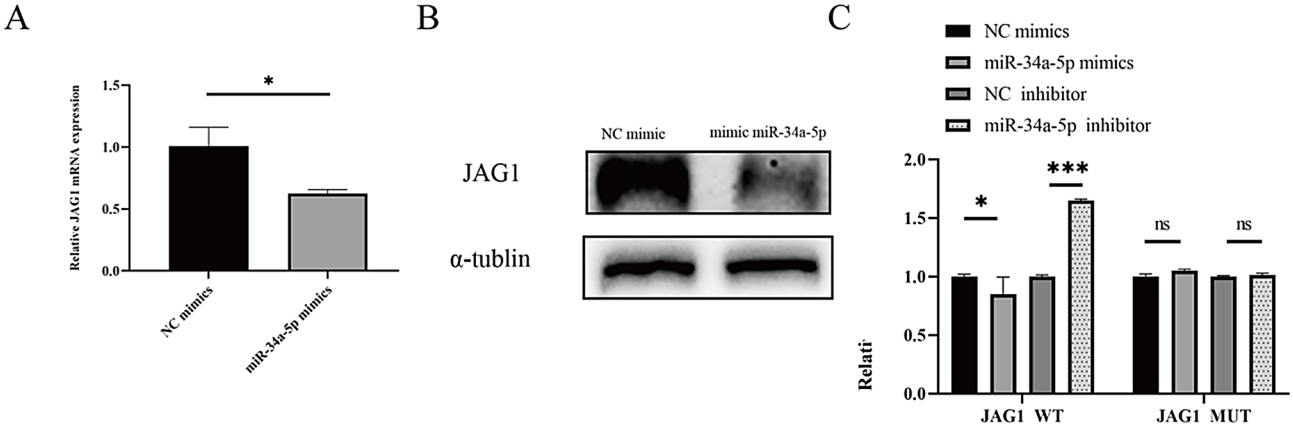

Fig. 4 miR-34a-5p has a targeting effect on JAG1. miR-34a-5p mimic, miR-34a-5p inhibitor, NC mimic, and NC inhibitor were introduced into 293T cells, respectively. A, B RT- qPCR and western blotting methods were used to detect JAG1 expression. C The targeting relationship between miR-34a-5p and JAG1 was determined by dual luciferase reporter gene assays *$p < 0.05$, **$p < 0.001$

