Increased Expression of Kindlin 2 in Luteinized Granulosa Cells Correlates With Androgen Receptor Level in Patients With Polycystic Ovary Syndrome Having Hyperandrogenemia

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Abstract

Hyperandrogenemia is the leading defect in patients with polycystic ovary syndrome (PCOS) and considered to be involved in the ovulation dysfunction of PCOS. During the process of ovulation, granulosa cells (GCs) undergo epithelial–mesenchymal transition (EMT), and integrin-interacting protein kindlin 2 is a well-known regulator in EMT. Therefore, our objective here was to compare the expression levels of kindlin 2 in luteinized GCs between patients with PCOS and control women and the relationship between kindlin 2 and PCOS pathogenesis. In this study, kindlin 2 expression was significantly increased in luteinized GCs from patients with PCOS, and kindlin 2 could be induced by testosterone both in vitro and in vivo. Meanwhile, kindlin 2 was positively correlated with androgen receptor (AR) in PCOS GCs. Taken together, kindlin 2 may play a role in luteinized GCs, especially in the case of excess androgen. Further studies are required to assess the specific role of kindlin 2 in follicular development and PCOS pathogenesis.

Keywords

polycystic ovary syndrome, kindlin 2, androgen, androgen receptor, luteinized granulosa cells

Introduction

Polycystic ovary syndrome (PCOS), with a prevalence of 5% to 10%, is the most common endocrine and metabolic dysfunction of reproductive-age women.^{1,2} Despite substantial efforts to define the pathogenesis of PCOS, the underlying mechanism has not been fully elucidated. High serum androgen level is the leading defect in patients with PCOS, and hyperandrogenemia is considered to be involved in the pathogenesis of follicular arrest.^{3,4} It has been considered that this syndrome resulted from abnormal regulation of steroidogenesis and specifically from androgen secretion by the ovary.⁵ Hyperandrogenemia is a salient feature of PCOS, and it has been argued that hyperandrogenemia represents a sine qua non for the diagnosis of this syndrome.⁶ At present, there is imminent concern that elevated serum androgen level has been associated with poor fertility in women with PCOS.⁷ Androgen receptor (AR) had also been suggested to regulate signaling pathways occurring in granulosa cells (GCs), and AR was overexpressed in GCs with PCOS.^{8,9}

Kindlin 2 (also called FERMT2) is an integrin-interacting protein that belongs to the kindlin family. Kindlin 2 has been well known as a novel coactivator of integrins.¹⁰ However,

recently we pinpointed new roles of kindlin 2 in regulating *Wnt* target genes and inducing epithelial–mesenchymal transition (EMT) in breast and lung cancer cells.^{11–15} Although it is considered to be an important step in tumor invasion and metastasis, EMT also occurs in embryogenesis and ovulation.¹⁶ During the process of ovulation, the epithelial nature of GCs is lost and GCs undergo EMT.^{17,18} So far there is no report concerning the

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role of kindlin 2 in female reproductive system, especially in follicular development and the process of ovulation.

In patients with PCOS, there was an increased density of small preantral follicles but no dominant follicle. These abnormalities in PCOS were further defined by abnormal GC proliferation and disparate growth of oocytes as well as surrounding GCs.¹⁹ Moreover, previous findings indicated that there were significant differences in the rate of cell death and proliferation in GCs with PCOS,²⁰ suggesting that PCOS GCs themselves may have intrinsic abnormalities. However, the dysfunction of GCs on fertility remains to be elucidated. In our pilot study, we were concerned with the effect of excess androgen on the pathogenesis of PCOS and found that kindlin 2 was expressed in GCs, especially expressed highly in GCs from patients with PCOS. To this end, the aims of the present study, utilizing the addition of exogenous androgen, were, first, to observe the alteration in kindlin 2 expression in human GCs using Western blot analysis and second to examine the underlying relationship between overexpression of kindlin 2 in GCs and PCOS occurrence.

Materials and Methods

Patients

All patients signed for informed consent before their inclusions in this study. This study was approved by the Clinical Ethics Committee of Peking University Second Hospital (FWA00001384).

