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Long non-coding RNAs regulate effects of β-crystallin B2 on mouse ovary development

QIAN GAO*, HANXIAO REN*, MINGKUN CHEN, ZIGUANG NIU, HAIBO TAO, YIN JIA, JIANRONG ZHANG and WENJIE LI

Department of Laboratory Diagnosis, Changhai Hospital, The Second Military Medical University, Shanghai 200433, P.R. China

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Abstract. β-crystallin B2 (*CRYBB2*) knockout mice exhibit morphological and functional abnormalities in the ovary. Long non-coding RNAs (lncRNAs) regulate gene transcription and translation, and epigenetic modification of genomic DNA. The present study investigated the role of lncRNAs in mediating the effects of *CRYBB2* in the regulation of ovary development in mice. In the current study, ovary tissues from wild-type (WT) and *CRYBB2* knockout mice were subjected to lncRNA and mRNA microarray profiling. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to group the differentially expressed lncRNAs into regulated gene pathways and functions. The correlation matrix method was used to establish a network of lncRNA and mRNA co-expression. Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) was used to verify expression of a number of these differentially expressed lncRNAs and mRNAs. There were 157 differentially expressed lncRNAs and 1,085 differentially expressed mRNAs between ovary tissues from WT and *CRYBB2* knockout mice. The GO and KEGG analyses indicated that these differentially expressed lncRNAs and mRNAs were important in Ca^{2+} signaling and ligand and receptor interactions. The correlation matrix method established an lncRNA and mRNA co-expression network, consisting of 53 lncRNAs and 45 mRNAs with 98 nodes and 75 connections. RT-qPCR confirmed downregulation of lncRNA A-30-P01019163 expression, which further downregulated its downstream gene purinergic receptor P2X, ligand-gated ion channel, 7 (P2rx7) expression in ovary tissues from *CRYBB2* knockout mice. In conclusion, CRYBB2 regulates expression of different lncRNAs to influence ovary development. lncRNA A-30-P01019163 may affect ovarian cell cycle and proliferation by regulating P2rx7 expression in the ovary.

Correspondence to: Dr Wenjie Li, Department of Laboratory Diagnosis, Changhai Hospital, The Second Military Medical University, 168 Changhai Road, Shanghai 200433, P.R. China
E-mail: wenjieli@pku.org.cn

*Contributed equally

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Introduction

β-crystallin B2 (CRYBB2) expression was initially reported in the eye lens where it functions to maintain lens transparency and refractive index. CRYBB2 deficiency was demonstrated to result in the generation of age-associated cataracts (1). However, a number of previous studies demonstrated that CRYBB2 is also expressed in the retina, brain, testis, and ovary (2-5). Our previous study observed that CRYBB2 was expressed in human and mouse ovaries, particularly in ovarian granulosa cells (6). *CRYBB2* knockout mice exhibited morphological and functional abnormalities in the ovary, including reduced ovarian index (ratio of ovary weight to total body weight) with increased follicle atresia, reduced mature follicles and dysregulated estrous cycle (7). Our data from a previous study also indicated a high level of estrogen in the diestrus and metestrus, but a low level of progesterone in the metestrus compared with wild-type (WT) mice (6). At the genetic level, expression of cell cycle and apoptosis-associated proteins, including B-cell lymphoma 2, cyclin-dependent kinase 4, and cyclin D2, were markedly lower in the *CRYBB2* knockout mice compared with WT mice (6). These data suggest that *CRYBB2* may be important in ovary development, however, the underlying molecular mechanism by which *CRYBB2* regulates ovarian development remains to be elucidated.