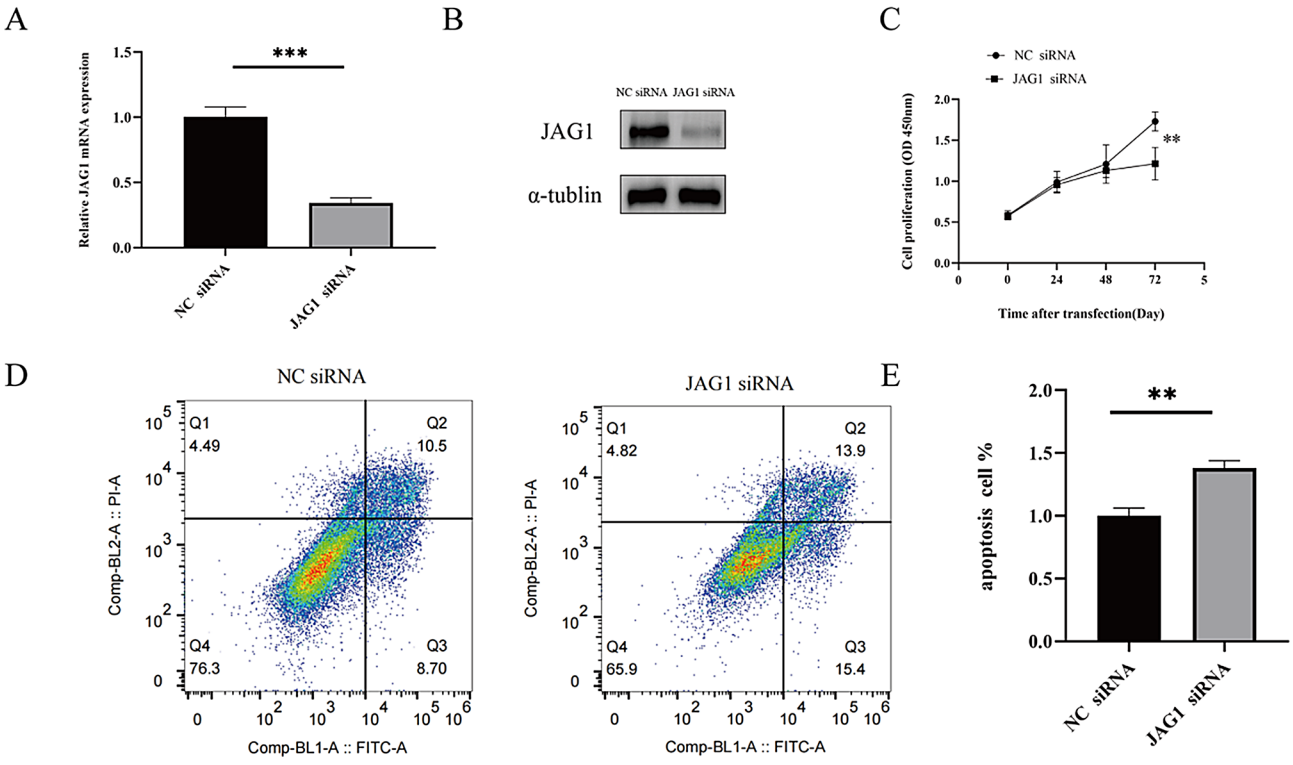

Fig. 5 JAG1 inhibits cell proliferation and effects apoptosis. A, B Verification of JAG1 siRNA or NC siRNA transfection into KGN cells using RT-qPCR and western blotting. C Cell proliferation was assessed by CCK8. D Apoptosis was detected using flow cytometry

FF is essential for providing nutrients to oocytes, while GCs and oocytes interact closely to regulate oocyte development. Changes in the FF micro-environment and GCs function can significantly impact oocyte maturation. In PCOS patients, hormonal imbalances, such as elevated levels of LH and insufficient levels of FSH, severely impair GCs function, disrupting follicular maturation and resulting in follicular atresia [25]. Additionally, the excessive androgen levels often observed in PCOS inhibit GC proliferation, further hindering follicular development and accelerating atresia [26]. Emerging evidence also suggests that certain miRNAs play a critical role in promoting GC apoptosis, contributing to the dysregulation of follicular development in PCOS [25]. However, the specific role of miR-34a-5p in PCOS-related GCs dysfunction remains unclear.

To explore the effects of miR-34a-5p on ovarian GCs, we conducted in vitro experiments using the immortalized human ovarian GCs line KGN. The results showed that upregulation of miR-34a-5p inhibited cell

