O-217 miR-181a promotes mouse granulosa cell apoptosis by suppressing sirt1-mediated foxo1 deacetylation

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Study question: Granulosa cell apoptosis plays a vital role in follicular atresia and the aim of the present study is to investigate the function of miR-181a in granulosa cell apoptosis.

Summary answer: Our results suggest that Sirtuin 1 (Sirt1) is a target gene of miR-181a, and by down-regulating Sirt1, miR-181a promotes Forkhead box O1 (FoxO1) acetylation and mouse granulosa cell (mGC) apoptosis.

What is known already: Previous studies have demonstrated that miR-181a promotes the apoptosis of several types of cells by suppressing target gene expression. Sirt1, one of the target genes of miR-181a, was found to bind to and deacetylate the pro-apoptotic modulator FoxO1, leading to decreased nuclear accumulation of FoxO1 and reduced promoting effect of FoxO1 on cell apoptosis.

Study design, size, duration: mGC was treated with adenovirus containing miR-181a (Ad-miR-181a), Ad-LacZ, antisense oligonucleotides of miR-181a (Ad-miR-181a inhibitor), or negative control for 48 h. HEK293T cells were transfected with the firefly and Renilla luciferase reporter plasmid together with or without the plasmid containing miR-181a for 48 h.

Participants/materials, setting, methods: mGC was collected from 21-day-old ICR mouse ovaries. Cell Death Detection ELISA was performed to test mGC apoptosis. Western blotting was used to measure caspase-3, cleaved-caspase-3, Sirt1, FoxO1, Acetylated FoxO1, and FasL levels. miR-181a's effect on Sirt1 expression of miR-181a for the future research.

Main results and the role of chance: miGC apoptosis was significantly increased by 5-fold (P < 0.01) after overexpression of miR-181a. Western blot results revealed that cleaved-caspase-3 level was apparently increased after overexpression of miR-181a and decreased by miR-181a inhibitor, but cleavage-3 had little change.

Luciferase assay results showed that miR-181a targeted the 3'UTR of Sirt1 mRNA. Sirt1 protein level in mGC was reduced after miR-181a overexpression and increased by miR-181a inhibitor, indicating Sirt1 being a target gene of miR-181a.

Limitations, reason for caution: As all our experiments are performed in vitro, the effect of miR-181a on granulosa cell apoptosis and follicle development in vivo is to be further investigated, and we will construct mouse model for overexpression of miR-181a for the future research.

Wider implications of the findings: The study of miR-181a in mGC apoptosis helps us understand the mechanisms of follicle development at a post-transcriptional level. Our results indicate that aberrant expression of miR-181a in the ovaries may result in accelerated follicular atresia, which will lead to ovarian dysfunction, such as premature ovarian failure. Thus, reduction of miR-181a may provide a potential new strategy for treating ovarian dysfunction.

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Trial registration number: ClinicalTrials.gov study number 01773603
Summary answer: The present study reveals a novel role of Shh in steroidogenesis in human placentas and the possible mechanism governing Shh-induced P4 and E2 production, providing with potentially crucial roles of Shh signaling in pregnant maintenance.

What is known already: In primate placentas, the production of steroid hormones, such as progesterone (P4) and estradiol (E2), is crucial for maintenance of pregnancy and fetal development. Hedgehog (Hh) signaling plays a key role in organogenesis of reproductive system.

Study design, size, duration: In vitro, identity Shh/Gli signaling is involved in human hormones production.

in vivo, study morphology in nude mice (blank, control group, low-dose inhibitor group, high-dose inhibitor group).

Participants/materials, setting, methods: cell/tissue culture, RT-PCR, qRT-PCR, Western Blotting, H&E staining, IHC, Luciferase Assay, Promoter Construct, IF, transfection, lentivirus-mediated gene knock down, retrovirus-mediated gene over-express, nude mice model, ELISA, embryo transplant, human cytotrophoblast isolation and culture, ChiP-Seq, Co-IP, In situ hybridization, protein purification, mass spectrometry, HPLC.