Study participants were patients who received in vitro fertilization-embryo transfer between July 2011 and December 2012 in the Center for Reproductive Medicine. All patients underwent controlled ovarian hyperstimulation with a combination of gonadotropin-releasing hormone agonist and recombinant human follicle-stimulating hormone (FSH) therapy. Pretreatment of the PCOS group was prescribed with an oral Diane 35 (ethinylestradiol and cyproterone acetate tablets). In all, 56 infertile women with regular menstrual cycles and 40 infertile women with PCOS were included in this study. Infertility of all women in the control group was due to sperm abnormalities.

All patients with PCOS were diagnosed according to the Rotterdam 2003 consensus criteria,²¹ in which at least 2 of the following 3 criteria were met: (1) oligo- or anovulation; (2) signs of clinical hyperandrogenemia and/or biological signs of hyperandrogenemia; and (3) polycystic ovaries on ultrasonography. In particular, women with possible androgen-secreting tumors, congenital adrenal hyperplasia, and thyroid diseases were excluded. Based on the long-term clinical trial and the suggested standard for hyperandrogenemia by the assay used, we regarded testosterone concentration greater than 2.6 nmol/L in the blood as the standard for hyperandrogenemia in the patients in this study. Since hirsutism was not frequently observed in Asian women with PCOS, thus this parameter was not used for scoring. However, a recent study has suggested that even in Asian women hirsutism should be considered as a parameter for scoring PCOS.²² We thus take this parameter into account in the future enlarged experiment.

Follicular Fluid Collection and Luteinized GC Preparation

Follicular fluid (FF) samples were obtained from individual follicles under general anesthesia 36 to 38 hours after injection of human chorionic gonadotropin (hCG), and transvaginal oocyte aspiration was performed with ultrasound guidance. Only blood-free, yellow-colored, undiluted FF samples were obtained from the patients. Each sample was collected into a sterile tube with culture medium and immediately centrifuged for 8 minutes at 800 rpm, resuspended the sediment with 0.01 mol/L phosphate-buffered saline (PBS), and then digested with 0.25% trypsin (Invitrogen, Carlsbad, California) for 10 min. The GCs were isolated from blood cells with 50% Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation. After washing twice with PBS, the GCs were resuspended in cells with a density of 2×10^5 for different application and then cultured at 37°C at 5% CO₂ in Dulbecco-modified Eagle medium (DMEM)/F12 (Invitrogen) supplemented with 10% fetal bovine serum (FBS; Gibco), penicillin (100 U/mL; Invitrogen), and streptomycin (100 µg/mL; Invitrogen). To minimize the individual difference, the control GCs were mixed together and then cultured for different application. However, GCs of each patient with PCOS were cultured separately.

Control GCs Treated With Testosterone

To demonstrate the effect of excess testosterone on kindlin 2 and AR expression, control GCs were seeded in 6-cm cell culture dishes and cultured for 24 hours. Then the medium was substitute with increasing concentrations of testosterone (molecular weight: 456.71; Testosterone Undecanoate Injection, Zhejiang Xian Ju Pharmaceutical Co Ltd, Zhejiang, China) at 0, 1, 15, 20, 100, and 150 µg/mL for 3 days. In addition, another group of GCs from control women were incubated with 20 µg/mL testosterone for 1 to 6 days.

In Vivo Animal Experiment

Animal care and handling were conducted in accordance with the Institutional Animal Welfare and Ethics Committee of Peking University (No. LA2011-73).

Six-week-old ICR female mice were bred in a room with 12-hour/12-hour light–dark cycles and given food and water ad libitum. The control group mice ($n = 7$) were injected intraperitoneally with bean oil (Sigma, St. Louis, Missouri), and the testosterone group mice ($n = 5$) were injected intraperitoneally with 1.3 mg/kg testosterone. The duration was 7 days for both the groups. Because serum testosterone concentration in PCOS group was about 2-fold than the control group according to our data, we selected 2-fold dose to elevate the serum testosterone concentration of mice in order to mimic the clinical features of PCOS. Then all of the mice were induced to superovulate by administering pregnant mare serum gonadotropin (PMSG; serum gonadotropin injection; Chifeng BO EN Pharmaceutical CO, LTD, Inner Mongolia, China) 10 IU followed by hCG (chorionic gonadotropin

injection; Ningbo Pharmaceutical Co, Ltd, Zhejiang, China) 10 IU 48 hours later. The metaphase II (MII) oocytes were obtained from the oviductal ampullae of superovulated mice killed 14 to 16 hours after hCG injection. The proteins of the mice ovaries were used to detect the expression of kindlin 2 and AR by Western blot analysis. To minimize the individual difference, a mixture of seven mice ovaries were used as the control group for Western blot analysis. However, the ovary protein of each mouse in the testosterone group was extracted separately. The dose conversion and injection frequency of the testosterone used in mouse were based on drug instruction and previous publication.²³

