European Review for Medical and Pharmacological Sciences 2019; 23: 7217-7223

MicroRNA-486-5p inhibits ovarian granulosa cell proliferation and participates in the development of PCOS *via* targeting MST4

X.-M. HAN[1], P.-Y. TIAN[2], J.-L. ZHANG[3]

[1]Reproductive Health Division, Zoucheng Maternal and Child Health and Family Planning Service Center, Zoucheng, China
[2]Department of Out-Patient, The People's Hospital of Zoucheng, Zoucheng, China
[3]Department of Gynecology, The People's Hospital of Zoucheng, Zoucheng, China

Abstract. – OBJECTIVE: To explore whether microRNA-486-5p affected the proliferation of ovarian granulosa cells by targeting MST4 (silk/threonine protein kinase 4), thereby promoting the development of polycystic ovary syndrome (PCOS).

MATERIALS AND METHODS: The level of microRNA-486-5p in PCOS tissues and adjacent normal tissues was detected by quantitative real-time polymerase chain reaction (qRT-PCR). After microRNA-486-5p up-regulation in KNG cells, the mRNA and protein level of related genes was examined using qRT-PCR and western blot assay, respectively. Meanwhile, cell proliferation and cell cycle were analyzed by cell counting kit-8 (CCK-8) assay and flow cytometry. After insulin treatment of KNG cells, expressions of microRNA-486-5p and MST4, cell proliferation as well as cell cycle, were detected by qRT-PCR, CCK-8 and flow cytometry, respectively. Furthermore, cell proliferation and cycle situation were examined after simultaneous up-regulation of MST4 and microRNA-486-5p *in vitro*.

RESULTS: MicroRNA-486-5p expression in PCOS tissues was significantly lower than that of normal tissues. In KNG cells, up-regulation of microRNA-486-5p significantly inhibited cell proliferation and cell cycle. The levels of cycle-associated proteins including CDK2 and CCNB1 decreased significantly. The results of dual-luciferase reporter gene assay showed that microRNA-486-5p could bind to MST4. After up-regulating microRNA-486-5p, both the mRNA and protein levels of MST4 decreased remarkably. MST4 expression was found significantly elevated in PCOS tissues as well. After overexpression of MST4, cell proliferation was enhanced, cell cycle was promoted, and expressions of cycle-related proteins increased. After treatment with different concentrations of insulin in KNG cells, the expression level of microRNA-486-5p decreased in a concentration-dependent manner. However, opposite results were observed in MST4 level. Meanwhile, the proliferation ability and cell cycle of insulin-treated cells were significantly enhanced. In addition, the inhibitory effect of microRNA-486-5p on cell proliferation and cell cycle could be partially reversed by simultaneous up-regulation of MST4 and microRNA-486-5p.

CONCLUSIONS: MicroRNA-486-5p can bind to MST4 in a targeted manner and inhibit the proliferation of ovarian granulosa cells, thereby inhibiting the development of PCOS.

Key Words:
Polycystic ovary syndrome, Ovarian granulosa cells, MicroRNA-486-5p, MST4.

Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease, the prevalence of which in women of childbearing age is 4% to 18%[1]. PCOS is characterized by hyperandrogenemia, insulin resistance, rare ovulation or ovulation without ovulation, and polycystic changes of the ovary. At the same time, it is accompanied by clinical symptoms of hypertrichosis, acne and other androgen[2]. Previous studies have shown that PCOS is related to infertility. Moreover, it can also increase the risk of metabolic disorders, such as diabetes mellitus, obesity, dyslipidemia, and cardiovascular disease[3]. Therefore, it seriously affects women's physical and mental health. PCOS is a primary follicular disease. The proliferation and apoptosis of granulosa cells are the underlying causes of follicular atresia and development[4]. Therefore, the research on the proliferation mechanism of ovarian granulosa cells is of great significance for the clinical diagnosis and treatment of PCOS.

