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Long non-coding RNA NEAT1 promotes ovarian granulosa cell proliferation and cell cycle progression via the miR-29a-3p/IGF1 axis

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Abstract

Background Granulosa cell proliferation and survival are essential for normal ovarian function and follicular development. Long non-coding RNAs (lncRNAs) have emerged as important regulators of cell proliferation and differentiation. Nuclear paraspeckle assembly transcript 1 (NEAT1) has been implicated in various cellular processes, but its role in granulosa cell function remains unclear.

Methods We investigated the function of lncRNA NEAT1 in human ovarian granulosa-like tumor cells (KGN). The effects of NEAT1 overexpression or silencing on cell proliferation and cell cycle were evaluated using CCK-8 assays and flow cytometry. The interaction between NEAT1, miR-29a-3p, and IGF1 was examined using dual-luciferase reporter assays, qRT-PCR, and Western blot analysis.

Results NEAT1 promoted granulosa cell proliferation and cell cycle progression by indirectly upregulated IGF1 expression through acting as a molecular sponge for miR-29a-3p. Cell proliferation and G2/M phase proportions were increased by overexpression of NEAT1, whereas cell proliferation and G2/M phase proportions decreased with NEAT1 silencing. The effects of NEAT1 on cell proliferation and cell cycle-related proteins (CCNB1 and CDK2) were partially reversed by miR-29a-3p mimic, while miR-29a-3p inhibitor rescued the effects of NEAT1 silencing.

Conclusion LncRNA NEAT1 could promote ovarian granulosa cell proliferation and cell cycle progression via the miR-29a-3p/IGF1 axis in polycystic ovary syndrome. Further investigation of this mechanism in clinical samples may have implications for understanding ovarian physiology and pathology.

Keywords Long non-coding RNA, NEAT1, miR-29a-3p, IGF1, Granulosa cells

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Introduction

Granulosa cells are essential orchestrators of ovarian function, controlling follicular development, steroidogenesis, and oocyte maturation. The precise regulation of granulosa cell proliferation and survival is critical for maintaining reproductive homeostasis, with dysregulation leading to various ovarian disorders. Understanding the molecular mechanisms governing granulosa cell function therefore has significant implications for reproductive health.

Long non-coding RNA (lncRNA) have emerged as critical regulators of cellular processes [1]. The dysregulation of lncRNAs has been implicated in reproductive disorders [2]. Among them, nuclear paraspeckle assembly transcript 1 (NEAT1) has garnered particular attention for its role in cell cycle control and endocrine regulation. While NEAT1's functions have been studied in various contexts [3], its specific regulatory mechanisms in granulosa cells remain unexplored, which represents an important knowledge gap, as granulosa cells possess unique molecular characteristics and regulatory networks distinct from other cell types. Several studies have demonstrated that lncRNAs play critical roles in post-transcriptional regulation of gene expression by sponging microRNAs (miRNAs), another class of non-coding RNAs that regulate gene expression post-transcriptionally [4, 5]. This intricate regulatory network adds another layer of complexity to gene expression control and cellular homeostasis. The interaction between NEAT1 and miR-29a-3p has been previously reported in several pathological conditions [6, 7]. Additionally, miR-29a-3p has been shown to target Insulin-like growth factor 1 (IGF1), a key player in ovarian function, promoting granulosa cell proliferation and steroidogenesis, in various biological contexts [8–11]. However, the potential regulatory mechanisms involving lncRNAs and miRNAs in modulating IGF1 expression in the context of ovarian granulosa cells remain to be elucidated [12]. Understanding this regulatory axis could reveal previously unknown mechanisms controlling granulosa cell function and ovarian development.

Cell cycle dysregulation plays a crucial role in granulosa cell proliferation and PCOS pathogenesis. Cyclin B1 (CCNB1) and Cyclin-dependent kinase 2 (CDK2) are key regulators of cell cycle progression. CCNB1 is essential for G2/M phase transition, while CDK2 primarily controls G1/S transition and S phase progression. Previous studies have shown that these proteins are dysregulated in various reproductive disorders, suggesting their potential involvement in PCOS. Considering the role of non-coding RNAs and the potential interplay between lncRNAs, miRNAs, and key regulatory factors, we hypothesized that NEAT1 might play a crucial role in ovarian granulosa cell function. In this study, we aimed to investigate the potential regulatory axis involving NEAT1, miR-29a-3p, and IGF1 in ovarian granulosa cells and its implications for cell proliferation and cycle progression.

Methods

Cell culture and transfection

Human ovarian granulosa-like tumor cell line KGN was obtained from RIKEN BioResource Center (Tsukuba, Japan). Cells were cultured in DMEM/F-12 medium (11330032, Gibco, USA) supplemented with 10% fetal bovine serum (10099141, FBS, Gibco) and 1% penicillin/streptomycin (15140122, Invitrogen, USA) at 37 °C in a humidified atmosphere with 5% CO_2.

For NEAT1 overexpression, the full-length NEAT1 sequence was cloned into the pcDNA3.1 vector (V79020, Invitrogen, USA). NEAT1 silencing was achieved using small interfering RNA (siRNA) targeting NEAT1. The miR-29a-3p mimic, inhibitor, and their respective negative controls were synthesized by GenePharma (Shanghai, China). Transfections were performed using Lipofectamine 3000 (L3000015, Invitrogen, USA) according to the manufacturer's instructions. To identify the most effective siRNA for silencing lncNEAT1, we screened multiple siRNA sequences (Figure s1) and selected the optimal sequence for subsequent functional studies. For NEAT1 overexpression, we constructed a pcDNA3.1(+)-flag-NEAT1 plasmid (9244 bp) containing CMV promoter, CMV enhancer, ampicillin resistance gene (AmpR), and multiple restriction sites (Figure s2A). KGN cells were transfected with either empty vector (vec), NEAT1 overexpression vector (vec-NEAT1), scrambled control (Scrambled), or NEAT1 siRNA (si-NEAT1) using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. The transfection efficiency was verified by qRT-PCR analysis (Fig. s2B).

