別紙様式８

研　究　主　論　文　抄　録

論文題目：マウス生後精巣において精原細胞の増殖及び減数分裂開始に対するニューレグリンとレチノイン酸の役割

（Roles of Neuregulins and Retinoic Acid in Spermatogonial Proliferation and Meiotic Initiation in Neonatal Mouse Testis）

熊本大学大学院自然科学研究科　　複合新領域科学専攻　　生命環境科学　講座

（　主任指導　　安部　眞一教授　）

論文提出者　　張　継東

（by　Jidong Zhang　）

主論文要旨

The differentiation of germ cells into spermatozoa occurs within a tubular seminiferous epithelium, and depends on a complex paracrine dialogue of germ cells with somatic cells such as Sertoli cells and Leydig cells. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) act on Sertoli cells and Leydig cells, respectively, and stimulate gene transcription and secretion of peptides that promote spermatogonia differentiation.

Meiosis is a process that produces gametes with extensive genetic diversity and the transition from mitosis to meiosis is unique to germ cells. In testes and ovaries spermatogonia and oogonia, respectively, undergo species-specific rounds of mitotic divisions followed by the initiation of meiosis. In the mammalian ovary, germ cells enter meiosis during embryogenesis, whereas those in the testis do not until puberty. Initiation of meiosis in developing ovaries, but not in testes, may involve environment cues. In murine embryonic ovaries and juvenile testes, retinoic acid (RA) induces meiosis via the *Stimulated by Retinoic Acid Gene 8* (*Stra8*), but its molecular pathway requires elucidation: How RA induces meiosis, and whether RA acts directly on germ cells or indirectly through Sertoli cells are unknown. Neuregulin (NRG)1 was identified in newt as a novel

FSH-upregulated gene homologous to mouse NRG1 which is known to control cell proliferation, differentiation and survival in various tissues (Falls, 2003). In vitro studies in newt testes showed that newt NRG1 plays a pivotal role in promoting spermatogonial proliferation by both direct effect on spermatogonia and indirect effect via somatic cells (Oral et al., 2008).

To analyze the role of NRG1 in neonatal mouse spermatogenesis, we generated Sertoli cell-specific conditional *Nrg1*$^{Ser-/-}$ mutant mice by crossing homozygous floxed mice (*NRG1*$^{loxP/loxP}$) with C57BL/6 mice carrying the transgene for a Cre recombinase-mutated estrogen receptor (Cre-ER^{TM}) (Metzger and Chambon, 2001) under the control of mouse *MIS* promoter that is specifically active in Sertoli cells (Dutertre et al., 1997). Tamoxifen (TAM) injection into 14 day-post-partum (dpp) *Nrg1*$^{Ser-/-}$ mutant mice induced testis degeneration, suppressed BrdU incorporation into spermatogonia and preleptotene primary spermatocytes, decreased the number of STRA8-positive cells and increased TUNEL-positive cells, respectively. In testicular organ cultures from 5-6-dpp wild-type mice and cultures of their re-aggregated spermatogonia and Sertoli cells, FSH, RA {all-trans-retinoic acid (ATRA), AM580, 9-cis-RA} and NRG1 promoted spermatogonia proliferation and meiotic initiation. However, TAM treatment of testicular organ cultures from the *Nrg1*$^{Ser-/-}$ mutants suppressed spermatogonia proliferation and meiotic initiation that was promoted by FSH or AM580. In re-aggregated cultures of purified spermatogonia, NRG1, NRG3, ATRA and 9-cis-RA promoted their proliferation and meiotic initiation, but neither AM580 nor FSH did. Also, FSH, RAs and NRG1 promoted *Nrg1* and *Nrg3* mRNA expression in Sertoli cells.

Furthermore, we have generated spermatogonia-specific *ErbB4* dominant negative (DN) conditional transgenic mice by crossing transgenic mice carrying the transgene for a mutant ErbB4 under the control of CAG promoter with mice carrying the transgene for a Cre recombinase-mutated estrogen receptor (Cre-ER^{TM}) under the control of mouse *VASA* promoter that is specifically active in germ cells (Tanaka et al., 2000). Injection of TAM into 14 dpp spermatogonia-specific *ErbB4 DN* conditional transgenic mice induced testis degeneration, suppressed BrdU incorporation into

spermatogonia and preleptotene primary spermatocytes. In organ culture of 5 dpp testes from spermatogonia-specific *ErbB4* DN conditional transgenic mice, TAM treatment inhibits the spermatogonial proliferation and meiotic initiation in all the cases when FSH, RAs or NRGs were added.

These results indicate that in juvenile testes RA and FSH induced meiosis indirectly through Sertoli cells when NRG1 and NRG3 were upregulated, as NRG1 amplified itself and NRG3. The amplified NRG1 and NRG3 directly induced meiosis in spermatogonia via ERBB4. In addition, ATRA and 9-cis-RA activated spermatogonia directly and promoted their proliferation and eventually meiotic initiation.