Long non-coding RNA-X-inactive specific transcript inhibits cell viability, and induces apoptosis through the microRNA-30c-5p/Bcl2-like protein 11 signaling axis in human granulosa-like tumor cells

Yichuan Guo\textsuperscript{ab}, Xueping Peng\textsuperscript{ab}, Ran Cheng\textsuperscript{abc}, Hui Chen\textsuperscript{abc}, and Xiaoyan Luo\textsuperscript{abc*}

\textsuperscript{a}Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China; \textsuperscript{b}Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, Chengdu, Sichuan, China; \textsuperscript{c}Reproductive Endocrinology and Regulation Laboratory, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China

**ABSTRACT**

The role of long noncoding RNAs (lncRNAs) is being actively explored in polycystic ovary syndrome (PCOS). Recent research has shown that long non-coding RNA (lncRNA) X–inactive Specific Transcript (XIST) is overexpressed in patients with PCOS and is associated with poor pregnancy outcomes. However, the precise function and mechanism of action of lncRNA XIST in PCOS are unknown. We aimed to determine whether lncRNA XIST contributes to PCOS by modulating ovarian granulosa cell physiology. We also investigated any potential molecular regulatory mechanisms. In this study, we discovered that the lncRNA XIST was significantly downregulated in human ovarian granulosa-like tumor (KGN) cells. Notably, overexpression of lncRNA XIST decreased miR-30c-5p expression in KGN cells, inhibited proliferation, and induced apoptosis in KGN cells. However, cotransfection with amiR-30c-5p mimic significantly reduced these effects. Additionally, we discovered that the miR-30c-5p mimic effectively inhibited Bcl2-like protein 11 (BCL2L11) expression, a critical apoptotic promoter, whereas silencing of miR-30c-5p increased BCL2L11 expression, inhibited KGN cell proliferation, and induced apoptosis. In contrast, cotransfection of BCL2L11 with siRNA significantly reversed these effects. In conclusion, this study established that lncRNA XIST plays a critical role in PCOS by modulating the miR-30c-5p/BCL2L11 signaling axis and regulating ovarian granulosa cell physiology.

**Highlights**

- LncRNA XIST inhibits KGN cell proliferation and induces apoptosis by reducing miR-30c-5p expression;
- MiR-30c-5p inhibits BCL2L11 expression in KGN cells;
- MiR-30c-5p gene silencing inhibits KGN cell proliferation and induces apoptosis through upregulation of BCL2L11 expression.
**Introduction**

Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic condition that affects about 5 to 20% of women of reproductive age [1,2]. Endocrinological and reproductive investigations involving anovulation, polycystic ovarian morphology, hyperandrogenism without adrenaline, and androgenic or pituitary conditions, are used to diagnose PCOS [3]. These are the common symptoms of PCOS, which can lead to menstrual disorders and result in infertility [4,5]. The risk of developing obesity, diabetes, cardiovascular disease, metabolic syndrome, and cancer also increases with PCOS [6]. Ovarian hyperstimulation syndrome and miscarriages are more likely to occur in pregnant women with PCOS [5]. Therefore, PCOS remains a significant risk factor for pregnancy and is closely linked to pregnancy outcomes. Several studies have shown that the altered apoptotic activity of granular cells contributes to PCOS development, although the exact cause of PCOS remains unknown [7,8]. Further studies have shown that genetic and epigenetic factors are responsible for the pathogenesis of PCOS by modulating granulosa cell apoptosis [9–11]; however, the underlying mechanism is not clear. Furthermore, PCOS cannot be cured with available treatment options, and therefore lifelong management is required [12]. This means that understanding the etiology of PCOS is important for the clinical management of this condition.

Long noncoding RNAs (lncRNAs) are a diverse group of RNAs that play important roles in a variety of biological processes, including gene expression, cell division, differentiation, and apoptosis [11]. Moreover, lncRNAs play a crucial role in several diseases, including diabetes [13], cancer [14], inflammation [15], and neurological diseases [16]. In addition, lncRNAs play a significant role in embryogenesis and fertility [17]. Several studies have shown that lncRNAs play a role in PCOS development [11,18]. The profile of lncRNAs in the serum, granulosa cells, and follicular fluid differ between the general population and patients with PCOS [19,20]. PCOS is characterized by abnormal expression of the lncRNAs, growth arrest-specific transcript 5 (GAS5) and B-Raf proto-oncogene, serine/threonine kinase-activated non-protein coding RNA (BANCR), which regulate cell proliferation and apoptosis [10,21]. Moreover, it has been shown that silencing lncRNAs can alleviate PCOS in mice [22,23]. lncRNA XIST is a critical regulator, which is involved in various human diseases [24,25]. Recent research has shown that lncRNA XIST is expressed at low levels in patients with PCOS and is associated with adverse pregnancy outcomes [26]. However, the underlying mechanism of lncRNA XIST in PCOS remains to be elucidated. Furthermore, lncRNAs function as sponges for miRNAs and influence gene expression [27]. miRNAs are a class of noncoding RNAs that play important roles in gene regulation. Additionally, miRNAs are implicated in the pathogenesis and progression of PCOS [28–30].