RNA extraction and qRT-PCR

Total RNA was extracted from KGN cells using TRIzol reagent (15596026, Invitrogen, USA) following the manufacturer's protocol. For mRNA and lncRNA analysis, cDNA was synthesized using the PrimeScript RT reagent Kit (RR037A, Takara, Japan). For miRNA analysis, cDNA was synthesized using the Mir-X miRNA First-Strand Synthesis Kit (638315, Takara, Japan). The sequences of primers used for qRT-PCR are listed in Table 1. qRT-PCR was performed using SYBR Premix Ex Taq II (RR820A, Takara, Japan) on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA). GAPDH and U6 were used as internal controls for mRNA/lncRNA and miRNA, respectively. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1 Primer sequence

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
IGF1	5′CCATGTCCTCCTCGCATCTC3′	5′ACCCTGTGGGCTTGTTGAAA3′
miR-29a-3p	5′TAGCACCATCTGAAATCGGTTA3′	5′GCTGTCAACGATACGCTACG3′
lncNEAT1	5′TAAGAGGACCCTGAGGTGGG3′	5′CCCACCACCCACACTGTATC3′
U6	5′CTCGCTTCGGCAGCACA3′	5′AACGCTTCACGAATTTGCGT3′
GAPDH	5′AACGGATTTGGTCGTATTGGG3′	5′CCTGGAAGATGGTGATGGGAT3′
CDK2	5′CGGATCTTTCGGACTCTGGG3′	5′ACTGGCTTGGTCACATCCTG3′
CCNB1	5′AGGCGAAGATCAACATGGCA3′	5′ACCAATGTCCCCAAGAGCTG3′

Western blot analysis

Cells were lysed in RIPA buffer (P0013B, Beyotime, China) supplemented with protease inhibitors. Protein concentrations were determined using the BCA Protein Assay Kit (23225, Thermo Fisher Scientific, USA). Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (IPVH08130, Millipore, USA). After blocking with 5% non-fat milk, membranes were incubated with primary antibodies against CCNB1 (ab32053, 1:1000, Abcam, UK), CDK2 (ab32147, 1:1000, Abcam, UK), IGF1 (ab9572, 1:1000, Abcam, UK), and GAPDH (ab8245, 1:2000, Abcam, UK) overnight at 4 °C. After washing, membranes were incubated with HRP-conjugated secondary goat-anti-rabbit antibodies (SA00001-2, 1:2000, proteintech, USA) or goat-anti-mouse antibodies (SA00001-1, 1:2000, proteintech, USA). Protein bands were visualized using an enhanced chemiluminescence detection system (Bio-Rad, USA). Uncropped gels and blots images can be found in the supplement file.

Cell proliferation assay

Cell proliferation was assessed using the Cell Counting Kit-8 (CK04, CCK-8, Dojindo, Japan) due to its high sensitivity and lower cytotoxicity compared to traditional MTT assay. Briefly, transfected KGN cells were seeded in 96-well plates at a density of 3×10^3 cells/well. At 24-, 48-, and 72-hours post-transfection, CCK-8 solution was added to each well and incubated for 2 h at 37 °C. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad).

Cell cycle analysis

Transfected KGN cells were harvested, washed with PBS, and fixed in 70% ethanol overnight at 4 °C for cell cycle analysis. Cells were then stained with propidium iodide (PI) solution containing RNase A (C1052, Beyotime, China) for 30 min at 37 °C in the dark. Cell cycle distribution was analyzed using a FACSCalibur flow cytometer (BD Biosciences, USA) and ModFit LT software (Verity Software House, USA).

Dual-luciferase reporter assay

The wild-type (WT) and mutant (MUT) 3'-UTR sequences of NEAT1 and IGF1 containing the predicted miR-29a-3p binding sites were cloned into the pmiRGLO dual-luciferase vector (Promega, USA). KGN cells were co-transfected with the luciferase reporter constructs and miR-29a-3p mimic or negative control using Lipofectamine 3000. Luciferase activities were measured 48 h post-transfection using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

Bioinformatics Analysis

Potential miR-29a-3p binding sites in the IGF1 3'UTR were predicted using TargetScan (www.targetscan.org) and miRWalk (mirwalk.umm.uni-heidelberg.de) databases. The interaction between NEAT1 and miR-29a-3p was predicted using the StarBase database (starbase.sysu.edu.cn).

Statistical analysis

All experiments were performed in triplicate and data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 8.0.3 software (GraphPad Software, USA). Differences between two groups were analyzed using Student's t-test, while one-way ANOVA followed by Tukey's post hoc test was used for multiple group comparisons. $P < 0.05$ was considered statistically significant.

Results

NEAT1 promotes KGN cell proliferation and regulates cell cycle progression

We investigated the role of NEAT1 in ovarian granulosa cells by overexpressing or silence NEAT1 in KGN cells. Based on the screening of three siRNA sequences targeting lncNEAT1 (Fig. s1), the most effective siRNA, series s340, was identified and subsequently used for experiments. We first assessed the expression of NEAT1 in each group, and the results showed that both overexpression and silencing of NEAT1 had significant effects. (Fig. s2B). NEAT1 overexpression significantly promoted cell proliferation, while silencing NEAT1 significantly reduced proliferation compared to the control group (Fig. 1A).

