IDENTIFICATION AND CHARACTERIZATION OF A NOVEL 20BETA-HSD IN THE NILE TILAPIA

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Introduction:

Steroidogenic shift from estrogen (E2) production during oocyte growth to 17alpha, 20beta-DP production in the oocytes final maturation stage is a prerequisite during reproductive cycle in teleosts. The dramatic increase in the capacity of postvitellogenic follicles to produce 17alpha, 20beta-DP is correlated with the down-regulation of E2 producing steroidogenic enzymes, i.e. P450c17-I and aromatase, up-regulation of P450c17-II and carbonyl reductase/20beta-hydroxysteroid dehydrogenase. Vast investigation has been carried out in fish steroidogenesis, however, the molecular mechanism of steroidogenic shift controlling the reproductive cycle is far from clear. In our present studies, a novel 20beta-HSD (named as 20beta-HSD-II), which showed high expression in the follicular cells during oocyte maturation stages, was characterized in tilapia.

Methods:

Animals- 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 26^0C under natural photoperiod until use. *Molecular cloning*- Partial sequence of tilapia 20beta-HSD-II was obtained from the EST database. Subsequently four primers were designed to amplify a full-length cDNA sequence by 5'-rapid amplication of cDNA ends (RACE) and 3'-RACE. Finally, two gene specific primers were designed to amplify the full cDNA sequence. *RT-PCR*- Total RNA was extracted, cDNA was synthesized, and RT-PCR was carried out to check the expression levels of tilapia 20beta-HSD-II in various tissues. Positive and negative controls were set up with plasmid DNA and water, respectively. A fragment of β-actin was amplified (as internal control) from tilapia to test the quality of the cDNAs used in the PCR. *In situ hybridization*- The whole bodies of XX and XY fry at 2, 5, 11, 20, 60 day after hatching and gonads of adult tilapia were fixed in 4% paraformaldehyde in 0.85XPBS at 4℃ to check the expression of 20β-HSD -I and -II in the gonad by ISH. Similarly, gonads from regularly spawning fish were sampled and fixed at d 1, 3, 5, 8, 10, 12, and 14 of the spawning cycle. Probes of sense and antisense digoxigenin-labeled RNA strands were transcribed in vitro with an RNA labeling kit from plasmid DNA containing ORFs of tilapia 20beta-HSD, 20beta-HSD-II.

Results and discussion:

The predicted amino acid sequences of 20beta-HSD-II showed the highest homology to tilapia and medaka 20beta-HSD-I (80.2% and 78.9%, respectively), but relatively low similarity to 20beta-HSD-I of human (65.5%) and chicken (65.8%). Tissue distribution by RT-PCR showed that 20beta-HSD-II was expressed in various tissues including gill, liver, intestine, kidney, muscle, ovary and testis. *In situ* hybridization (ISH) analysis demonstrated that 20beta-HSD-II was expressed in the follicular cells and cytoplasm of oocyte, as well as in the interstitial cells in the testis. Additionally, the expression of 20beta-HSD-II was also detected in both the epithelial cells of intestine and interrenal cells of the head kidney. However, the expression of 20beta-HSD-I was only detected in the early vitellogenic oocytes. No expression of 20beta-HSD-I could be detected in the testis, intestine and head kidney by ISH.

Interestingly, two type of 20beta-HSDs displayed distinct spatial and temporal expression profiles during the reproductive cycle. 20beta-HSD-I was exclusively expressed in the previtellogenic ooctyes and no expression was detected in the follicular layer cells. Interestingly, the expression of 20beta-HSD-II was dominantly expressed in the both theca cells and granulosa cells from 4 day after spawning to 12 day after spawning and was restricted to theca cells in degenerated follicular cells after ovulation. The expression profiles suggested that 20beta-HSD-II, but not 20beta-HSD-I, might be involved in the production of 17alpha, 20beta-DP.

Ontogenic expression by ISH showed that 20beta-HSD-I was only detected in germ cells in the XX gonads and no expression was found in the XY gonads. Importantly, the expression of 20beta-HSD-II in both somatic cells and germ cells was initiated from 5dah in XX gonads, while it was delayed to 20dah in the XY gonads. The ontogenic expression pattern revealed that 20beta-HSD-II might be responsible for 17alpha, 20beta

production which is required for the initiation of meiosis in fish during early development stages.

Conclusion:

A novel 20beta-HSD (20beta-HSD-II), which showed the highest homology with tilapia 20beta-HSD-I, was cloned from the Nile tilapia. The spatial and temporal expression profile of 20beta-HSD-I and –II during reproductive cycle strongly suggested that 20beta-HSD-II, but not 20beta-HSD-I, might be involved in the biosynthesis 17alpha, 20beta-DP in tilapia.