Using the web software starBase (http://starbase. sysu. edu. cn/index. php), our results demonstrated that miR-30c-5p is the downstream target of lncRNA XIST, which was also confirmed by a dual-luciferase reporter assay. Therefore, we aimed to investigate the contribution of lncRNA XIST and its association with miR-30c-5p to PCOS.

In the present study, we hypothesized that lncRNA XIST regulates the miR-30c-5p/BCL2L11 signaling axis in PCOS development. Therefore, in this study, we explored the potential role of lncRNA XIST in PCOS and analyzed its molecular mechanism.

**Materials and methods**

**Cell lines and treatment**

Human granulosa-like tumor (KGN) cells (Procell Life Science&Technology Co., Ltd., Wuhan, China) were cultured in dulbecco’s modified easy medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and antibiotics (100 μg/ml penicillin and 100 μg/ml streptomycin) at 37°C and 5% CO₂. Normal ovarian surface epithelial (IOSE80) cells were used as a control in this study, and were grown in DMEM supplemented with 10% FBS. KGN and IOSE80 cells were harvested at 70%–80% confluence.

**RNA isolation and quantitative real-time PCR (qRT-PCR)**

Total RNA was extracted using an RNeasy kit (Qiagen, Duesseldorf, Germany) and then reverse transcribed into cDNA using a reverse transcription
kit (Invitrogen). Quantitative RT-PCR (Applied Biosystems) and the SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd.) were used to quantify the levels of lncRNA XIST, miR-30c-5p, and BCL2L11 according to the manufacturer’s protocol. We used U6 for miRNA and GAPDH for mRNA as internal controls. The relative gene expression levels were determined using the 2^−Δ∆Cq method [31]. Primer sequences were listed as following:

lncRNA XIST forward, 5'-GGTGGGACATGTGGCGTCA-3';
reverse 5'-CAGCCACGTAATCCAGATGAT-3';
miR-30c-5p forward, 5'-GCCGCTGTAACACCTCCTACACT-3';
reverse 5'-GTGCAGGGTCCGAGGT-3';
BCL2L11, forward 5'-TAAGGTCTGAGTGTGACATGGAGGTCTTTTTCCGAG-3';
reverse 5'-CCAGCCCATGATGGTTCTGAT-3';
miR-30c-5p reverse, 5'-GCCGCTGTAAACATCCTACACT-3';
lncRNA XIST, forward 5'-CATCATCCCTGCCTCT-3',
reverse 5'-TAAGTTCTGAGTGTGA-3';
miR-30c-5p forward, 5'-GGTGGGTCATGTGTGTACATGGAGGTCTTTTTCCGAG-3';
reverse 5'-CCGGAGAGGTCTTTTTCCGAG-3';
Bax, forward 5'-CCCGAGAGGTCTTTTTCCGAG-3';
reverse 5'-GCCGCTGTAAACATCCTACACT-3';
Bcl-2, forward 5'-GGTGGGCTGCTGATGGTTCTGAT-3';
reverse 5'-CCAGCCCATGATGGTTCTGAT-3';
GAPDH, forward 5'-CATCATCCCTGCCTCTACTGGA-3';
reverse 5'-GTCCTGCTCTGAGGAGGTAGG-3';
U6 S 5'-GGAAACGATACAGAGAAGATTAGC-3';
Stem-loop-R 5'-CTCAACTGTTGCTGGAAGTTAGC-3';

Cell transfection

KGN cells were plated in a 12-well plate at a density of 5 \times 10^5 cells/well and cultured overnight. According to the manufacturer’s instructions, KGN cells were transected with 0.5 µg of oligonucleotides (control-plasmid or XIST-plasmid; mimic control or miR-30c-5p mimic; XIST-plasmid+mimic control or XIST-plasmid+miR-30c-5p mimic; inhibitor control or miR-30c-5p inhibitor; control-siRNA or BCL2L11-siRNA; and miR-30c-5p inhibitor+control-siRNA or miR-30c-5p inhibitor+BCL2L11-siRNA) using 0.6 µL of Lipofectamine 2000 (Invitrogen). Transfected KGN cells were collected 48 h after transfection and transfection efficiency was confirmed using qRT-PCR.