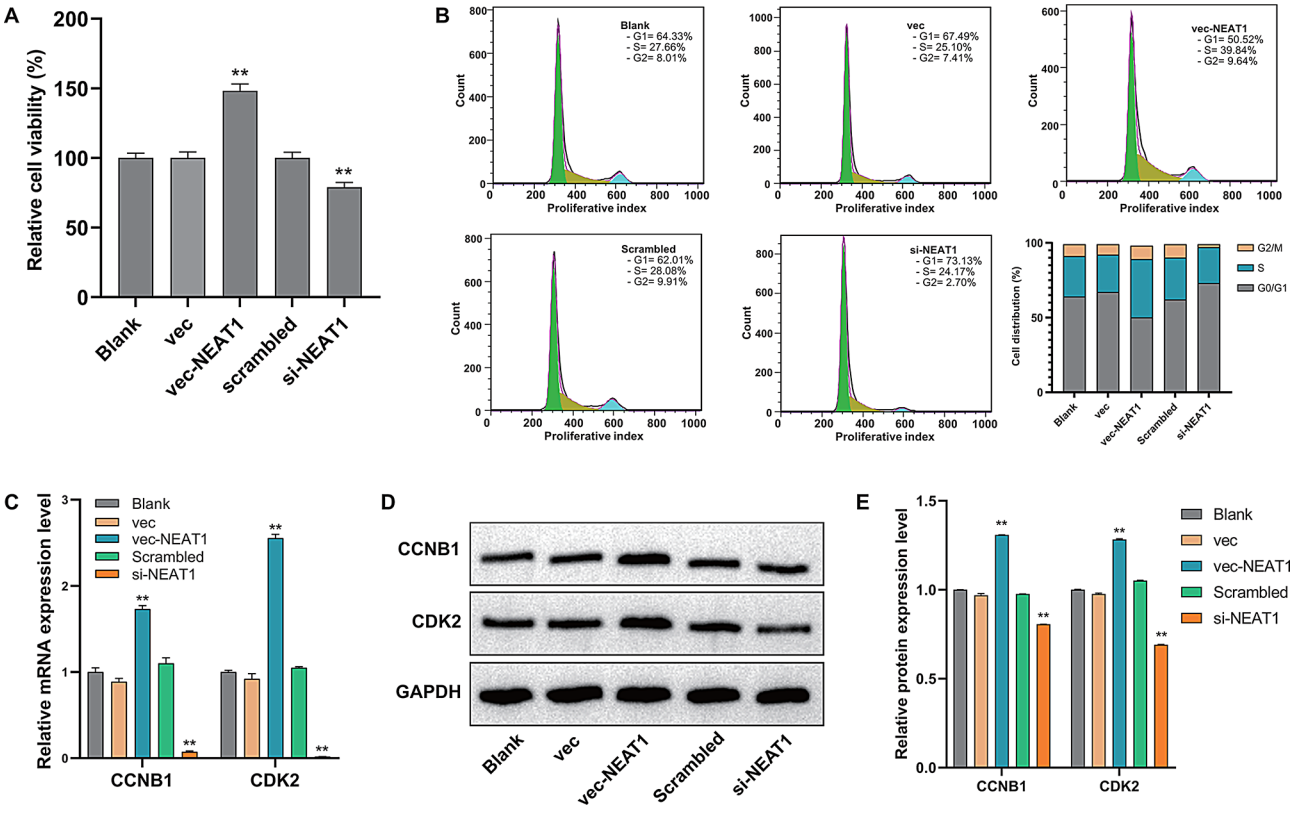

Fig. 1 NEAT1 promotes KGN cell proliferation and regulates cell cycle progression. **A** CCK-8 results showing the effects of NEAT1 overexpression and silencing on KGN cell proliferation; **B** Flow cytometry plots showing cell cycle distribution in KGN cells with NEAT1 overexpression, silencing, and their respective controls, with the quantification of cell cycle phase distribution (G0/G1, S, G2/M); **C** qRT-PCR analysis of CCNB1 and CDK2 mRNA expression levels in KGN cells with NEAT1 overexpression, silencing, and their controls; **D** Western blot analysis of CCNB1 and CDK2 protein levels in KGN cells with NEAT1 overexpression, silencing, and their controls; **E** Quantification of CCNB1 and CDK2 protein levels from Western blot analysis. Data is presented as mean ± SD. $p < 0.05$ and $^{**}p < 0.01$ compared to control groups

Cell cycle distribution was analyzed by flow cytometry to explore the mechanism of NEAT1 on cell proliferation. NEAT1 overexpressing increased the proportion of cells in S, G2/M phases with a corresponding decrease the proportion in G0/G1 phases. In contrast, NEAT1 silencing resulted in G0/G1 phase arrest and decreased proportion of cells in S and G2/M phases (Fig. 1B). Furthermore, qPCR and Western blot analyses showed that NEAT1 overexpression increased the expression of CCNB1 and CDK2, while NEAT1 silencing decreased expression of these proteins (Fig. 1C-E).

NEAT1 directly interacts with miR-29a-3p

Bioinformatics analysis using StarBase revealed that NEAT1 caused miR-29a-3p to bind to the sequence, elucidating the mechanism responsible for its effects (Fig. 2A). Dual-luciferase reporter assay was conducted to validate the interactions. The results demonstrated that miR-29a-3p mimic significantly reduced the luciferase activity of the wild-type NEAT1 reporter construct, but without effect on the mutant NEAT1 reporter (Fig. 2B).

NEAT1 regulates KGN cell proliferation through miR-29a-3p

qPCR analysis revealed that NEAT1 overexpression decreased miR-29a-3p expression, while miR-29a-3p levels were increased when NEAT1 was silenced (Fig. 3A). Examination of NEAT1 and miR-29a-3p effects on cell cycle-related proteins showed that NEAT1 overexpression increased CCNB1 and CDK2 protein levels, while miR-29a-3p mimic reversed this effect. Conversely, NEAT1 silencing decreased CCNB1 and CDK2 expression, which was partially rescued by miR-29a-3p inhibitor (Fig. 3B-D).

Cell proliferation and cell cycle analyses were performed to confirm NEAT1's functional relationship with miR-29a-3p. CCK-8 assays demonstrated that miR-29a-3p mimic attenuated the pro-proliferative effect of NEAT1 overexpression, while miR-29a-3p inhibitor partially rescued the anti-proliferative effect of NEAT1 silencing (Fig. 3E). Flow cytometry analysis revealed that miR-29a-3p mimic counteracted the cell cycle-promoting effects of NEAT1 overexpression, while miR-29a-3p inhibitor partially reversed the cell cycle arrest caused by NEAT1 silencing (Fig. 3F).

