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LncRNA-LET inhibits cell viability, migration and EMT while induces apoptosis by up-regulation of TIMP2 in human granulosa-like tumor cell line KGN

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disease characterized by hyperandrogenism, irregular menses, and polycystic ovaries. Several long non-coding RNAs (lncRNAs) are aberrantly expressed in PCOS patients; however, little is known about the effects of the lncRNA-low expression in tumor (lncRNA-LET) on PCOS. We aimed to explore the effects of lncRNA-LET on human granulosa-like tumor cell line, KGN.

Methods: Expression of lncRNA-LET in normal IOSE80 cells and granulosa cells was determined by qRT-PCR. KGN cell viability, apoptosis and migration were measured by trypan blue exclusion method, flow cytometry assay and wound healing assay, respectively. TGF-β1 was used to induce epithelial-mesenchymal transition (EMT) process. LncRNA-LET expression and mRNA expressions of TIMP2 and EMT-related proteins were measured by qRT-PCR. Western blot analysis was used to measure the protein expression of apoptosis-related proteins, EMT-related proteins, TIMP2, and the proteins in the Wnt/β-catenin and Notch signaling pathways.

Results: lncRNA-LET was down-regulated in KGN cells, and its overexpression inhibited cell viability and migration, and promoted apoptosis in KGN cells. Overexpression of lncRNA-LET increased the expression of E-cadherin and decreased the expressions of N-cadherin and vimentin in KGN cells. These effects of lncRNA-LET on KGN cells were reversed by TIMP2 suppression. Overexpression of TIMP2 inhibited cell viability, migration and EMT process, and increased apoptosis by activating the Wnt/β-catenin and Notch pathways.

Conclusion: Overexpression of lncRNA-LET inhibits cell viability, migration and EMT process, and increases apoptosis in KGN cells by up-regulating the expression of TIMP2 and activating the Wnt/β-catenin and notch signaling pathways.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive and endocrine disorder characterized by hyperandrogenism, irregular menses, and polycystic ovaries. The prevalence of PCOS ranges from 6% to 9% as per the National Institutes of Health/National Institute of Child Health and Human Disease criteria. Clinical features include oligomenorrhea, amenorrhea or prolonged erratic menstrual bleeding, hirsutism, and infertility. Type 1, type 2, and gestational diabetes have been identified as risk factors for PCOS. Women with PCOS are more likely to have insulin resistance, obesity, and mental health disorders including depression and anxiety. Treatment options for PCOS include oral contraceptives for menstrual irregularities and hirsutism; spironolactone and finasteride for androgen excess; and clomiphene, laparoscopic ovarian drilling, gonadotropins and assisted reproductive technology for infertility [1]. As a primary step, understanding the mechanism of PCOS is crucial to find effective diagnostic and therapeutic targets.

Although the etiology of PCOS remains unclear, several studies have suggested the multifactorial nature of this syndrome. Of these, genetic factors tend to play a key role in the development and maintenance of PCOS [2,3]. Salilew-Wondim et al. reported the up-regulation of 573 genes and down-regulation of 430 genes in the ovaries of PCOS patients. Most of the down-regulated genes were associated with the biosynthesis and metabolism of steroids, cholesterol and lipids, whereas the up-regulated genes were associated with cell proliferation,

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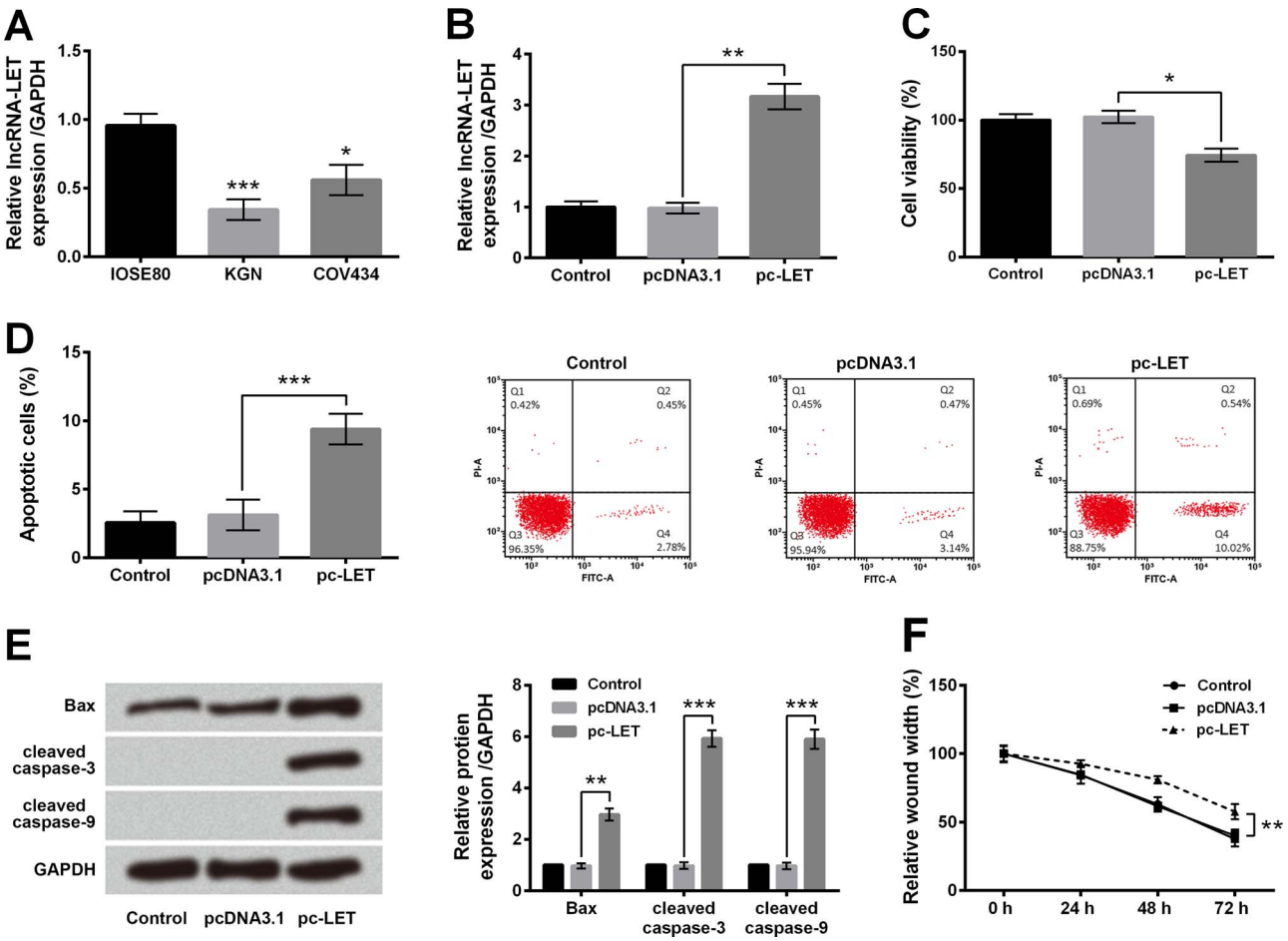