To identify and assess the role of *CRYBB2* in ovary development, differentially expressed long non-coding RNAs (lncRNAs) were profiled in *CRYBB2* knockout mice. lncRNAs are a class of non-coding RNAs with nucleotides >200 bp and transcribed by RNA polymerase II. Functionally, lncRNAs may regulate gene transcription, protein translation, and epigenetic modification of genomic DNA. Altered expression and regulation of lncRNAs has been associated with human diseases and aging (8-12). For example, previous studies have suggested that overexpression of lncRNA HOX transcript antisense RNA associated with the recurrence of hepatocellular

carcinoma, poor prognosis in colorectal cancer, and malignant behaviors of gastrointestinal stromal tumors (13-15). lncRNAs modulate cell functions by regulating expression of targeted downstream genes (16), which may in turn affect embryo development (17), inactivate the X chromosome, and regulate genomic imprinting (18). Thus, the current study assessed whether these lncRNAs mediate the functions of CRYBB2 in ovary development and investigated the underlying mechanisms. Microarray profiling between ovarian tissues from *CRYBB2* knockout and WT mice was conducted and bioinformatic analysis of differentially expressed lncRNAs and mRNAs was performed. A number of these differentially expressed lncRNAs and mRNAs were verified using quantitative reverse transcription-polymerase chain reaction (RT-qPCR).

Materials and methods

Animals. Male and female C57BL/6 mice were obtained from the Experimental Animal Center of the Second Military Medical University (Shanghai, China). *CRYBB2* knockout mice were generated by the Ingenious Targeting Laboratory (Ronkonkoma, NY, USA), as described previously (19). All mice were maintained on a 12 h light/dark cycle with a temperature of 21±1˚C and humidity of 50~70% in a pathogen-free facility with access to food and water *ad libitum*. Bedding material and a plastic house or tube was placed in the cage for environmental enrichment. Daily examinations were performed on all animals throughout the experimental period. Humane euthanasia of mice was performed under isoflurane anesthesia using intracardiac injection of pentobarbitone (150 mg/kg). The present study was conducted in accordance with institutional guidelines and approved by the Animal Care and Use Committee of Changhai Hospital (Shanghai, China).

A total of three 8-9-week-old (weight, 18.0±2.0 g) female *CRYBB2* knockout mice and three age-matched WT female mice were obtained. Ovary tissues were collected from mice following a 10-day experimental period.

RNA isolation, cDNA synthesis and labeling, and hybridization. Ovarian tissues were homogenized on ice and total cellular RNA was isolated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol and then quantified using NanoDrop ND-1000 (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and agarose gel electrophoresis. RNA samples were further purified using an RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA) and reverse transcribed into cDNA with fluorescent labeling for microarray hybridization using using the AffinityScript QPCR cDNA Synthesis kit (Agilent Technologies, Inc., Santa Clara, CA, USA). These labeled cDNA probes were then hybridized to Agilent mouse expression profiling (8x60K) microarray using the Gene Expression Hybridization kit (Agilent Technologies, Inc.) according to the manufacturer's protocol. The arrays were scanned into a file and analyzed using Feature Extraction software, version v10.7.3.1 (Agilent Technologies, Inc.). The arrays were scanned at 5 μm/pixel resolution using an Axon GenePix 4000B scanner