Main results and the role of chance: Shh stimulated the steroidogenic enzymes levels, and induced P4 and E2 production. Infection with smoothoomed-expressing retroviruses increased not only the expression of steroidogenic enzymes, but also the production of P4 and E2, whereas cycloamine markedly decreased the Shh-induced expression of steroidogenic enzymes and steroid hormone production.

By using gene-reporter constructs and CHiP-seq assay, we further identified Shh transactivated aromatase and HSD3b1 gene through Gli2, and Shh transactivated CYP11A1 gene through Gli3. In vivo, Female nude mice were ovariectomized, and subcutaneously inoculated with JEG-3 cells at the left and right flanks of mice. Subcutaneous injection with HH signaling inhibitor, cycloamine, did not affect the weights of JEG-3 cells-derived tumors, but dose-dependently decreased huamn P4 and E2 levels in circulation and uterine tissue responses to E2 and P4.

Limitations, reason for caution: cell transfection, mice treating, virus packaging, embryo transplant.

Wider implications of the findings: The present study provides with potentially crucial roles of Shh signaling in pregnant maintenance.

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Trial registration number: nude mice:150

O-220 MicroRNA 132 promotes estradiol release from mouse granulosa cells by transcriptional repression of Nr4a2

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Study question: This study was to validate the transcriptional induction of microRNA 132 (miR-132) by 8-Br-cAMP treatment in cultured mouse granulosa cells (mGCs) in vitro, and to investigate the effect on estradiol (E2) release from mGCs stimulated by miR-132 as well as the molecular mechanism underlying.

Summary answer: E2 biosynthesis in mGCs could be promoted by miR-132, a cAMP-response microRNA, due to the up-regulation of cytochrome P450 aromatase (Cyp19a1), which was regulated by miR-132 through directly suppressing the expression of an orphan nuclear receptor-Nr4a2.

What is known already: Ovarian steroid hormones such as E2 play important roles in many biological progresses including follicular development, oocyte maturation and endometrial proliferation, while the dysfunction of estrogen synthesis is implicated in the development of polycystic ovary syndrome and premature ovarian failure. MicroRNAs such as miR-132, which are short non-coding RNAs of 20–24 nucleotides, were indentified to regulate the proliferation, apoptosis and differentiation of mGCs through transcriptional repression of mRNAs by targeting the 3′-untranslated region.

Study design, size, duration: mGCs were collected from ovaries of 21-day-old ICR mice through follicle puncture. Cultured mGCs were treated with 8-Br-cAMP for 24 h to detect miR-132 levels or transfected with miR-132 mimics, NR4A2-specific siRNA oligonucleotides, Flag-NR4A2 plasmids and negative controls to compare the hormone secretion and expression changes of related genes.

Participants/materials, setting, methods: Concentrations of E2 and progesterone in culture medium were determined by a chemiluminescence-based assay. Quantitative RT-PCR and western blot were performed respectively to identify the expression levels of miR-132 after 8-Br-cAMP treatment and the effects of miR-132 on Cyp19a1, Cyp11a1 and Nr4a2 in mGCs.

Main results and the role of chance: The expression of miR-132 in cultured mGCs was continuously induced by 8-Br-cAMP and reached the peak level at 12 h (~5-fold increased, P < 0.05). The release of E2 from mGC significantly promoted more than 70% (P < 0.01) after over-expressing of miR-132. However, no significant alteration of progesterone was detected. Real-time PCR exhibited an induction of Cyp19a1 (2.8-fold increased, P < 0.01) by miR-132 instead of Cyp11a1, a key gene for progesterone synthesis. Nr4a2, which represses Cyp19a1 expression thought its PH domain, was down-regulated by miR-132 in protein level without significant transcriptional change. Knock-down of Nr4a2 attenuated the effect of miR-132, ectopic expression of Flag-NR4A2 abrogated the stimulation effect of miR-132 on E2 release.

These findings further supported the induction of E2 synthesis by miR-132 through the repression of Nr4a2.

Limitations, reason for caution: Our in vitro study was limited to the functions of miR-132 in mGC using transient transfection. Further experiments will be needed to determine the effect on human luteinized granulosa cells and whether miR-132 is involved in proliferation or apoptosis. Reproductive functions of miR-132 in vivo remain to be elucidated.