Corresponding Author: Jianling Zhang, MM; e-mail: yszjl0156@tom.com

MicroRNA is a kind of non-coding small RNA, which is mainly involved in post-transcriptional regulation in the organism. It combines with the 3'-UTR of target mRNAs and negatively regulates gene expression at post-transcriptional level. This may eventually lead to the degradation or translation inhibition of related genes[5]. Studies have shown that multiple microRNAs are involved in the regulation of ovarian granulosa cell proliferation and apoptosis[6,7]. They also play critical regulatory roles in ovarian reproductive and endocrine functions. Abnormal expression or dysfunction of ovarian microRNA may affect follicular development and atresia, as well as, hormone synthesis and metabolism. Ultimately, this leads to the occurrence of ovarian diseases such as PCOS[8]. MicroRNA-486-5p is a newly discovered microRNA. Navon et al[9] have confirmed the down-regulated expression of microRNA-486-5p in pancreatic cancer, colon cancer, liver cancer, lung cancer, lymphoma, ovarian cancer, prostate cancer, and testicular cancer using microarray method. It has been reported[10] that microRNA-486-5p is down-regulated in the middle phase of PCOS. The above findings provide a new idea for the clinical diagnosis and treatment of PCOS. Furthermore, microRNA-486-5p is expected to be a new molecular target for PCOS treatment.

MST4 (silk/threonine protein kinase 4) is located at Xq26.2. The gene product is composed of 416 amino acids with a molecular weight of 46 529Da. The protein kinase is mainly located in the Golgi, which is specifically activated by linking to the Golgi matrix protein GM130[11]. As a member of the protein kinase family, MST4 is a key regulator of cell apoptosis, proliferation, and differentiation[12]. Studies[13,14] have demonstrated that MST4 is able to promote tumor cell proliferation. The expression level of MST4 has been found extremely high in colon and hepatoma cell lines. Yu et al[15] have found that microRNA-593-5p inhibits the development of gastric cancer through targeted regulation of MST4. According to relevant literature at home and abroad, few researches have investigated the correlation between PCOS and MST4 or microRNA-486-5p. Therefore, the aim of this study was to explore the effect of microRNA-486-5p and MST4 on ovarian granulosa cells, and to reveal the mechanism of microRNA-486-5p and MST4 in the occurrence and development of PCOS. Our findings might help to provide new ideas for the clinical diagnosis and target of PCOS.

Materials and Methods

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Detection

Total RNA in cells was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The relative expression levels of microRNA-486-5p and MST4 were detected using a miRNA PCR detection kit (Thermo Fisher Scientific, Waltham, MA, USA). Primer sequences used in this study were as follows: microRNA-486-5p Forward: 5'-TGGGATCCATGAGGAAGGGACATGAAGA-3'; Reverse 5'-ACCGAAGCTTAAAAAAGCTCGGTCCCAGAGTCAG-3'. MST4 Forward 5'-TCACATTTGAGACCCTGTGTTTG-3'; Reverse 5'-ACCTATCCTTGGGTAAATGATCTTG-3'. CDK2, Forward: 5'-CCAGGAGTTACTTCTATGCCTGA-3'; Reverse: 5'- TTCATCCAGGGGAGGTACAAC-3'. CCNB1, Forward; 5'-CAGGAGGCATTGCTGATGAT-3'; Reverse 5'-GAAGGCTGGGGCTCATTT-3'. Glyceraldehyde 3-phosphate dehydrogenase (GADPH), Forward: 5'-CCCAGCCTCAAGATCATCAGCAATG-3'; Reverse: 5'-ATGGACTGT GGTCATGAGTCCTT-3'.

Cell Culture

KNG cells were cultured in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12) (Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) in an incubator with 5% CO_2 at 37°C. At the time of passage, the cells were first digested with 0.25% trypsin (containing EDTA). After, the cell suspension was evenly distributed into a cell culture flask, with the whole medium supplemented to 6 mL per flask. Subsequently, cell culture was continued for subsequent experiments.

Cell Transfection

2×10^5 cells with good growth were first plated into 6-well plates. When the degree of cell fusion reached 80%, cell transfection was performed according to the instructions of Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). 6-8 h after transfection, culture medium was replaced. After 48 h, the medium was replaced with fresh medium containing 2 μg/mL puromycin, followed by cell culture for 72 h. The cells were then transferred to new 6-well plates. When cell clones appeared, monoclone was picked and transferred to another 6-well plate. After 2 days, the cells were transferred to a new flask to establish stable cell lines.

Cell Counting kit-8 (CCK-8) Assay

After transfection, cells in log phase were trypsinized and re-suspended in complete medium. 100 μL of cell suspension was added in culture plate at a density of 5×10^3 cells/per well. After culture for 0, 24, 48, 72, and 96 h, respectively, 100 μL of DMEM solution containing 10% CCK-8 reagent (Dojindo, Kumamoto, Japan) was added to each well, followed by incubation in an incubator for 2 h in dark. Lastly, absorbance at 450 nm was detected using a preheated microplate reader.

