PREVIEW

The Regulation of Gonadal Somatic Cell Differentiation in Humans

Min Chen [1], Fei Gao [2,3,4,5],*

[1] *Guangdong and Shenzhen Key Laboratory of Male Reproductive Medicine and Genetics, Institute of Urology, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Shenzhen 518036, China*
[2] *State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China*
[3] *Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing 100101, China*
[4] *Beijing Institute for Stem Cell and Regenerative Medicine, Beijing 100101, China*
[5] *University of Chinese Academy of Sciences, Beijing 100049, China*

In mammals, both male and female gonads are derived from the bipotential gonadal primordium, *i.e.*, the genital ridge. Primordial germ cells (PGCs) are the germ cell progenitors that arise from the extraembryonic ectoderm, migrate through the hindgut endoderm, and reach the genital ridge before sex determination [1]. Both Sertoli cells and granulosa cells are derived from somatic cells in undifferentiated genital ridges. The differentiation of germ cells and gonadal somatic cells during gonad development is regulated by various factors, including transcription factors, epigenetic regulators, and environmental factors. The interactions between germ cells and somatic cells also play critical roles in gonad development and PGC differentiation. The differentiation of PGCs is affected by the signals derived from the surrounding somatic environment, indicating that the surrounding gonadal microenvironments may tightly regulate the behaviors of

PGCs [2–4]. The regulation of PGC and gonadal somatic cell development in mouse models has been extensively investigated previously [5–7]. Several research groups have described the developmental transcriptomic landscapes of human fetal germ cells (FGCs) and spermatogenesis, with studies mainly focusing on the development of germ cells [8–17]. A variety of germ cell types and states have been well demonstrated throughout the development in human fetal, neonatal, infant, and adult stages [9,11,12,18]. However, the regulation of gonadal somatic cell differentiation and the interactions between germ cells and gonadal somatic cells in humans still remain incompletely understood.

Recently, Wang et al. [19] systematically analyzed the fetal germ cells (FGCs) and gonadal somatic cells in human embryos and fetuses using a time-series single-cell RNA sequencing (scRNA-seq) strategy. They also analyzed the development of both germ cells and gonadal somatic cells in a Turner syndrome embryo (45, XO). This study is the first to systematically analyze the gene expression patterns and cell type compositions in monosomy X (45, XO) gonads compared with normal female (46, XX) and male (46, XY) embryos at the same developmental stage (7 weeks of gestation; 7 W).

---

* Corresponding author.
E-mail: gaof@ioz.ac.cn (Gao F).

The results suggest that the lack of one copy of X chromosome has different influences on germ cells and gonadal somatic cells. It is proposed that the XO embryo is likely originated from an XX zygote (specifically losing one copy of X chromosome). X chromosome inactivation (XCI) is an essential mechanism to compensate for different dosages of the X-linked genes between female (XX) and male (XY) cells. After specification, germ cells in normal female gonads reactivate the inactivated X chromosome, which will remain active during FGC development. On the other hand, in the gonadal somatic cells, the inactivated X chromosome will be maintained at the inactivated state permanently. Therefore, X chromosome monosomy would cause different outcomes in germ cells and somatic cells. In germ cells, it leads to the loss of one active X chromosome (all of the genes on the active X chromosome are 'active', permitting being expressed). However, in gonadal somatic cells, it leads to the loss of one inactive X chromosome (majority of the genes on the inactive X chromosome are epigenetically silenced with only dozens of genes escaping the inactivation process and keeping being actively expressed). This may partially explain the cell type-specific abnormalities in the gonads of Turner syndrome embryos. Furthermore, X chromosome monosomy leads to the depletion of Purkinje cell protein 4-positive and tachykinin precursor 1-negative (PCP4$^+$TAC1$^-$) cell type in the Turner syndrome embryo. Alternatively, if the XO embryo is originated from an XY zygote (specifically losing Y chromosome), the phenotypes would suggest that the genes on Y chromosome are not essential for the development of germ cells and some types of gonadal somatic cells.

In this study, Wang et al. [19] validated the presence of some abnormal cells that simultaneously expressed classical markers of gonadal somatic cells and germ cells at three different developmental stages (8 W, 16 W, and 21 W) with three different combinations of antibodies, POU class 5 homeobox 1/delta like non-canonical Notch ligand 1 (POU5F1/DLK1), synaptonemal complex protein 3/WT1 transcription factor (SCP3/WT1), and DEAD-box helicase/forkhead box L2 (DDX4/FOXL2) [19], which has not been reported previously. What will be the final fate of these abnormal cells? Will these abnormal cells gradually disappear with development, or will they develop into some teratoma 'progenitor' cells in the adult? Follow-up investigations are needed to answer these interesting questions.

