Single-nuclei Sequencing Reveals Compositional Alterations in Ovarian Cell Types of AMH Deficient Mice

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Anti-Müllerian hormone (AMH), produced by granulosa cells (GC) of growing follicles, plays a crucial role in regulating folliculogenesis. AMH acts as a gatekeeper of follicle growth by inhibiting primordial follicle recruitment and follicular atresia, as well as inhibiting FSH sensitivity of small antral follicles. However, the precise cell type-specific mechanisms through which AMH regulates follicular development remain largely unknown. Due to the heterogeneous composition of ovary tissue, our previous bulk RNA sequencing analyses were unsuccessful to identify AMH regulated genes. Therefore, we conducted single-nuclei RNA sequencing to identify transcriptional changes in a cell type- and follicle-stage specific manner.

Ovaries from 4-month-old AMH deficient (AMHKO) mice (n=5 per group) and wild type (WT) littermates (n=6 per group) were isolated at diestrous. Chromium Single Cell Multiome ATAC + Gene Expression (10x Genomics) was performed. Following ambient RNA removal and filtering, we performed normalization and doublet removal, then analyzed the combined data using Seurat. Transcriptional cellular clusters and GC subclusters were profiled using relevant literature-based markers. Subsequently, we evaluated whether differences in ovarian cellularity reflected variations in ovarian composition between WT and AMHKO mice by analyzing the cell percentage of cell clusters per sample. After quality control analyses, we included 63,236 cells from AMHKO mice and 73,313 cells from WT mice for characterization.

Unbiased clustering and uniform manifold approximation and projection (UMAP) analysis revealed eight distinct clusters. The two largest clusters comprised GC (n=49,459 cells) and mesenchymal cells of the ovarian stroma (n=46,703 cells). Furthermore, two clusters of corpora lutea (CL) were identified: one active CL cluster (n=21,867 cells) and one regressing CL cluster (n=9,101 cells). In addition, four smaller clusters of other cell types were identified, including endothelial cells, ovarian surface epithelial cells and two clusters of immune cells.

The GC cluster segregated into five distinct subclusters, including preantral (n=5,596 cells), antral-mural (n=16,175 cells), mitotic (n=13,073 cells), atretic (n=14,172 cells), and an unknown subcluster (n=443 cells). This unknown subcluster was characterized by high expression of *Ki67* and *Cenpf*, involved in cell proliferation, along with other granulosa cell markers (*Fst*, *Slc18a2*, *Nr5a2*, *Inha*). Apart from previously established markers, further transcriptomic profile analysis revealed potential novel gene signatures within each identified GC subcluster, necessitating further verification.

Analysis of the cellular composition revealed a ~50% ($P<0.01$) and ~30% ($P<0.01$) increase in AMHKO-derived preantral and mitotic GC compared to WT mice, which may be consistent with the increased number of healthy small antral follicles in AMHKO mice. Also, the percentage of AMHKO-derived atretic GC was significantly increased compared to WT mice. In contrast, the percentage of AMHKO-derived active CL cells was decreased by ~35% ($P<0.01$) compared to WT mice, suggestive of a suboptimal CL function.

Investigations into single-cell transcriptional differences within each GCs cluster between WT and AMHKO mice are ongoing, as well as analysis of intercellular signaling networks using CellChat.

In conclusion, our study shows that loss of AMH signaling alters the ovarian cellular distribution, which will aid in identifying differentially expressed gene in a cell-cluster specific manner.