Flow Cytometry

Cells were first cultured in 0.5% Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) for 48 h. Then the medium was replaced with 10% RPMI-1640 medium for another 48 h. Subsequently, the cells were collected in 3 mL tubes with approximately 2.0×10^6 cells per tube. After washing twice with phosphate-buffered saline (PBS) solution, the cells were fixed with 70% alcohol and placed in a refrigerator at -20°C for 24-48 hours. After conventional PI staining, the DNA content of cells was measured by a Switzerland Paetec flow cytometer (Partec AG, Arlesheim, Switzerland). Finally, the percentage of each phase of cell cycle was analyzed by software.

Western Blot Assay

200 μL of protein lysate (containing 2 μL of protease inhibitor and 2 μL of phosphatase inhibitor) was first added to each well. The mixture was stirred well on ice until the cells were completely lysed. 5×loading buffer containing β-mercaptoethanol was added and mixed, boiled for 15 min, and stored at -20°C for use. Extracted protein samples were separated by 10% polyacrylamide gel and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% bovine serum albumin (BSA) for 2 h, the membranes were incubated with primary antibodies overnight. On the next day, the membranes were incubated with corresponding secondary antibody for 1 h. Finally, immunoreactive bands were exposed by enhanced chemiluminescence (ECL) method. The experiment was repeated for three times.

Luciferase Reporter Gene Assay

Bioinformatics prediction website was used to predict the binding fragment of microRNA-486-5p to MST4. Target genes of microRNA-486-5p were predicted using target gene websites, including microRNAbase.org, targetscan.org, and microrna.org. Meanwhile, target gene function was searched online as well. The amplified MST4 3'-UTR sequence was inserted into microRNA-Report Luciferase plasmid to construct the MST4 3'-UTR luciferase reporter vector MST4-WT (wild type) and the mutant vector MST4-MUT (mutant type). Luciferase reporter vector and mutant vector were co-transfected into cells with microRNA NC or microRNA-486-5p. Finally, luciferase activity was detected in strict accordance with Dual-Luciferase Reporter Kit (Promega, Madison, WI, USA).

Cellular Insulin Treatment

Cells in log phase were digested with 0.25% trypsin and seeded into 96-well plates at a density of 2×10^4 cells/well. 6 replicated wells were set in each group. After culture for 24 h, the cultured medium was replaced with 200 μL complete medium containing 1 ng / m L, 10 ng / m L, or 100 ng / m L insulin. A negative control group was set up at the same time.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for all statistical analysis. *t*-test was used to compare the differences between two groups. One-way analysis of variance (ANOVA) was applied to compare the differences among different groups, followed by Post-Hoc Test LSD (Least Significant Difference). $p<0.05$ was considered statistically significant: $*p<0.05$, $**p<0.01$, $***p<0.001$.

Results

MicroRNA-486-5p was Lowly Expressed in PCOS Tissues

QRT-PCR showed that the expression of microRNA-486-5p in PCOS tissues was significantly lower than that of the control group (Figure 1A.). Further analysis of the interaction between microRNA-486-5p and PCOS showed that the expression of microRNA-486-5p was significantly up-regulated in KNG cells as well. These results suggested good transfection efficiency (Figure 1B). After upregulation of microRNA-486-5p *in vitro*, cell proliferation was inhibited in a time-dependent manner (Figure 1C). The number of cells remaining in the

