The Identification And Functional Analysis Of Circrnas In Endometrial Receptivity Of Mice With Polycystic Ovary Syndrome

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Abstract

Background: The disorders of endometrial receptivity and ovulatory dysfunction are both important causes of infertility of patients with Polycystic Ovary Syndrome(PCOS) .The study aimed to investigate the expression profile and functional analysis of circRNAs in the endometrial receptivity of mice with PCOS.

Methods: Twenty-four 4-week-old female C57BL/6 mice were randomly divided into two groups(PCOS group and Normal control group,n=12) and PCOS group was subcutaneously with DHEA 6mg/kg for 20 days.The circRNA expression profile in the endometrium tissue of two groups on the 4th day of gestation was screened by gene chip technique.Bioinformatics analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were performed to preliminary understand the functions of circRNAs. The expression of selected circRNA was verified by RT-PCR and and circRNA–microRNA(miRNA)–messenger RNA (mRNA) negative correlation network analyses was construct by Cytoscape.

Result: Compared with mice in NC group,there are 205 differentially expressed circRNAs significantly in mice of PCOS group,including 147 up-regulated and 58 down-regulated circRNAs(fold change [FC] $\geq$ 1.5 and p value < 0.05). GO analysis demonstrated that the host genes of differentially regulated circRNAs were mainly related to protein binding,cellular process and cellular anatomical entity.KEGG analysis revealed that these source genes were engaged in the Proteoglycans in cancer,Cell junctions (focal adheren and gap junction) ,Pathways in cancer,Ras signaling pathway,Chemokine signaling pathway autophagy and PI3K-Akt signaling pathway.qRT-PCR results showed that the expression levels of circRNA_38548 (P<0.05), circRNA_001686(P<0.05) and circRNA_38550(P<0.01) in PCOS group were significantly higher than those in normal group,while the expression level of circRNA_27938 in PCOS group was significantly lower than that in control group (P<0.05). The expression of circRNA_29710 (P=0.051) and circRNA_34429 (P=0.892) showed no significant difference between the two groups.We used target prediction software(TargetScan and miRanda) to predict the target genes of circRNA_38548, circRNA_001686, circRNA_38550 and circRNA_27938.The circRNA-miRNA-mRNA regulatory network diagram was constructed using Cytoscape software,involving 4 circRNAs (circRNA_38548, circRNA_38550, circRNA_001686, circRNA_27938) and 17miRNAs (miR-214-3p,miR-532-5p and miR-432,etc.) and 136mRNAs(Lifr, FOXK1, FOXO1, HOXA10, etc.).

Conclusions: The circRNAs were aberrantly expressed in the implantation window of PCOS mice,suggesting that differentially expressed circRNA may play a role in the endometrial receptivity of PCOS. We validated four circRNAs(circRNA_38548, circRNA_38550, circRNA_001686 and circRNA_27938),which may contribute to the abnormal endometrial receptivity of PCOS by regulating the expression of Lifr, FOXK1, FOXO1, HOXA10 and other genes through targeting mir-214-3p, mir-532-5p, mir-423 and other miRNAs. These four circRNAs are worthy targets for further study of the related mechanisms.

Introduction

Polycystic ovary syndrome (PCOS), the incidence of which is 5%~10%, is a common reproductive endocrine and metabolic disease. It is characterized by oligomenorrhea or anovulation, polycystic ovaries and hyperandrogenism, usually accompanied by insulin resistance and obesity[1]. Ovulation disorders/anovulation is an important cause of PCOS-related infertility, affecting 40% of women of reproductive age all over the world[2]. With the development of technology, PCOS patients can obtain high-quality fertilized eggs with assisted reproductive technology (ART). However, studies found that the success rate of transplantation are lower and the spontaneous abortion are higher among the women with PCOS comparing to non-PCOS patients[3-4]. Unexpectedly, using the oocytes donated by PCOS women will not lower fertilization, implantation and clinical pregnancy rates[5]. Several pathophysiological changes in PCOS, like oligomenorrhea, hyperandrogenism, hyperinsulinemia, abnormal luteinizing hormone (LH) secretion and inflammation has been confirmed to compromise endometrial health in the long term, including dysfunctional endometrial receptivity (ER)[6-7]. Endometrial receptivity refers to the status of the uterus when the endometrium is in synchrony with development of the blastocyst and is available to accept the blastocyst for adhesion, invasion and implantation[8]. The decrease of endometrial receptivity is one of the most important causes of infertility or pregnancy loss.