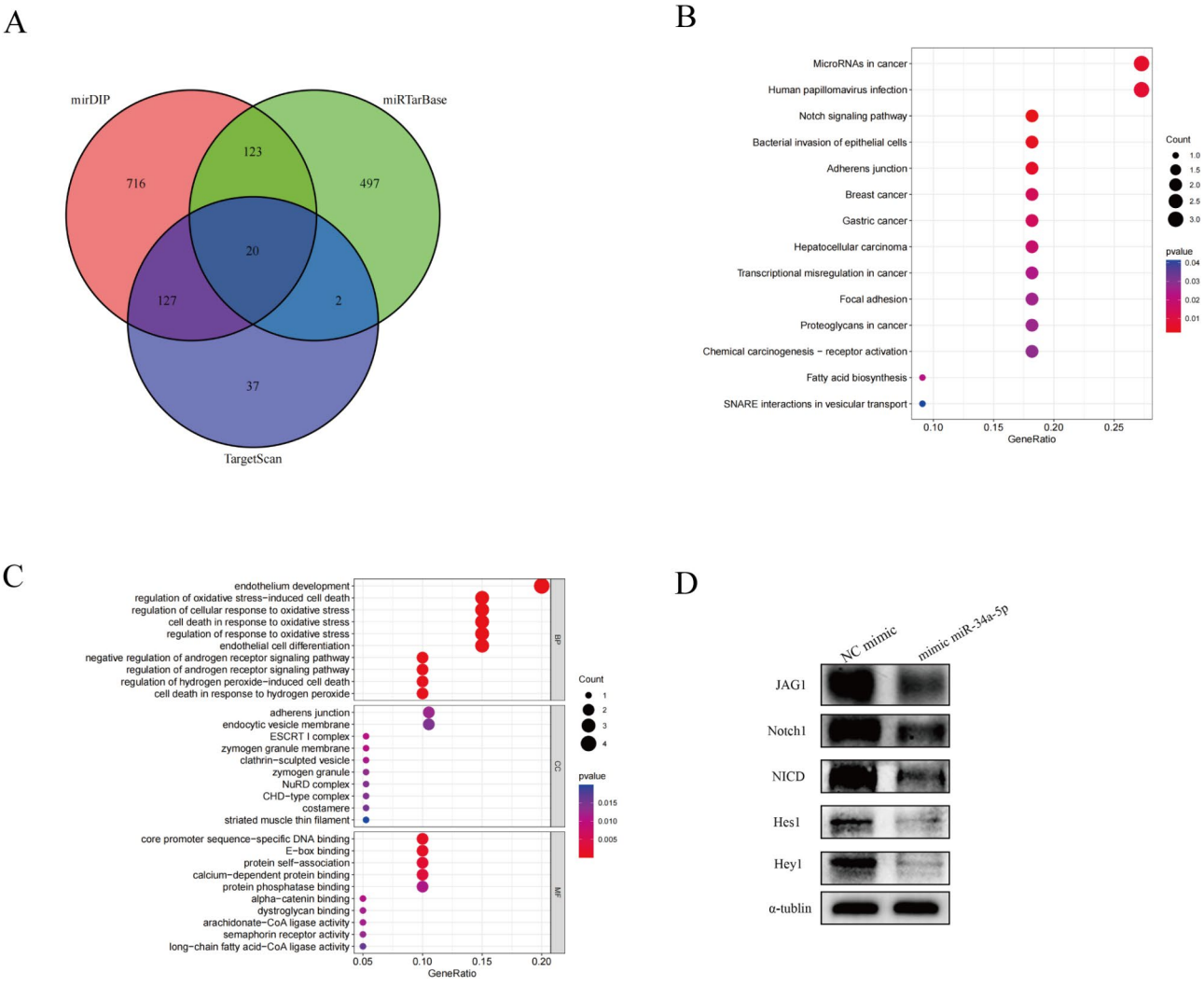

Fig. 6 miR-34a-5p target gene prediction and enrichment analysis. A mirDIP, miRTarBase, TargetScan website to predict miR-34a-5p target genes. B, C KEGG and GO pathway analysis. D Detection of related protein expression by western blotting

proliferation and induced apoptosis in KGN cells. Conversely, inhibition of miR-34a-5p expression enhanced cell proliferation and reduced apoptosis. Apoptosis is characterized by the activation of caspase family proteins and the balance between pro-apoptotic and anti-apoptotic proteins [27]. In this study, miR-34a-5p expression was positively correlated with pro-apoptotic proteins (e.g., Caspase-3, Caspase-9, and Bax) and negatively correlated with the anti-apoptotic protein Bcl-2, suggesting that miR-34a-5p plays a significant role in regulating GC survival.

miRNAs regulate gene expression by binding to the 3' untranslated regions (3' UTR) of specific target genes, thereby influencing transcriptional or translational activities [28]. Identifying the target genes of miRNAs is therefore crucial to understanding their role in disease pathogenesis [29]. Through bioinformatic predictions, we identified JAG1 as a potential target gene of miR-34a-5p. This finding is consistent with previous studies that reported miR-34a directly regulates JAG1 expression in cervical and choriocarcinoma cells [30, 31]. JAG1 is known to be overexpressed in various cancers, where it promotes cell proliferation, growth, and tumor progression [32]. In ovarian cancer, JAG1 overexpression not only enhances cell proliferation and metastasis but also promotes cancer stem cell-like properties [33]. Similarly, in hepatocellular carcinoma, JAG1 expression correlates positively with tumor nodule formation and an increase in cancer stem cell populations [34, 35]. In our study, dual-luciferase reporter analysis confirmed that miR-34a-5p directly binds to specific sequences of JAG1. Furthermore, miR-34a-5p overexpression suppressed JAG1 mRNA and protein expression in KGN cells, suggesting that miR-34a-5p functions through the regulation of JAG1. Downregulation of JAG1 significantly impaired the proliferative capacity of KGN cells, while co-treatment

