Received: 16 March 2018 | Accepted: 19 April 2018

DOI: 10.1002/mrd.22988

Molecular Reproduction & Development

CORRESPONDENCE

Selective deletion of WLS in peritubular myoid cells does not affect spermatogenesis or fertility in mice

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Funding information
Academician Workstation Support, Grant/Award Numbers: Changsha and Shandong, Shenyang; National Natural Science Foundation of China, Grant/Award Numbers: 31471352, 31501198; Young Elite Scientists Sponsorship Program by CAST, Grant/Award Number: YESS20160118; Clinical Capability Construction Project for Liaoning Provincial Hospitals, Grant/Award Numbers: LNCCC-D50-2015, LNCCC-C09-2015

Whether wingless-related MMTV integration site (WNT) signaling is necessary for spermatogenesis is controversial. Genetic knockout of the *Wls* gene, which is responsible for the secretion of various WNTs (Banziger et al., 2006), makes it possible to study the overall effect of WNT signaling (both canonical and noncanonical) and total WNTs in testes. Recently, we found that the conditional knockout of wntless WNT ligand secretion mediator (WLS) in Sertoli cells (using *Amh*-Cre) or in caput epididymis (using *Lcn5*-Cre) had no apparent influence on male fertility, whereas loss of WLS in germ cells (using *Mvh*-Cre or *Stra8*-Cre) disrupted spermatogenesis in an age-dependent manner via elevating reactive oxygen species and triggering germ cell apoptosis in mice (Chen et al., 2016; Cheng et al., 2018). Peritubular myoid (PM) cells, the main cellular components of the wall of seminiferous tubules, appear to participate in tubule contractility, spermatogonial stem cell self-renewal, and male fertility. To explore the potential role of WNTs secreted from PM cells, we generated PM-specific WLS-knockout (PM-WLSKO), conditional knockout (CKO), mice and further examined the male fertility.

In CKO male mice, the recombination of the locus of X-over P1 (loxP) sites using *Myh11*-Cre will result in the removal of exon 3 of *Wls* gene predominantly in PM cell population (Figure 1a). It should be noted that *Myh11*-Cre is expressed in all kinds of smooth muscle cells, including, but not limited to, PM cells. PM cells were isolated using Percoll discontinuous gradients and further identified by immunostaining of the PM cell marker alpha-smooth muscle actin (α-SMA) (Figure 1b). At least 90% of the isolated cells were identified as α-SMA positive (Figure 1c). We observed a significant reduction (approximately 87%) of *Wls* messenger RNA (mRNA) level in the PM cells isolated from CKO mice (Figure 1d). Surprisingly, there were no overt abnormalities in testis weight (Figure 1e), pregnancy rate (Figure 1f), and litter size (Figure 1g) of CKO males. Furthermore, the morphology of testis and epididymis of CKO mice was indistinguishable from that of control mice for 10 weeks (Figure 1h), suggesting that fertility and spermatogenesis are not disrupted by the deletion of WLS protein in PM cells. The same conclusion was further drawn from the normal expression of PM cell-specific marker, α-SMA; Sertoli cell marker, sex determining region Y-box 9 (SOX9); Leydig cell maker, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (HSD3B1); spermatogonia maker, zinc finger and BTB domain containing 16 (PLZF); round spermatid maker, Calmegin; and elongated spermatid maker, aquaporin 3 (AQP3), by immunofluorescence staining (Figure 1i).

In summary, our study demonstrates that the blockage of WNT ligand secretion from PM cells is independent of spermatogenesis and final fertility by a PM-WLSKO mouse model. We cannot fully exclude the possibility that the phenotype may be derived from a little residual WLS expression. Although it is a negative study, a series of WLS conditional knockout mice generated by us conclude that germ cell-secreted WNTs seem to be sufficient to support spermatogenesis in mice. It is essential to explore whether or not WLS protein is essential for spermatogenesis in humans.