Circular RNA (circRNA) is a class of new-found endogenous noncoding RNA. Different from linear RNA molecules, circRNA is a covalently closed continuous loop without 5' caps and 3' poly A tails[9-10]. For its special molecular structure, circRNA is more stable and conserved, making it a more suitable biomarker than other RNA types[11-12]. Recent evidences have demonstrated that circRNAs have many biological functions, including regulation of host gene expression in a cis-acting manner, regulation of miRNA target genes by serving as miRNA sponges, translating proteins, or interacting with proteins. circRNAs have been reported to be involved in a series of pathological and physiological processes, such as various malignant cancer, cardiovascular disease and endocrine dysfunctions[13-14]. Recently, studies have revealed that circRNA plays a critical role in the construction of endometrial receptivity and embryo implantation. Yuxuan Song found that a number of circRNAs were differentially expressed in the endometrium of dairy goats and suggested circRNAs might be potential regulators of receptive endometrium at the post-transcriptional level[15]. Zhang L et al. demonstrated that the apoptosis and proliferation of endometrial epithelial cell is regulated by a ciR8073-miR181a-Neurotensin Pathway during Embryo Implantation[16]. Besides, circ-8073 was found to regulate CEP55 by sponging miR-449a to promote EEC proliferation via the PI3K/AKT/mTOR pathway in dairy goats, suggesting that circRNAs could function as a regulator in the development of endometrial receptivity[17]. On the whole, circRNAs are involved in endometrial receptivity, but the expression profile and functional analysis of circRNAs have not been reported in the endometrial receptivity of PCOS.

Considering the difficulty of obtaining the endometrium of PCOS patients at the implantation window, and the mouse uterus on the fourth day of pregnancy is in the opening period of embryo implantation window and is a good model for the study of endometrium receptivity. Hence, we identified and characterized the expression profiling of circRNAs in the endometrial tissues in early pregnant PCOS mice on Day 4. We also

conducted a series of bioinformatic analyses, such as Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes(KEGG), and constructed the ceRNA network analysis to help us explore potential functions and search for the key circRNAs in the endometrial receptivity of polycystic ovary syndrome.

Methods And Materials

Animal experiment

Four-week-old female C57BL/6 mice were provided by ZhaoYan (Suzhou,China) and kept in a pathogen-free environment with a animal facility ($25 \pm 1^{\circ}\text{C}$, 14-hr light–10-hr dark cycle) of Soochow University. All mice had free access to water and received standard laboratory chow. In this experiment, 24 female mice were randomly divided into two groups of 12 rats each, including PCOS group (P) and NC group(N). The PCOS group mice were subcutaneously injected with DHEA ($6\text{mg}\cdot 100\text{g}\cdot 1\cdot \text{day}^{-1}$) dissolved in the sterile soybean oil solution(0.2ml) for 20 consecutive days[18]. The NC group were subcutaneously injected with sterile soybean oil(0.2ml) for 20 consecutive days.

From the tenth day of establishing the PCOS model, vaginal smears were collected daily and the determination of estrous cycle was evaluated microscopically with hematoxylin-eosin (HE) staining. On the 21 day, all mice were mated with fertile males(ZhaoYan Suzhou,China) of the same strain to induce pregnancy at five in the afternoon. The identification of vaginal plug or the presence of spermatozoa in the vaginal smears was considered to indicate Day 0 of pregnancy. Upon the corroboration of pregnancy, female mice were separated from their male partners. On Day 4 of pregnancy, the mice were killed by the cervical dislocation to remove the embryo ,collect endometrial samples by squeezing and obtain ovaries. The ovaries were fixed in 10% paraformaldehyde for 48h, and then embedded in paraffin, sectioned at $5\mu\text{m}$, stained with hematoxylin and eosin and analyzed using an light microscopy. One sample set is mixed by the same endometrial samples of 3 mice. These samples were immediately cryopreserved in liquid nitrogen overnight and then stored in -80°C refrigerator for gene chip sequencing and RT-PCR verification.