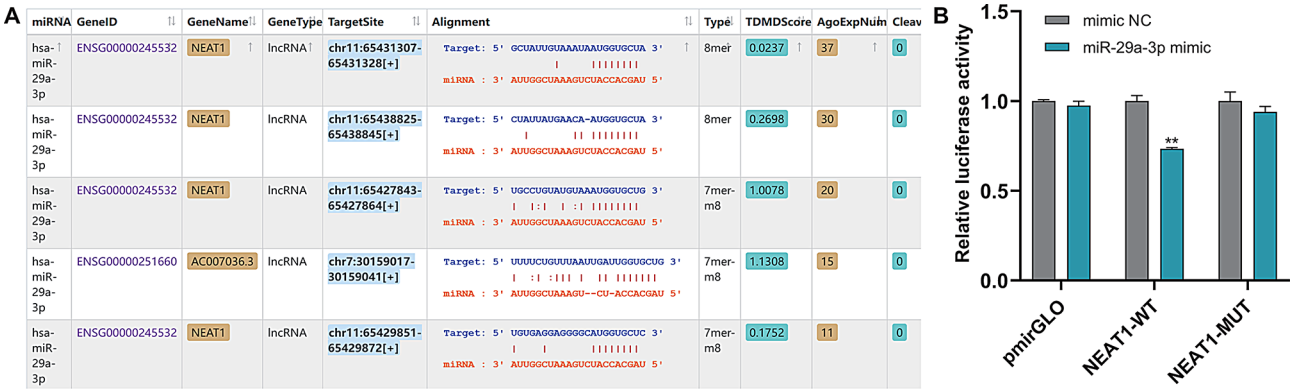

Fig. 2 NEAT1 directly interacts with miR-29a-3p. **A** Bioinformatics prediction (StarBase) of the potential binding site between NEAT1 and miR-29a-3p; **B** Dual-luciferase reporter assay results showing the interaction between NEAT1 and miR-29a-3p. Wild-type (WT) or mutant (MUT) NEAT1 constructs were co-transfected with miR-29a-3p mimic or negative control (NC) into KGN cells. Relative luciferase activity was measured 48 h post-transfection. Data are presented as mean ± SD from three independent experiments. $p < 0.05$ and $^{**}p < 0.01$ compared to control groups

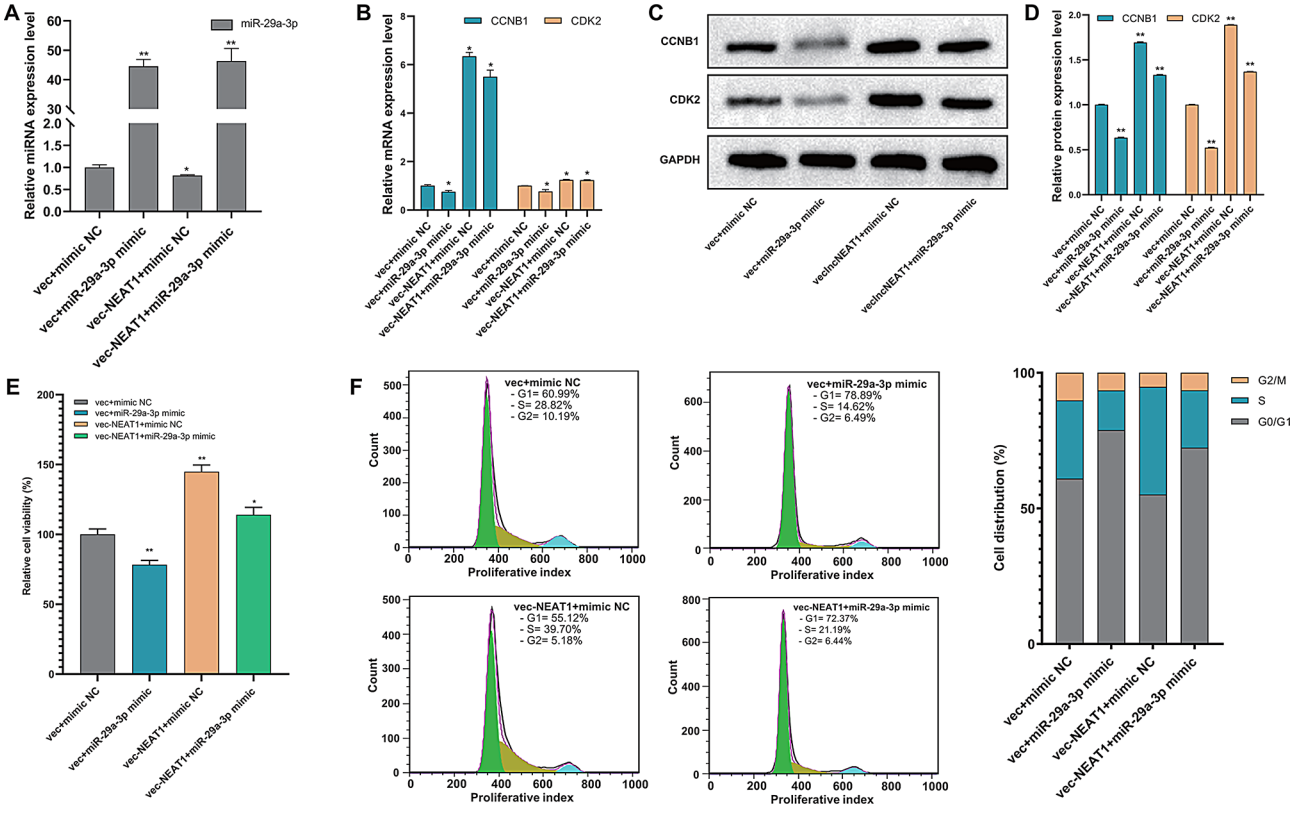

Fig. 3 NEAT1 regulates KGN cell proliferation through miR-29a-3p. **A** qRT-PCR analysis of miR-29a-3p expression levels in KGN cells transfected with miR-29a-3p mimic, NEAT1 + mimic NC, and NEAT1 + miR-29a-3p mimic; **B** qRT-PCR analysis of CCNB1 and CDK2 mRNA levels in KGN cells transfected with different constructs; **C** Western blot analysis showing the effects of miR-29a-3p and NEAT1 on CCNB1 and CDK2 protein expression; **D** Quantification of CCNB1 and CDK2 protein levels from Western blot analysis; **E** CCK-8 results showing the effects of miR-29a-3p and NEAT1 on KGN cell proliferation; **F** Flow cytometry plots showing cell cycle distribution in KGN cells under different treatment conditions, with quantification of cell cycle phase distribution (G0/G1, S, G2/M) shown in the bar graph. Data is presented as mean ± SD. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to control groups

