TRANSCRIPTOME ANALYSIS OF ARTIFICIALLY INDUCED SEX REVERSAL IN THE NILE TILAPIA

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Introduction:

The Nile tilapia, a worldwide cultured fish, is a good model for the study of sex determination and differentiation, due to the availability of monosex offsprings and short (14days) spawning cycle. In order to elucidate the molecular mechanism for induced sex reversal, transcriptome analysis of gonads (testis and ovary) from 3-month-old tilapia was carried out by RNA sequencing.

Methods:

Animal- All genetic female (XX) and male (XY) tilapia (*Oreochromis niloticus*) were obtained by artificial fertilization of eggs from normal females (XX) with sperm from either sex-reversed males (XX) or super males (YY), respectively. Tilapias were reared in 0.5-ton tanks with re-circulating aerated fresh water at 26ºC under natural photoperiod until 3 months old. Total RNA was extracted from female and male gonad, respectively, using TRIzol reagent (TaKaRa, Japan). The Illumina RNA-seq was performed by BGI (Beijing Genomics Institute). *Drug treatment-* The aromatase inhibitor Letrozole was dissolved in 95% ethanol, and then added to the feed for long-term treatment of all 3-month-old genetic female tilapia until sex reversal. *Histological studies-* After 0, 10, 20, 45, 70, 90 days of Letrozole treatment, the gonads were dissected out and fixed in Bouin's solution for 24 h at room temperature, and subsequently dehydrated, embedded in paraffin, and then serially sectioned at 6μm thickness. The sections were stained with hematoxylin and eosin. *Real time PCR-* Gonads from Letrozole treated group and control group were sampled at 0, 10, 20, 45, 70, 90 days after treatment Total RNA was extracted from all the samples and cDNA was prepared for the real-time PCR.

Results and Discussion:

Totally about 70000 unigenes were found to be expressed in 3 month old gonads. Out of 11765 annotated unigenes, about 10935 were expressed in both gonads, while 193 and 593 were found to be expressed specifically in the ovary and testis, respectively. Comparative study of the transriptome data from the testis and ovary revealed 14066 differentially expressed genes, in the 3-month-old tilapia gonads, with 10111 expressed higher in testis, while 3955 were expressed higher in ovary. Therefore, more genes are expressed in much higher levels in the testis compared with the ovary. Functional classification of unigenes was done by COG and KEGG. Predominant classifications like cell cycle control, posttranslational modification, ribosomal structure, energy production and conversion, suggest that the fundamental metabolism and development were the main processes in the 3-month-old tilapia gonads. The predominant classes of genes expressed in the ovary were fatty acid-binding protein, Cyclin, Actin and Zona pellucida sperm-binding protein, indicating it as the nutritive and energy accumulation period for oocyte growth. While, in the testis, the predominant classes were NADH-ubiquinone oxidoreductase, ATP-dependent DNA helicase and Cell differentiation factor, indicating an active cell division phase for spermatogenesis in the 3-month-old gonad. Analysis of the expression profiles of the steroidogenic enzyme genes in the transcriptome revealed that only the estrogen synthesis related genes, such as *Cyp19a1a*, *Cyp19a1b, 3beta-HSD-II* and *17 beta -HSD-I* were expressed higher in the ovary than in the testis, while the majority of steroidogenic enzymes, such as *Cyp11b1, P450scc, Star1, Star2, 3beta-HSD-I, 11 beta -HSD-II* and *17beta-HSD12-II*, were expressed higher in the testis. This indicates the differential synthesis of steroid hormone between ovary and testis, with much active steroidogenesis in the testis. Some important gene families were analyzed thoroughly with the transcriptome data. For example, 29 Fox genes were found to be expressed in the 3-month-old tilapia gonad, with 12 and 17 genes being expressed much higher in the ovary and testis, respectively, indicating that like Foxl2, these Fox genes might also play important roles in fish gonad differentiation and development.

In addition, sex reversal from ovary to functional testis was successfully induced by long term treatment of 3-month-old female tilapia with aromatase inhibitor, Letrozole. Histological examination of the gonad samples fixed at different time during treatment revealed that sex reversal was a gradual process, which started from ventral to dorsal and from posterior to anterior of the gonad. Real time PCR was employed to check the profiles of genes which were found in the transcriptome and were known to play important roles in fish sex determination and differentiation. The results showed that *Sf-1* and *Cyp19a1a* were significantly up-regulated during the early period of treatment (0-45 dat, days after

treatment), followed by a moderate expression levels in the remaining period, which was in consistence with the aromotase protein level, as revealed by immunohisto-staining with tilapia aromatase anti-sera. Foxl2 was found to be down regulated, while Dmrt1 was up-regulated gradually during the treatment period. It is worth noting that the un-regulation of *Cyp11b1*, the key enzyme responsible for 11KT production, was up-regulated only at the late treatment period. The transcriptome analysis of the sex reversal process is still in progress.

Conclusion:

Our data indicate that estrogen is not only critical for the ovarian differentiation, but also for the maintenance of the ovary, while androgen might be the consequence, instead being the reason of the Letrozole induced sex reversal.