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Transcriptome response of previtellogenic ovary in *Anguilla japonica* after artificial hormone injection

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ABSTRACT

In this study we investigate the intra-ovarian pathways underlying early follicle development in Japanese eels, *Anguilla japonica*. We conducted high-throughput transcriptome analyses in the initial development of the ovary via the next-generation sequencing (NGS). Japanese eels were treated with three weekly salmon pituitary homogenate (SPH) injections. Using RNA-seq, we obtained 29,117,237 and 41,867,557 reads from the control and the SPH-injected groups, respectively. Combining these RNA-seq datasets, we acquired a total of 101,711 unigenes (N50 = 1,517 bp) after performing de novo assembly. After differentially expressed gene (DEG) analysis, 4,211 and 7,059 annotated genes showed upregulation and downregulation respectively in SPH-injected ovarian tissues. Furthermore, functions of annotated genes were classified by GO and KEGG analyses. The PTEN/PI3K-Akt pathway, Tsc/mTOR signaling, oocyte meiosis and reproduction functions were found in data of differentially expressed genes.

1. Introduction

The Japanese eel, *Anguilla japonica* is a catadromous teleost that spawns in the ocean and its larvae migrate to the coasts of eastern Asian countries. In both natural settings and aquaculture farms, the female reproduction is usually blocked at the early stage of puberty (Dufour et al., 1988). In the initial quiescent state of vertebrate ovary, the oocytes are arrested at the meiosis prophase I in perinucleolus stage. Once recruitment from primordial to primary ovarian follicles occurs, oocytes are enclosed by monolayer proliferating granulosa cells, and basement lamina is secreted to separate it from stromal cells (Selman et al., 1993). Artificial hormone manipulation is needed to force the eel gonad to enter into the growth and maturation pathway (Ohta et al., 1997; Yamamoto and Yamauchi, 1974). Variations in gonadal development prior to salmon pituitary homogenate (SPH) injections have always been observed and reported among female eels (Ijiri et al., 1998), and ovarian follicles develop into asynchronous clutches after SPH injections alone was also observed in our laboratory. These problems could be related to the poor studies on the teleost primary growth including primordial-primary ovarian follicle recruitment, cytoplasm variations and early follicular cell development over the long-term previtellogenic stage. Although recent studies have reported on initial development of the mammalian primordial follicle (Adhikari and Liu, 2009), there is still a need to investigate the early previtellogenic development in the semelparous eel.

To understand the transcriptomic profile in gonad development, different experimental designs of transcriptome analysis have been used in mammals and non-mammals (Duan et al., 2015; Katz-Jaffe et al., 2009). Recently, a draft genome of the adult female eel (Henkel et al., 2012), and transcriptomes of the wild glass eels including the early stage embryos (Hsu et al., 2015) have been reported. Nevertheless, the mechanisms of previtellogenic ovarian development are still unclear in teleost. In this study, transcriptomic profiles at the previtellogenic stage of ovarian growth were established after SPH injection, and differentially expressed genes were further identified. The results of the transcriptomic profiles provide more information and further understanding of previtellogenic development in the gonad of female Japanese eels.

2. Data description

2.1. Induction of initial ovarian development by SPH injection

Japanese eels were purchased from an eel culture farm in Lukang, Taiwan. The environmental conditions of the samples are compliant with the MIGS standard in a briefly description (Table 1). The eels were 3–4 years old, weighed 550–650 g.

Eels were then kept in a 200 l tank with a recirculating seawater system. Water temperature was maintained at 20 ± 1 °C. The eels were

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Table 1
Data descriptions of environmental conditions in compliance with the MIGS standard.

Item	Description
Investigation_type	Eukaryote
Project_name	Transcriptome response of previtellogenic ovary in *Anguilla japonica* after artificial hormone injection
Lat_lon	24.0842 N 120.4455 E
Country	Taiwan
Geo_loc_name	Lukang, Taiwan
Collection_date	18-July-2014
Env_biome	ENVO:00002030
Env_feature	ENVO:00000294
Env_material	ENVO:00002011
Ploidy	Diploid
Num_replicons	NA
Estimated_size	1.02E9 (Henkel et al., 2012)
Propagation	Sexual
Isol_growth_condt	Aquaculture farm
Seq_meth	Illumina Hiseq2000
Assembly	Trinityrnaseq v 2.0.6
Finishing_strategy	Contigs

induced by three weekly intraperitoneal injections of SPH at a dosage concentration of 20 mg/0.5 ml/kg, while the control eels were injected with saline solution. The experiment was reviewed by the Animal Research Committee of the National Taiwan University. The approval number is NTU-103-EL-74.

