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The Luteinizing Hormone-Testosterone Pathway Regulates Mouse Spermatogonial Stem Cell Self-Renewal by Suppressing WNT5A Expression in Sertoli Cells

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SUMMARY

Spermatogenesis originates from self-renewal of spermatogonial stem cells (SSCs). Previous studies have reported conflicting roles of gonadotropic pituitary hormones in SSC self-renewal. Here, we explored the role of hormonal regulation of SSCs using *Fshb* and *Lhcgr* knockout (KO) mice. Although follicle-stimulating hormone (FSH) is thought to promote self-renewal by glial cell line-derived neurotrophic factor (GDNF), no abnormalities were found in SSCs and their microenvironment. In contrast, SSCs were enriched in *Lhcgr*-deficient mice. Moreover, wild-type SSCs transplanted into *Lhcgr*-deficient mice showed enhanced self-renewal. Microarray analysis revealed that *Lhcgr*-deficient testes have enhanced WNT5A expression in Sertoli cells, which showed an immature phenotype. Since WNT5A was upregulated by anti-androgen treatment, testosterone produced by luteinizing hormone (LH) is required for Sertoli cell maturation. WNT5A promoted SSC activity both in vitro and in vivo. Therefore, FSH is not responsible for GDNF regulation, while LH negatively regulates SSC self-renewal by suppressing WNT5A via testosterone.

INTRODUCTION

Spermatogonial stem cells (SSCs) are the founder cell population of spermatogenesis (de Rooij and Russell, 2000; Meistrich and van Beek, 1993). Although their number in testes is very small (0.02%–0.03% of total germ cells), they are the only stem cells in the germline that have the unique ability to undergo self-renewal division to produce numerous progenitor cells. SSCs are thought to develop from gonocytes in the neonatal testis during the perinatal stage (Shinohara et al., 2001). SSCs rapidly expand their number during sexual maturation and maintain a constant population size in adults. The SSC population size is determined based on the number of niches (Oatley et al., 2011), which likely develop through complex interactions among Sertoli cells, Leydig cells, peritubular myoid cells, and macrophages (DeFalco et al., 2015; Meng et al., 2000; Oatley et al., 2009; Spinnler et al., 2010). Sexual maturation is accompanied by dynamic changes in hormonal milieu, and development of these cell types is influenced by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secreted from the pituitary gland. However, little is known regarding the roles of these hormones in SSC and niche development.

SSC research was revolutionized by the development of spermatogonial transplantation in 1994 (Brinster and Zimmermann, 1994). This technique provided the first functional assay for SSCs and has been widely used to characterize SSCs. In initial attempts to improve the colonization efficiency of SSCs, suppression of hypothalamus signaling was found to confer beneficial effects on transplantation. Treatment of recipient animals with leuprolide, a gonadotropin-releasing hormone (GnRH) analog, resulted in enhanced colonization of donor cells. The donor cell colony count increased by approximately 2.7-fold, which was suggestive of enhanced homing of SSCs (Ogawa et al., 1998). Moreover, leuprolide appeared to enhance self-renewal division because the colonized area in leuprolide-treated recipients increased by 5.3-fold (Dobrinski et al., 2001). Prolonged administration of leuprolide suppresses the secretion of LH and FSH from the pituitary gland, which induces testosterone production by Leydig cells and stimulates the proliferation of Sertoli cells, respectively (Karashima et al., 1988). Although a GnRH analog is generally suppressive to spermatogenesis in wild-type (WT) mice, it protects SSCs from damage by chemical or radiation treatment (Meistrich, 1998). This suppressive effect of the gonadotropic pituitary hormones on SSC self-renewal was confirmed in another study, which showed that SSCs underwent more extensive self-renewal divisions in hypophysectomized hosts (Kanatsu-Shinohara et al., 2004). Several possibilities, including the direct action of GnRH on germ cells, reduction of intratesticular testosterone, or increased kit ligand (KITL) expression, are believed to be involved. However, further studies are required (Ogawa et al., 1998). These studies performed