Fig. 1. Overexpression of lncRNA-LET inhibits KGN cell viability and migration, and promotes apoptosis.
(A) The relative expression of lncRNA-LET in normal ovarian surface epithelial cells (IOSE80 cells) and granulosa cells (KGN and COV434 cells) was measured by qRT-PCR. KGN cells were transfected with either pcDNA3.1 or pc-LET, and untransfected KGN cells were served as control. (B) The relative expression of lncRNA-LET was measured using qRT-PCR. (C) Cell viability was measured using trypan blue exclusion method. (D) Cell apoptosis was measured using flow cytometry assay. (E) Expression of apoptosis-related proteins was measured by Western blot analysis. (F) Cell migration was measured using wound healing assay, and relative wound width in the wounded cells were measured at 0, 24, 48, and 72 h after the wound injury. Data are presented as the mean ± SD (n = 3). *P < .05, **P < .01, ***P < .001. GAPDH: glyceraldehyde 3-phosphate dehydrogenase; qRT-PCR: quantitative reverse transcription polymerase chain reaction.

differentiation, adhesion, and blood vessel development [4]. In another study, 770 genes were found to be relevant to follicular development, cell survival and apoptosis [5].

Long non-coding RNAs (lncRNAs) are a large and diverse class of non-protein-coding transcripts that are longer than 200 nucleotides [6]. LncRNAs regulate various biological processes, including cell invasion and metastasis, apoptosis, DNA damage, angiogenesis, microRNA silencing, tumor development, embryonic development, inflammation, and immune cell development [7–10]. LncRNAs are also involved in several regulatory mechanisms, such as cell cycle control, gene imprinting, mRNA degradation, splicing regulation, chromatin remodeling, translocational regulation, and epigenetic regulation [6]. It has been reported that several lncRNAs are aberrantly expressed in cumulus cells of PCOS patients, which may contribute to the occurrence of PCOS and in turn affects oocyte development [11].

The lncRNA-low expression in tumor (lncRNA-LET) is reported to be under-expressed in hepatocellular carcinoma, colorectal cancer, and squamous-cell lung carcinoma tissues [12]. However, little is known about the functions of lncRNA-LET in PCOS. A previous study has reported that lncRNA-LET could affect accumulation and stability of hypoxia-inducible factor 1 alpha subunit (HIF-1α) [12]. Another study also proved that the HIF-1α/endothelin (ET)-2 signaling pathway was inhibited in the ovaries of PCOS rats [13]. Therefore, we hypothesized that there might be a correlation between lncRNA-LET and PCOS. In the present study, we explored the effects of lncRNA-LET on human granulosa-like tumor cell line, KGN, which possesses the physiological characteristics of ovarian cells and is the main cell type producing androgen in the ovaries.

2. Materials and methods

2.1. Cell culture and treatment

Human immortalized ovarian surface epithelial cell line IOSE80 and human granulosa cell line COV434 were obtained from Cell Bank of the Chinese Academy of Science (Shanghai, China) and Sigma-Aldrich (St. Louis, MO, USA), respectively. Human granulosa-like tumor cell line KGN was obtained from Suer (Shanghai, China). IOSE80 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Carlsbad, CA, USA), and COV434 and KGN cells were cultured in DMEM/F-12 medium (Gibco), in a humidified atmosphere with 5% CO_2 at 37 °C. Culture medium for all cells was supplemented with 10% fetal bovine serum (Gibco), 100 U/mL penicillin G, and 0.1 mg/mL streptomycin sulfate (Invitrogen, Carlsbad, CA, USA). Transforming growth factorbeta1 (TGF-β1; 10 ng/mL) was used to induce epithelial-mesenchymal transition (EMT) process in the cells.