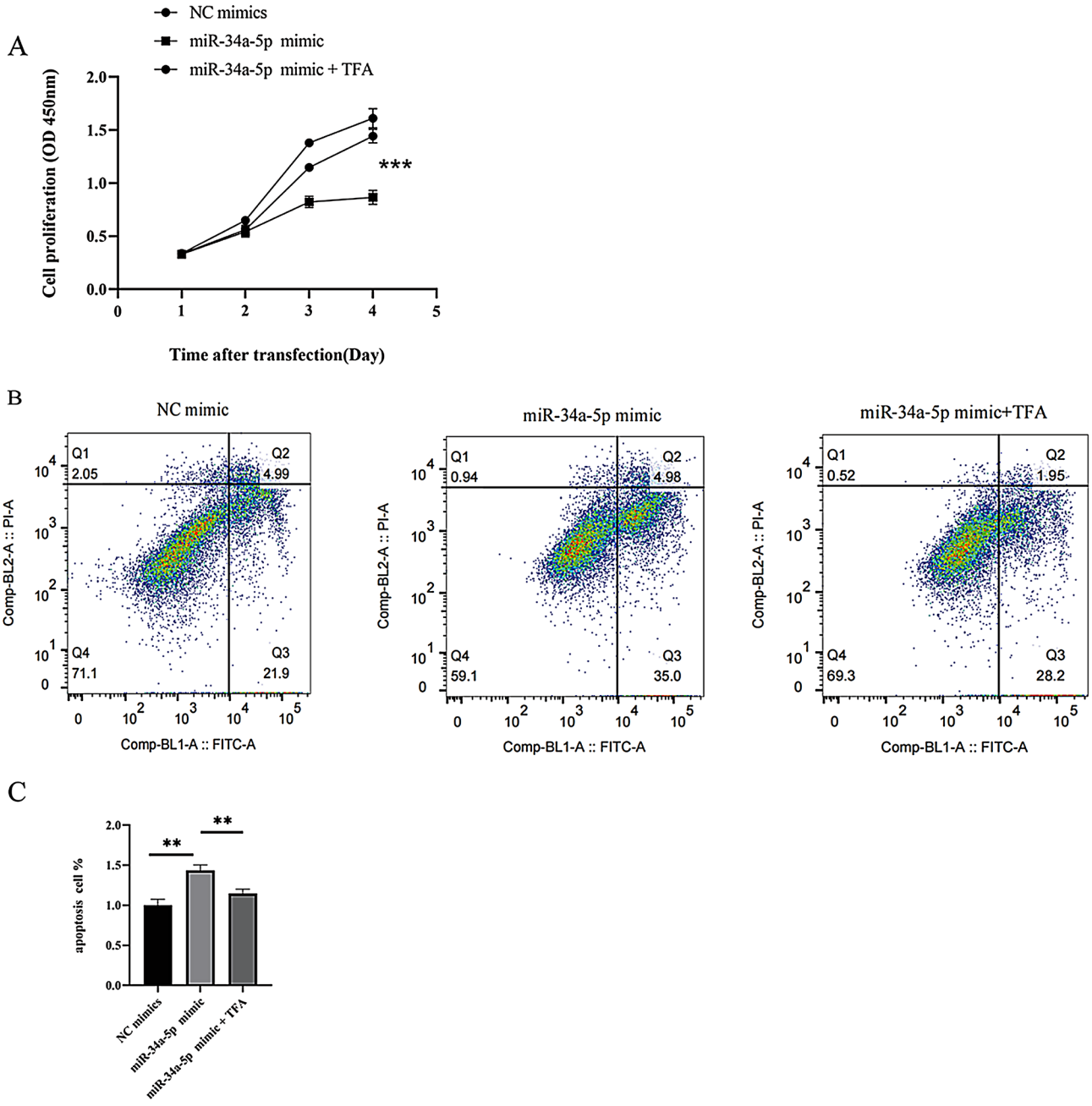

Fig. 7 Effect on cell proliferation and apoptosis after addition of pathway agonists. A CCK8 assay for evaluating cell proliferation. B Flow cytometry analysis for detecting cell apoptosis. ***$p < 0.001$

with a JAG1 agonist and miR-34a-5p mimic reversed this effect. These findings suggest that miR-34a-5p regulates GC proliferation through the JAG1 signaling pathway.

