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Gdnf-Gfra1 pathway is expressed in a spermatogenetic dependent manner and is regulated by Fsh in a fish testis

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What makes the spermatogonial stem cells (SSCs) self-renew or differentiate to produce spermatozoa is barely understood, in particular in non-mammalian species. Our research explores possible regulations of the spermatogonial stem cell niche in teleost, locally by paracrine factors and peripherally by hormonal regulation. In the present study, we focus on the Gdnf/Gfra1 pathway that plays a major role in the regulation of SSC self-renewal in mammals. Using qPCR measurements in isolated testicular cell populations, the *gdnfb* paralog was found expressed in testicular somatic cells and in A spermatogonia. In contrast, the transcript of the *gdnf* receptor, *gfra1a,* was preferentially expressed in a population of undifferentiated A-spermatogonia (*und* A-Spg) purified by centrifugal elutriation. This particular cell population also demonstrated high “stemness” potential in transplantation studies, and preferentially expressed nanos2, a putative SSC marker in trout [Bellaiche et al 2014]. Furthermore, by flow cytometry we find that only a sub-fraction of the und-A-Spg (<20%) expressed the *gdnf* receptor. In trout, spermatogenesis develops along a strict annual cycle. We show that *gdnfb* and its receptor were expressed in a spermatogenetic activity dependent manner. Interestingly, a dramatic increase of the *gdnfb* transcript coincided with the progressive cessation of rapid spermatogonial proliferation and of meiosis towards the end of the reproductive cycle. These results suggest that, in trout, Gdnfb is involved in the repression of *und* A-Spg differentiation. In rodents, the Follicle Stimulating Factor, Fsh, is an endocrine regulator of the SSCs self-renewal through the up regulation of *Gdnf*. We demonstrate that in trout, in vitro Fsh treatment stimulated the expression of the *gfra1a1*, but not of its ligand, *gdnfb.* Fsh treatment also stimulated the proliferation of *und* A-Spg co-cultured with testicular somatic cells. Based on those results we propose that the Gfra1 positive cells correspond to the putative SSCs in rainbow trout and that the balance between SSC self-renewal and differentiation during the trout spermatogenetic cycle is possibly under paracrine regulation by Gdnfb and under peripheral regulation by Fsh via the control of *gfra1* expression.

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Androgenic modification through GtH- induced development of previtellogenic ovarian follicles in Japanese eel, *Anguilla japonica* : the gene expression of ARα, ARβ, FSHR, jeGDF-9 and jeKi-67.

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In Japanese eel, the development of ovarian follicles is promoted by using SPH induction. According to our laboratory *in vivo* data, when induction was combined SPH with androgen (17α-methyltestosterone, MT) might to maintained the survival number of ovarian follicles. Generally, the ovary development suspend in primary ovarian follicle stage in the pubertal female eels. In this state, the folliculogenesis is one of the most important progressions to initial ovarian development. We hypothesize that the androgen receptors (ARs) actions in granulosa cells interact with the paracrine oocyte-secreted growth differentiation factor 9 (GDF-9). This cell-to-cell interaction will control the rhythm of folliculogenesis to maintain most of the ovarian follicles. Therefore, two experiments were designed to verify it. The first experiment in our study, ARα, ARβ, FSHR and jeGDF-9 mRNA were cloned in previtellogenic ovary. Progressively, we design *in vivo* experiments by intraperitoneal injecting with SPH, SPH+MT for 3 weeks and, SPH, MT, SPH+flutamide and MT+flutamide for 2 weeks. RT-qPCR was use to analyze the mRNA expression copies. The expression copies of ARα and ARβ mRNA were maintained in the same level between seawater control and flutamide injecton group. The data showed a significant high level ($P < 0.05$) of ARα and ARβ mRNA in SPH+MT injections for 3 weeks. In addition, ARα and ARβ mRNA in SPH only or MT only injection were shown 3-5 times higher than seawater control. The FSHR and jeGDF-9 mRNA of hormone-injected groups were also shown higher levels than seawater control. Moreover, MT treatments showed significantly high level of these two genes. However, these mRNA expression conditions were decline significantly in flutamide injections. In the second part of experiment, the relationship between proliferation and androgenic actions in ovarian follicles was observed cocurrently by *in vitro* ovarian tissue culture system and drug treatments. Nine female eels were separated into initial freshwater, seawater control and positive control (three injections of SPH). Ki-67 is an extensive indicator for cell proliferation. Thereby, jeKi-67 was cloned in female eel ovary. The mRNA expression data of ARα, ARβ, FSHR, jeGDF-9 and jeKi-67 showed different mRNA expression patterns between GSI < 1% and GSI > 1% in previtellogenic ovary. Likewise, further data will show more details of the relationship among androgens, its antagonists in folliculogenesis. Consequently, the data from two part of experiment reveal that GtHs are prerequisite to actuate ovarian growth and development. However, this GtH- induced process might be modified by androgens to enhance the survival of ovarian follicles in previtellogenic stage,this survival mechanism may maintain cell proliferation in folliculogenesis.