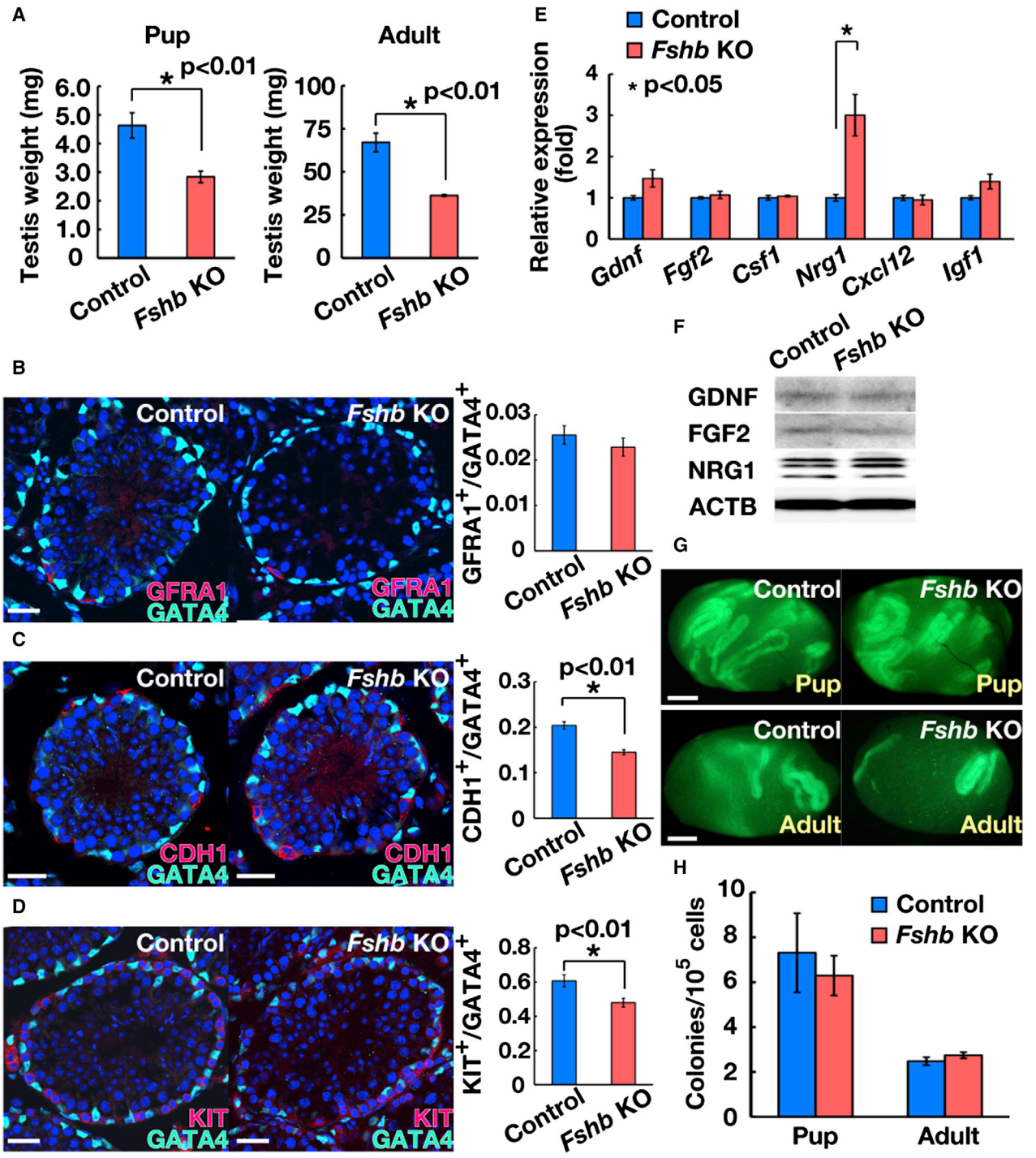

Figure 1. Functional Analysis of SSCs in *Fshb* KO Mice

(A) Testis weight of 8-day-old and 6-week-old mice (n = 4 testes).

(B–D) Immunohistochemistry and quantification of indicated spermatogonia markers in *Fshb* KO adult mouse testes. At least 200 cells in four testes were counted.

(E) Real-time PCR analysis of busulfan-treated adult mouse testes (n = 7 experiments).

(F) Western blot analysis of busulfan-treated adult mouse testes.

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