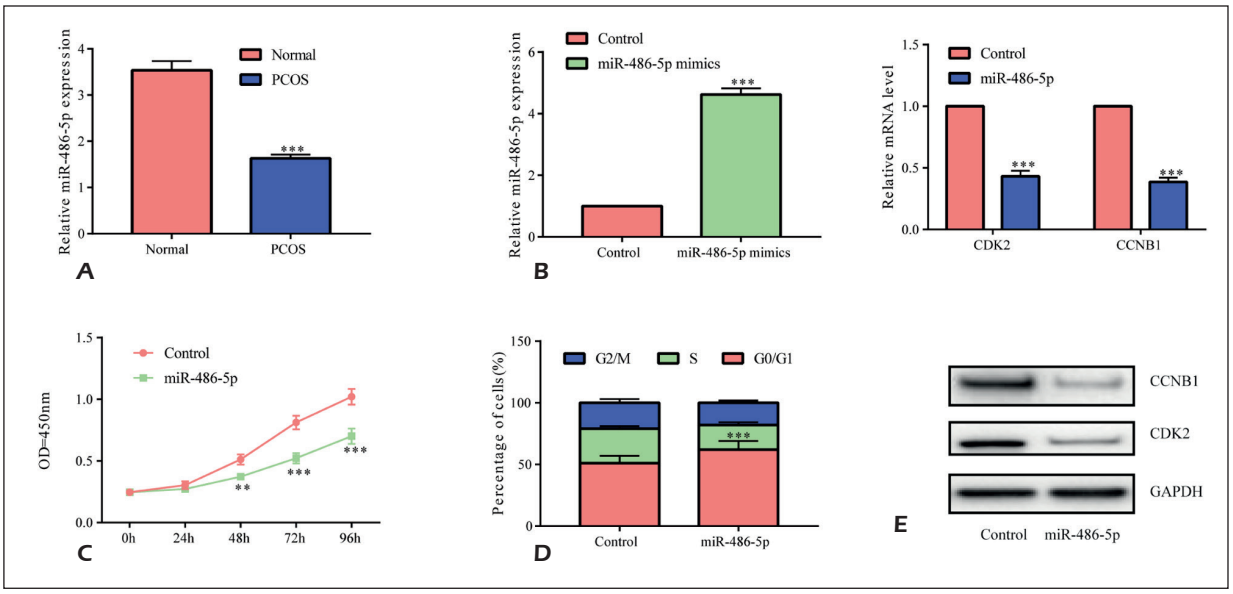

Figure 1. MicroRNA-486-5p was lowly expressed in PCOS. ***A,*** The expression of microRNA-486-5p in PCOS tissues was significantly lower than that of control group. ***B,*** Transfection efficiency of microRNA-486-5p mimics in KNG cells; ***C,*** In KNG cells, up-regulated microRNA-486-5p significantly inhibited cell proliferation; ***D,*** In KNG cells, up-regulated microRNA-486-5p significantly inhibited cell cycle; ***E,*** After upregulation of microRNA-486-5p, the expression of cyclin-related proteins CDK2 and CCNB1 decreased significantly.

G0/G1 phase increased, while the number in the S phase decreased (Figure 1D), indicating suppressed cell cycle. In addition, the expressions of cyclin-related proteins, such as CDK2 and CCNB1, were significantly down-expressed (Figure 1E). The above results revealed that microRNA-486-5p expression level was correlated with the occurrence of PCOS.

MST4 Was a Potential Target Gene of microRNA-486-5p

Bioinformatics analysis revealed the existence of binding sites between microRNA-486-5p and MST4 (Figure 2A). Luciferase reporter gene assay showed that wild-type MST4 could fluoresce quenched microRNA-486-5p. As shown in Figure 2B, microRNA-486-5p could bind to MST4

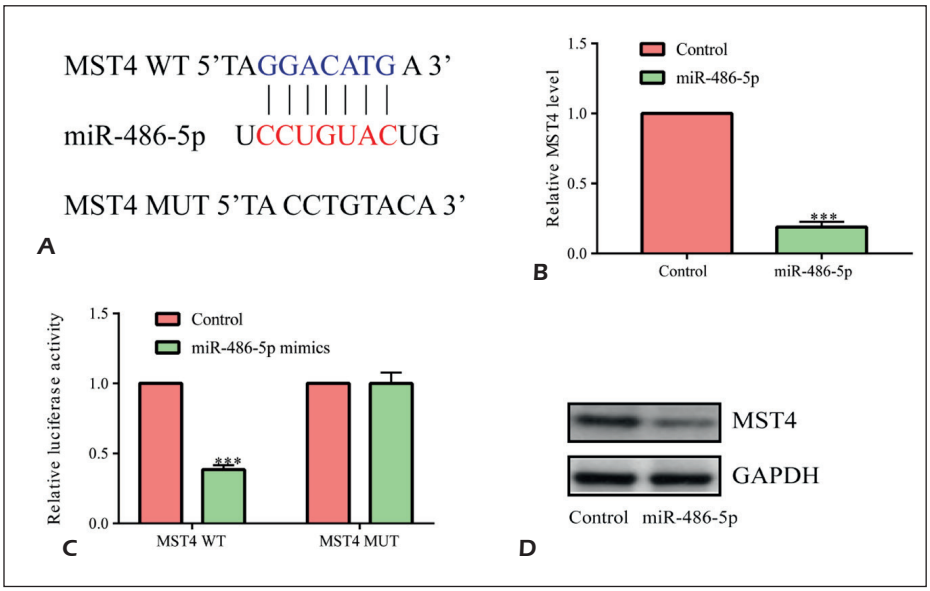

Figure 2. MST4 was the potential target gene of microRNA-486-5p. ***A,*** There was a potential binding site for miR-486-5p and MST4; ***B,*** Dual luciferase reporter gene showed a binding relationship between the two molecules; ***C-D,*** After up-regulation of miR-486-5p expression in KNG cells, the expression of MST4 was significantly reduced.