CircRNA microarray analysis

Using TRIzol reagent to isolate the total RNAs from endometrial samples (15,596,026, Life Technologies, Carlsbad, USA). Liner RNAs were digested with Rnase R(Epicentre, Inc.) to enrich circular RNAs. We utilized a random priming method (Arraystar Super RNA Labeling Kit; Arraystar) to amplify the enriched circular RNAs and transcribe it into fluorescent cRNA. The cRNAs were hybridized onto the Arraystar Mouse circRNA Array V2 ($8\times 15\text{K}$, Arraystar) and scanned by the Agilent Scanner G2505C. The array images were analyzed by Agilent Feature Extraction software (version 11.0.1.1). The quantile normalization and subsequent data processing were performed by the R software limma package. Differentially expressed circRNAs with statistical significance between two groups were identified through Volcano Plot filtering. Hierarchical Clustering was performed to show the distinguishable circRNAs expression pattern among samples.

Quantitative real-time polymerase chain reaction (qRT-PCR) validation

In this study, six randomly selected differentially expressed circRNAs were validated by qRT-PCR, and the primer sequences used are listed in Table 1. Endometrial samples were treated using TRI Reagent (Sigma: T9424) for total RNA extraction. Then, using SuperScript™ III Reverse Transcriptase kit (Invitrogen: 18080-044) for reverse transcription. All primers were synthesized by Generay (Shanghai, China). The expression of the circRNAs was confirmed by qRT-PCR using ViiA 7 Real-time PCR System (Applied Biosystems). Glyceraldehyde phosphate dehydrogenase (GAPDH) was amplified as an internal control, and the relative expression level of each circRNA was calculated with $-\Delta\Delta 2^{-Ct}$.

Bioinformatics Analysis

The GO analysis was performed to acquire meaningful annotation of genes and gene products in a wide variety of organisms. The ontology covers three domains: Biological Process, Cellular Component and Molecular Function. KEGG pathway analysis is a functional analysis to reveal the circRNA-mediated biological processes and signaling pathways. Evidences have demonstrated that circRNA plays a crucial role in fine tuning the level of miRNA-mediated regulation of gene expression by sequestering the miRNAs. Their interaction with disease-associated miRNAs indicates that circRNAs are important for disease regulation. To infer the potential biological functions of circRNAs, the host genes of differentially expressed circRNAs were used for Gene Ontology (<http://www.geneontology.org/>) and KEGG pathway analysis (<http://www.genome.jp/kegg/>). The circRNA-miRNA and miRNA-mRNA interaction was predicted with Arraystar's home-made miRNA target prediction software based on TargetScan & miRanda. The ceRNA networks were visualized by Cytoscape.

Statistical analysis

Data analysis in this study was conducted using SPSS 23.0. Student's t-test analysis was performed for the comparisons between P group and C group. $P < 0.05$ was considered as the statistically significant difference. All data was shown as the $\bar{x} \pm s$.

Results

Characterization of PCOS by histological analysis

According to the results of vaginal smears and the characteristics of the vulva during different estrus cycles, mice estrous cycles include proestrus, estrus, metestrus, and diestrus. The estrous cycle of the NC group showed regular periodic changes, while the PCOS group is continuously in the diestrus. (Fig. 1). Compared with the control group, the volume of ovary in model animals increased and the surface of ovary appeared polycystic change by visual inspection. Histological observation showed that the number of atretic follicles, vesicular follicles and degenerated granulosa cells was significantly higher, and the corpus luteum and dominant follicles was obviously reduced in the PCOS group (Fig. 2), which was consistent with relevant literature reports [18].