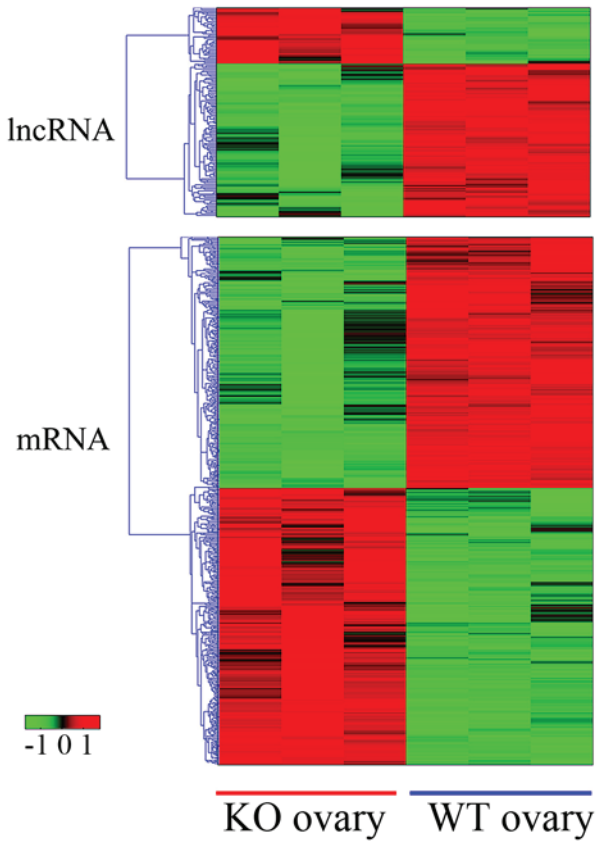

Figure 1. Cluster analysis of differentially expressed lncRNAs and mRNAs in ovary tissues from WT and β-crystallin B2 knockout mice. The horizontal axis represents the sample group and the vertical axis represents the differentially expressed mRNAs or lncRNAs. Upregulated genes are red and downregulated genes are green. Included are 157 lncRNAs (42 downregulated vs. 115 upregulated) and 1,085 mRNAs (570 downregulated vs. 515 upregulated) KO, knockout; WT, wild-type; lncRNA, long non-coding RNA.

(Molecular Devices, LLC, Sunnyvale, CA, USA) piloted by GenePix Pro 6.0 software (Molecular Devices, LLC) and then imported into NimbleScan software (version 2.5; Roche NimbleGen, Inc., Madison, WI, USA) for grid alignment and expression data analysis. Expression data were normalized using quantile normalization and the Robust Multichip Average algorithm included in the NimbleScan software. The probe level files and mRNA level files were generated following normalization. All mRNA level files were imported into Agilent GeneSpringGX software (version 11.0; Agilent Technologies, Inc.) for further analysis. Differentially expressed lncRNAs and mRNAs were identified via fold change filtering.

Functional analysis of microarray data. Gene Ontology (GO; www.geneontology.org) was performed to group differentially expressed lncRNAs and their targeted genes into biological processes, cellular components, and molecular features of the biological functions. Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg) analysis was performed to identify roles of the target genes and group them into gene pathways. The correlation matrix method was used to establish a diagram of lncRNA and mRNA co-expression regulatory networks. Prior to functional analyses, the

Table I. Differentially expressed long non-coding RNAs in ovary tissues from β-crystallin B2 knockout mice.

Probe name	P-value	FC (abs)	Regulation
A_30_P01032506	0.001900622	2.244835	Down
A_30_P01029732	0.026376737	2.3413253	Down
A_30_P01020038	0.020382054	2.3550973	Down
A_30_P01019163	0.008867065	2.3823233	Down
A_30_P01017808	0.003141625	2.3902857	Down
A_30_P01028465	0.026580842	2.3909771	Down
A_30_P01024278	0.010205811	2.4727218	Down
A_30_P01031572	0.036648612	2.5956807	Down
A_30_P01027590	0.034441914	2.6343288	Down
A_30_P01022135	0.04770927	3.120179	Down
A_30_P01031631	0.001265911	3.135137	Down
A_30_P01032372	0.009654216	3.4001627	Down
A_30_P01032133	0.012271924	3.434039	Down
A_30_P01024342	$2.97x10^{-4}$	3.7296832	Down
A_30_P01024108	0.004697592	3.8198798	Down
A_30_P01022445	$2.66x10^{-5}$	3.8623033	Down
A_30_P01022656	$2.08x10^{-4}$	4.069888	Down
A_30_P01023636	0.02164327	4.4273615	Down
A_30_P01022538	$9.77x10^{-4}$	5.492365	Down
A_30_P01033546	0.004913332	5.9940124	Down
A_30_P01019023	0.024659809	3.7894382	Up
A_30_P01024108	0.004697592	3.8198798	Up
A_30_P01017880	0.034943227	3.8256395	Up
A_30_P01022445	$2.66x10^{-5}$	3.8623033	Up
A_30_P01026472	0.022304738	3.8728366	Up
A_30_P01030900	0.023211088	3.9997435	Up
A_30_P01022656	$2.08x10^{-4}$	4.069888	Up
A_30_P01018745	$1.84x10^{-5}$	4.266393	Up
A_30_P01033353	0.03583063	4.398616	Up
A_30_P01021636	0.01934708	4.4207296	Up
A_30_P01023636	0.02164327	4.4273615	Up
A_30_P01022538	$9.77x10^{-4}$	5.492365	Up
A_30_P01019825	0.006051722	5.5902176	Up
A_30_P01033546	0.004913332	5.9940124	Up
A_30_P01024788	$1.11x10^{-5}$	7.118162	Up
A_30_P01024270	$6.38x10^{-5}$	8.226261	Up
A_30_P01027087	0.003292078	8.981714	Up
A_30_P01031162	0.011685452	9.579193	Up
A_30_P01021698	$1.31x10^{-5}$	10.52238	Up
A_30_P01020936	$4.52x10^{-4}$	22.788074	Up