in a targeted manner. To further verify whether there was a regulatory effect between the two molecules, we overexpressed microRNA-486-5p in KNG cells. The expression of MST4 was found decreased significantly after overexpression of microRNA-486-5p (Figure 2C, 2D). The above results verified that the expression of MST4 was negatively regulated by microRNA-486-5p.

MST4 Promoted the Proliferation and Cell Cycle of KNG Cells

QRT-PCR and Western blot analysis indicated that both mRNA and protein levels of MST4 increased significantly in PCOS tissues (Figure 3A). To further clarify the role of MST4 in KNG cells, we transfected MST4 overexpression plasmid *in vitro*. Afterwards, MST4 was stably up-regulated (Figure 3B), indicating that successful transfection efficiency. After up-regulating MST4, cell proliferation ability was found significantly enhanced (Figure 3C). The number of cells in the G0/G1 phase decreased, while the number of S phase increased (Figure 3D). This indicated that cell cycle was accelerated. In addition, the levels of cycle-related proteins CDK2 and CCNB1 were also significantly elevated (Figure 3E). The above results suggested that MST4 was able to promote cell proliferation.

MicroRNA-486-5p Played its Role by Regulating MST4

In order to clarify the regulatory association between microRNA-486-5p and MST4 in PCOS development, we treated KNG cells with different concentrations of insulin. Results found that microRNA-486-5p level decreased in a concentration-dependent manner (Figure 4A). However, MST4 level showed the opposite trend (Figure 4B). Subsequent experiments showed that the proliferative ability of KNG cells treated with insulin was significantly enhanced (Figure 4C), and cell cycle was promoted (Figure 4D). Simultaneous up-regulation of MST4 and microRNA-486-5p in KNG cells partially reversed the inhibitory effect of microRNA-486-5p on cell proliferation and cell cycle (Figure 4E, 4F). The above results revealed that the effect of microRNA-486-5p on KNG cell proliferation was achieved through its regulation on MST4.

Discussion

PCOS is a common endocrine disorder. It mainly occurs in infertile patients without ovulation, accounting for about 70%. Follicular stage is an endocrine manifestation of PCOS patients.