Identification of differentially expressed circRNAs

In total, 14,193 differentially expressed circRNAs were identified in mice uterus samples from two groups. There are 206 circRNAs with exhibited significant differences ($FC \geq 1.5, p < 0.05$), among which 148 and 58 were up- and down-regulated. The box plot demonstrates that the intensities of the 6 samples were nearly the same after normalization (Fig. 3a). Distinguishable circRNA expression patterns were displayed through a volcano plot (Fig. 3b) and hierarchical clustering plot (Fig. 3c). The distribution of the circRNAs on the chromosomes was depicted (Fig. 3d). The circRNAs are classified into 5 types: "exonic", "intronic", "antisense", "sense overlapping" and "intergenic", most of the differentially expressed circRNAs in uterus during implantation window arise from the exons of the linear transcript (Fig. 3e). Based on the absolute value of FC, the top 10 upregulated and downregulated circRNAs were shown in Table 2.

qRT-PCR validation

According to P-value, fold change value and the genomic origin of the circRNAs, we chose six circRNAs (circRNA_38548, circRNA_38550, circRNA_001686, circRNA_27938, circRNA_22927 and circRNA_34429) for further RT-PCR verification. The results showed that the expression levels of circRNA_38548 ($P < 0.05$), circRNA_001686 ($P < 0.05$) and circRNA_38550 ($P < 0.01$) in PCOS group were significantly higher than those in normal group, while the expression level of circRNA_27938 in PCOS group was significantly lower than that in control group ($P < 0.05$). The expression of circRNA_29710 ($P = 0.051$) and circRNA_34429 ($P = 0.892$) showed no significant difference between the two groups. (Fig. 4).

Bioinformatics Analysis

GO and KEGG pathway enrichment analysis were performed on the host genes of differentially expressed circRNA (Fig. 5a). The result shows that the top 10 enriched BP, CC, MF terms and top 12 KEGG pathways. The enriched BP terms were mainly related to cellular process, transport, biological regulation, cellular localization and metabolic process. The most enriched CC was the cellular anatomical entity, intracellular, cytoplasm, organelle, and intracellular organelle. For GO-MF terms, protein binding, binding, ion binding, anion binding, small molecule binding and identical protein binding were enriched. KEGG pathway enrichment analysis found that DEGs were mainly related to carcinogenic processes, such as Proteoglycans in cancer, Pathways in cancer, Focal adhesion, Pathways in cancer, Ras signaling pathway, PI3K-Akt signaling pathway, and so on (Fig. 5b). These pathways are involved in the establishment and maintenance of endometrial receptivity. Hence, the results implied that vital biological functions regulated by the host linear transcripts of differentially expressed circRNAs might be closely associated with the pathogenic mechanism of defective endometrial receptivity in PCOS patients.

Construction of circRNA-miRNA-mRNA negative correlation networks

We obtained a total of 1818 target miRNAs and 1841 target mRNAs from the miRanda and TargetScan databases and screened 17 miRNAs and 136 mRNAs from target genes of circRNA_38548

circRNA_38550 circRNA_001686 and circRNA_27938. To visualize the interactions between molecules, we built circRNA-miRNA-mRNA negative correlation networks based on bioinformatics. The results suggest that circRNAs may be involved in the pathogenesis of impaired endometrial receptivity through interactions with these miRNAs (Fig. 6).

Discussion

Impaired endometrial receptivity is one of the most important causes of PCOS-related infertility [19]. Some of the pathophysiological changes associated with PCOS have been shown to be associated with reduced endometrial receptivity [20]. However, it has never received the same attention as ovulation dysfunction and the exact mechanism still remains unclarified. Our study is the first to profile the expression patterns of circRNAs in the receptive endometrium in the PCOS mice. The results showed a change in the expression patterns of circRNAs between the two groups. There were 3090 circRNAs were abnormally expressed in the endometrial receptivity of PCOS mice, and 205 circRNAs were significantly different ($P < 0.05$ and FC value ≥ 1.5). Compared with the control group, there were 147 up-regulated and 58 down-regulated circRNAs, suggesting that these circRNAs may be closely related to the infertility with PCOS. Six randomly selected circRNAs (circRNA_38548, circRNA_38550, circRNA_001686, circRNA_27938, circRNA22927 and circRNA34429) were validated by RT-PCR validation. The result presented that relative expression levels of this circRNAs (circRNA_38548 circRNA_38550 circRNA_001686 circRNA_27938 circRNA22927 and circRNA34429) were consistent with the results of the original microarray analysis, and circRNA_38548 circRNA_38550 circRNA_001686 circRNA_27938 expressed with statistical significance.