LncRNA target analysis and luciferase reporter assay

The bioinformatics software starBase 2.0 (http://starbase.sysu.edu.cn/index.php) was used to determine the association between lncRNA XIST and miR-30c-5p [32]. A dual-luciferase reporter assay was performed to confirm this relationship [32]. Wild-type or mutant lncRNA XIST fragments containing miR-30c-5p binding sites were synthesized and placed into the pGL3-basic plasmid (Sangon) to generate lncRNA XIST-wild-type (WT) or lncRNA XIST-mutated (Mut). The reporter plasmid was cotransfected with 50 ng of Renilla luciferase reporter plasmid using Lipofectamine 2000. Following an overnight incubation period, relative luciferase activity was determined using a dual-luciferase assay kit (Promega). The relative luciferase activity was compared to that of the internal control Renilla luciferase.

MTT assay

KGN cells were seeded at a density of 5 \times 10^4 cells/well in 96-well plates and incubated at 37°C for 24 h. The cells were then transfected with the appropriate oligonucleotides and incubated for 0, 24, 48, and 72 h. Thus, the MTT assay [33] was performed at four different time points. After incubation, 10 µL of MTT solution was added to each well and incubated at 37°C for 4 h. To solubilize the formazan product, 100 µL of DMSO was added to each well and incubated at 37°C for 3 h. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad Laboratories, Inc.). The optical density of each group was compared with that of the control group to determine cell viability.

Flow cytometry analysis

KGN cells were harvested after transfection, plated in a 6-well plate at a density of 6 \times 10^4 cells/mL, and incubated for 24 h. Further, the cells were trypsinized and stained with annexin V-FITC and propidium iodide. A BD flow cytometer (BD Biosciences, USA) was used to measure cell apoptosis, which was then analyzed using the Kaluza analysis software (version 2.1.1.20653; Beckman Coulter, Inc.) [34].
Western blot analysis

Protein levels were determined using western blot assay [35]. Total protein was extracted from KGN cells using RIPA lysis buffer and protease and phosphatase inhibitors. Before separation on a 10% SDS-PAGE gel, protein samples were denatured at 100°C. After separation, proteins were then transferred to a polyvinylidene difluoride membrane. The membrane was then blocked with 5% skimmed milk for 1.5 h and incubated with primary antibodies against Bax (cat. no. 5023; 1: 1000; Cell signaling Technology, Beverly, MA, USA), Bcl-2 (cat. no. 4223; 1: 1000; Cell signaling Technology, Beverly, MA, USA), BCL2L11 (cat. no. 2933; 1: 1000; Cell signaling Technology, Beverly, MA, USA), and GAPDH (cat. no. 5174; 1: 1000; Cell signaling Technology, Beverly, MA, USA). After washing with PBS, the membranes were incubated with secondary antibody IgG-HRP (cat. no. 7074; 1: 2000; Cell signaling Technology, Beverly, MA, USA) at 37°C for 2 h. Finally, protein bands were identified using the Immobilon ECL Ultra Western HRP Substrate and images were captured using a G-Box system. Images were then analyzed using ImageJ software version 1.8.0 (NIH, Bethesda, MD, USA).

Statistical analysis

SPSS 19.0 (SPSS Inc., Chicago, IL) was used for statistical analysis, and data were presented as the mean ± standard deviation (SD). The student’s t-test was used to determine the difference between two groups, and one-way analysis of variance was used to make multiple comparisons. P < 0.05 was considered statistically significant.

Results

Direct binding site exists between lncRNA XIST and miR-30c-5p

To reveal the interactions between miR-30c-5p and lncRNA XIST, bioinformatics tool, starBase, was used. The results predicted a direct binding site between lncRNA XIST and miR-30c-5p (Figure 1(a)). Using a dual-luciferase reporter gene assay, we confirmed that lncRNA XIST binds to miR-30c-5p, as shown in Figure 1(b).

