Picturing the dynamics of trophoblast differentiation in per-implantation stage human embryos by single cell RNA sequencing
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Introduction
Single cell RNA sequencing performed on manually picked cells from trypsin-dissociated embryos, has enabled us to define the transcriptomic landscape of primitive placental trophoblast (TB) that exists before the villous placenta forms and that surrounds the epiblast and associated embryonic tissues during the enigmatic D8-D12 phase of human development when embryonic loss is high. We were able to follow two sub-lineages that emerged from proliferating cytoTB (CTB) at the periphery of the conceptus. A unique form of cytoTB with features of syncytoTB (STB), named as Pre-STB, was detectable at D8 while mature STB was at its zenith at D10. A form of migratory TB (MTB), some with a mixed MTB/CTB phenotype (named as Pre-MTB) also arose around D10. By D12, STB generation was in decline, CTB had entered a new phase of proliferation, and mature MTB cells had begun to travel away from the main body of the conceptus. These studies on human embryos are providing new insights into human implantation and establishment of pregnancy.

Figure 1. Isolation of trophoblast cells from extended cultured human embryos. (A) Workflow for culturing human embryos and isolating cytotrophoblast (CTB), syncytiotrophoblast (STB) and migratory trophoblast (MTB) for single cell RNA-seq. (B) Morphologies of D5 human blastocyst after zona pellucida removal and embryos at embryonic D8, D10, and D12, respectively. Pink circles outline MTB seen at D12 but rarely earlier. Scale bar, 200 µm. (C) Beta-human chorionic gonadotropin (CG) immunofluorescence at embryo D8, D10, and D12. Scale bar, 200 µm. (D) Levels of hCG in cell culture medium at embryo D8-D12 (Mean ± S.E.M.). (E) Morphologies of CTB, STB and MTB after single cell enzyme digestion. Scale bar, 200 µm.

Figure 2. Expression of epiblast marker POU5F1, TB markers KRT7 and GATA3 in human D10 embryos. (A) The 3D montage of a D10 human embryo demonstrating the multi-nucleated syncytium located on the periphery (indicated by arrows), and POU5F1 positive epiblast cells confined to the central area of the embryo. (B) POU5F1 positive epiblast cells surrounded by the prospective yolk sac labelled by Phalloidin staining (Left panel); Expression of KRT7 and GATA3 in human D10 embryos (Middle and right panel).

Figure 3. Identification of cell type specific markers for CTB, STB and MTB. (A) Principal component analysis (PCA) of trophoblast cells showing discrete clusters based on cell type. (B) Unsupervised clustering analysis reveal subsets of CTB that had a partial STB and STB signatures, classified as Pre-STB and Pre-MTB, respectively. (C) Average FPKM for CTB, STB and MTB marker gene expression in each cell type. (D) Expression, based on least likelihood of false discovery of the top 10 marker genes for CTB, STB and MTB in each cell type. (E) Clustering analysis of each cell type achieved by using panels cell-type marker genes and (F) a correlation map indicating strong correlations between each cell type and their marker genes.

Figure 4. Gene ontology and pathway analysis of cell type specific genes for CTB, STB, and MTB.

Figure 5. Dynamics of CTB differentiation. (A) Diagram to illustrate the switch of cell functions of CTB at different developmental stages as revealed by GO terms and pathway analysis. (B) Diagram to illustrate the switch of cell functions of CTB as they undergo differentiation to STB (upper panel) and MTB (bottom panel) as revealed by GO terms and pathway analysis.

Conclusion
• We successfully captured transcriptome dynamics in trophoblast cells occurring within the primitive placenta between D8 and D12 post-fertilization, a time that in vivo corresponds to the first five days after the embryo begins to implant into the uterine wall.
• The transcriptome profiles of a mitotic population of stem cells (CTB), the primitive syncytiotrophoblast (STB), and a population of migratory cells (MTB) demonstrated here for the first time, were identified and described.
• Two sub-lineages of CTB with features of STB or MTB were identified and characterized, illustrating the unique transient stages during early trophoblast differentiation.
• Our data suggest that the primitive placenta, though short-lived, is able to promote highly specialized cell functions at very specific time points throughout the implantation, a period when the familiar villous placenta has not yet emerged.
• A deeper studying of these transcriptome data will likely elucidate additional insights into mechanisms that facilitate human implantation.