FC, fold change.

predicted potential lncRNA targets were integrated with the differentially expressed mRNAs using a cut-off value of ≥2.0 fold change or P<0.05.

RT-qPCR. Total RNA was isolated from granulosa cells from the ovaries of WT and *CRYBB2* knockout mice using TRIzol reagent and reverse transcribed into cDNA using the PrimeScript RT Reagent Kit (Takara Biotechnology, Co., Ltd., Dalian, China) according to the manufacturer's protocols. These cDNA samples were then subjected to qPCR analysis using a Applied Biosystems 7300 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the SYBR Green PCR Master Mix kit (Takara Biotechnology, Co., Ltd.). qPCR amplification was conducted at 95˚C for

Table II. Differentially expressed mRNAs in ovary tissues from β-crystallin B2 knockout mice.

Gene	P-value	FC (abs)	Regulation
Prkar2b	0.040216	2.1857524	Down
Lrp11	0.006975	2.9040618	Down
P2rx7	$4.11x10^{-4}$	4.6213336	Down
Calml3	0.011701	5.126569	Down
Dclre1c	$3.39x10^{-4}$	5.1453366	Down
Lpcat2	0.002695	5.828531	Down
Stk32a	0.001242	5.833555	Down
Cyp19a1	0.034041	6.1799	Down
Megf10	0.045621	6.1827664	Down
Plcxd1	$1.04x10^{-4}$	6.82413	Down
Gm5103	0.005622	8.389297	Down
Fermt1	0.001363	8.656997	Down
Mlxip	$3.10x10^{-4}$	8.746344	Down
Slc6a2	0.046581	8.750824	Down
Gm7969	0.012795	9.970471	Down
Ces2a	0.028129	10.472376	Down
Dclre1c	$1.23x10^{-4}$	10.645219	Down
Ostn	$9.07x10^{-6}$	11.019576	Down
Jph4	0.001674	12.722166	Down
Onecut2	0.048542	13.894124	Down
Ces2a	0.028128909	10.472376	Up
Dclre1c	$1.23x10^{-4}$	10.645219	Up
Jph4	0.001673621	12.722166	Up
Onecut2	0.04854157	13.894124	Up
Itln1	$5.97x10^{-5}$	14.339393	Up
Arsk	$7.13x10^{-5}$	16.06716	Up
Sfrp4	0.023781205	17.065975	Up
Plekhg4	0.047409292	17.067673	Up
Nuf2	$6.05x10^{-5}$	17.872303	Up
Adh7	0.0064019	18.950857	Up
Itln1	$4.63x10^{-5}$	19.05549	Up
Ptgfr	0.032550577	20.059986	Up
Pou6f1	$1.71x10^{-4}$	23.766891	Up
Saa2	0.023944996	25.063555	Up
Wnt10b	0.02046059	27.648937	Up
Ifi202b	0.001051159	28.72067	Up
Dcpp1	0.014649089	40.37458	Up
Cd5l	$4.12x10^{-4}$	42.74245	Up
Dcpp2	0.012848383	45.13117	Up

FC, fold change.

2 min, followed by 40 cycles of 95˚C for 15 sec, and 60˚C for 30 sec. The relative expression of each target gene compared to β-actin was calculated using the $2^{-\Delta\Delta Cq}$ method (20). Specific primers used were as follows: Forward, 5'-AGCCATGTACGTAGCCATCC-3' and reverse, 5'-CTCTCAGCTGTGGTGGTGAA-3' for β-actin; forward, 5'-CGAGTTGGTGCCAGTGTGGA-3' and reverse, 5'-CCTGCTGTTGGTGGCCTCTT-3' for *P2rx7*; and forward, 5'-TCCACTCAGGAAGAGCTGGT-3' and reverse, 5'-TAGCACCCTCGGGATATCTG-3' for lncRNAA-30-P01019163.