Of the top 10 GO terms, we found the enriched BP terms were mainly related to cellular process, transport, biological regulation, cellular localization and metabolic process, the most enriched CC was the cellular anatomical entity, intracellular, cytoplasm, organelle, and intracellular organelle. For GO-MF terms, protein binding, binding, ion binding, anion binding small molecule binding and identical protein binding were enriched most. The KEGG analysis results indicated that the target genes contributed to many pathways, the top 15 of which are pathways in cancer, cell junctions (focal adhesion and gap junction), chemokine signaling pathway, autophagy, PI3K-Akt signaling pathway and Ras signaling pathway. Some signaling pathways have been found to be involved in regulating endometrial receptivity and embryo implantation. Endometrium periodically changes during the menstrual cycle, which is regulated by the gap junction between the endometrial cells [21]. The gap junction proteins of endometrial stromal cells are the most important units of gap junction, involving in many important cellular events, such as cell growth, differentiation, tumorigenesis, neoangiogenesis, apoptosis, etc [22]. The abnormal expression of connexins in the endometrial stromal cells might lead to abnormal differentiation of uterine stromal cells and impaired production of several key angiogenic factors, including vascular endothelial growth factor (VEGF), leading to the endometrial dysfunction and embryo implantation failure [23]. Elevated levels of inflammatory mediators like IL-6 and TNF- α were reported in endometrium of PCOS women [24], phosphorylating insulin receptor substrate-1 serine through PI3K-Akt pathway, hinder insulin signal transduction and affect endometrial glucose metabolism [25], which may be one of the reasons for impaired endometrial receptivity in PCOS. Except for the example we gave

above, MAPK pathway [26] and Ras pathway [27] were both found to be related to embryo implantation and pregnancy maintenance. Their role in PCOS endometrial receptivity disorder needs further study. Having said all of above, we can speculate that the abnormally expressed circRNAs, whose host genes are concerned with the pathways KEGG analysis mentioned, may damage endometrial homeostasis and impact endometrial receptivity in PCOS patients significantly.

Based on the results of qRT-PCR analysis, we constructed a novel circRNA-related ceRNA network containing 4 circRNAs, 17 miRNAs and 136 mRNAs. Recently, since it has been found abnormality expressed in many tumors and closely related to invasion, metastasis and prognosis of the malignancy, miR-214-3p, miR-532-5p and miR-432 might be regarded as a clinical biomarker and therapeutic target [28-30]. A foreign study [31] showed that there is a similar molecular mechanism between the embryo implantation and cancer metastasis in many mammals, including human, involving cell proliferation and apoptosis, immune avoidance and angiogenesis stimulation. In combination with the signaling pathways involved in the above biological analysis, we suspected that circRNAs whose host genes may affect endometrial receptivity by regulating the capabilities of proliferation, apoptosis, migration and permeability of endometrium.