Small-Interfering RNA Transfection

Kindlin 2 and AR small-interfering RNA (siRNA) for in vitro experiments were designed according to the human complementary DNA sequence. The sense targeting sequence of kindlin 2 is as follows: AAG CUG GUG GAG AAA CUC G and that of AR is as follows: GAC CUA CCG AGG AGC UUU C. In addition, a nonsense sequence with no similar match to any known sequence was designed as control. Cultures of GCs were transfected with siRNA using Lipofectamine RNAiMAX Reagent (Invitrogen). Briefly, 10 μ L of Lipofectamine RNAiMAX and 500 μ L of Opti-MEM (Invitrogen) were incubated at room temperature for 5 minutes, then again incubated with both 200 pmol siRNA and 500 μ L Opti-MEM at room temperature for 20 minutes. The transfection complex was added to the cells with 1 mL 5% FBS DMEM/F12 at 37°C. After 6 hours, the medium was drawn and the new DMEM/F12 containing 10% FBS was added. After 72 hours, the cells were washed and proteins extracted.

In addition, after transiently transfecting with kindlin 2 or AR siRNA for 72 hours, the GCs were cocultured with testosterone (20 μ g/mL). After 72 hours, the cells were washed and proteins extracted. Similarly, after testosterone (20 μ g/mL) was added to the medium for 72 hours, the GCs were transfected with kindlin 2 siRNA. After 72 hours, the cells were washed and the proteins extracted.

Western Blot Analysis

The lysates of hGCs and mouse ovaries were extracted using the PBS TDS lysis buffer containing a 10% protease inhibitor cocktail, incubated on ice for 30 minutes and then centrifuged at 12 000 rpm for 15 minutes at 4°C. Gel electrophoresis was performed on 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotted onto polyvinyl difluoride membrane. The transferred membrane was blocked for 1 hour at room temperature with 5% nonfat milk in Tris-buffered saline Tween and incubated with primary antibody overnight at 4°C, kindlin 2 (1:2000 dilution; MAB2617; Millipore, Burlington, California), AR (1:3000 dilution; 3165-1; Epitomics, Temecula, California), and β -actin (1:2000 dilution; sc-47778; Santa Cruz Biotechnology, Santa Cruz, California). After washing, the membrane was incubated with its corresponding

Table 1. Comparison of Clinical Parameters Between Control Women and Patients With PCOS.^a

	Control	PCOS	P Value
N	56	40	
Age, years	31.87 $\pm$ 4.83	30.28 $\pm$ 3.48	.510
BMI, kg/m ²	21.52 $\pm$ 2.64	24.38 $\pm$ 4.10	.001
Day 2 testosterone, nmol/L	1.75 $\pm$ 0.75	2.81 $\pm$ 0.99	.000
Day 2 LH, IU/L	3.36 $\pm$ 2.20	3.62 $\pm$ 2.70	.906
Day 2 FSH, IU/L	5.50 $\pm$ 2.96	4.77 $\pm$ 2.43	.231
Number of big follicles	11.55 $\pm$ 6.52	15.75 $\pm$ 7.48	.005
Number of oocytes	14.86 $\pm$ 9.54	18.63 $\pm$ 9.46	.039

Abbreviations: BMI, body mass index; big follicles, follicles that are larger than 14 mm on the day of hCG injection; day 2, the second day of menstrual cycle before Diane 35 treatment; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PCOS, polycystic ovarian syndrome; SD, standard deviation.

^aData are given as mean $\pm$ SD. The nonparametric Mann-Whitney test was carried out.

secondary antibody for 1 hour at 4°C. Immobilized antibodies were detected by enhanced chemiluminescence (Pierce Chemical Co, Rockford, Illinois), with the relative level in each sample normalized to β -actin. Densitometric analysis was performed with ImageJ software (National Institutes of Health, Bethesda, Maryland). Data shown were representative of at least 3 independent experiments with similar results.