Opposite expression patterns of lncRNA XIST and miR-30c-5p in ovarian cell lines

To investigate the expression patterns of lncRNA XIST and miR-30c-5p in granulosa cells, qRT-PCR was performed on IOSE80 and KGN cells. The KGN cells expressed significantly less lncRNA XIST than IOSE80 cells, although miR-30c-5p expression was significantly higher in the KGN cells (Figure 2(a,b)).

lncRNA XIST inhibits KGN cell proliferation and induces apoptosis by reducing miR-30c-5p expression

To explore the mechanism of lncRNA XIST in PCOS, KGN cells were transfected with a control-plasmid or an XIST-plasmid, a mimic control or a miR-30c-5p mimic, an XIST-plasmid +mimic control or an XIST-plasmid+miR-30c-5p mimic to determine whether XIST regulates miR-30c-5p expression in granulosa cells. Compared with the control plasmid, the lncRNA XIST-plasmid significantly increased XIST expression in the KGN cell line. (Figure 3(a)). Similarly, in KGN cells, the miR-30c-5p mimic notably increased miR-30c-5p expression (Figure 3(b)). Overexpression of lncRNA XIST markedly
reduced the expression of miR-30c-5p in the KGN cell line. In contrast, cotransfection of the miR-30c-5p mimic reversed this effect (Figure 3(c)), indicating that lncRNA XIST inhibits miR-30c-5p expression in the KGN cell line.

To determine the effect of XIST on miR-30c-5p, KGN cells were transfected with the control plasmid, XIST-plasmid, XIST-plasmid+miR-30c-5p mimic, or XIST-plasmid+miR-30c-5p mimic. Compared with the control plasmid, the XIST-plasmid significantly decreased KGN cell viability (Figure 4(a)), promoted apoptosis in KGN cells (Figure 4(b,c)), increased the concentration of apoptosis-related Bax protein and its mRNA expression (Figure 4(d,e)), and decreased the concentration of Bcl-2 protein and its mRNA expression (Figure 4(d,f)). Conversely, cotransfection of the miR-30c-5p mimic significantly reduced these effects. These results suggest that lncRNA XIST inhibits KGN cell proliferation and induces apoptosis in KGN cells by lowering miR-30c-5p expression.

**MiR-30c-5p inhibits BCL2L11 expression in KGN cells**

To understand whether miR-30c-5p can regulate BCL2L11 in KGN cells, we first determined the expression of BCL2L11 in IOSE80 and KGN cells, the evidence suggests that the expression of BCL2L11 was significantly lower in the KGN cells than in the IOSE80 cells, as shown in Figure 5(a,b). The MiR-30c-5p mimic, mimic control, inhibitor control, or miR-30c-5p inhibitor were transfected into KGN cells. The MiR-30c-5p mimic significantly increased miR-30c-5p expression in KGN cells.
cells compared with the mimic control group (Figure 5(c)), while decreasing BCL2L1 expression (Figure 5(d,e)). Compared with the inhibitor control, the miR-30c-5p inhibitor significantly reduced miR-30c-5p expression (Figure 5(f)) and increased BCL2L11 expression (Figure 5(g,h)) in the KGN cells.

**miR-30c-5p gene silencing inhibits KGN cell proliferation and induces apoptosis through upregulation of BCL2L11 expression.**

To explore whether miR-30c-5p affect KGN cells via regulating BCL2L11 expression, KGN cells were transfected with control siRNA, BCL2L11-siRNA, inhibitor control, miR-30c-5p inhibitor, miR-30c-5p inhibitor+control siRNA, or miR-30c-5p inhibitor+BCL2L11-siRNA.

Compared with the control siRNA group, BCL2L11-siRNA significantly reduced BCL2L11 expression in the KGN cells (Figure 6(a,b)). The miR-30c-5p inhibitor significantly increased BCL2L11 expression in the KGN cells compared with the inhibitor control group, and this increase was significantly reversed by BCL2L11-siRNA (Figure 6(c,d)).

Compared with the inhibitor control group, the miR-30c-5p inhibitor significantly reduced the viability of KGN cells (Figure 7(a)), promoted apoptosis in KGN cells (Figure 7(b,c)), increased the amount of Bax protein and its mRNA expression (Figure 7(d,e)), and decreased the amount of Bcl-2 protein and its mRNA expression (Figure 7(d,f)). These effects were significantly inhibited by BCL2L11-siRNA cotransfection. These findings suggest that miR-30c-5p regulates the BCL2L11 gene to control KGN cell proliferation and apoptosis.