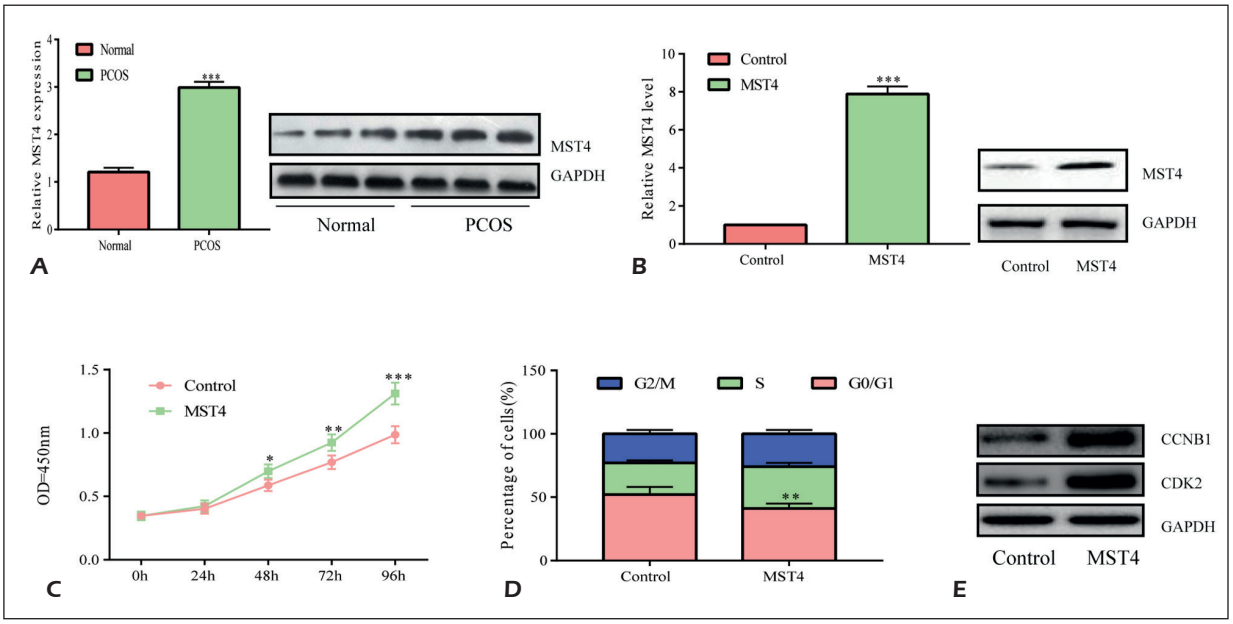

Figure 3. MST4 promoted the proliferation and cell cycle of KNG cells. ***A,*** In PCOS, the expression of MST4 increased significantly; ***B,*** After transfection of the overexpression plasmid of MST4 in KNG cells, MST4 could be stably expressed; ***C,*** After up-regulating the expression of MST4 in KNG cells, the proliferation ability of cells was significantly enhanced; ***D,*** In KNG cells, MST4 significantly promoted cell cycle; ***E,*** After up-regulation of MST4, the expression of cycle-related proteins CDK2 and CCNB1 was significantly up-regulated.

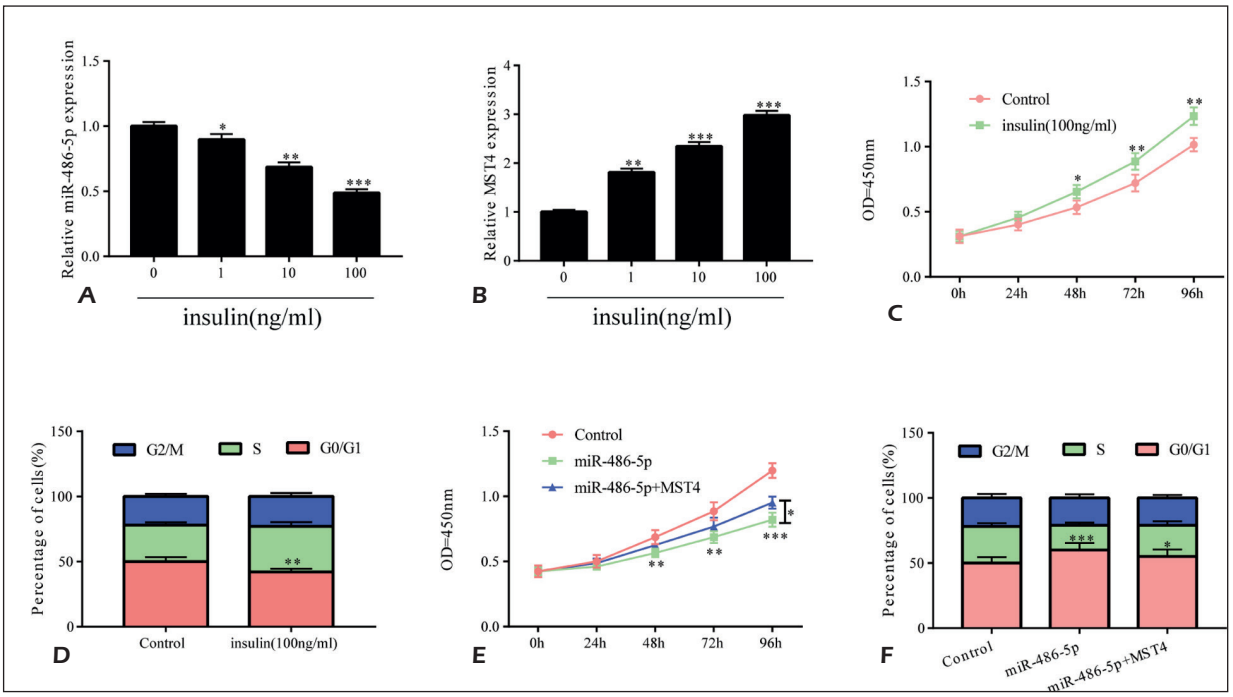

Figure 4. MiR-486-5p functioned via regulating MST4. ***A,*** After treatment with different concentrations of insulin in KNG cells, the expression of miR-486-5p decreased in a concentration-dependent manner. ***B,*** After treatment with different concentrations of insulin in KNG cells, the expression of MST4 increased and was correlated with concentration; ***C,*** After treatment, the proliferation ability of KNG cells was significantly enhanced; ***D,*** Insulin significantly promoted cell cycle of KNG cells; ***E,*** Simultaneous up-regulation of MST4 in KNG cells could partially reverse the inhibitory effect of miR-486-5p on cell proliferation; F, Simultaneous up-regulation of MST4 in KNG cells could partially reverse the inhibition of miR-486-5p on cell cycle.