Using KEGG and GO functional enrichment analyses, we identified that the target genes of miR-34a-5p were enriched in the Notch signaling pathway, which plays a critical role in PCOS-related reproductive dysfunction [36]. For example, the Notch signaling pathway is essential for GC proliferation and normal follicular development [37]. Disruption of ovarian Notch signaling has been shown to promote GC apoptosis and impair follicular growth [38, 39]. Since the Notch signaling pathway is closely related to embryo quality and oocyte fertilization [40]. Its inhibition may further exacerbate PCOS-related reproductive abnormalities. Our study found that miR-34a-5p overexpression in KGN cells downregulated JAG1, reducing NICD release and decreasing Notch signaling activity. This was associated with the downregulation of Notch-related proteins, including NICD, p21, Hes1, and Hey1. These results suggest that miR-34a-5p

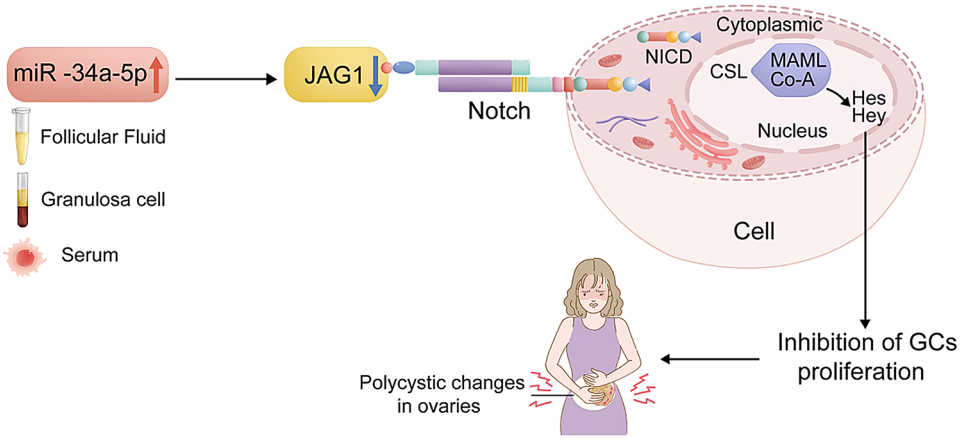

Fig. 8 Overview of the role of miR-34a-5p in GCs. miR-34a-5p is elevated in serum and FF in PCOS. Elevated miR-34a-5p negatively regulates JAG1 protein and also affects Notch pathway-related proteins, which upregulates the expression of pro-apoptosis-related proteins and ultimately affects GCs proliferation

overexpression in ovarian GCs may inhibit follicular maturation through suppression of the Notch pathway, leading to the accumulation of immature follicles and ultimately causing ovulatory dysfunction and infertility.

In summary, as illustrated in Fig. 8, miR-34a-5p may promote GCs apoptosis by regulating both apoptosis-related proteins (e.g. Caspase-3, Caspase-9, Bax, Bcl-2) and Notch signaling-related proteins (e.g. NICD, p21, Hes1, Hey1). These findings suggest that miR-34a-5p regulates GC proliferation and apoptosis through the JAG1/Notch signaling pathway, underscoring the importance of GC-oocyte interactions for oocyte maturation. Our study provides new insights into how GC dysfunction affects follicular development in PCOS and highlights miR-34a-5p as a potential therapeutic target to improve GC activity in PCOS patients.

However, this study has several limitations. First, our investigation was limited to GCs from mature follicles, which may not represent all follicular stages. Additionally, the high expression of miR-34a-5p in GCs from PCOS patients has not been fully confirmed due to the small sample size. Future studies with larger samples are needed to clarify its role. Second, while we demonstrated that miR-34a-5p affects JAG1 expression, we have not fully elucidated whether JAG1 pathway agonists regulate cell proliferation and apoptosis specifically through the Notch pathway. Finally, further animal studies are required to determine whether reducing ovarian miR-34a-5p levels can promote GC proliferation and ameliorate PCOS symptoms. These limitations will be addressed in future research.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-025-01623-4.

Supplementary Material 1

Acknowledgements
We are very grateful to Xiaomeng Wang and Fang Liu for the collection of FF samples.

Author contributions
Kexin Zhang performed most of the data analysis. Kexin Zhang conducted the validation experiments. Xiaomeng Wang and Fang Liu help collect the clinical samples. Kexin Zhang wrote the manuscript with inputs from all co-authors. Hong Lin, Yan Wang, Min Zhao and Xiaofei Wang help edited and revised the manuscript.Yijing Chu and Lin Xu designed the experiments and supervised the project.