**Discussion**

XIST is a novel lncRNA that is overexpressed in patients with PCOS and has been linked to poor pregnancy outcomes. However, the precise function and role of XIST in PCOS are unknown. The goal of this study was to determine the role and mechanism of XIST in PCOS. In patients with PCOS patients, IncRNA XIST has been shown to influence the physiology of ovarian granulosa cells by modulating the miR-30c-5p/BCL2L11 signaling axis.

PCOS is a complex endocrine disorder that affects a large percentage of women of reproductive age worldwide. It has a significant negative impact on the quality of life in women, particularly premenopausal women [11]. Although several causes have been identified, the pathophysiology of PCOS remains unclear [4]. New evidence suggests that changes in noncoding RNA expression, particularly lncRNAs, are linked to the

---

**Figure 4.** lncRNA XIST affects KGN cell proliferation and apoptosis via miR-30c-5p

KGN cells were transfected with either control plasmid, XIST-plasmid, XIST-plasmid+mimic control, or XIST-plasmid+miR-30c-5p mimic for 48 h. The MTT assay was used to determine cell proliferation (a), cell apoptosis was measured using flow cytometry (FCM) (b), and the resulting apoptosis rates were presented statistically (c). The protein levels of Bax and Bcl-2 were detected with western blotting (d), and the mRNA levels of Bax (e) and Bcl-2 (f) were detected using RT-qPCR. **p < 0.01 vs. control-plasmid; #p < 0.01 vs. XIST-plasmid+mimic control.
GAS5, SRLR, and PVT1 are examples of differentially expressed lncRNAs that have been shown to play a role in the pathophysiology of PCOS and regulate ovarian granulosa cell death [36–38]. Abnormal or altered apoptosis of granulosa cells has also been linked to PCOS development [8,39].

The lncRNA XIST has been linked to a variety of human diseases [14,26,40,41], and its expression varies in patients with PCOS patients. However, the role of XIST in PCOS remains unclear. In particular, lncRNA XIST has been implicated in cell proliferation and as a complement to the repressive complex on the female X chromosome [42]. Furthermore, previous findings indicated that XIST is involved in X chromosome inactivation suggest that XIST could be involved in PCOS [43]. According to this study, the lncRNA XIST is much less common in human ovarian KGN cells than in normal IOSE80 cells. Furthermore, XIST expression is lower in PCOS and is linked to poor pregnancy outcomes according to a previous study. As a result, comprehensive research into the function of XIST and its molecular mechanism may aid in its identification as a potential PCOS marker.

Figure 5. miR-30c-5p negatively regulated BCL2L11 expression in KGN cells (a and b) The mRNA and protein expression of BCL2L11 in normal ovarian surface epithelium (IOSE80) and human ovarian granulosa cell-like (KGN) cells were determined using qRT-PCR and a western blot assay. (c) The level of miR-30c-5p in KGN cells transfected with the mimic control or miR-30c-5p mimic was detected using RT-qPCR. (d and e) The mRNA and protein expression of BCL2L11 in KGN cells transfected with the mimic control or miR-30c-5p mimic were determined using qRT-PCR and a western blot assay. (f) The level of miR-30c-5p in KGN cells transfected with inhibitor control or miR-30c-5p inhibitor was detected using RT-qPCR. (g and h) The mRNA and protein expression of BCL2L11 in KGN cells transfected with inhibitor control or miR-30c-5p inhibitor were determined using qRT-PCR and a western blot assay. **p < 0.01 vs. IOSE80; ##p < 0.01 vs. mimic control; &&p < 0.01 vs. inhibitor control.
Figure 6. BCL2L11-siRNA reversed miR-30c-5p inhibitor induced up-regulation of BCL2L11 in KGN cell (A and B) The mRNA and protein expression of BCL2L11 in KGN cells transfected with control-siRNA or BCL2L11-siRNA were determined using qRT-PCR and a western blot assay. (C and D) The mRNA and protein expression of BCL2L11 in KGN cells transfected with the inhibitor control, miR-30c-5p inhibitor, miR-30c-5p inhibitor+control-siRNA, or miR-30c-5p inhibitor+BCL2L11-siRNA were determined using qRT-PCR and western blot assay. **p<0.01 vs. control-siRNA; ##p<0.01 vs. inhibitor control; &&p<0.01 vs. miR-30c-5p inhibitor+control-siRNA.

