Single Cell Insights into Epithelial Morphogenesis in the Developing Neonatal Mouse Uterus

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The uterus is vital for successful reproduction in mammals, and two different types of epithelia (luminal and glandular) are essential for embryo implantation and pregnancy establishment. At birth, the uterus is neither fully developed nor differentiated in domestic and laboratory animals as well as humans. On postnatal day (PND) 0, the mouse uterine horns are characterized by a central lumen lined by simple epithelium that is surrounded and supported by undifferentiated mesenchyme cells. Between PNDs 0 and 5, epithelial invaginations (buds) appear in the epithelium that are gland primordia, and the mesenchyme segregates into two layers with the inner forming the stroma of endometrium and the outer forming the myometrium. By PND 10, the epithelium undergoes specification, delineating into uterine-type luminal epithelium (LE), alongside the emergence of bud- and teardrop-shaped epithelial invaginations, marking the initiation of epithelial cell bifurcation and differentiation into FOXA2-positive glandular epithelium (GE) cells. Subsequently, these FOXA2-positive GE cells to develop into the stroma, forming mature glands by PND15. Despite extensive characterization of the uterus, the intrinsic cellular and molecular mechanisms governing epithelial morphogenesis in the developing neonatal uterus are little understood. Here, a combination of single-cell RNA-sequencing (scRNA-seq) and fluorescent in-situ hybridization (ISH) was utilized to discover intrinsic cellular and molecular mechanisms directing uterine epithelium morphogenesis during a critical window of postnatal development. The undifferentiated epithelium (PNDs 1 and 5) and determined uterine epithelium (PND 12 and 15) were heterogeneous and contained several different cell clusters based on single cell transcription profiles. Characterization of cell types were based on marker genes for LE (*Calb1, Gsto1, Tacstd2*), GE (*Cxcl15, Foxa2, Sox9*), mesenchyme/stroma (*Vim*), proliferation (*Hmgb2, Mki67, Pcna*), and putative stem cells (*Axin2, Gstm7, Lgr5, Top2a*). The expression pattern of putative markers (*Aldh1a1*, *Aldh1a3*, and *Cited4*) of GE progenitor cells identified by scRNA-seq were validated by ISH. *Aldh1a1* and *Aldh1a3* expression began on PNDs 1 and 5 localized to the epithelia lining the lumen, and by PNDs 12 and 15 expression was localized specifically to GE cells. Expression of *Cited4* was detected specifically in the GE at all developmental time points (after PND 5). Interestingly, *Cited4* is not regulated by *Foxa2* and is expressed in the LE of *Foxa2* conditional knockout mice, indicating a role in uterine epithelial development. Taken together, these findings reveal dynamic expression patterns suggesting the specification of epithelial cells down the GE lineage prior to the expression of *Foxa2*. These findings provided foundational insights for ongoing mechanistic studies on epithelial specification and GE development using mouse genetic models. This work was supported by NIH Grant R01HD096266.