Abstract # 1760

Single Cell Interrogation of The Uterine-Placental Interface. Regan L. Scott, University of Kansas Medical Center, USA

During a healthy pregnancy, a special lineage of placental cells, referred to as invasive trophoblast cells, exit the placenta and invade into the uterus where they restructure the uterine parenchyma and facilitate remodeling of spiral arteries. Invasive trophoblast cells help anchor the placenta, modulate immune cell populations, and facilitate nutrient delivery to the fetus. These trophoblast-directed uterine modifications are essential for a healthy pregnancy. Insufficient trophoblast invasion and abnormal cross-talk at the uterine-placental interface are major contributors to obstetrical complications such as early pregnancy loss, preeclampsia, intrauterine growth restriction, and pre-term birth. In humans, these events transpire during early gestation, thus their investigation represents a significant ethical challenge. In vitro analyses can provide insights into trophoblast cell potential but fall short as tools to understand the physiology of the invasive trophoblast cell lineage. Implementation of in vivo models to test hypotheses regarding mechanisms underlying the development and function of the invasive trophoblast cell lineage are essential to advance the field. Rodents exhibit hemochorial placentation similar to humans. While the mouse displays shallow trophoblast invasion, the rat exhibits deep intrauterine trophoblast invasion and extensive uterine spiral artery remodeling, comparable to what is observed in the human. In this study, we sought to phenotype cells at the uterine-placental interface of the rat with the goal of identifying conserved candidate regulators of the invasive trophoblast cell lineage. Single-cell RNA sequencing (scRNA-seq) and single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq) were used to interrogate cells at the uterine-placental interface on gestation days 15.5 and 19.5 of the Holtzman Sprague-Dawley rat. Single cell suspensions were prepared by enzymatic digestion of the uterine-placental interface. Single cell libraries were then constructed using the 10X Genomics platform and sequenced with an Illumina NovaSeq 6000 system. Computational analysis with the Cellranger pipeline led to the identification a number of unique cell clusters defined by their transcript profiles, including invasive trophoblast cells (e.g. Prl5a1, Prl7b1, Tpbpa, Plac1, Tfap2c, Igf2, Cdkn1c, Tfpi), endothelial (e.g.Egfl7, Adgrl4, Rasip1, Sox17, Nos3), vascular smooth muscle (e.g. Acta2, Myl9, Tagln, Myh11), natural killer (e.g. Nkg7,Prf1, Gzmb, Gzmm), and macrophage (e.g. Fcgr3a, Lyz2, Aif1, Tyrobp, Cybb) cell clusters. A prominent subset of rat invasive trophoblast cell transcripts is conserved in the invasive extravillous trophoblast cell lineage of first trimester human placenta (e.g. Igf2, Cdkn1c, Tfpi, Ascl2, Mmp12, Cited2, etc).

Nuclei were also isolated from the single cell preparations of the uterine-placental interface, libraries prepared, and sequenced. Analysis with the Cellranger-ATAC pipeline identified unique clusters based on chromatin accessibility, including invasive trophoblast, endothelial cell, vascular smooth muscle, natural killer cell, and macrophage clusters. ASCL2, AP1, TFAP2C, and ATF1 DNA binding motifs were most abundant in accessible regions of the invasive trophoblast cell clusters. These findings provide a foundational dataset to identify and interrogate key conserved regulatory mechanisms essential for development and function of an important compartment within the hemochorial placentation site that is essential for a healthy pregnancy.
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