Figure 7. miR-30c-5p inhibitor affects KGN cell proliferation and apoptosis via BCL2L11 KGN cells were transfected with the inhibitor control, miR-30c-5p inhibitor, miR-30c-5p inhibitor+control-siRNA, or miR-30c-5p inhibitor+BCL2L11-siRNA for 48 h. The MTT assay was used to determine cell proliferation (a), cell apoptosis was measured using FCM (b), and the resulting apoptosis rates are presented statistically (c). The protein levels of Bax and Bcl-2 were detected using a western blot assay (d), and the mRNA levels of Bax (e) and Bcl-2 (f) were detected using RT-qPCR. **p < 0.01 vs. inhibitor control; ###p < 0.01 vs. miR-30c-5p inhibitor+control-siRNA.
IncRNAs influence biological processes by acting like a sponge for microRNAs. This encourages cancer cell proliferation and metastasis by modulating IncRNA XIST miR-186-5p [44]. XIST has also been linked to cell proliferation and apoptosis as well as to the regulation of several other miRNAs [45]. We used bioinformatics software (starBase) to identify a direct binding site between IncRNA XIST and miR-30c-5p. To confirm this binding further, we used a dual-luciferase reporter gene assay. We identified that IncRNA XIST and miR-30c-5p have opposing expression patterns in ovarian cell lines. The IncRNA XIST was downregulated in human ovarian granulosa cells, whereas miR-30c-5p expression was significantly increased. Therefore, we can speculate that IncRNA XIST could influence miR-30c-5p expression. To determine whether IncRNA XIST regulates miR-30c-5p expression, we transfected KGN cells with either a control plasmid, XIST-plasmid, mimic control, miR-30c-5p mimic, XIST-plasmid+mimic control, or XIST-plasmid+miR-30c-5p mimic. XIST significantly reduced the expression of miR-30c-5p in the KGN cells. Further, KGN cells were transfected with the control plasmid, XIST-plasmid, XIST-plasmid+mimic control, or XIST-plasmid+miR-30c-5p mimic to understand the impact of XIST on granulosa cells. According to our findings, the XIST-plasmid reduced cell viability and accelerated apoptosis in KGN cells. Overexpression of IncRNA XIST increased the apoptosis-related Bax protein and its mRNA expression, and decreased anti-apoptotic Bcl-2 protein and its mRNA expression. In contrast, cotransfection of the MiR-30c-5p mimic significantly reduced these effects. Overall, these findings show that IncRNA XIST inhibited KGN cell proliferation and accelerated apoptosis by lowering miR-30c-5p expression.

By inactivating the anti-apoptotic protein BCL-2 and activating the apoptotic protein Bax, BCL2L11 participates in the regulation of cell proliferation and apoptosis. We further examined the role of miR-30c-5p in BCL2L11 regulation in KGN cells. We transfected KGN cells with either control-siRNA, BCL2L11 siRNA, miR-30c-5p inhibitor, miR-30c-5p inhibitor+control-siRNA, miR-30c-5p inhibitor+BCL2L11 siRNA, miR-30c-5p inhibitor+BCL2L11 siRNA, miR-30c-5p inhibitor+BCL2L11 siRNA, or miR-30c-5p inhibitor+1B1L2CBit. Inhibition of miR-30c-5p with siRNA reduced cell function and accelerated apoptosis in KGN cells. Furthermore, it increased expression of Bax protein and its mRNA, whereas Bcl-2 decreased the expression of Bax protein and its mRNA. Conversely, the effects of BCL2L11-siRNA cotransfer were significantly altered. According to our findings, KGN regulates cell proliferation and apoptosis via the miR-30c-5p BCL2L11 gene.

Conclusions

In summary, the miR-30c-5p/BCL2L11 signaling axis is required for cell proliferation and apoptosis in KGN cells. In granulosa cells, IncRNA XIST was significantly downregulated. We have shown the function of IncRNA XIST in granulosa cells for the first time, suggesting that it could be a viable therapeutic target for PCOS.