2.2. Tissue collection, RNA extraction and cDNA library construction

The eels were sacrificed 48 hours after the last injection. Whole ovaries were taken for gonadal somatic index (GSI) measurements. Ovarian tissues containing twenty ovarian lamellae were collected from the control and the SPH-injected female eels, respectively. Ovarian lamellae were treated with RNAlater (Thermo Fisher Scientific, USA) at 4°C to inhibit RNase activity. Total RNA was extracted from ovarian lamellae of the two groups by using the TRIzol total RNA extraction reagent following the manufacturer's protocol (Thermo Fisher Scientific, USA). The quality and integrity of ovarian total RNA were determined via a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). The 260/280 absorbance ratio of ovarian total RNA was about 1.9, and the RIN value ≥ 8. Total RNAs were pooled from the control and the SPH-injected samples in equal amounts for cDNA library construction, respectively. The RNA sample was treated with DNase I. The mRNA was isolated from the quantified total RNA via oligo-attached poly-T magnetic beads (Thermo Fisher Scientific, USA) and subsequently disrupted into fragments using divalent cations and heat treatments. The first-strand cDNA was synthesized via reverse transcriptase with small fragments of mRNA as templates, and the second-strand cDNA was synthesized via DNA polymerase I. The double-stranded cDNA was synthesized using the Genomic Sample Prep Kit (Illumina, USA) and ligated with an Illumina PE Adaptor (Illumina, USA).

2.3. Illumina sequencing, de novo assembly and annotation

Adaptor-ligated cDNA fragments were purified and sequenced on an Illumina HiSeq 2000 platform (Illumina, USA) producing 90 bp paired-end reads. A total of 29,117,237 and 41,867,557 reads were obtained from the control and the SPH injected groups, respectively. Raw reads were filtered via SolexaQA software (Cox et al., 2010). Sequence information from all analyses (12 Gb) was used to construct contigs, and relative transcript abundance was subsequently estimated using the RPKM (reads per kilobase of sequence per million reads) method. De novo assembly of the transcriptome was conducted with a short reads assembling program, Trinityrnaseq v 2.0.6 (Grabherr et al., 2011), and assembled sequences were selected for annotation analysis. The clean reads were combined and assembled into 101,711 unigenes, and the N50 length was 1,517 bp (Suppl. table 1).

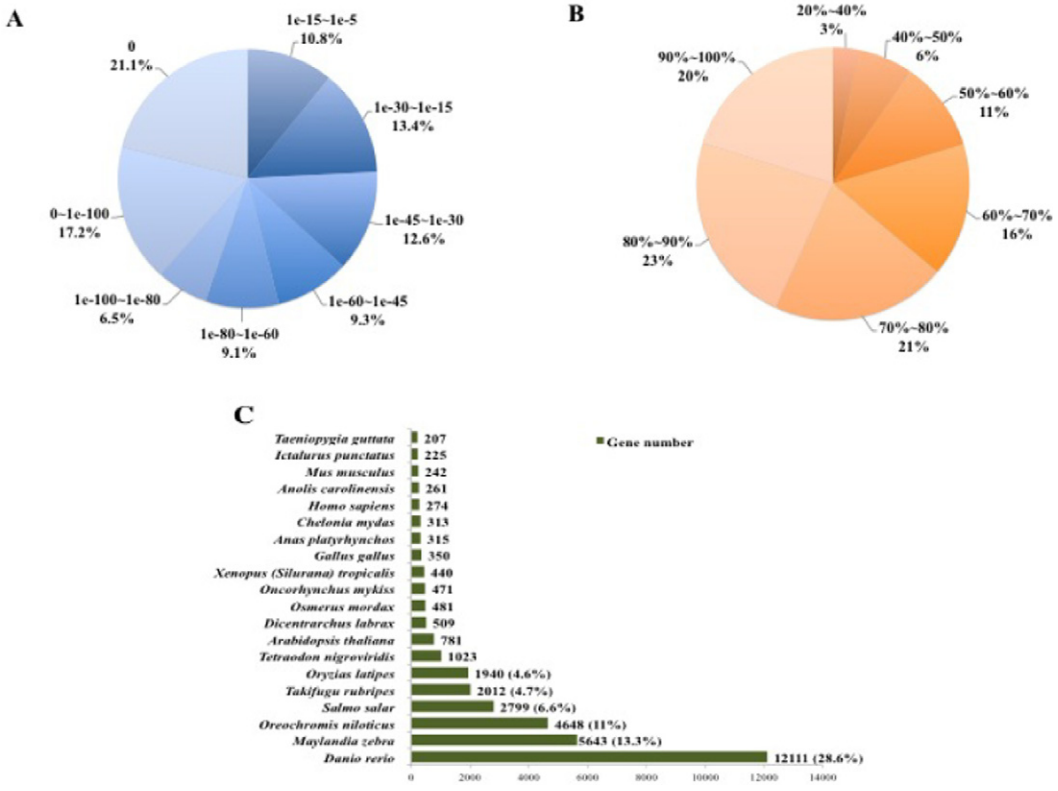

Fig. 1. Nr database BLAST hits in the Japanese eel ovary. A: E-value distribution of nr BLAST matched unigenes; B: Sequences similarity and distribution of nr BLAST matched unigenes; C: Species similarity of nr BLAST hits.