In addition, Wang et al. [19] also identified a new subtype of germ cells that highly expressed secreted protein acidic and rich in cysteine (SPARC), a master gene regulating cell migration. According to previous studies, germ cell migration occurs from the hindgut along the gut and across to dorsal mesentery to reach gonads at around 4–5 W in humans, thus most of the germ cells have already completed the migration process at around 5 W. However, the SPARC$^+$ germ cells can be found in the gonads of human fetuses up to 23 W, suggesting that after entering the gonad, some of the germ cells may still maintain the migration potential and could migrate within the gonad to optimize their spatial distributions there.

Wang et al. [19] have found that the bone morphogenetic protein (BMP) signaling pathway has developmental stage-dependent functions for male germ cells, and the BMP signaling activity specifically promotes the expression of ALDH1A2 [a gene encoding a retinoic acid (RA)-synthesizing enzyme] in male germ cells at relatively late developmental stage (15 W). Further studies have demonstrated that the BMP signaling pathway plays vital roles in the gonocyte-to-spermatogonium transition (GST) process.

This study has also revealed the functional crosstalk between the BMP signaling pathway and RA signaling pathway in male gonadal somatic cells. BMP signaling activity inhibits the expression of ALDH1A3 (a gene encoding a RA-synthesizing enzyme) in all testicular somatic cell types, Sertoli cells, Leydig cells, and keratin 19-positive (KRT19$^+$) cells, at both the early (7 W) and late (15 W) developmental stages. Notably, different gonadal somatic cells exhibit different responses to BMP inhibition. BMP and RA signaling pathways play critical roles in regulating the meiosis of germ cells [6,20–22]. These results would provide important information for better understanding the regulation of meiosis initiation in germ cells.

Furthermore, Wang et al. [19] have also assessed the developmental origins of the granulosa cells in females and those of the steroidogenic lineages (Leydig cells in males and theca cells in females) in both genders. The results indicate that DLK1$^+$ cells in early developmental stages of male and female embryos may further differentiate into Leydig cells in testes and theca cells in ovaries. DLK1 was continually expressed in Leydig cells of the testes, while its expression disappeared in theca-like cells of ovaries. The origin of steroidogenic cells is a controversial question with a lot of debates in the literature. The results indicate that DLK1$^+$ cells are probably a new type of progenitors of steroidogenic cells. They have also proposed that TAC1$^+$ cells at 7 W may further differentiate into granulosa cells in later development stages. However, these conclusions need to be validated by lineage tracing experiments in other animal models.

Finally, Wang et al. [19] have found that Sertoli cells, rather than Leydig cells, express HSD17B3 during fetal and neonatal periods. Leydig cells do not express HSD17B3 until neonatal stages and the ratio of cells that express HSD17B3 gradually increases afterward. These results indicate that the androstenedione is produced by Sertoli cells, but not by fetal Leydig cells during fetal stages, which is consistent with the findings in mouse model [23–25], suggesting that the regulation of steroidogenesis in fetal male gonads is conserved in mice and humans.