Data Availability Statement

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

[1] Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol. 2018;14:270–284.
[2] Azziz R, Carmina E, Chen Z, et al. Polycystic ovary syndrome. Nat Rev Dis Primers. 2016;2:16057.
[3] Azziz R, Carmina E, Dewailly D, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006;91:4237–4245.
[4] Ehrmann DA. Polycystic ovary syndrome. N Engl J Med. 2005;352:1223–1236.
[5] Norman RJ, Dewailly D, Legro RS, et al. Polycystic ovary syndrome. Lancet. 2007;370:685–697.
[6] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group., Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81:19–25.
[7] Song WJ, Shi X, Zhang J, et al. Akt-mTOR signaling mediates abnormalities in the proliferation and apoptosis of ovarian granulosa cells in patients with polycystic ovary syndrome. Gynecol Obstet Invest. 2018;83:124–132.
[8] Mikaeli S, Rashidi BH, Safa M, et al. Altered FoxO3 expression and apoptosis in granulosa cells of women with polycystic ovary syndrome. Arch Gynecol Obstet. 2016;294:185–192.
[9] Shi Y, Zhao H, Shi Y, et al. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. Nat Genet. 2012;44:1020–1025.
[10] Wang W XX, Jiao JZ, Jiang J, et al. Genome-wide DNA methylation and gene expression patterns provide insight into polycystic ovary syndrome development. Oncotarget. 2014;5:6603–6610.
[11] Mu L, Sun X, Tu M, et al. Non-coding RNAs in polycystic ovary syndrome: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2021;19:10.
[12] Jin P, Xie Y. Treatment strategies for women with polycystic ovary syndrome. Gynecol Endocrinol. 2018;34:272–277.
[13] Li Y, Yuan X, Shi Z, et al. LncRNA XIST serves as a diagnostic biomarker in gestational diabetes mellitus and its regulatory effect on trophoblast cell via miR-497-5p/FOXO1 axis. Cardiovasc Diagn Ther. 2021;11:716–725.
[14] Liu J, Yao L, Zhang M, et al. Downregulation of LncRNA-XIST inhibits non-small cell lung cancer by activating miR-335/SOD2/ROS signal pathway mediated pyroptotic cell death. Aging (Albany NY). 2019;11:7830–7846.
[15] Du M, Yuan L, Tan X, et al. The LPS-inducible LncRNA Mirt2 is a negative regulator of inflammation. Nat Commun. 2017;8:2049.
[16] Ma N, Tie C, Yu B, et al. Identifying lncRNA-miRNA-mRNA networks to investigate Alzheimer’s disease pathogenesis and therapy strategy. Aging (Albany NY). 2020;12:2897–2920.
[17] Goudarzi M, Berg K, Pieper LM, et al. Individual long non-coding RNAs have no overt functions in zebrafish embryogenesis, viability and fertility. Elife. 2019;8. DOI:10.7554/eLife.40815
[18] Chen L, Kong CX. LINCO0173 regulates polycystic ovarian syndrome progression by promoting apoptosis and repressing proliferation in ovarian granulosa cells via the microRNA-124-3p (miR-124-3p)/jagged canonical Notch ligand 1 (JAG1) pathway. Bioengineered. 2022;13:10373–10385.
[19] Murri M, Insenser M, Fernández-Durán E, et al. Effects of polycystic ovary syndrome (PCOS), sex hormones, and obesity on circulating miRNA-21, miRNA-27b, miRNA-103, and miRNA-155 expression. J Clin Endocrinol Metab. 2013;98:E1835–E1844.
[20] Naji M, Aleyasin A, Neekoonam S, et al. Differential Expression of miR-93 and miR-21 in granulosa cells and follicular fluid of polycystic ovary syndrome associating with different phenotypes. Sci Rep. 2017;7:14671.
[21] Yang R, Chen J, Wang L, et al. LncRNA BANCR participates in polycystic ovary syndrome by promoting cell apoptosis. Mol Med Rep. 2019;19:1581–1586.
[22] Li Y, Zhao W, Wang H, et al. Silencing of LncRNA steroid receptor RNA activator attenuates polycystic ovary syndrome in mice. Biochimie. 2019;157:48–56.
[23] Jiang B, Xue M, Xu D, et al. Down-regulated LncRNA HOTAIR alleviates polycystic ovaries syndrome in rats by reducing expression of insulin-like growth factor 1 via microRNA-130a. J Cell Mol Med. 2020;24:451–464.
[24] Chen DL, Ju HQ, Lu YX, et al. Long non-coding RNA XIST regulates gastric cancer progression by acting as a molecular sponge of mir-101 to modulate EZH2 expression. J Exp Clin Cancer Res. 2016;35:142.
[25] Wei W, Liu Y, Lu Y, et al. LncRNA XIST promotes pancreatic cancer proliferation through miR-133a/EGFR. J Cell Biochem. 2017;118:3349–3358.
[26] Liu M, Zhu H, Li Y, et al. Expression of serum LncRNA-Xist in patients with polycystic ovary syndrome and its relationship with pregnancy outcome. Taiwan J Obstet Gynecol. 2020;59:372–376.
[27] Gao Y, Zhang R, Wei G, et al. Long non-coding rna maternally expressed 3 increases the expression of neuron-specific genes by targeting mir-128-3p in all-trans retinoic acid-induced neurogenic differentiation from amniotic epithelial Cells. Front Cell Dev Biol. 2019;7:342.
[28] Chen B, Xu P, Wang J, et al. The role of MiRNA in polycystic ovary syndrome (PCOS). Gene. 2019;706:91–96.
[29] Xue Y, Lv J, Xu P, et al. Identification of microRNAs and genes associated with hyperandrogenism in the follicular fluid of women with polycystic ovary syndrome. J Cell Biochem. 2018;119:3913–3921.
[30] Chen HX, Fu YF, Guo ZX, et al. MicroRNA-29c-3p participates in insulin function to modulate polycystic ovary syndrome via targeting Forkhead box O 3. Bioengineered. 2022;13:4361–4371.
[31] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25:402–408.
[32] Zhou XQ, Chang YZ, Zhu LR, et al. LINCO0839/miR-144-3p/WTAP (WT1 Associated protein) axis is involved in regulating hepatocellular carcinoma progression. Bioengineered. 2021;12:10849–10861.
[33] Kumar P, Nagarajan A, Uchil PD. Analysis of Cell Viability by the MTT Assay. Cold Spring Harb Protoc. 2018;2018:469–471.
[34] Telford WG. Multiparametric analysis of apoptosis by flow cytometry. Methods Mol Biol. 2018;1678:167–202.