Statistical Analysis

All data analyses were performed using SPSS (version 13.0; SPSS Inc) and SAS statistical software package (version 9.1; SAS Institute Inc). Statistical comparisons between control women and patients with PCOS were performed with nonparametric Mann-Whitney test. Univariate analysis of correlation between the expression of kindlin 2 and the AR was determined by nonparametric Spearman test. The results were considered statistically significant at $P < .05$ level.

Results

Comparison of Clinical Parameters Between Control Women and Patients With PCOS

Main clinical and endocrinal parameters in control women and patients with PCOS are summarized in Table 1. No difference between the 2 groups was found for age, the luteinizing hormone, and FSH serum levels on day 2 of menstrual cycle. However, body mass index, testosterone serum level on day 2 of menstrual cycle, number of big follicles, and number of oocytes were significantly higher in patients with PCOS than that in control women ($P = .001$, $P = .000$, $P = .005$, and $P = 0.039$, respectively).

Increased Expression of Kindlin 2 and AR in GCs is Correlated With PCOS

The expression of kindlin 2 and AR in GCs from 56 control women and 40 patients with PCOS was analyzed by

Figure 1. Kindlin 2 and AR expressions were significantly increased in GCs of patients with PCOS. PCOS group $n = 40$, control women $n = 56$. A, Of the 40 patients with PCOS, 6 representative GCs were analyzed for kindlin 2 and AR expression using Western blot analysis. Of the 56 control women, 2 representative GCs were used as the control. Both PCOS and normal control GC proteins were extracted from the mono-layer of GCs incubated for 48 hours. The expression levels of kindlin 2 and AR were detected by Western blot analysis. B, The different expression levels between control and PCOS GCs were statistically analyzed using nonparametric Mann-Whitney test. *** $P < .001$ is considered significant. AR, androgen receptor; PCOS, polycystic ovarian syndrome; GCs, granulosa cells.

Western blot analysis. The expression levels of kindlin 2 and AR were found 2.5- and 2.3-fold separately higher in patients with PCOS than in control women (Figure 1). Although the expression level of kindlin 2 was significantly increased in PCOS GCs, the expression level of each patients with PCOS was different. In addition, AR expression level was overexpressed simultaneously. However, it should be pointed out that our data about different expression levels of kindlin 2 and AR in each PCOS GCs may be due to various serum androgen level and the disease course of each patient with PCOS.

Given that the expression levels of both kindlin 2 and AR were elevated in PCOS GCs as indicated earlier, we hypothesize that there was a relationship between kindlin 2 and AR. To this end, we performed a nonparametric Spearman test and found that no correlation was found between the protein levels of kindlin 2 and AR in control GCs (Spearman $\rho = .2446$, $P = .1282$). However, a significantly positive correlation was seen in the protein levels of kindlin 2 and AR in PCOS GCs (Spearman $\rho = .7109$, $P < .001$; Table 2).

Kindlin 2 and AR are Upregulated by Testosterone in GCs From Control Women

Interestingly, both kindlin 2 and AR were upregulated in the presence of testosterone in a dose-dependent manner. In addition, in a time course study both kindlin 2 and AR expression were upregulated with the addition of 20 $\mu\text{g/mL}$ testosterone in a time-dependent manner (Figure 2).

Table 2. Univariate Correlations Between the Expression of Kindlin 2 and AR in Control Women and Patients With PCOS.^a

	Kindlin 2 (Control)	Kindlin 2 (PCOS)
AR (control)	$R = .2446$, $P = .1282$	$R = .7109$, $P < .001$
AR (PCOS)		

Abbreviations: R, Spearman ρ ; AR, androgen receptor; PCOS, polycystic ovarian syndrome.

^aGrouping method: each group was divided into 4 subgroups with each half maximum value of Western blot analysis, the relative level in each sample normalized to β -actin. Then univariate analysis of correlation between the expression of kindlin 2 and AR was determined by nonparametric Spearman test. Expression of kindlin 2 was not correlated with AR in control women but positively correlated with AR in patients with PCOS.

Upregulation of Kindlin 2 and AR in Mouse Ovary by Testosterone With Concomitant Reduction in MII Oocytes

Similar to the upregulation in GCs from human GCs, both kindlin 2 and AR were highly induced in the ovary of testosterone-treated mice (Figure 3A). However, the number of MII oocytes in oviductal ampullae of testosterone-treated mice was significantly reduced than that in the control group (Figure 3B). The average number of MII oocytes in the control group was about 51, whereas in testosterone group it was about 21.

