Regulation of gonocytes-to-spermatogonia transition (GST) in the murine testes

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Introduction

Spermatogenesis is a highly coordinated process of sperm production that occurs throughout life in male animals. This continuity is supported by the spermatogonial stem cells (SSCs). Thus the establishment of SSCs in the testes represents a crucial developmental event in male reproduction. The SSCs originate from the primordial germ cells (PGCs). In the mouse embryos, PGCs are formed at embryonic day (E) 7.25. These embryonic germ cells migrate and reach the developing gonad by E11 after which they are enclosed by somatic cells. Once PGCs become residents in the gonad, they are referred to as gonocytes. In the gonads, gonocytes experience exponential proliferation before they progressively enter mitotic arrest from E13.5. By E15.5, most gonocytes have arrested at G0/G1 of the cell cycle. At E18.5 (a day prior to birth), these quiescent germ cells begin to relocate from the central region to the inner periphery of the seminiferous cords. At postnatal day (P) 1.5 (one day after birth) gonocytes resume the cell cycle. During this perinatal period, gonocytes-to-spermatogonia transition (GST) occurs. However, the precise timing of GST is unclear due to the lack of a definitive distinction between gonocytes and nascent spermatogonia. At GST, the gonocytes may choose between two alternative cell fates – the SSCs or differentiating spermatogonia. However it is unknown how these cell fate decisions are regulated in the gonocytes during GST.

Results

In the first part of my thesis, I determined the precise timing of GST and investigated extrinsic signalings involved in regulating GST. For this purpose, I generated developmental expression profiles of known adult spermatogonial stem cell (SSC) marker genes (*Gfra1*, *Ret*, *Plzf* and *Nanos2*) and differentiating spermatogonial marker genes (*Stra8*, *Ngn3* and *cKit*) during gonocytes development from E13.5 to P5.5 using qRT-PCR and immunohistology. I found that a subset of gonocytes up-regulated spermatogonial markers as early as E18.5. These nascent spermatogonia correlated with the previously observed relocating and reproliferating gonocytes in the perinatal testes. Thus spermatogonial genes expression serves as a definitive marker of GST. To identify signalings that are involved in regulating GST, I evaluated the requirement of glial cell line-derived neurotrophic factor (GDNF),

retinoic acid (RA), bone morphogenetic protein (BMP), Nodal and X signalings using conditional gene ablation *in vivo* or organ culture *in vitro*. I found that signaling X was required for the expression of both SSC and differentiating spermatogonial marker genes, and for the relocation and reproliferation of gonocytes. I showed that pharmacological inhibition of signaling X (Xi) suppressed the expression of GDNF and RA receptors in cultured testes. Xi mainly suppressed the MEK/ERK pathway. MEK inhibitor (MEKi) recapitulated Xi's inhibition of SSC markers expression and gonocytes relocation. Whereas inhibition of PI3K/Akt pathway (a known mediator of GDNF and RA signalings) suppressed differentiating spermatogonial markers expression and gonocytes reproliferation. Taken together, I proposed that signaling X functions upstream of GDNF and RA signaling to regulate SSC and differentiating spermatogonial markers expression, and the relocation and reproliferation of gonocytes during GST.

In the second part of my thesis, I investigated intrinsic regulation of GST. For this purpose, I focused on a protein Y which is functionally required in both the gonocytes and SSCs. I found that gonocytes expressed heterogeneous levels of protein Y during and prior to GST. I hypothesized that Y^{High} gonocytes give rise to SSCs and Y^{Weak} gonocytes give rise to differentiating spermatogonia. To test this hypothesis, I examined the emergence of SSCs or differentiating spermatogonia in double transgenic mice carrying fluorescent reporters (*Y-CHERRY* and *Gfra1-GFP* or *Stra8-EGFP*) during GST. Unexpectedly, SSCs (Gfra1-GFP^{+}) emerged from Y-$CHERRY^{Weak}$ gonocytes in addition to differentiating spermatogonia (Stra8-$EGFP^{+}$). Immunohistological analyses revealed that *Stra8*$^{+}$ differentiating spermatogonia constituted a subset of $GFRA1^{+}$ SSCs in the perinatal testes. Lineage-tracing experiments confirmed that E18.5 *Gfra1*$^{+}$ nascent spermatogonia (by *Gfra1-Ert2Cre*) gave rise to both SSCs and differentiating spermatogonia; while E18.5 *Stra8*$^{+}$ nascent spermatogonia (by *Stra8-Ert2Cre*) exclusively gave rise to differentiating spermatogonia. On the other hand, Y-$CHERRY^{High}$ gonocytes up-regulated Gfra1-GFP but not Stra8-EGFP in P3.5 testes. Conditional ablation and overexpression of *Y* in gonocytes demonstrated that the expression levels of protein Y is an intrinsic regulator of GST timing and cell fate decisions of gonocytes.

Conclusion

In conclusion, I defined the precise initiation of GST in E18.5 mouse testes using spermatogonial marker genes expression. I revealed that signaling X is required during GST for the expression of spermatogonial genes and gonocytes relocation and reproliferation. Finally, I demonstrated that the timing of GST and cell fate decisions of gonocytes are intrinsically regulated by the expression levels of protein Y. My study contributes to the understanding of cell fate regulation of gonocytes in the murine testes.