miR-29a-3p targets IGF1 to regulate KGN cell proliferation

Bioinformatics analysis using TargetScan and miRWalk predicted IGF1 as a potential target of miR-29a-3p, with binding sites in its 3'UTR region (Fig. 4A). A dual-luciferase reporter assay was conducted to validate this prediction, and it was found that miR-29a-3p mimic reduced the luciferase activity of wild-type as well as mutant IGF1 3'UTR reporters (Fig. 4B).

The functional significance of the miR-29a-3p/IGF1 axis in KGN cells was investigated through qPCR and

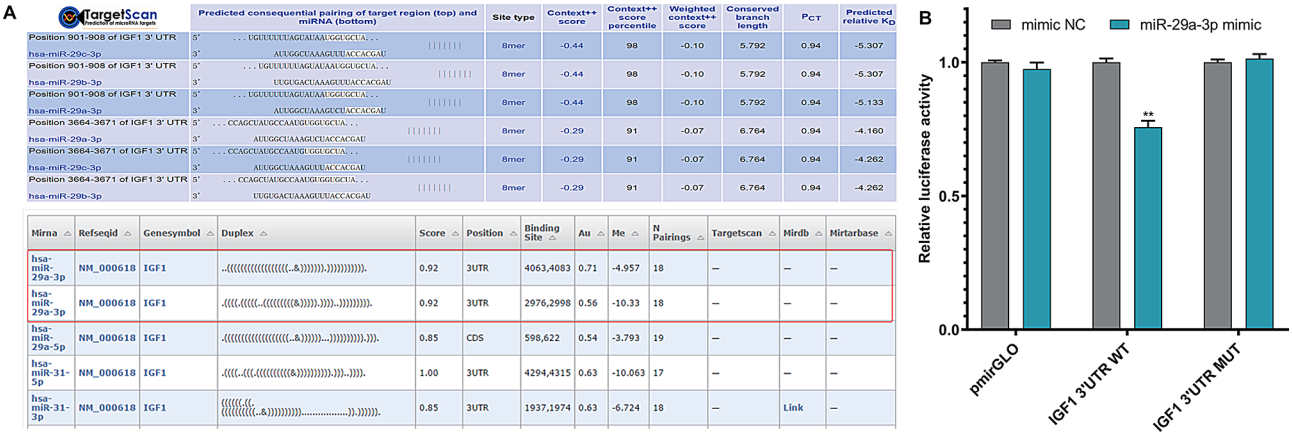

Fig. 4 miR-29a-3p targets IGF1 to regulate KGN cell proliferation. **A** Bioinformatics prediction (TargetScan for upper one and miRWalk for lower one) of the potential binding sites between miR-29a-3p and IGF1 3'UTR; **B** Dual-luciferase reporter assay results confirming the interaction between miR-29a-3p and IGF1. Wild-type (WT) or mutant (MUT) IGF1 3'UTR constructs were co-transfected with miR-29a-3p mimic or negative control (NC) into KGN cells. Relative luciferase activity was measured 48 h post-transfection. Data is presented as mean ± SD. $p < 0.05$ and $^{**}p < 0.01$ compared to control groups

Western blot analyses, which revealed that miR-29a-3p mimic decreased the expression of IGF1, CCNB1, and CDK2 (Fig. 5A-C). As shown using CCK-8 and flow cytometry assays, miR-29a-3p mimics inhibited cell proliferation and induced G0/G1 phase arrest in KGN cells, while overexpressing IGF1 reversed these effects (Fig. 5D and E).

NEAT1 regulates IGF1 expression through miR-29a-3p

In order to determine the complete regulatory axis of NEAT1/miR-29a-3p/IGF1, we examined the effects of NEAT1 on IGF1 expression. qPCR and Western blot analyses demonstrated that NEAT1 overexpression increased IGF1 expression, while IGF1 levels decreased when NEAT1 was silenced (Fig. 6). Furthermore, these effects were partially reversed by miR-29a-3p mimic and inhibitor, respectively. To summarize these findings, we propose a regulatory model in which NEAT1 modulates KGN cell proliferation through the miR-29a-3p/IGF1 axis (Fig. 7).

Discussion

Our study reveals a previously uncharacterized regulatory network in granulosa cells, demonstrating that NEAT1 functions as a competing endogenous RNA by sequestering miR-29a-3p to modulate IGF1 expression. While these molecular interactions have been observed in other contexts, their specific role in granulosa cells is particularly significant given their specialized functions in follicular development and ovarian function. NEAT1 relieves the inhibitory effect of miR-29a-3p on IGF1, thereby promoting cell proliferation and cell cycle progression by this mechanism.

The investigation of NEAT1 in granulosa cells was based on its established roles in cell proliferation and endocrine regulation. Previous studies have demonstrated NEAT1's involvement in steroid hormone signaling and cell cycle control in various tissues, making it a promising candidate for studying granulosa cell function. Our results showing that NEAT1 promotes granulosa cell proliferation and cell cycle progression are consistent with previous studies in other cell types and disease models. For instance, Yuan et al. reported that NEAT1 promotes proliferation and invasion in endometrial cancer [13]. Similarly, Qi et al. found that NEAT1 regulates cell proliferation and apoptosis in non-small cell lung cancer [14]. These findings extend to ovarian granulosa cells, suggesting a conserved function of NEAT1. Despite its conservation of function, NEAT1 continues to play an important role in cellular homeostasis and is potentially a therapeutic target as a result [15].