Statistical analysis. All data were presented as the mean ± standard deviation. The data were Log2-transformed and median centered by genes using Cluster software, version 3.0 (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm). Statistical analyses were performed using SPSS 17.0 (SPSS,

Table III. Gene ontology analysis of differentially expressed genes.

Term	Number of genes (%)	P-value
Biological process		
Cell cycle and proliferation	3 (4)	0.479979
Stress response	4 (6)	0.205102
Transport	5 (7)	0.563981
Developmental processes	6 (9)	0.459856
RNA metabolism	7 (10)	0.315857
Other metabolic processes	12 (17)	0.007676
Cell organization and biogenesis	6 (9)	0.193590
Cell-cell signaling	1 (1)	0.502761
Signal transduction	11 (16)	0.120754
Protein metabolism	6 (9)	0.502818
Death	1 (1)	0.827556
Other biological processes	8 (11)	0.990425
Cellular component		
Cytosol	1 (2)	0.505969
Mitochondrion	1 (2)	0.862146
Endoplasmic reticulum/golgi	3 (5)	0.460760
Other cytoplasmic organelle	1 (2)	0.604395
Nucleus	10 (18)	0.306385
Plasma membrane	6 (1)	0.438812
Other membranes	14 (26)	0.661329
Translational apparatus	1 (2)	0.330956
Non-structural extracellular	2 (4)	0.830334
Other cellular component	15 (28)	0.354726
Molecular function		
Enzyme regulator activity	3 (5)	0.141417
Transcription regulatory activity	4 (7)	0.174629
Transporter activity	2 (4)	0.623057
Signal transduction activity	11 (20)	0.082302
Nucleic acid binding activity	7 (13)	0.252320
Kinase activity	2 (4)	0.503159
Other molecular function	26 (47)	0.535279

Inc., Chicago, IL, USA). The data were statistically analyzed using an unpaired Student's t-test or by one-way analysis of variance followed by Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Microarray profiling of differentially expressed lncRNAs and mRNA in ovary tissues from wild type and CRYBB2 knockout mice. Microarray data on differential lncRNA and mRNA expression levels were analyzed using Cluster software, version 3.0 with the cut-off values set as ≥2-fold difference or $P<0.05$. The data indicated 157 differentially expressed lncRNAs (42 downregulated vs. 115 upregulated) and 1,085 differentially expressed mRNAs (570 downregulated vs. 515 upregulated; Fig. 1). The genes were selected according to a software forecast associated with the processes of ovarian development, and the lncRNAs associated with these genes were selected (Tables I and II).

Bioinformatic analysis of differentially expressed lncRNAs and mRNA in ovary tissues from WT and CRYBB2 knockout mice. GO analysis was performed for functional annotation of differentially expressed lncRNAs and mRNAs and it was observed that they were predominantly involved in cell cycle regulation, cell proliferation, metabolism, and signal transduction (Table III). One particular gene, P2rx7, localized in the cytoplasm, functions to regulate cell cycle progression and proliferation, and the signal transduction process.

Subsequently, KEGG analysis was conducted to group differentially expressed lncRNAs and mRNA into gene pathways. The data from the present study indicated that the genes predominantly formed signaling pathways associated with the regulation of Ca^{2+} signaling, ligand and receptor interactions, and other cell signaling pathways (Table IV). For example, P2rx7 was identified to be predominantly involved in Ca^{2+} signaling.

Table IV. KEGG pathways analysis of differentially expressed genes.