When the proliferation of granulosa cells is inhibited, immature follicle atresia and luteinization will occur. This can seriously affect the normal development of follicles, leading to poor quality of ovum and embryos[16]. Therefore, in-depth study on the mechanism of PCOS and the discovery of new targets are of great significance.

MicroRNA has been reported highly conserved in evolution. MicroRNA is involved in the normal growth, metabolism, and development of the body, which also plays a pivotal role in multiple biological processes[17]. Yao et al[18] have shown that microRNAs, including microRNA-712, microRNA-224, microRNA-764-3p, microRNA-143, microRNA-383, and microRNA-320, can participate in the proliferation, differentiation and development of ovarian granule cells by regulating TGF-B/SMAD4. MicroRNA-143 in the ovary of rats with PCOS can block the formation of primordial follicles by inhibiting the proliferation of pre-granulosa cells[19]. The expression of microRNA-145 is down-regulated in granulosa cells of PCOS patients. Meanwhile, its targeting to insulin receptor substrate 1 inhibits granulosa cell proliferation by regulating P13/Akt and MAPK/ERK signaling pathways[20]. Previous studies have demonstrated that microRNA-486-5p is closely related to the occurrence and development of malignant tumors. Wang et al[21] have found that microRNA-486-5p is down-regulated in lung cancer tissues. In this study, we found that microRNA-486-5p was lowly expressed in PCOS. Furthermore, down-regulation of microRNA-486-5p affected the proliferation of ovarian granulosa cells, thereby inhibiting the occurrence and development of PCOS.

MST-4 gene is localized in many disease-associated chromosomal regions, and there is a strong linkage imbalance in the gene promoter region[12]. Recent studies have reported that MST4 can regulate cell proliferation and promote pituitary tumors through kinase sequences and downstream signaling pathways[12]. It has also been demonstrated that microRNA-4728 is a tumor-inhibiting microRNA that controls MAPK signal transduction by targeting MST4[22]. We similarly found that microRNA-486-5p could bind to MST4 in a targeted manner. In addition, microR-

NA-486-5p inhibited the proliferation of ovarian granulosa cells, thus affecting the occurrence and development of PCOS.

We found that microRNA-486-5p could inhibit the proliferation of ovarian granulosa cells by regulating MST4. Our findings provided a theoretical basis for the pathogenesis and treatment of PCOS and broadened the thinking for further research of PCOS as well.

Conclusions

MicroRNA-486-5p is low expressed in PCOS tissues. Overexpression of microRNA-486-5p can inhibit the proliferation of ovarian granulosa cells through negatively regulating MST4 expression. Our findings suggest a new targeted therapy of PCOS.

Conflict of Interests

The authors declared no conflict of interest.