Mutual Regulation Between Kindlin 2 and AR in GCs

Knockdown of kindlin 2 led to a remarkable downregulation of AR protein in GCs from both control women and patients with

Figure 2. Expression of kindlin 2 and androgen receptor (AR) were induced by testosterone in granulosa cells (GCs) from control women. A-C, Control GCs were incubated with increasing concentrations of testosterone at 0, 1, 15, 20, 100, and 150 µg/ml for 3 days. Results showed that kindlin 2 and AR expression levels were induced in a dose-dependent manner. D-F, Control GCs were incubated with 20 µg/ml testosterone for 1 to 6 days as indicated. Results showed that kindlin 2 and AR expression levels were induced in a time-dependent manner. *** $P < .001$ is considered significant.

PCOS (Figure 4A). In contrast, knockdown of AR led to a marked kindlin 2 upregulation in GCs from both control women and patients with PCOS (Figure 4B). Importantly, testosterone could upregulate AR expression, while simultaneous knockdown of kindlin 2 could reduce AR even with the addition of testosterone, suggesting that androgen-stimulated AR expression required the presence of kindlin 2 (Figure 4C). In agreement with this tendency, knockdown of kindlin 2 followed by addition of testosterone still could not restore the AR expression to the control level (Figure 4C), again indicating that kindlin 2 was required for androgen-induced AR upregulation. After knockdown of AR, adversely, kindlin 2 was still upregulated by testosterone, suggesting that androgen-induced kindlin 2 upregulation was independent of AR (Figure 4D). Taken together, these data indicated that kindlin 2 and AR were mutually regulated in GCs, and kindlin 2 was required for the maintenance of AR.

Discussion

The main finding of this study using Western blot analysis was that expression of kindlin 2 was significantly increased in luteinized GCs from patients with PCOS compared to the control women; meanwhile, kindlin 2 could be induced by testosterone both in vitro and in vivo. In PCOS GCs, kindlin 2 expression was positively correlated with AR. In addition, there was a mutual regulation between kindlin 2 and AR. Kindlin 2 was required for androgen-induced AR upregulation, but androgen-induced kindlin 2 upregulation was independent of AR.

It was well known that high serum androgen level was one of the most common clinical features in patients with PCOS. Impaired oocyte maturation and ovarian dysfunction in patients with PCOS may be related to abnormal endocrine factors and extra- and intrafollicular microenvironment during folliculogenesis, including hyperandrogenemia, insulin resistance, and hyperinsulinemia.^{7,24,25} Moreover, some researchers considered

Figure 3. Kindlin 2 and androgen receptor (AR) expressions were induced by testosterone in mouse ovary followed by reduction of metaphase II (MII) oocytes. A, To minimize the individual difference, the control group comprised a mixture of 7 ovaries from mice, which were used for Western blot analysis. Five mice in testosterone group were injected intraperitoneally with testosterone at a dose of 1.3 mg/kg. The kindlin 2 and AR expressions were apparently increased in testosterone-treated mice. B, The number of MII oocytes in the control group was 51 ± 20.85 and in the testosterone group was 21.4 ± 4.04 . Data are given as mean $\pm$ standard deviation. $**P < .01$ is considered significant. In the control group, the number of MII oocytes was 67, 42, 35, 30, 82, 33, and 68; while in testosterone-treated group, the number of MII oocytes was 23, 26, 21, 22, and 15.

that excess androgen might be both the consequence and the cause of the ovarian abnormality.²⁶ The gene expression profiles of ovaries from long-term, androgen-treated female-to-male transsexuals demonstrated a considerable overlap with PCOS; this finding provided supportive evidence that androgen played an important role in the pathogenesis of PCOS.²⁷ The PCOS was characterized by arrested follicular maturation with excessive ovarian production of androgens, but the origins and underlying mechanisms of PCOS remained unclear.²⁸ In the present study, we focused on the effect of excess androgen on PCOS occurrence. First of all, we found that the expression of kindlin 2 was significantly increased in PCOS GCs. To our knowledge, this is the first study to report that kindlin 2 expression was raised in PCOS GCs from women with stimulated preovulatory follicles. An interesting finding in our study was that in control GCs, kindlin 2 expression was upregulated in both dose- and time-dependent manners after treating with testosterone *in vitro*. Based on the aforementioned facts, increased kindlin 2

expression in control GCs treated with testosterone suggested that this androgen was responsible for the alteration in kindlin 2 in patients with PCOS. These abnormalities might be related to the mechanism underlying PCOS. As the number of luteinized GCs from each patient with PCOS is limited, in the present study we could not perform testosterone addition experiment in PCOS GCs, but we inferred that the results would be the same in PCOS GCs, which require further validation.