Term	Count	P-value	Genes	Up	Down
Glutathione metabolism	1	0.106032	Gsta2	0	1
Metabolism of xenobiotics by cytochrome P450	1	0.143567	Gsta2	0	1
Drug metabolism-cytochrome P450	1	0.147434	Gsta2	0	1
Ribosome	1	0.172182	Rps3a	0	1
PPAR signaling pathway	1	0.143567	Adipoq	0	1
Calcium signaling pathway	3	0.00614	P2rx7, Ptger1, Ptafr	3	0
Cytokine-cytokine receptor interaction	1	0.45143	Cxcl1	0	1
Chemokine signaling pathway	1	0.353888	Cxcl1	0	1
Neuroactive ligand-receptor interaction	4	0.001745	P2rx7, Ptger1, Ptafr, Vipr1	4	0
Cell cycle	1	0.226819	Hdac1	0	1
Apoptosis	1	0.179664	Prkar2b	1	0
Notch signaling pathway	1	0.099971	Hdac1	0	1
Insulin signaling pathway	1	0.268146	Prkar2b	1	0
Adipocytokine signaling pathway	1	0.139685	Adipoq	0	1
Type II diabetes mellitus	1	0.099971	Adipoq	0	1
Huntington's disease	1	0.334126	Hdac1	0	1
Pathways in cancer	1	0.530933	Hdac1	0	1
Chronic myeloid leukemia	1	0.155119	Hdac1	0	1

Building the lncRNA-mRNA regulatory network. The lncRNA target predictions were superimposed into the lncRNA-mRNA correlation network. As presented in Fig. 2, the network has a total of 98 nodes, 75 connections, 53 lncRNAs and 45 mRNAs. Expression of glutathione S-transferase α 2 (Gsta2) and DEAD (Asp-Glu-Ala-Asp) box polypeptide 43 (Ddx43) was regulated by a total of five lncRNAs, while expression of coiled-coil domain containing 68 (Ccdc68), olfactory receptor 464 (Olfr464) and low density lipoprotein receptor-related protein 11 (Lrp11) were regulated by four lncRNAs. In addition, expression levels of protein kinase, cAMP dependent regulatory, type II β (Prkar2b), leucine-rich repeat-containing G protein-coupled receptor 6 (Lgr6) and adiponectin, C1Q and collagen domain containing (Adipoq) were regulated by a total of three lncRNAs.

Validation of differentially expressed lncRNAs and mRNAs in ovary tissues of WT and CRYBB2 knockout mice. RT-qPCR analysis of different lncRNAs and mRNAs was performed and it was demonstrated that expression levels of lncRNA A-30-P01019163 and P2rx7 were significantly different between10 cases of ovary tissues from WT and *CRYBB2* knockout mice ($P<0.05$; Fig. 3), whereas other lncRNAs and mRNAs did not indicate any difference between WT and *CRYBB2* knockout mouse ovary tissues (data not shown).

Discussion

CRYBB2 knockout mice exhibit abnormal morphological and functional mouse ovaries. The current study investigated the underlying molecular mechanisms by which *CRYBB2* affects expression of lncRNAs to alter mouse ovary structure and function. Certain aberrant lncRNA expression in the regulation of other genes was assessed. A total of 157 differentially expressed lncRNAs and 1,085 mRNAs were observed. The GO database analysis indicated that these altered gene expressions and were predominantly distributed in the biological process of metabolism, immune system, and signal transduction. The KEGG database pathway analysis suggested that the predominant signaling pathways were associated with Ca^{2+} signaling and ligand-receptor interaction.

Subsequent to establishing a correlation matrix lncRNA and mRNA co-expression network map, a total of 98 nodes, 75 connections, 53 lncRNAs, and 45 mRNAs were identified. Gsta2 and Ddx43 were regulated by five lncRNAs, Ccdc68, Olfr464 and Lrp11 by four lncRNAs, Prkar2b, Lgr6, and Adipoq by three lncRNAs. Gsta2 participates in cytochrome P450 metabolism and cytochrome P450 is important in the conversion of androgens to estrogens. P2rx7, Olfr464, Lrp11, Prkar2b, Lgr6, and Adipoq are involved in cellular signal transduction and P2rx7, Prkar2b, and Hdac1 are involved in regulation of the cell cycle and cell proliferation, while Prkar2b, Hdac1, and Dock7 are important in cell development. lncRNA A-30-P01019163 and P2rx7 were differentially expressed in ovarian tissues of *CRYBB2* knockout mice.

