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Genome -wide transcriptome profiling in ovaries of small-tail Han sheep during the follicular and luteal phases of the oestrous cycle

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ABSTRACT

Ovarian function, the control of which is predominantly integrated by the hypothalamic-pituitary-ovarian axis, is pivotal for maintaining reproductive efficiency in sheep. To understand the regulatory mechanism of the follicular-luteal phase transition in prolific sheep, the genome-wide expression patterns of microRNAs and genes in ovarian tissue of Small Tail Han sheep were examined during the follicular and luteal phases of the oestrous cycle. Differentially expressed genes (n = 450) were identified and a total of 139 known and 72 novel miRNAs were identified in the two libraries. It is suggested that differential abundance of miR-200a, 200b and 200c may have an important role in the follicular-luteal transition. A miRNA-regulated gene expression network was created for exploring the regulation of the follicular-luteal transition and quantitative real-time PCR verified the reliability of the RNA-seq data.

1. Introduction

The processes of ovarian follicle development, ovulation and luteal formation and regression occur repeatedly during reproduction in mammals (McBride et al., 2012). The ovary is an organ composed of germ and somatic cells. The major function of the ovary is to differentiate ovarian follicles and produce oestrogen and progesterone (Richards and Pangas, 2010). The sheep reproductive cycle is determined by the integrated hypothalamic-pituitary-ovarian axis in the breeding season (Bartlewski et al., 2011). Briefly, the production of GnRH by the hypothalamus and subsequent secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary gland, with peak occurrence before ovulation, is evoked and sustained by decreased progesterone secretion by the corpus luteum (CL) and increased oestradiol secretion during the follicular phase of the oestrous cycle. The 16- to 17-day-long oestrous cycle of sheep can be divided into follicular and early luteal phases. Rhythmic LH pulses in the plasma prevail at all reproductive states in ewes (Rawlings and Cook, 1993). The quantities of CL-secreted progesterone increased until day 7 (day 0 = day of initiation of oestrus), and then, the circulating progesterone concentration in plasma remained constant until day 12 during the luteal phase. The FSH pulse frequency increased during the growth phase of large antral follicle development during early dioestrus (Bartlewski et al., 2011). The pattern of changes in hormone synthesis suggested pivotal roles during the luteal-follicular transition. Meanwhile, steroidogenesis and the expression of antral follicle development genes and presence of proteins in sheep ovaries also have important roles in ovarian functions (Juengel et al., 2002).

The microRNAs (miRNAs), which are small non-coding RNAs that contain approximately 22 nucleotides, are widely distributed in

Abbreviations: STH, small-tailed Han sheep; RT-PCR, real-time PCR; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes

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plants, animals and some viruses (Bartel, 2004). These RNAs modulate gene expression by inhibiting mRNA translation and regulating mRNA degradation during the post-transcriptional period. The microRNAs are involved in many physiological processes in vertebrates. Recent studies have resulted in exploration of the regulatory functions of small RNAs in ovarian cells in the context of apoptosis, ovulation, steroidogenesis, gonadal development and CL development (Baley and Li, 2012; Imbar and Eisenberg, 2014). Some miRNAs appear to be important regulators of ovarian follicular and luteal development (Donadeu et al., 2012).

The small-tail Han sheep (STH) is a famous native breed with multiple kidding rates in northern China. To explore the roles of miRNAs in mediating post-transcriptional regulation in ovarian follicle recruitment in prolific sheep, it is necessary to identify the differentially abundant miRNAs and mRNAs in ovaries during the follicular (1st day of oestrus, tested by ram) and luteal (12th day) phases of the oestrous cycle. Genome-wide gene expression profiles in ovaries between the two reproductive phases were conducted using the Illumina high-throughput sequencing technology.

2. Materials and methods

2.1. Ethics statement

All animal procedures strictly followed the recommendations of the relevant national and/or local animal welfare bodies. All experimental procedures were approved by the Animal Care and Use Committee of Hebei Province, China. The owner of the sheep approved of the animal study. No protected or endangered species were used in this study.

2.2. Sample preparation

The STH sheep were selected from a farm in Hebei province, China. Three ovaries from the same sheep were collected in the follicular phase (tested by ram, oestrus, 1st day) and luteal (12th day) phases with the surgery method during the breeding season (September–October). All ovaries were immediately frozen in liquid nitrogen and then stored at – 80 °C for isolation of total RNA and construction of RNA libraries. All the selected sheep were 3 years old and multiparous. Total RNA was isolated from ovaries using TRIzol reagent (Ambion, Thermo Fisher Scientific) according to the manufacturer's instructions.

2.3. Construction of RNA libraries and sequencing

As input material, 3 μg of total RNA per sample were used for construction of the RNA library at Beijing Novogene Bioinformatics Technology (Tianjin, China). For the small RNA library, the 3′ and 5′ end adapters were ligated, and then, the first-strand cDNA was synthesized using M-MULV reverse transcriptase (RNase H-). Small RNAs that were 10–30 nt in length were generated and purified on an 8% polyacrylamide gel. The mRNA sequencing libraries were generated using the NEBNext® Ultra™ RNA Library Prep Kit (New England Biolabs, USA). To preferentially select cDNA fragments that were 150–200 bp in length, the library fragments were purified with the AMPure XP system (Beckman Coulter, Beverly, USA). All the sequencing processes were completed on an Illumina HiSeq 2500 platform.

2.4. Analysis of miRNA sequencing data

The raw reads were processed with custom Perl and Python scripts to remove adapter sequences and low-quality reads. The small RNA tags were subsequently mapped to the reference sequence by Bowtie (Langmead et al., 2009). Mapped small RNA reads were aligned with miRBase 20.0 using mirdeep2 software (Friedlander et al., 2012). Furthermore, the abundances of miRNA were compared with DESeq in the R package (1.8.3). Relative abundances were analysed by TPM (transcripts per million) (Zhou et al., 2010): Normalization expression = mapped read count/total reads * 1,000,000. The Benjamini and Hochberg method was used for adjusted *P*-values (Storey and Tibshirani, 2003). The differential abundances of miRNAs were identified with q-value < 0.01 and log2 (fold change) > 1. Target gene candidates of miRNAs with differential abundances were predicted with Targetscan, PicTar and miRanda. The predicted target gene candidates were subjected to gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The GO enrichment analysis was modified by the GOseq-based Wallenious non-central hypergeometric distribution to adjust for gene length bias. The statistical enrichment of KEGG pathways was tested with KOBAS software (Mao et al., 2005).

2.5. Analysis of mRNA sequencing data

All clean data (clean reads) were obtained by removing reads containing adapter sequences, reads containing poly-N sequences and low-quality reads from the raw data. Paired-end clean reads were aligned to the sheep reference genome (oar3.1) using the TopHat program. The number of mapped reads for each gene was normalized and calculated using the RPKM (reads per kb per million reads) method. Differential abundances analyses were performed using the DESeq R package (1.10.1). The differential abundances were determined using a model based on the negative binomial distribution. The *P*-values were adjusted using the Benjamini and Hochberg approach for controlling the false discovery rate. Corrected *P*-values of 0.005 and a log2 (fold change) of 1 were set as the threshold for significant differential abundances. The gene function can be clustered by three categories (biological process, cellular component and molecular function) in Gene Ontology (GO), an international standardized classification system. The