Somatic Cell Fate Specification and Separation in the Fetal Ovary

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Proper differentiation of somatic cell types in the fetal ovary lays the foundation for future ovarian function in the adult. Defects in cell type differentiation lead to diseases including polycystic ovarian syndrome (PCOS), primary ovarian insufficiency (POI), and infertility in women. Understanding how each cell type is established is crucial in developing methods to intervene in ovarian diseases caused by cellular dysfunction. The two major somatic cell lineages in the ovary include supporting cells that secrete hormones and provide nutrients to the germ cell, and interstitial cells that produce hormones and maintain ovarian structural integrity. While it is known that supporting cells and interstitial cells originate from a common somatic progenitor within the fetal ovary, a critically unanswered question is how interstitial cells are specified apart from the supporting cells during ovarian development. To address this question, we performed single-cell mRNA sequencing of mouse ovarian cells at the beginning of ovary formation. We discovered two major somatic populations, one positive for the nuclear receptor *Nr2f2*, and the other positive for the nuclear receptor *Nr5a1*. To investigate the differences between these two populations, we performed differential gene expression analysis and identified that the two cell populations express different components of an important signaling pathway, the Notch pathway. Specifically, the *Nr2f2*$^+$ population expresses Notch receptor *Notch3* and the downstream transcription co-activators, whereas the *Nr5a1*$^+$ population expresses high level of Notch antagonist *Numb*. This suggests that Notch pathway is active in the *Nr2f2*$^+$ population, and conversely, is repressed in the *Nr5a1*$^+$ population. To test whether Notch signaling specifies somatic fate, we performed lineage tracing experiments by labeling early Notch-active cells in the fetal ovary. We found that these Notch-active cells later become interstitial cells in the adult ovaries. Single-cell mRNA sequencing of lineage-traced ovaries identified that the early Notch-active cells co-express *Nr2f2* and were predicted to be endothelial cells and pericytes upon cell type analysis. Finally, to explore whether Notch signaling directs interstitial fate determination, we ectopically activated Notch pathway in the *Nr5a1*$^+$ population and observed significant increase of interstitial-to-supporting cell ratio and a disrupted morphology in the fetal ovary. This suggests that a balanced Notch pathway is critical in determining the somatic composition during ovarian development. In conclusion, our results support a model in which active Notch signaling in *Nr2f2*$^+$ cells specifies the interstitial cell lineage and separates them from the *Nr5a1*$^+$ supporting cell lineage at the beginning of ovary formation.