Crybb2 not only functions to maintain lens transparency and refractive index, but also affects ovary development in mice. At the gene level, Crybb2 may mediate Ca^{2+} signal transduction (21,22). The current study demonstrated that Crybb2 also regulates expression of lncRNAs in ovarian tissues, which may be a novel area of research on Crybb2-regulation of gene expression. Previous studies

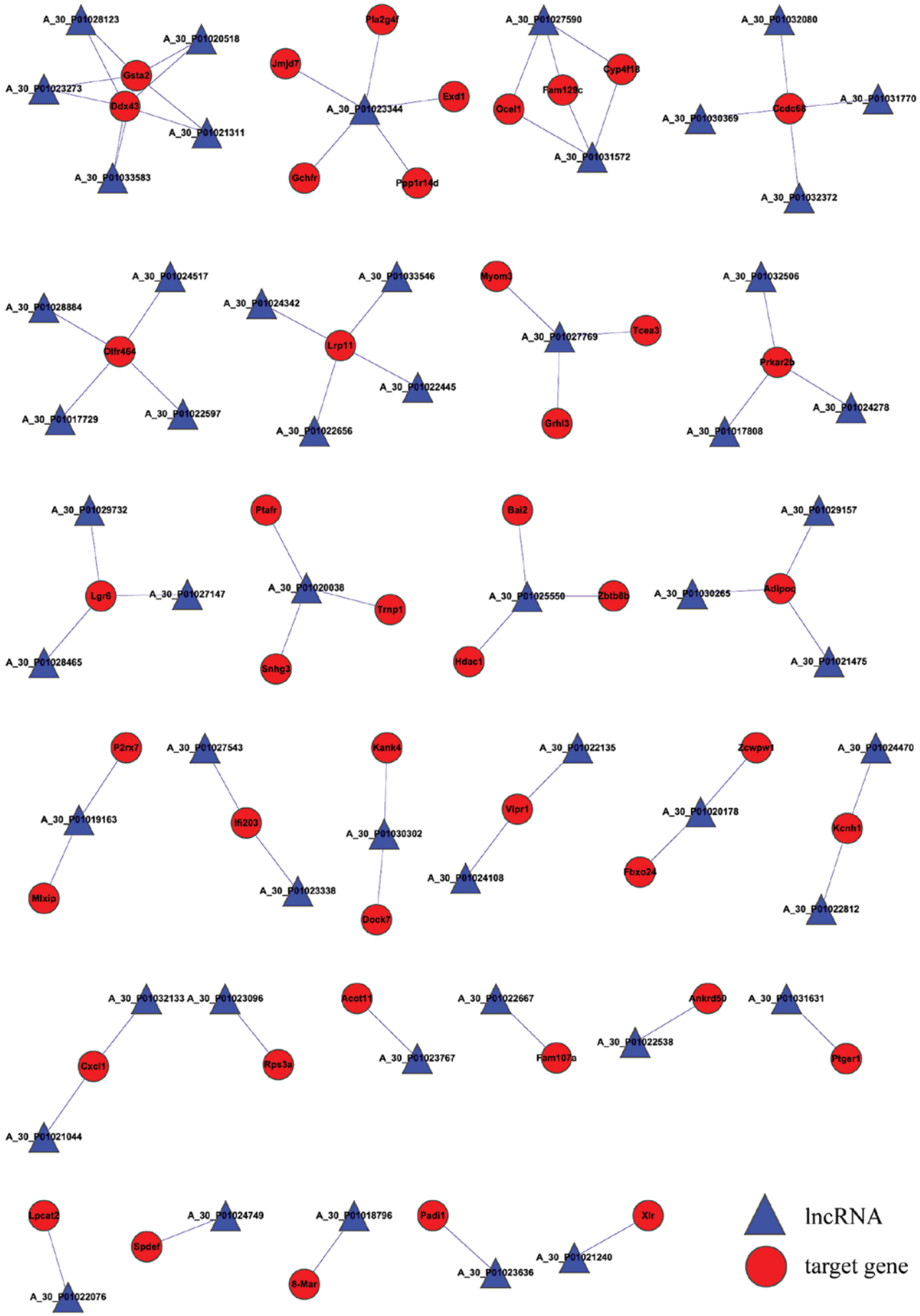

Figure 2. lncRNA-mRNA regulatory network. Circles represent genes and triangles represent lncRNAs; lines represent the association between the two types of regulation (solid line represents a *cis*-regulation, the dashed line represents the *trans*-regulation). lncRNA, long non-coding RNA.