[35] Han D, Yu ZJ, Zhang H, et al. Microenvironment-associated gene HSD11B1 may serve as a prognostic biomarker in clear cell renal cell carcinoma: a study based on TCGA, RT-qPCR, Western blotting, and immunohistochemistry. Bioengineered. 2021;12:10891–10904.

[36] Wang C, Yue S, Jiang Y, et al. LncRNA GAS5 is upregulated in polycystic ovary syndrome and regulates cell apoptosis and the expression of IL-6. J Ovarian Res. 2020;13:145.

[37] Li L, Zhu J, Ye F, et al. Upregulation of the lncRNA SRLR in polycystic ovary syndrome regulates cell apoptosis and IL-6 expression. Cell Biochem Funct. 2020;38:880–885.

[38] Liu G, Liu S, Xing G, et al. lncRNA PVT1/microRNA-17-5p/PTEN Axis regulates secretion of E2 and P4, proliferation, and apoptosis of ovarian granulosa cells in PCOS. Mol Ther Nucleic Acids. 2020;20:205–216.

[39] Cui X, Jing X, Wu X, et al. Abnormal expression levels of BMP15/Smad1 are associated with granulosa cell apoptosis in patients with polycystic ovary syndrome. Mol Med Rep. 2017;16:8231–8236.

[40] Chanda K, Mukhopadhya D. LncRNA Xist X-chromosome instability and alzheimer’s disease. Curr Alzheimer Res. 2020;17:499–507.

[41] Cheng Q, Wang L. LncRNA XIST serves as a ceRNA to regulate the expression of ASF1A, BRWD1M, and PFKFB2 in kidney transplant acute kidney injury via sponging hsa-miR-212-3p and hsa-miR-122-5p. Cell Cycle. 2020;19:290–299.

[42] Penny GD, Kay GF, Sheardown SA, et al. Requirement for Xist in X chromosome inactivation. Nature. 1996;379(6561):131–137.

[43] Mukherjee S. Pathomechanisms of polycystic ovary syndrome multidimensional approaches. Front Biosci (Elite Ed). 2018;10(3):384–422.

[44] Wang H, Shen Q, Zhang X, et al. The long non-coding RNA XIST controls non-small cell lung cancer proliferation and invasion by modulating miR-186-5p. Cell Physiol Biochem. 2017;41(6):2221–2229.

[45] Liu H, Deng H, Zhao Y, et al. LncRNA XIST/miR-34a axis modulates the cell proliferation and tumor growth of thyroid cancer through MET-PI3K-AKT signaling. J Exp Clin Cancer Res. 2018;37:279.

[46] Essafi A, Fernández de Mattos S, Hassen YA, et al. Direct transcriptional regulation of Bim by FoxO3a mediates ST1571-induced apoptosis in Bcr-Abl-expressing cells. Oncogene. 2005;24:2317–2329.

[47] Meng S, Hu Y, Zhu J, et al. miR-30c-5p acts as a therapeutic target for ameliorating myocardial ischemia-reperfusion injury. Am J Transl Res. 2021;13:2198–2212.