References

1) Teede HJ, Misso ML, Boyle JA, Garad RM, McAllister V, Downes L, Gibson M, Hart RJ, Rombauts L, Moran L, Dokras A, Laven J, Piltonen T, Rodgers RJ, Thondan M, Costello MF, Norman RJ. Translation and implementation of the Australian-led PCOS guideline: clinical summary and translation resources from the International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome. Med J Aust 2018; 209: S3-S8.
2) Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet 2007; 370: 685-697.
3) Guzick DS. Polycystic ovary syndrome. Obstet Gynecol 2004; 103: 181-193.
4) Zhou JY, Zhang XY, Yu ML, Lu SF, Chen X. [Effect of transcutaneuos acupoint electrostimulation on serum sex hormone levels and expression of ovarian steroid hormone metabolic enzymes in polycystic ovary syndrome rats]. Zhen Ci Yan Jiu 2016; 41: 11-17.
5) Jiang YC, Ma JX. The role of MiR-324-3p in polycystic ovary syndrome (PCOS) via targeting WNT2B. Eur Rev Med Pharmacol Sci 2018; 22: 3286-3293.
6) Sirotkin AV, Laukova M, Ovcharenko D, Brenaut P, Mlyncek M. Identification of microRNAs controlling human ovarian cell proliferation and apoptosis. J Cell Physiol 2010; 223: 49-56.
7) Sirotkin AV, Ovcharenko D, Grossmann R, Laukova M, Mlyncek M. Identification of microRNAs controlling human ovarian cell steroidogenesis via a genome-scale screen. J Cell Physiol 2009; 219: 415-420.
8) Sirotkin AV, Laukova M, Ovcharenko D, Brenaut P, Mlyncek M. Identification of microRNAs controlling human ovarian cell proliferation and apoptosis. J Cell Physiol 2010; 223: 49-56.
9) Navon R, Wang H, Steinfeld I, Tsalenko A, Ben-Dor A, Yakhini Z. Novel rank-based statistical methods reveal microRNAs with differential expression in multiple cancer types. PLoS One 2009; 4: e8003.
10) Shi L, Liu S, Zhao W, Shi J. miR-483-5p and miR-486-5p are down-regulated in cumulus cells of metaphase II oocytes from women with polycystic ovary syndrome. Reprod Biomed Online 2015; 31: 565-572.
11) Ten KJ, Jansen M, Yuan J, Oorschot V, Begthel H, Di Giacomo V, Colland F, de Koning J, Maurice MM, Hornbeck P, Clevers H. Mst4 and Ezrin induce brush borders downstream of the Lkb1/Strad/Mo25 polarization complex. Dev Cell 2009; 16: 551-562.
12) Ling P, Lu TJ, Yuan CJ, Lai MD. Biosignaling of mammalian Ste20-related kinases. Cell Signal 2008; 20: 1237-1247.
13) Xiong W, Matheson CJ, Xu M, Backos DS, Mills TS, Salian-Mehta S, Kiseljak-Vassiliades K, Reigan P, Wierman ME. Structure-based screen identification of a mammalian Ste20-like kinase 4 (MST4) inhibitor with therapeutic potential for pituitary tumors. Mol Cancer Ther 2016; 15: 412-420.
14) Ma X, Zhao H, Shan J, Long F, Chen Y, Chen Y, Zhang Y, Han X, Ma D. PDCD10 interacts with Ste20-related kinase MST4 to promote cell growth and transformation via modulation of the ERK pathway. Mol Biol Cell 2007; 18: 1965-1978.
15) Yu H, Wei W, Cao W, Zhan Z, Yan L, Wu K, Xie D, Cai B, Xie Y, Xiao Q. Regulation of cell proliferation and metastasis by microRNA-593-5p in human gastric cancer. Onco Targets Ther 2018; 11: 7429-7440.
16) Kuang Y, Chen Q, Hong Q, Lyu Q, Ai A, Fu Y, Sho-ham Z. Double stimulations during the follicular and luteal phases of poor responders in IVF/ICSI programmes (Shanghai protocol). Reprod Biomed Online 2014; 29: 684-691.
17) Roderburg C, Trautwein C. Cell-specific functions of miRNA in the liver. J Hepatol 2017; 66: 655-656.
18) Yao G, Yin M, Lian J, Tian H, Liu L, Li X, Sun F. MicroRNA-224 is involved in transforming growth factor-beta-mediated mouse granulosa cell proliferation and granulosa cell function by targeting Smad4. Mol Endocrinol 2010; 24: 540-551.
19) Zhang J, Ji X, Zhou D, Li Y, Lin J, Liu J, Luo H, Cui S. miR-143 is critical for the formation of primordial follicles in mice. Front Biosci (Landmark Ed) 2013; 18: 588-597.
20) Cai G, Ma X, Chen B, Huang Y, Liu S, Yang H, Zou W. MicroRNA-145 negatively regulates cell proliferation through targeting IRS1 in isolated ovarian granulosa cells from patients with polycystic ovary syndrome. Reprod Sci 2017; 24: 902-910.
21) Wang J, Tian X, Han R, Zhang X, Wang X, Shen H, Xue L, Liu Y, Yan X, Shen J, Mannoor K, Deepak J, Donahue JM, Stass SA, Xing L, Jiang F. Downregulation of miR-486-5p contributes to tumor progression and metastasis by targeting protumorigenic ARHGAP5 in lung cancer. Oncogene 2014; 33: 1181-1189.
22) Schmitt DC, Madeira DSL, Zhang W, Liu Z, Arora R, Lim S, Schuler AM, McClellan S, Andrews JF, Kahn AG, Zhou M, Ahn EY, Tan M. ErbB2-intronic microRNA-4728: a novel tumor suppressor and antagonist of oncogenic MAPK signaling. Cell Death Dis 2015; 6: e1742.