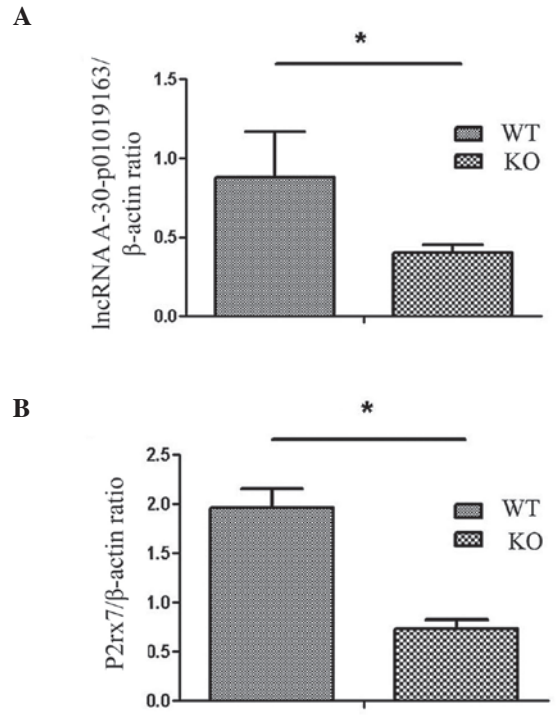

Figure 3. Validation of differentially expressed lncRNAs and mRNAs in ovary tissues from WT and *CRYBB2* knockout mice. (A) RT-qPCR indicated lncRNA A-30-P01019163 was downregulated in ovary tissues from *CRYBB2* KO mice; (B) RT-qPCR indicated P2rx7 was downregulated in ovary tissues from the *CRYBB2* KO mice. *P<0.05. RT-qPCR, reverse transcription-quantitative polymerase chain reaction; KO, knock out; WT, wild-type.

using large scale cDNA library sequencing and next generation sequencing demonstrated abundant lncRNAs in mammals, however, not all lncRNAs are functional and only a relatively small proportion have been demonstrated to be biologically relevant (11,23). For example, as of mid-2014, 197 lncRNAs have been functionally annotated in lncRNA databases (24). However, other lncRNAs may be translated into proteins (25). In addition, lncRNAs, unlike miRNAs, can down- or upregulate gene expression by targeting transcriptional activators or repressors (10), in addition to post-transcriptional regulation of protein expression.

In the current study differentially expressed lncRNAs in ovary tissues of *CRYBB2* knockout mice were profiled and a total of 157 differentially expressed lncRNAs were observed. Furthermore, differentially expressed mRNAs were profiled and a total of 1,085 differentially expressed mRNAs in ovary tissues of *CRYBB2* knockout mice were observed. GO and KEGG pathway analyses were performed to determine associations between these lncRNAs and mRNAs, and a number of these were subsequently verified using RT-qPCR. It was observed that lncRNA A-30-P01019163 and P2rx7 were significantly differentially expressed between10 cases of ovary tissues from WT and *CRYBB2* knockout mice. Thus, *CRYBB2* knockout could downregulate expression of lncRNA A-30-P01019163 and, subsequently, suppress expression of the downstream gene P2rx7 and affect ovarian cell signal transduction, cell cycle, and ultimately ovarian development.

The current study is a proof-of-principle study and there are certain limitations. For example, it was not confirmed how lncRNA A-30-P01019163 regulates P2rx7 expression and the mechanistic investigation into how lncRNA A-30-P01019163-regulated P2rx7 expression mediates the effects of *CRYBB2* on ovary development could be expanded on.

In conclusion, CRYBB2 regulates expression of different lncRNAs to influence ovary development. lncRNA A-30-P01019163 may affect ovarian cell cycle and proliferation by regulating P2rx7 expression in the ovary.

Acknowledgements

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