Together, Wang et al. [19] have systematically analyzed the regulation of FGC and gonadal somatic cell development in humans. They have identified and characterized new types of FGCs and gonadal somatic cells, and verified all of them by systematic immunofluorescent staining. They have proposed that the DLK1$^+$ cell population is a progenitor population of the steroidogenic cell lineage and the TAC1$^+$ cell population is a progenitor population of granulosa cells (**Figure 1**). Notably, they have demonstrated the crosstalk between BMP and RA signaling pathways by functional assays. The single-cell omics studies are greatly helping advancing the human developmental biology, filling the gaps between the functional studies in mouse model and cell atlas
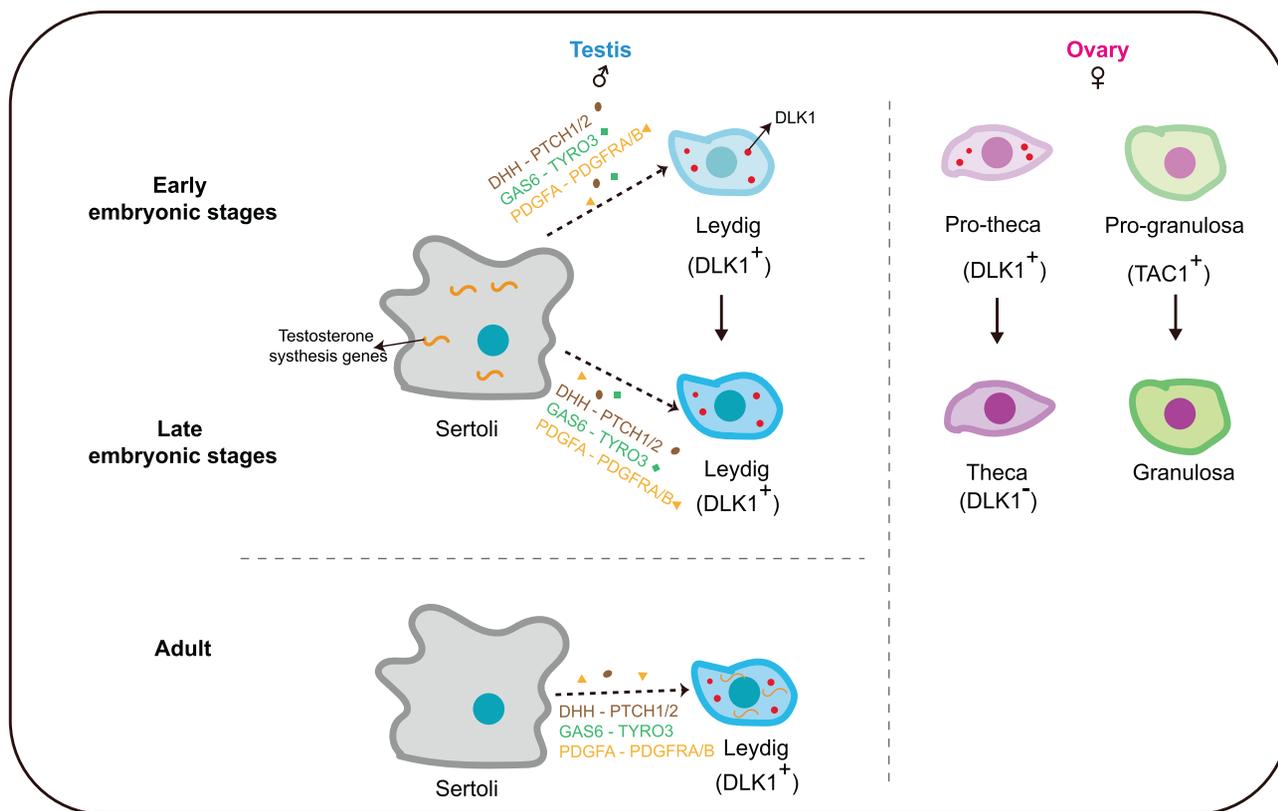
Figure 1   The regulation of gonadal somatic cell differentiation in humans

studies in humans. This study provides a complex but highly ordered development and interaction network for the human FGCs and gonadal cells.

CRediT author statement

Min Chen: Writing - original draft, Writing - review & editing. **Fei Gao:** Funding acquisition, Writing - original draft, Writing - review & editing. Both authors have read and approved the final manuscript.

Competing interests

The authors have declared no competing interests.

Acknowledgments

ORCID

ORCID 0000-0001-6577-6705 (Min Chen)
ORCID 0000-0002-4029-6411 (Fei Gao)

References

[1] Anderson R, Copeland TK, Schöler H, Heasman J, Wylie C. The onset of germ cell migration in the mouse embryo. Mech Dev 2000;91:61–8.

[2] Childs AJ, Kinnell HL, Collins CS, Hogg K, Bayne RA, Green SJ, et al. BMP signaling in the human fetal ovary is developmentally regulated and promotes primordial germ cell apoptosis. Stem Cells 2010;28:1368–78.

[3] Kurek M, Albalushi H, Hovatta O, Stukenborg JB. Human pluripotent stem cells in reproductive science—a comparison of protocols used to generate and define male germ cells from pluripotent stem cells. Int J Mol Sci 2020;21:1028.

[4] She ZY, Yang WX. *Sry* and *SoxE* genes: how they participate in mammalian sex determination and gonadal development? Semin Cell Dev Biol 2017;63:13–22.

[5] Gancz D, Lengil T, Gilboa L. Coordinated regulation of niche and stem cell precursors by hormonal signaling. PLoS Biol 2011;9: e1001202.

