conservation of somatic cell replacement during early development of mouse female germline cysts

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Abstract

In mice fetal ovary, Wnt4-expressing somatic cells we term "escort-like cells (ELCs)" interact with early developing cysts of both sexes. After E12.5, Lgr5+ pre-granulosa cells ingress from the ovarian surface epithelium, and our lineage tracing showed that they replace escort-like cells in the cortical region to establish the wave 2 granulosa cell population supporting primordial follicles. In contrast, lineage marking of ELCs at E10.5 showed that early somatic cells are not heavily replaced in the medullar ovarian region that give rise to wave 1 follicles. Reflecting their distinct somatic cellular origins, second wave follicles were ablated by diptheria toxin treatment of Lgr5-DTR-EGFP mice at E16.5, while first wave follicles developed normally and supported fertility. These findings argue that somatic cell replacement in mice and Drosophila has been evolutionarily conserved, and while not essential, likely aids female gamete development.

Results

Using our single cell RNA sequencing data (not shown here), wnt-expressing escort-like cells (ELCs) were identified. Then we used double in situ hybridization, immunofluorescence, immunohistochemistry and electron micrograph to investigate the cellular localization of ELCs in early gonads. The results clearly show that ELCs express high levels of Wnt, and BMP target genes, and tightly wrap individual cysts/nests.

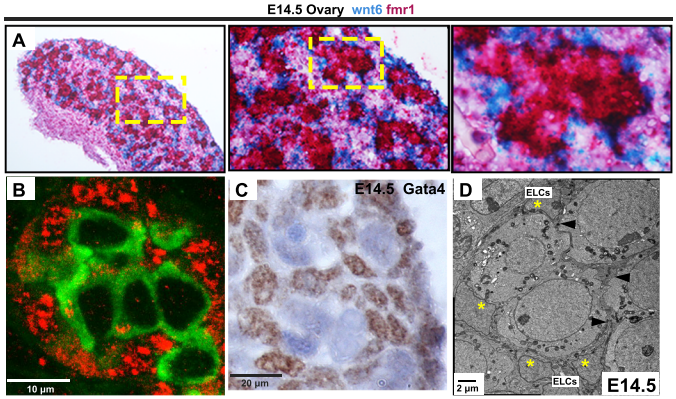

Figure 1 (A) In situ hybridization (ISH) analysis shows Wnt6 (blue) and Fmr1 (red) mRNA expression in the E14.5 ovaries. (B) Cellular localization of Id1 in E14.5 ovaries. Ovaries were stained for Id1, and the oocyte marker DDX4 at E14.5 by immunofluorescence. (C) Cellular localization of Gata4 in E14.5 ovaries by immunohistochemistry. (D) Electron micrograph of E14.5 ovary showing part of a germline cyst surrounded by ELCs (yellow asterisks). Squamous membranes of ELCs surrounding the germ cells are indicated by arrowheads.

To gain further insight into the developmental fates of ELCs and surface derived pregranulosa cells, we carried out developmental trajectory analysis of the somatic cell populations (single cell RNA sequencing data). This yielded a curve that connected E12.5 ELCs and epithelial cells with two distinct populations of E18.5 pregranulosa cells. In addition, in situ analysis also showed the cortical Lgr5+ pregranulosa cells represent surface epithelium-derived cells that have migrated inward and replaced ELCs in cysts at the ovarian cortex.

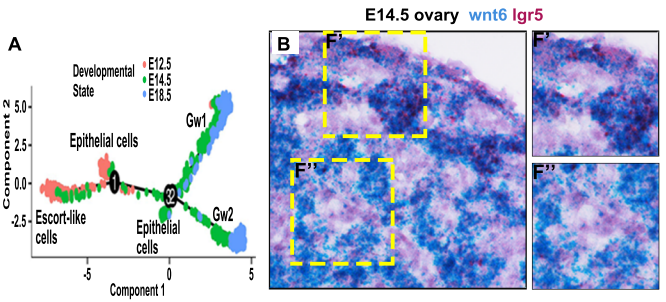

Figure 2 (A) Differentiation trajectory of E12.5, E14.5, and E18.5 ovary beginning with escort-like cells and epithelial cells and splitting into two branches (Gw1, Gw2) constructed by Monocle. Cell colored by the developmental state. (B) In situ hybridization analysis shows Wnt6 (blue) and Lgr5 (red) mRNA expression in the E14.5 ovary. Lgr5+ pregranulosa cells were detected in the cortical but not in the deeper subregions.