The selection of CCNB1 and CDK2 as downstream markers was based on their established roles in cell cycle regulation, with CCNB1 controlling G2/M transition and CDK2 regulating G1/S progression. The coordinated changes in these cell cycle regulators following NEAT1 manipulation suggest that the NEAT1/miR-29a-3p/IGF1 axis may influence granulosa cell proliferation through modulating multiple cell cycle progression [6, 9, 10]. The interaction between NEAT1 and miR-29a-3p, as well as the targeting of IGF1 by miR-29a-3p, have been previously reported in other biological contexts [10, 11]. This was also revealed by our luciferase reporter assays added to the growing body of evidence supporting the ceRNA hypothesis. This mechanism, first proposed by Salmena et al. [16], suggests that lncRNAs can regulate miRNA function by acting as molecular sponges. Our findings are in line with recent studies showing similar interactions between NEAT1 and other miRNAs. For example, Studies demonstrated that NEAT1 sponges miR-214 to promote proliferation and invasion in ovarian cancer [17] and multiple myeloma [18]. The consistency of these

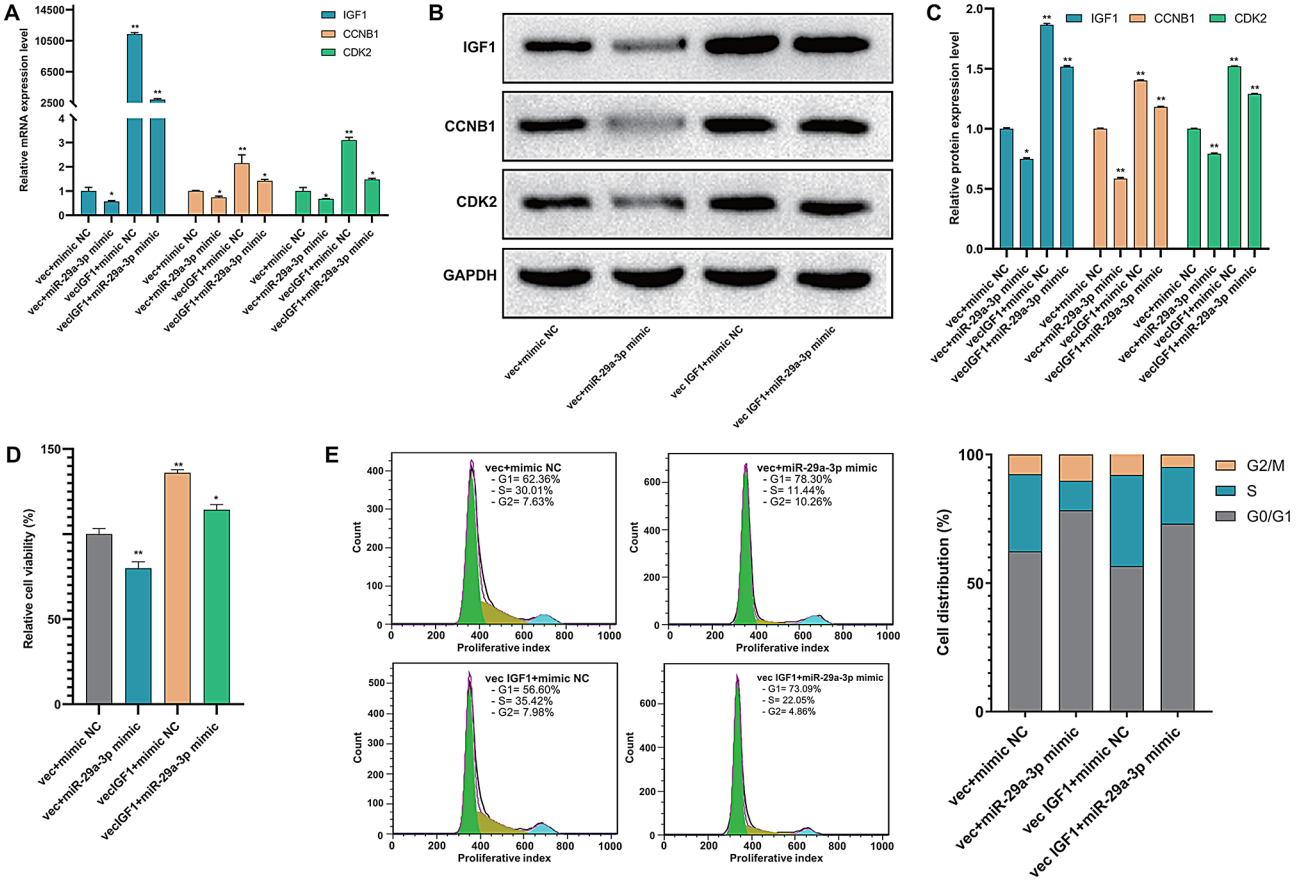

Fig. 5 miR-29a-3p regulates KGN cell proliferation through IGF1. **A** qRT-PCR analysis of IGF1, CCNB1, and CDK2 mRNA expression levels in KGN cells transfected with miR-29a-3p mimic, IGF1 + mimic NC, or IGF1 + miR-29a-3p mimic; **B** Western blot analysis of IGF1, CCNB1 and CDK2 protein levels in KGN cells transfected with miR-29a-3p mimic, IGF1 + mimic NC, or IGF1 + miR-29a-3p mimic; **C** Quantification of IGF1, CCNB1, and CDK2 protein levels from Western blot analysis; **D** CCK-8 results showing the effects of miR-29a-3p and IGF1 on KGN cell proliferation; **E** Flow cytometry plots showing cell cycle distribution in KGN cells under different treatment conditions (miR-29a-3p mimic with or without IGF1 overexpression), with quantification of cell cycle phase distribution (G0/G1, S, G2/M) shown in the bar graph. Data is presented as mean ± SD. *$p < 0.05$ and **$p < 0.01$ compared to control groups

findings across different cellular contexts suggests that the ceRNA function of NEAT1 may be a fundamental mechanism of gene regulation.

