Supplemental Discussion

The Expression Characteristics of Ribosome-associated Genes and RNA-binding Proteins

Moreover, we analyzed the expression characteristics of ribosome-associated genes in FGCs and their niche cells, which are usually considered 'housekeeping' genes. For most of these ribosome genes, we found that mitotic FGCs in both female and male embryos expressed these genes at very high level, as expected. However, they were expressed at much lower levels in female meiotic FGCs and male mitotic arrest FGCs. Ribosome-associated genes were generally expressed at a lower level in gonadal somatic cells than in mitotic FGCs (Figure S5B). This pattern indicates that the translational machinery is generally different between mitotic and meiotic cells and between FGCs and their niche cells. Interestingly, *RPS6KA3* and *RPS6KA6* were specifically up-regulated in female meiotic prophase FGCs. It was reported that *RPS6KA6* might participate in cell cycle arrest signaling pathway and *RPS6KA3* was expressed in prophase spermatocytes, but now it seems that *RPS6KA3* is implicated in meiosis. The expression level of *RPL39L* and *RPS6KC1* was significantly higher in FGCs than in gonadal somatic cells. *B2M* was expressed more highly in late stage FGCs than in mitotic FGCs. *TRPS1* was specifically up-regulated in oogenesis FGCs and mitotic arrest FGCs rather than early stage FGCs (Figure S5C). This finding is consistent with our preceding GO analysis of human FGCs and their niche cells, where the translation activity of FGCs declines with their development process. This finding also indicates that housekeeping genes are not always expressed at a very similar level in different cell types.

Furthermore, we analyzed the expression pattern of RNA-binding proteins. microRNA processing genes, *DICER1*, *DROSHA*, and *AGO1-4* were expressed in both FGCs and gonadal somatic cells, whereas piRNA-interacting genes, PIWIL1-4 and *DND1*, were specifically expressed in FGCs, as expected. *PIWIL1* was expressed more highly in mitotic phase FGCs in both female and male embryos. *PIWIL2* was expressed more highly in female RA responsive phase FGCs, meiotic prophase FGCs

and male gonadal mitotic phase FGCs. *PIWIL3* was specifically expressed in oogenesis phase FGCs and marginally expressed in male gonadal mitotic FGCs. *PIWIL4* was expressed at the highest level in male gonadal mitotic FGCs (Figure S5D).

Species specifically expressed genes in mouse and human FGCs and gonad niche cells

We focused on the expression pattern of genes that play critical roles in mouse FGC and gonadal development. Intriguingly, we identified some gene expression divergence between mice and humans.

Wnt3 is expressed in post-implantation epiblasts and is critical for FGC specification and migration in mice (Aramaki et al., 2013; Tanaka et al., 2013). However, we found that *WNT3* was expressed in female mitotic FGCs, meiotic prophase FGCs and all three phases of male FGCs in humans, implying that the WNT signaling pathway may play an important part throughout the development process of gonads (Figure S3C). *Ifitm1* and *Ifitm3* are expressed in mouse migrating FGCs (Lange et al., 2008; Tanaka et al., 2005). We found that although *IFITM1* and *IFITM3* were expressed in early FGCs, they were expressed at an even higher level in gonadal somatic cells, suggesting that *IFITM1* and *IFITM3* may play roles in gonadal somatic cells (Figure S3C).

Cbx2 (also known as *M33*) is expressed in the mouse genital ridge, and loss-of-function mutation of *Cbx2* leads to gonadal dysgenesis (Katoh-Fukui et al., 2011). However, we found that in humans, *CBX2* was expressed not only in female endothelial cells and early granulosa cells but also in all phases of FGCs (Figure S3C). *Kdr* is expressed in epithelial cells, and pharmacological inhibition of KDR impairs endothelial migration and testis cord formation in male mice (Bott et al., 2010; Bott et al., 2006; Cool et al., 2011). However, we found that in human gonadal somatic cells,

KDR was expressed in endothelial cells and granulosa cells of female gonads but not in the Sertoli cells or Leydig cells of male gonads (Figure S3C).

In summary, although the FGC development has many similar features between mice and humans, these species also show specific features that are very different from each other. This finding reminds us that although the mouse is a highly valuable model organism to investigate mammalian germ cell development, we also need FGCs from scarce human samples *in vivo* and FGC-like cells differentiated *in vitro* to determine the species-specific features of human germ cells.

Supplemental Reference

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