We validated the trajectory analysis using lineage marking of progenitors of the two major somatic cell populations. Axin2+ cell tracing experiments show that ELCs are initially found in association with all germ cell cysts, but are lost in the cortical region as surface-derived cells invade. In contrast, the great majority of the tdTomato+ cells clonally related to the Lgr5+ cells labeled at E13.5 were still found at the ovarian surface or were observed contacting germ cells in the outer cortical region only.

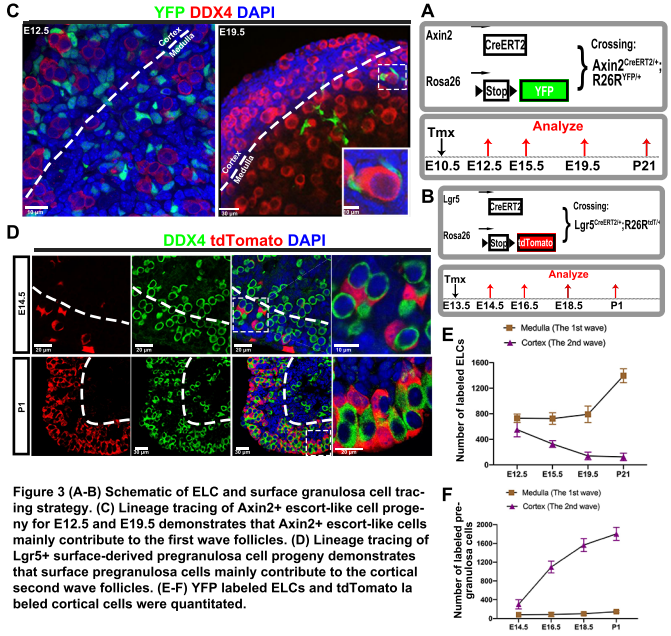

Figure 3 (A-B) Schematic of ELC and surface granulosa cell tracing strategy. (C) Lineage tracing of Axin2+ escort-like cell progeny for E12.5 and E19.5 demonstrates that Axin2+ escort-like cells mainly contribute to the first wave follicles. (D) Lineage tracing of Lgr5+ surface-derived pregranulosa cell progeny demonstrates that surface pregranulosa cells mainly contribute to the cortical second wave follicles. (E-F) YFP labeled ELCs and tdTomato la beled cortical cells were quantitated.

We then used the Lgr5-DTR-EGFP mice to ablate Lgr5+ cells during fetal follicle development by treatment with diphtheria toxin (DT) to test the prediction of our studies that only second wave (cortical) follicles should be affected. In control animals, a robust population of nearly 4,000 primordial follicles was observed at P5 in the cortical region, however, pregnant females carrying the construct that were DT treated at e16.5 and examined at P5, contained less than 200 primordial follicles, while the number of wave one primary follicles was unchanged.

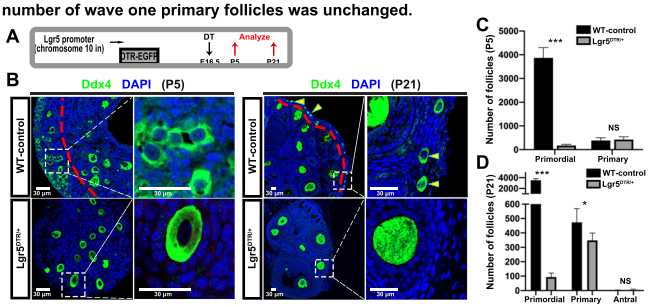

Figure 4 (A) Experimental strategy to ablate Lgr5-expressing cells using the Lgr5-DTR-EGFP mouse model. (B) Histological analysis of the ovary from wild-type mice and Lgr5DTR/+ animals at P5 and P21. (C-D) Follicle quantification in the ovary at P5 and P21 after DT administration at E16.5. NS, Not significant. *P < 0.05, ***P < 0.001 (t-test). Scale bars: 30 um.

Discussion

In Drosophila, like the mouse, the first follicles are made from germ cells derived directly from primordial germ cells (and not from stem cells). These first germ cells are wrapped by escort cells that are probably not replaced since follicle cell stem cells have not yet appeared. The first 100 or so follicles produced by young Drosophila females may be coated with epithelial cells that rapidly differentiated using only escort cell precursors, a striking parallel to the wave 1 population in the mouse.