A mechanistic link between non-coding RNA regulation and a key growth factor implicated in granulosa cell has been established in our study by identifying IGF1 as a direct target of miR-29a-3p. Francoeur et al. [19] shown that IGF1 plays an important role in follicular development, steroidogenesis, and the function of granulosa cells. According to our findings, granulosa cell may manifest a dysregulation of IGF1 signaling caused by the NEAT1/miR-29a-3p axis. This is particularly significant given that previous studies have reported altered IGF1 levels in ovarian function disorders [20–22]. The ability of NEAT1 to indirectly regulate IGF1 through miR-29a-3p adds a new dimension to our understanding of growth factor regulation in the ovary.

Molecular understanding of granulosa cell may be influenced by the regulatory network we have uncovered. The NEAT1/miR-29a-3p/IGF1 axis may contribute to follicular arrest and anovulation by increasing granulosa cell proliferation. The regulatory network we have uncovered provides novel insights into how non-coding RNAs integrate with known regulatory pathways in granulosa cells. The NEAT1/miR-29a-3p/IGF1 axis specifically controls proliferation through modulation of CCNB1 and CDK2, which is crucial for normal follicular development. This is consistent with the work of Franks et al. [23], who proposed that aberrant follicular development in polycystic ovary syndrome is due to dysregulated granulosa cell proliferation and differentiation. Moreover, the NEAT1/miR-29a-3p/IGF1 axis may contribute to follicular arrest and anovulation by increasing granulosa cell proliferation. Diamanti-Kandarakis and Dunaif also highlighted the importance of insulin/IGF signaling in androgen excess [24]. Our findings suggest that similar approaches targeting NEAT1 or miR-29a-3p could normalize granulosa cell function and IGF1 signaling. This is particularly relevant given the increasing interest in RNA-based therapeutics, as exemplified

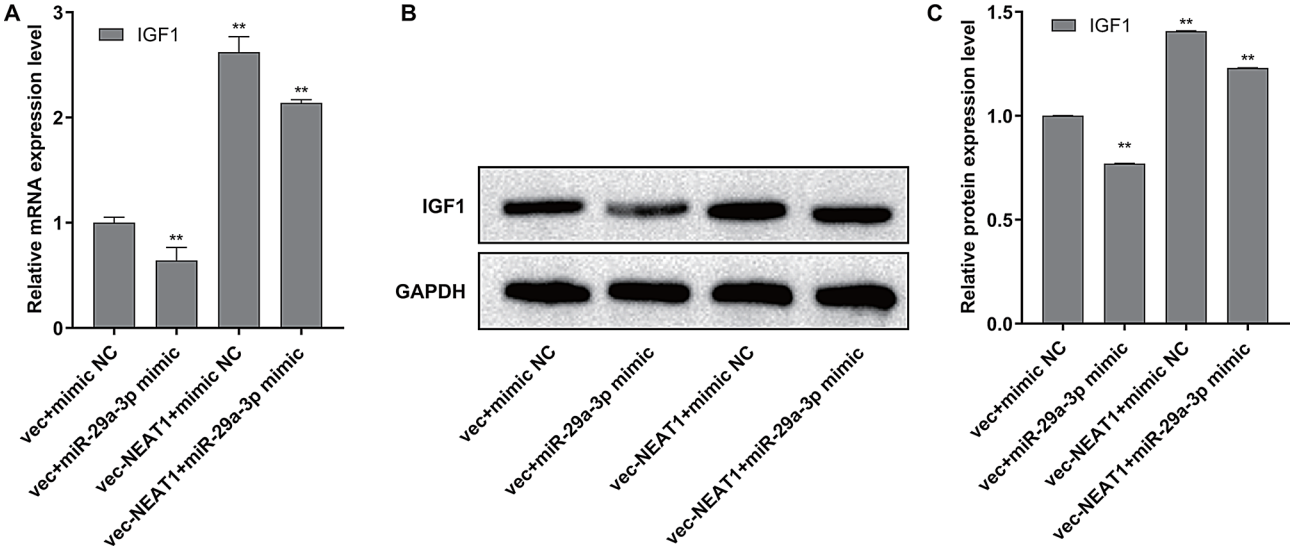

Fig. 6 NEAT1 regulates IGF1 expression through miR29a-3p. **A** qRT-PCR analysis of IGF1 mRNA expression levels in KGN cells with NEAT1 overexpression or silencing, combined with miR-29a-3p mimic or inhibitor; **B** Western blot analysis of IGF1 protein levels in KGN cells with NEAT1 overexpression or silencing, combined with miR-29a-3p mimic or inhibitor; **C** Quantification of IGF1 protein levels from Western blot analysis. Data is presented as mean ± SD. $p < 0.05$ and $^{**}p < 0.01$ compared to control groups

