LETTER

Myh11-Cre is not limited to peritubular myoid cells and interaction between Sertoli and peritubular myoid cells needs investigation

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Glial cell line-derived neurotrophic factor (GDNF) is a well-defined paracrine factor that promotes spermatogonial stem cell (SSC) self-renewal and maintenance, as shown both in vivo and in vitro. Previously, Sertoli cells were considered the only source of GDNF within mouse testes. In a recent article, Liang-Yu Chen et al. describe the role of peritubular myoid (PM) cells in GDNF secretion and SSC pool maintenance by disrupting the *Gdnf* gene in PM cells (1). In their previous study, these authors found that testosterone (T) stimulates GDNF expression in adult mouse PM cells in vitro (2). Accordingly, they conclude that T acts through PM cells to modulate the level of GDNF in the testis niche to optimize renewal of the SSC pool.

However, PM cell-specific gene knockout is impossible because no markers exist to identify PM cells yet. The myosin heavy polypeptide 11 smooth muscle-Cre (*Myh11*-Cre) mouse strain was used to remove *Gdnf* within PM cells in this study (1). However, *Myh11*-Cre is expressed in all kinds of smooth muscle cells (3), including, but not limited to PM cells. From figure S4D in ref. 1, we observed some positive staining of GDNF in vascular smooth muscle cells in the interstitial space. Given that vasculature is a potential contributor for SSC niche (4), we suggest that loss of GDNF from blood vessels (or other smooth muscle cells) should also be considered in the *Gdnf* cKO murine model.

Surprisingly, *Gdnf* expression did not differ significantly between T-treated and untreated cultures containing mixtures of PM and Sertoli cells (2). The effect of T on *Gdnf* levels was apparently dampened by the presence of Sertoli cells in vitro. Therefore, the effect of interactions (antagonistic, synergetic, or independent) between Sertoli cells and PM cells on *Gdnf* expression should also be determined in vivo. In addition to a PM cell-specific knockout, Sertoli cell-specific knockout of *Gdnf* is particularly needed to reveal the interaction between Sertoli cells and PM cells in GDNF production and SSC self-renewal, otherwise their studies (in vivo vs. in vitro) may cause confusion.

Although Sertoli cell numbers were comparable in wild-type and *Gdnf* cKO mice, the function of Sertoli cells might be affected. Collapse of spermatogenesis and loss of undifferentiated spermatogonia in *Gdnf* cKO males might be partially a result of the indirect effects of Sertoli cell impairment. Those phenomena occurred in androgen receptor (*Ar*) cKO males, because Sertoli cell function was impaired, and expression of some androgen-dependent Sertoli cell genes was reduced (5). It is worth investigating whether PM cell knockout of *Gdnf* affects the function of other cell populations within the testes, including Sertoli cells, Leydig cells, and the PM cells themselves.

We agree with Chen et al. (1) that GDNF produced by PM cells signals to undifferentiated spermatogonia and regulates their maintenance, but *Myh11*-Cre is not expressed specifically in PM cells. Further observation of PM cell-specific markers and generation of PM cell-specific Cre transgenes could be helpful. Revelation of interaction between Sertoli cells and PM cells in GDNF production and SSC self-renewal urgently requires a Sertoli cell-specific knockout of *Gdnf*.

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