Mammalian target of rapamycin (mTOR) is a highly conserved serine/threonine kinase that modulates cell metabolism, growth and proliferation in humans [32]. Rapamycin-insensitive companion of mTOR (Rictor), the host gene of circRNA_38548, is a core ligand for mTOR. Rictor is highly expressed in several malignancies, like hepatocellular carcinoma (HCC) [33]. A recent study found that Rictor is a key factor in the establishment of uterine receptivity, reduced expression of Rictor is closely associated with disorders of endometrium epithelial transformation and fluid absorption [34]. Insulin-like growth factor 1 receptor (IGF1R) is expressed in the epithelium and stroma of the endometrium [35] and plays important roles in mediating the growth and differentiation of endometrial cells. It has been reported that IGF1R is upregulated in the endometrial epithelium at the time of endometrial receptivity and reduced expression of IGF1R in epithelial cells destabilizes embryo attachment [36]. IGF1R also has been discovered decreased in the endometrium of women with endometriosis, leading to decreased proliferation of endometrial stromal cells [37]. This explains why endometriosis patients have impaired endometrial preparation and receptivity. The results of qRT-PCR showed that circRNA_27938 was downregulated in PCOS mice endometrium during implantation window. We speculated that one reason for the defective endometrial receptivity of PCOS may be related to decreased IGF1R caused by downregulated circRNA_27938. It would be worthwhile to further explore the role of circRNA_27938 in endometrial lesions of PCOS. We also found tumor necrosis factor (TNF), homeobox protein (HOX) A10, factor Forkhead Box O1 (FOXO1) and leukemia-inhibitory factor (LIF) contained in the ceRNA network, all of which have been regarded as endometrial receptivity markers [38-39]. These indicators also have complicated changes in patients with PCOS. One recent study showed decreased gene expression of HOXA-10, HOXA-11, and LIF in endometrial receptivity of patients with PCOS, which might contribute PCOS-related infertility [40].

Conclusion

In general ,we analyzed the circRNA landscape by microarray analysis in PCOS mice uterus during implantation window.Four circRNAs were verified by RT-PCR and ceRNA network was constructed,which laid a foundation for further research on the regulation and control mechanism of circRNAs in infertility caused by PCOS.

Declarations

Ethics approval and consent to participate

The care and experimental treatment of the animals were approved by the Animal Research Committee of Soochow University(Approval No.SUDA20211218A01) and all applicable institutional and governmental regulations concerning the ethical use of animals were followed.We promise that the study is reported in accordance with ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Zi-wei Zhao, Da-wei Li and Nan Wang designed the study and performed the data analyses.

Zi-wei Zhao carried out the experiments and wrote the manuscript;

Yan ping Pan, Li Xu and Yu-jing Weng contributed significantly to the experiments, data collection and manuscript preparation;

Wei-qin Zhou, Cai-ping Mao helped perform the analysis with constructive discussions.

All authors reviewed the manuscript.

Zi-wei Zhao, Da-wei Li and Nan Wang contributed equally to this work.

Data availability

The RNA-Seq data generated during the current study are available in the GEO database (GSE 195605) (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE195605>). All other relevant data is available within the manuscript or its accompanying supplementary material.

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References

1. Nawrocka J, Starczewski A. *Effect of metformin treatment in women with polycystic ovarian syndrome depends on insulin resistance*. Gynecol Endocrinol. 2007;23 (4):231–237.
2. Eden JA. *The polycystic-ovary-syndrome presenting as resistant acne successfully treated with cyproterone-acetate*. Med J Aust. 1991;155(10):677–80.
3. Steiner N, et al. *A comparison of IVF outcomes transferring a single ideal blastocyst in women with polycystic ovary syndrome and normal ovulatory control*. Arch Gynecol Obstet. 2020;8.
4. Tang Kefu et al. *In vitro fertilization outcomes in women with polycystic ovary syndrome: A meta-analysis*. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2021; 259: 146–152.
5. George Queiroz Vaz, Alessandra Viviane Evangelista, Cassio Alessandro Paganoti Sartorio, Maria Cecilia Almeida Cardoso, Maria Cecilia Erthal, Paulo Gallo, Marco Aurelio Pinho Oliveira. *Are Patients with Polycystic Ovarian Syndrome Ideal Candidates for Oocyte Donation?*. BioMed Research International, 2016; Article ID 5701609.
6. Terhi T. Piltonen, *Polycystic ovary syndrome: Endometrial markers*. Best Practice & Research Clinical Obstetrics & Gynaecology. 2016;37:66–79.
7. Bai XC, Zheng LW, Li DD and Xu Y. *Research progress of endometrial receptivity in patients with polycystic ovary syndrome: a systematic review*. Reproductive biology and endocrinology. 2021; 19(1):122–122.
8. Lessey BA, Young SL. *What exactly is endometrial receptivity?* Fertil Steril. 2019; 111(4):611–617.
9. Kolakofsky D. *Isolation and characterization of Sendai virus DI - RNAs*. Cell, 1976;8(4):547–555.
10. Chen LL. *The biogenesis and emerging roles of circular RNA*. Nat Rev Mol Cell Biol. 2016;17:205–211.
11. Jeck WR, Sorrentino JA, Wang K, et al. *Circular RNAs are abundant, conserved and associated with ALU repeats*. RNA. 2013;19:141–157.
12. Lasse S. Kristensen et al. *The biogenesis, biology and characterization of circular RNAs*. Nature Reviews Genetics. 2019;20(11):675–691.
13. Bao C, Lyu D, Huang S. *Circular RNA expands its territory*. Mol Cell Oncol. 2016;3:e1084443
14. Wang, Y., Liu, J., Ma, J. et al. *Exosomal circRNAs: biogenesis, effect and application in human diseases*. Mol Cancer. 2019;18:116.