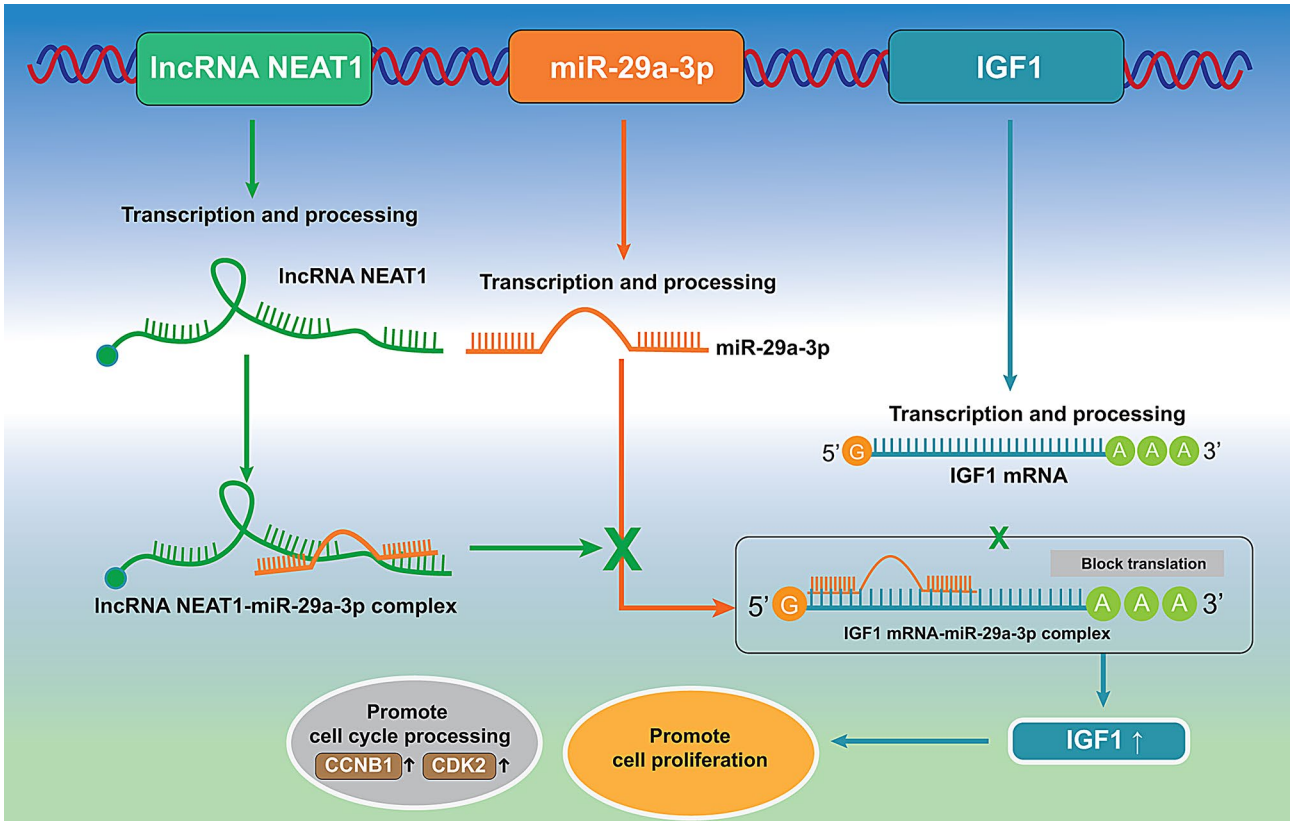

Fig. 7 Regulatory model of the NEAT1/miR-29a-3p/IGF1 axis in KGN cells. NEAT1 functions as a ceRNA by sponging miR-29a-3p, thereby preventing miR-29a-3p-mediated suppression of IGF1 mRNA translation. This regulation promotes IGF1 expression, which in turn enhances cell proliferation and cell cycle progression by upregulating cell cycle-related proteins CCNB1 and CDK2

by recent successes in other fields of medicine [25, 26]. For instance, the approval of patisiran for the treatment of hereditary transthyretin-mediated amyloidosis demonstrates the clinical potential of RNA interference therapies [27, 28]. This study also raises issues about the broader network of molecular interactions based on the interaction between NEAT1, miR-29a-3p, and IGF1. It would be interesting to explore whether other miRNAs known to be dysregulated in ovarian function disorders also interact with NEAT1 or other lncRNAs. While the NEAT1/miR-29a-3p/IGF1 axis appears to intersect with broader regulatory networks, our findings establish a direct mechanistic link in granulosa cells, highlighting a specific pathway that may serve as a therapeutic target. This study lays the groundwork for investigating how these interactions integrate into complex ovarian regulatory systems.

However, some limitations exist in our study. Although we have demonstrated an axis between NEAT1/miR-29a-3p/IGF1 in granulosa cells, our findings are primarily based on the KGN cell line, which, while widely used as a granulosa cell model, may not fully recapitulate all aspects of primary granulosa cell function. The use of primary granulosa cells would provide additional validation of our findings and potentially reveal cell type-specific variations in this regulatory network. In addition, this regulatory network likely interacts with other important pathways in granulosa cells. For instance, the NEAT1/miR-29a-3p/IGF1 axis may crosstalk with steroid hormone signaling pathways, given that both NEAT1 and IGF1 are known to be hormone-responsive, it is plausible that the NEAT1/miR-29a-3p/IGF1 axis interacts with steroid hormone signaling pathways to influence granulosa cell function. Future studies could explore whether hormonal regulators, such as FSH and LH, modulate this axis to coordinate follicular development and ovulation. Recent work by Vaasjo et al. [29] has shown that lncRNA expression can be regulated by DNA methylation and histone modifications, suggesting that investigation of epigenetic mechanisms in NEAT1 regulation could provide additional insights. To further delineate the mechanistic role of the NEAT1/miR-29a-3p/IGF1 axis, future studies incorporating pathway-specific inhibitors could clarify the causality of these interactions and identify potential nodes for therapeutic intervention. Furthermore, in vivo studies would be valuable to understand the physiological significance of this regulatory axis in follicular development and ovarian function. The potential involvement of additional regulatory mechanisms, including other miRNAs and downstream targets, also deserves exploration in future studies.

In conclusion, our study reveals a novel regulatory mechanism in granulosa cells, where the NEAT1/miR-29a-3p/IGF1 axis controls cell proliferation through specific effects on cell cycle progression. Future validation in primary cells and in vivo models will further establish the physiological significance of this pathway in ovarian function.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-025-01588-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Author contributions

Conceptualization, LN. H. and W. C.; methodology, W. C. and ZW. Q.; software, W. C.; validation, Q. X. and YH. F.; formal analysis, LN. H. and W. C.; investigation, YH. F., Q. X. and L. H.; resources, J. L. and X. R.; data curation, X. R.; writing- original draft preparation, LN. H.; writing- review and editing, W. C.; visualization, LN. H.; supervision, W. C., ZW. Q. and J. L.; project administration, LN. H, and W. C.; funding acquisition, LN. H, and W. C. All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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