Funding
No funding.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
We express our gratitude to the Reproductive Center of Qingdao University Hospital for providing the samples. The experiment was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. (Ethics No. QYFY WZLL 28336).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Obstetrics, The Affiliated Hospital of Qingdao University, 59 Haier Road, Qingdao 266000, China
[2]Qingdao University, Qingdao, China
[3]Department of Gynecology and Obstetrics, Dazhou Dachuan District People's Hospital (Dazhou Third People's Hospital), Dazhou, Sichuan Province 635000, China
[4]Qingdao Laoshan District Maternal and Child Health Care Family Planning Service Center, Qingdao 266000, China
[5]Department of Obstetrics, The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266000, China

Received: 5 October 2024 / Accepted: 12 February 2025
Published online: 13 March 2025

References

1. Sadrzadeh S, Hui EVH, Schoonmade LJ, et al. Birthweight and PCOS: systematic review and meta-analysis. Hum Reprod Open. 2017;20172017:hox010.
2. Belenkaia LV, Lazareva LM, Walker W, et al. Criteria, phenotypes and prevalence of polycystic ovary syndrome. Minerva Ginecol. 2019;7171:211–23.
3. Li R, Zhang Q, Yang D, et al. Prevalence of polycystic ovary syndrome in women in China: a large community-based study. Hum Reprod (Oxford England). 2013;2828:2562–9.
4. Deng Y, Li H, Song Y, et al. Whole genome transcriptomic analysis of ovary granulosa cells revealed an Anti-Apoptosis regulatory gene DLGAP5 in polycystic ovary syndrome. Front Endocrinol. 2022;1313:781149.
5. Barbieri RL, Ryan KJ. Hyperandrogenism, insulin resistance, and acanthosis nigricans syndrome: a common endocrinopathy with distinct pathophysiologic features. Am J Obstet Gynecol. 1983;147147:90–101.
6. Haoula Z, Salman M, Atiomo W. Evaluating the association between endometrial cancer and polycystic ovary syndrome. Hum Reprod (Oxford England). 2012;2727:1327–31.
7. Barry JA, Azizia MM, Hardiman PJ. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction update. 2014; 2020:748– 58.
8. Guan C, Zahid S, Minhas AS, et al. Polycystic ovary syndrome: a risk-enhancing factor for cardiovascular disease. Fertil Steril. 2022;117117:924–35.
9. Zhu T, Cui J, Goodarzi MO. Polycystic ovary syndrome and risk of type 2 diabetes, coronary heart disease, and stroke. Diabetes. 2021;7070:627–37.
10. Fiorentino G, Cimadomo D, Innocenti F, et al. Biomechanical forces and signals operating in the ovary during folliculogenesis and their dysregulation: implications for fertility. Hum Reprod Update. 2023;2929:1–23.
11. Xiao S, Du J, Yuan G et al. Granulosa Cells-Related MicroRNAs in Ovarian Diseases: Mechanism, Facts and Perspectives. Reproductive sciences (Thousand Oaks, Calif.). 2024.
12. Tong C, Wu Y, Zhang L, et al. Insulin resistance, autophagy and apoptosis in patients with polycystic ovary syndrome: association with PI3K signaling pathway. Front Endocrinol. 2022;1313:1091147.
13. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nature reviews. Genetics. 2004;55:522–31.
14. Chen B, Xu P, Wang J, et al. The role of MiRNA in polycystic ovary syndrome (PCOS). Gene. 2019;706706:91–6.
15. Pan W, Chai B, Li L, et al. p53/MicroRNA-34 axis in cancer and beyond. Heliyon. 2023;99:e15155.
16. Li S, Wei X, He J, et al. The comprehensive landscape of miR-34a in cancer research. Cancer Metastasis Rev. 2021;4040:925–48.
17. Misir S, Hepokur C, Oksasoglu B, et al. Circulating serum miR-200c and miR-34a-5p as diagnostic biomarkers for endometriosis. J Gynecol Obstet Hum Reprod. 2021;5050:102092.
18. Li HL, Duan YA, Zhao N. MiR-34a-5p directly targeting TRIM44 affects the biological behavior of ovarian cancer cells. Eur Rev Med Pharmacol Sci. 2021;2525:1250–60.