Androgen activates AR, a key transcription factor mediating androgen-induced signaling.²⁹ The GCs-specific AR appears to promote preantral follicle growth and prevent follicular atresia; it is essential for normal follicular development and fertility.³⁰ Our findings confirmed that AR expression was significantly increased in PCOS GCs; this result was in agreement with Cateau-Jonard et al who found that AR was overexpressed in GCs from stimulated follicles of women with PCOS using quantitative real-time polymerase chain reaction.⁹ Moreover, the upregulation of AR in control GCs treated with testosterone was consistent with the previous study, which reported an increased AR expression in the GCs of testosterone-treated rhesus monkeys from preantral to large antral follicle stage.³¹ After treatment with testosterone, to a certain extent, increased expression of kindlin 2 and AR in control GCs was similar to the observations found in patients with PCOS, whose main clinical feature was hyperandrogenemia. In the present study, however, we demonstrated that kindlin 2 expression was strongly and positively correlated with AR in PCOS GCs. In addition, we first identified that there was a mutual regulation between kindlin 2 and AR in GCs. After knockdown of AR, kindlin 2 was still upregulated by testosterone, suggesting that androgen-induced kindlin 2 upregulation was independent of AR. In contrast, testosterone could upregulate AR expression while simultaneous knockdown of kindlin 2 could reduce AR even with the addition of testosterone, suggesting that kindlin 2 was required for androgen-induced AR upregulation. In agreement with this tendency, knockdown of kindlin 2 followed by the addition of testosterone still could not restore AR expression to the control level. These findings suggested that kindlin 2 may participate in the regulatory pathway of AR activated by androgen. Collectively, these results inferred that kindlin 2 may play an important role in GCs of control women and patients with PCOS. Kindlin 2 was required for the maintenance of AR, which was important for GCs function as well as in the occurrence of PCOS.

Whether the increased expression of kindlin 2 in PCOS GCs may disturb normal ovarian function in patients with PCOS was further studied by our experiment in female mice *in vivo*. The most compelling evidence is upregulation of kindlin 2 in mouse ovary by testosterone with concomitant reduction in MII oocytes. Overexpression of kindlin 2 may be involved in the disorder of follicular development or dysfunction of oocyte maturation. So the significance of our studies was to provide a plausible relationship between increased kindlin 2 level and ovarian dysfunction of PCOS.

In summary, in the present investigation, we found that increased expression of kindlin 2 in PCOS GCs and kindlin

Figure 4. Mutual regulation between kindlin 2 and AR in granulosa cells (GCs). The GCs were transiently transfected with small interfering RNA (siRNA); 72 hours later, the cells were subjected to protein extractions or continued the coculture with testosterone (T) for 72 hours. A, GCs were transfected with control or kindlin 2-specific siRNA as indicated from both control women and patients with PCOS. B, GCs were transiently transfected with control or androgen receptor (AR)-specific siRNA as indicated from both control women and patients with polycystic ovarian syndrome (PCOS). C, Control GCs were transfected with control or kindlin 2 siRNA as indicated and then cocultured with T. In the rightmost lane, control GCs were treated with T for 72 hours and then transfected with kindlin 2 siRNA. In order to clearly observe the difference between each lane, the exposure time was prolonged in kindlin 2 expression. D, Control GCs were transfected with control or AR siRNA and then cocultured with testosterone.

2 can be induced by androgen in both dose- and time-dependent manners. There was a mutual regulation between kindlin 2 and AR in GCs, suggesting that kindlin 2 and AR may work together to mediate dysregulated follicular development in PCOS. However, further studies will be necessary to fully elucidate the role of kindlin 2 in PCOS pathogenesis.

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