Wider implications of the findings: These findings cast light on the classical cAMP-dependent signaling cascades triggered by follicle-stimulating hormone, which induces estrogen biosynthesis through transcriptional up-regulation of Cyp19a1 in granulosa cells. The effect of miR-132 on E2 stimulation of granulosa cells reveals potential application of miR-132 in the non-steroid drug design for regulating reproduction and treating steroid-related disorders.

Study fundings/competing interest(s): State Key Development Program of Basic Research of China Grant (973 Project No. 2010CB945104); National Natural Science Foundation of China (Nos. 81070508, 30900727, 81070492, 81170570).

Trial registration number: None.
grown under normal oxygen. The present study also demonstrated that ANP potently decreased VEGF mRNA expression by greater than 2-folds and CNP increased in antral follicle growth which suggests a possible pathway for the increases in antral follicle number reported to be mediated by the PDE5-inhibitor, sildenafil citrate. This work also demonstrated a clear inhibitory effect of NPs on VEGF levels which may have important implications for understanding the roles of NPs and VEGF in OHSS and PCOS.

Study funding/competing interest(s): This research project is funded by the University of Nottingham and the Saudi Arabian Cultural Bureau and King Faisal Specialist Hospital and research center.

Trial registration number: No registration number. A trial registration number is only required for clinical trials.

O-223 Anti-mullerian hormone (AMH) has a direct regulatory role on theca cell function
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Study question: Do theca cells have functional AMH receptors whereby AMH may directly regulate thecal androgen production?

Summary question: This work concurs with earlier studies showing AMH regulation of androgen production in theca cells (TC) from a monovulatory species and extends them by demonstrating the expression of the AMH receptor type 2 (AMHR2) by theca cells and the existence of direct AMH/AMHR2 interactions in this cell type.

What is known already: AMH is a widely utilised biomarker of ovarian reserve which is expressed exclusively by the granulosa cells (GC) of growing follicles. The role of AMH during folliculogenesis is poorly understood despite the observation that AMH levels are elevated in women with polycystic ovarian syndrome (PCOS). Recent studies in monovulatory sheep, whilst confirming a negative association between AMH and GC aromatase activity, have also suggested a direct paracrine role for AMH in modulating thecal androgen production.

Study design, size, duration: AMHR2 and AMH mRNA expression levels were compared between freshly collected ovine TC and GC (control) (n = 4). In a parallel study, the AMHR2 and AMH-AMHR2 interactions were studied in theca cells that were cultured for 6 days.

Participants/materials, setting, methods: The expression of AMHR2 and AMH mRNA in TC and GC was determined utilising quantitative real-time (q)PCR. While, AMHR2 and AMH/AMHR2 interactions were visualised using Proximity Ligation Analysis (PLA) in theca cells. An enzyme preparation was used to disperse the theca cells and eliminate any possible contamination with granulosa cells.

Main results and the role of the change: AMHR2 mRNA expression was noted in theca cells and granulosa cells from freshly harvested sheep ovaries, although levels were 3-fold greater in granulosa cells (P < 0.05). AMH mRNA was also expressed in theca cells but again it was more than 15 times greater in granulosa cells (p < 0.01). Using the highly sensitive PLA approach the AMHR2 protein and the AMH ligand receptor interaction were also detected in theca cells.

Limitations, reason for caution: Although we have demonstrated the expression of mRNA for AMHR2 in theca cells and the presence of specific AMH/AMHR2 interactions, we have yet to demonstrate that AMH binding to this receptor activates a specific intra-cellular signalling cascade and these studies are underway.

Wider implications of the findings: These results concur with recent evidence of theca cell responsiveness to AMH, thus adding support to the contention that AMH may have an important role in the regulation of thecal cell function. Elucidation of this mechanism may have important implications for our understanding of the role of AMH during folliculogenesis as perturbation of this mechanism may help explain the link between elevated androgen levels and AMH in women with PCOS.

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Trial registration number: N/A