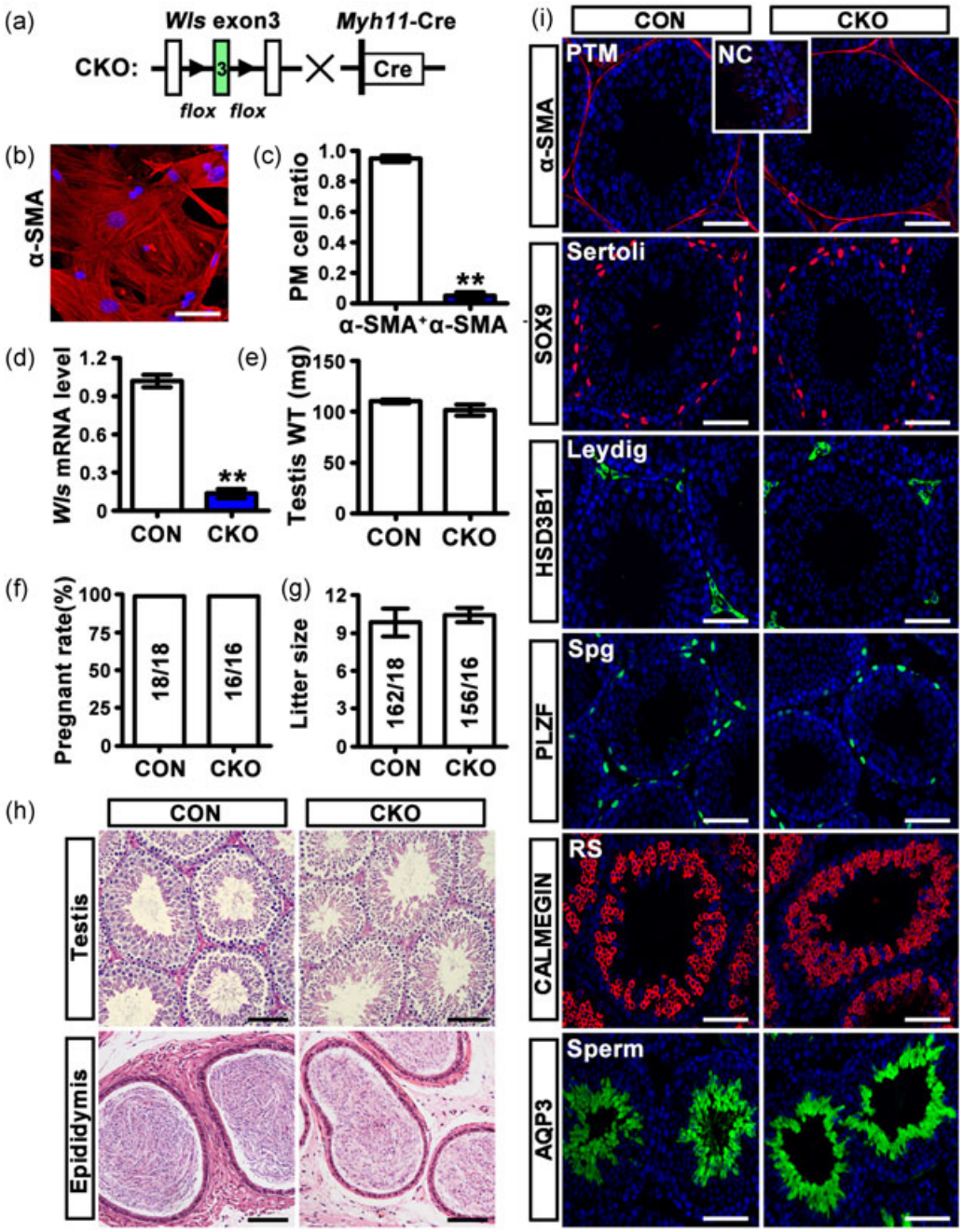

FIGURE 1 Deletion of WLS in PM cells is not essential for male fertility in mice. (a) Hybrid scheme used to generate CKO mice. The gene knockout was further confirmed by PCR genotyping. (b) Representative image of isolated cells stained with a PM cell marker, α-SMA. Scale bar, 10 μm. (c) Purity of isolated PM cells was indicated by the percentage of α-SMA-positive cells. (d) qRT-PCR analysis showing the significant reduction of *Wls* mRNA level in PM cell lysates of CKO mice, compared with that in control mice. *Gapdh* served as the internal control gene. (e) Average weight of one side testis. (f) The pregnancy rate was calculated as the ratio of the number of pregnant females to the number of successfully mating females. (g) The litter size of female mice mated with CKO or control males. The data in (c)–(g) are expressed as the mean ± standard error of the mean. $^{**}p < 0.01$. (h) Testis and cauda epididymis morphology of 8-week-old CKO mice as revealed by H&E staining. (i) Immunofluorescent staining of sections with specific markers, including α-SMA (ab5694; Abcam, Cambridge, MA), SOX9 (ab5535; Merck, Berlin, Germany), HSD3B1 (ab65156; Abcam, Cambridge, MA), PLZF (sc-28319; Santa Cruz, Dallas, TX), Calmegin (ab171971; Abcam, Cambridge, MA), and AQP3 (ab125219; Abcam, Cambridge, MA). No primary antibody NC was shown. Scale bars in (h) and (i), 100 μm. AQP3: aquaporin 3; CKO: conditional knockout; CON: control; H&E: haematoxylin and eosin stain; HSD3B1: hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1; mRNA: messenger RNA; NC: negative control; PCR: polymerase chain reaction; PLZF: zinc finger and BTB domain containing 16; PM: peritubular myoid; qRT: quantitative real-time; α-SMA: alpha-smooth muscle actin; SOX9: sex determining region Y-box 9; WLS: wntless WNT ligand secretion mediator [Color figure can be viewed at wileyonlinelibrary.com]

ACKNOWLEDGMENT

We thank Prof. Yang Xiao (Institute of Biotechnology, AMMS, Beijing, China) for her generous donation of *Wls*$^{+/flox}$ mice.

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