15. Song,Y., Zhang,L.,Liu,X.*et al.**Analyses of circRNA profiling during the development from pre-receptive to receptive phases in the goat endometrium.*J Animal Sci Biotechnol.2019;10(03):633–647.
16. Zhang L,Liu X,Che S,*et al.**Endometrial Epithelial Cell Apoptosis Is Inhibited by a ciR8073-miR181a-Neurotensis Pathway during Embryo Implantation.*Mol Ther Nucleic Acids.2019;14:262–273.
17. Xiaorui Liu, Lei Zhang, Yuexia Liu, Jiuzeng Cui, Sicheng Che, Xiaopeng An, Yuxuan Song, Binyun Cao.*Circ-8073 regulates CEP55 by sponging miR-449a to promote caprine endometrial epithelial cells proliferation via the PI3K/AKT/mTOR pathway.*Biochimica et Biophysica Acta (BBA) - Molecular Cell Research.2018,1865(8):1130–1147.
18. Li,SY.,Song,Z.,Song,MJ.*et al.**Impaired receptivity and decidualization in DHEA-induced PCOS mice.*Sci Rep.2016;6:38134.
19. Schulte MM, Tsai JH, Moley KH. *Obesity and PCOS: the effect of metabolic derangements on endometrial receptivity at the time of implantation.* Reproductive sciences. 2015;22(1):6–14.
20. Palomba S, Piltonen TT, Giudice LC. *Endometrial function in women with polycystic ovary syndrome: a comprehensive review.* Human reproduction update. 2020;27:584–618.
21. B. Risek, F.G. Klier, A. Phillips, D.W. Hahn, N.B. Gilula.*Gap junction regulation in the uterus and ovaries of immature rats by estrogen and progesterone.*J Cell Sci.1995;108:1017–1032.
22. Laws MJ, Taylor RN, Sidell N,*et al.**Gap junction communication between uterine stromal cells plays a critical role in pregnancyassociated neovascularization and embryo survival.*Development.2008, 135 (15):2659–2668.
23. Jie Yu,Sarah L.Berga,Wei Zou,He-Ying Sun,Erika Johnston-MacAnanny,*et al.**Gap Junction Blockade Induces Apoptosis in Human Endometrial Stromal Cells.*MOLECULAR REPRODUCTION AND DEVELOPMENT.2014,8(17):666–675.
24. Ebejer K, Calleja-Agius J. *The role of cytokines in polycystic ovarian syndrome.*Gynecol Endocrinol. 2013;29(6):536–40.
25. Oróstica L,Poblete C,Romero C,Vega M.*Pro-inflammatory markers negatively regulate IRS1 in endometrial cells and endometrium from women with obesity and PCOS.* Reprod Sci.2020;27(1):290–300.
26. Xie Ke-Ming,*et al.**NME1 at the human maternal-fetal interface downregulates titin expression and invasiveness of trophoblast cells via MAPK pathway in early pregnancy.*Reproduction.2010;139(4):799–808.
27. Lumbers Eugenie R,*et al.**Decidualisation of human endometrial stromal cells is associated with increased expression and secretion of prorenin.*Reproductive biology and endocrinology:RB&E.2015;13(1):129.
28. Fang YY, Tan MR, Zhou J, *et al.**miR-214-3p inhibits epithelial-to-mesenchymal transition and metastasis of endometrial cancer cells by targeting TWIST1.*Onco Targets Ther.2019;12:9449–9458.
29. Huang C, Yu W, Wang Q, Huang T, Ding Y.*CircANTXR1 Contributes to the Malignant Progression of Hepatocellular Carcinoma by Promoting Proliferation and Metastasis.*JOURNAL OF HEPATOCELLULAR CARCINOMA.2021;8:1339–1353.