[6] Miyauchi H, Ohta H, Nagaoka S, Nakaki F, Sasaki K, Hayashi K, et al. Bone morphogenetic protein and retinoic acid synergistically specify female germ-cell fate in mice. EMBO J 2017;36:3100–19.

[7] Wu Q, Fukuda K, Weinstein M, Graff JM, Saga Y. SMAD2 and p38 signaling pathways act in concert to determine XY primordial germ cell fate in mice. Development 2015;142:575–86.

[8] Li L, Dong J, Yan L, Yong J, Liu X, Hu Y, et al. Single-cell RNA-seq analysis maps development of human germline cells and gonadal niche interactions. Cell Stem Cell 2017;20:858–73.

[9] Guo J, Grow EJ, Mlcochova H, Maher GJ, Lindskog C, Nie X, et al. The adult human testis transcriptional cell atlas. Cell Res 2018;28:1141–57.

[10] Guo J, Nie X, Giebler M, Mlcochova H, Wang Y, Grow, EJ, DonorConnect,, et al. The dynamic transcriptional cell atlas of testis development during human puberty. Cell Stem Cell 2020;26:262–76.

[11] Hermann BP, Cheng K, Singh A, Roa-De La Cruz L, Mutoji KN, Chen IC, et al. The mammalian spermatogenesis single-cell transcriptome, from spermatogonial stem cells to spermatids. Cell Rep 2018;25:1650–67.

[12] Sohni A, Tan K, Song HW, Burow D, de Rooij DG, Laurent L, et al. The neonatal and adult human testis defined at the single-cell level. Cell Rep 2019;26:1501–17.

[13] Zhang Y, Yan Z, Qin Q, Nisenblat V, Chang HM, Yu Y, et al. Transcriptome landscape of human folliculogenesis reveals oocyte and granulosa cell interactions. Mol Cell 2018;72:1021–34.

[14] Fan X, Bialecka M, Moustakas I, Lam E, Torrens-Juaneda V, Borggreven NV, et al. Single-cell reconstruction of follicular remodeling in the human adult ovary. Nat Commun 2019;10:3164.

[15] Wagner M, Yoshihara M, Douagi I, Damdimopoulos A, Panula S, Petropoulos S, et al. Single-cell analysis of human ovarian cortex identifies distinct cell populations but no oogonial stem cells. Nat Commun 2020;11:1147.

[16] Jan SZ, Vormer TL, Jongejan A, Röling MD, Silber SJ, de Rooij DG, et al. Unraveling transcriptome dynamics in human spermatogenesis. Development 2017;144:3659–73.

[17] Wang M, Liu X, Chang G, Chen Y, An G, Yan L, et al. Single-cell RNA sequencing analysis reveals sequential cell fate transition during human spermatogenesis. Cell Stem Cell 2018;23:599–614.

[18] Guo F, Yan L, Guo H, Li L, Hu B, Zhao Y, et al. The transcriptome and DNA methylome landscapes of human primordial germ cells. Cell 2015;161:1437–52.

[19] Wang R, Liu X, Li L, Yang M, Yong J, Zhai F, et al. Dissecting human gonadal cell lineage specification and sex determination using a single-cell RNA-seq approach. Genomics Proteomics Bioinformatics 2022;20:223–45.

[20] Zhang L, Li Y, Hu Y, Chen M, Cen C, Chen M, et al. Somatic cell-derived BMPs induce premature meiosis in male germ cells during the embryonic stage by upregulating *Dazl* expression. FASEB J 2022;36:e22131.

[21] Griswold MD, Hogarth CA, Bowles J, Koopman P. Initiating meiosis: the case for retinoic acid. Biol Reprod 2012;86:35.

[22] Feng CW, Bowles J, Koopman P. Control of mammalian germ cell entry into meiosis. Mol Cell Endocrinol 2014;382:488–97.

[23] Shima Y, Miyabayashi K, Haraguchi S, Arakawa T, Otake H, Baba T, et al. Contribution of Leydig and Sertoli cells to testosterone production in mouse fetal testes. Mol Endocrinol 2013;27:63–73.

[24] Wen Q, Cheng CY, Liu YX. Development, function and fate of fetal Leydig cells. Semin Cell Dev Biol 2016;59:89–98.

[25] Whiley PAF, O'Donnell L, Moody SC, Handelsman DJ, Young JC, Richards EA, et al. Activin A determines steroid levels and composition in the fetal testis. Endocrinology 2020;161:bqaa058.