19. Zuo Y, Zheng W, Liu J, et al. MiR-34a-5p/PD-L1 axis regulates cisplatin chemoresistance of ovarian cancer cells. Neoplasma. 2020;6767:93–101.
20. Li Q, Xu J. [miR-34a-5p regulates viability, invasion and apoptosis of placental trophoblastic cells via modulating CDK6 and PI3K/AKT pathway]. Nan Fang Yi Ke Da Xue Xue Bao. 2020;4040:79–86.
21. Recchia K, Jorge AS, Pessôa LVF et al. Actions and roles of FSH in germinative cells. Int J Mol Sci. 2021; 2222.
22. Liu F, Wang X, Zhao M, et al. Ghrelin alleviates inflammation, insulin resistance, and reproductive abnormalities in mice with polycystic ovary syndrome via the TLR4-NF-κB signaling pathway. Discov Med. 2024;3636:946–58.
23. Shimizu K, Nakamura T, Bayasula, et al. Molecular mechanism of FSHR expression induced by BMP15 in human granulosa cells. J Assist Reprod Genet. 2019;3636:1185–94.
24. Tian-Min Y, Suxia L, Shufang D, et al. Combined transcriptomic and metabolomic analysis of women with polycystic ovary syndrome. Dis Markers. 2022;20222022:4000424.
25. Chu YL, Xu YR, Yang WX, et al. The role of FSH and TGF-β superfamily in follicle Atresia. Aging. 2018;1010:305–21.
26. Franks S, Hardy K. Androgen action in the ovary. Front Endocrinol. 2018;99:452.
27. Wong RS. Apoptosis in cancer: from pathogenesis to treatment. J Experimental Clin cancer Research: CR. 2011;3030:87.
28. Ma M, Li H, Yin S, et al. Overexpression of miR-92a attenuates kidney ischemia-reperfusion injury and improves kidney preservation by inhibiting MEK4/JNK1-related autophagy. Cell Mol Biol Lett. 2023;2828:20.
29. Oulas A, Boutla A, Gkirtzou K, et al. Prediction of novel MicroRNA genes in cancer-associated genomic regions–a combined computational and experimental approach. Nucleic Acids Res. 2009;3737:3276–87.
30. Pang RT, Leung CO, Ye TM, et al. MicroRNA-34a suppresses invasion through downregulation of Notch1 and Jagged1 in cervical carcinoma and choriocarcinoma cells. Carcinogenesis. 2010;3131:1037–44.
31. Gao Y, Yang M, Wei L, et al. miR-34a-5p inhibits cell proliferation, migration and invasion through targeting JAG1/Notch1 pathway in HPV-Infected human epidermal keratinocytes. Pathol Oncol Research: POR. 2020;2626:1851–9.
32. Simon DP, Giordano TJ, Hammer GD. Upregulated JAG1 enhances cell proliferation in adrenocortical carcinoma. Clin cancer Research: Official J Am Association Cancer Res. 2012;1818:2452–64.
33. Liu Z, Zhu Y, Li F, et al. GATA1-regulated JAG1 promotes ovarian cancer progression by activating Notch signal pathway. Protoplasma. 2020;257257:901–10.
34. Sun HW, Wu C, Tan HY, et al. Combination DLL4 with Jagged1-siRNA can enhance Inhibition of the proliferation and invasiveness activity of human gastric carcinoma by Notch1/VEGF pathway. Hepatogastroenterology. 2012;5959:924–9.
35. Li D, Masiero M, Banham AH, et al. The Notch ligand JAGGED1 as a target for anti-tumor therapy. Front Oncol. 2014;44:254.
36. Shen H, Liang Z, Zheng S, et al. Pathway and network-based analysis of genome-wide association studies and RT-PCR validation in polycystic ovary syndrome. Int J Mol Med. 2017;4040:1385–96.
37. George RM, Hahn KL, Rawls A, et al. Notch signaling represses GATA4-induced expression of genes involved in steroid biosynthesis. Reprod (Cambridge England). 2015;150150:383–94.
38. Guo S, Quan S, Zou S. Roles of the Notch signaling pathway in ovarian functioning. Reproductive sciences (Thousand Oaks, Calif.). 2021; 2828:2770–8.
39. Hubbard N, Prasasya RD, Mayo KE. Activation of Notch signaling by oocytes and Jag1 in mouse ovarian granulosa cells. Endocrinology. 2019;160160:2863–76.
40. Masoudi M, Yamini N, Salehi F, et al. Notch signaling pathway in cumulus cells reflecting zygote and embryo quality in polycystic ovary syndrome. Arch Gynecol Obstet. 2021;304304:1097–105.

Publisher's note