30. Wang,Yong; Xu,Wei; Zu,Maoheng; Xu, Hao.Circular RNA circ_0021093 regulates miR-432/Annexin A2 pathway to promote hepatocellular carcinoma progression,.Anti-Cancer Drugs.2021;32(5):484–495.
31. Kshitiz et al.Evolution of placental invasion and cancer metastasis are causally linked[J].Nature Ecology&Evolution.2019;3(12):1743–1753.
32. Harris TE, Lawrence JC Jr. TOR signaling. Sci STKE. 2003;2003(212): re15.
33. Dong X, Feng M, Yang H, Liu H, Guo H, Gao X, Liu Y, Liu R, Zhang N, Chen R, Kong R.Rictor promotes cell migration and actin polymerization through regulating ABLIM1 phosphorylation in Hepatocellular Carcinoma.IntJ Biol Sci.2020;16(15):2835–2852.
34. Yue Zhang,Xinman Du,Xuemei Chen,Hongyu Tang,Qin Zhou,Junlin He,Yubin Ding,Yingxiong Wang,Xueqing Liu,Yanqing Geng.Rictor/mTORC2 is involved in endometrial receptivity by regulating epithelial remodeling.FASEB JOURNAL.2021;35(7):e21731.
35. Zhou J.,Dsupin B. A, Giudice L. C., BondyC. A.Insulin-like growth factor system gene expression in human endometrium during the menstrual cycle.Clin. Endocrinol. Metab.1994;79: 1723–1734.
36. Youn-Jung Kang,Miranda Lees,LauraC. Matthews,Susan J. Kimber,Karen Forbes,John D.Aplin.miR-145 suppresses embryo–epithelial juxtacrine communication at implantation by modulating maternal IGF1R.J Cell Sci.2015;128 (4):804–814.
37. Sanaz Ghazal,Brett McKinnon,Jichun Zhou,Martin Mueller,Yi Men,Lihua Yang,Michael Mueller,Clare Flannery,Yingqun Huang,Hugh S Taylor.H19 lncRNA alters stromal cell growth via IGF signaling in the endometrium of women with endometriosis.EMBO Mol Med.2015;7:996–1003.
38. L.Wang,S.Lv,W.Mao,et al.Assessment of endometrial receptivity during implantation window in women with unexplained infertility.Gynecol Endocrinol.2020;36:917–921.
39. Nancy Ashary, Saniya Laheri and Deepak Modi.Homeobox genes in endometrium: from development to decidualization.INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY.2020;64: 227–237
40. Kara M,Ozcan SS,Aran T, Kara O,Yilmaz N.Evaluation of endometrial receptivity by measuring HOXA-10, HOXA-11 and leukemia inhibitory factor expression in patients with polycystic ovary syndrome. Gynecology and minimally invasive therapy.2019;8(3):118–122.

Tables

Tables 1-2 are available in the Supplemental Files section.

Figures

Figure 1

estrous cycle changes in PCOS group and NC group

Figure 2

Morphological observation of ovarian tissue in PCOS mice

a:NC group,b:PCOS group(HE×40)

c:NC group,d:PCOS group(HE×200)

Figure 3

Differentially expressed circRNAs in uterus of PCOS mice during implantation window (n=3).a:Box plot demonstrating the intensities of 6 samples after normalization;b c:Volcano plot and hierarchical clustering plot demonstrating a distinguishable circRNAs expression pattern in mice uterus samples from two groups. d The chromosomal distribution of all differentially expressed circRNAs e: The categories of differentially expressed circRNAs.

Figure 4

Real-time quantitative PCR verification

Figure 5

GO and KEGG pathway analyses of the host genes of the differentially expressed circRNAs