Controlled, efficient differentiation of human pluripotent stem cells into proliferative trophoblast

Jaroslav Slamecka, Carlos Tristan, Tao Deng, Pei-Hsuan Chu, Claire Malley, Joseph Ernest, Princy Francis, Vukasin Jovanovic, Seung Mi Ryu,

Jason Inman, Hyen Jong Hong, Pinar Ormanoglu, Elena Barnaeva, Yeliz Gedik, Anton Simeonov, Ilyas Singeç

National Center for Advancing Translational Sciences (NCATS), Stem Cell Translation Laboratory (SCTL), NIH, Rockville, MD, 20850

Abstract

The *in vivo* embryonic counterpart of cultured human pluripotent stem cells (hPSCs) are presumably the cells of the post-implantation epiblast, with a capacity restricted to giving rise to the embryo proper and no longer able to develop into trophectoderm (TE). Previous studies that reported TE differentiation from hPSCs remain controversial, in part due to incomplete differentiation or the use of undefined culture conditions. Here we describe highly efficient TE differentiation, followed by fusion of a subset of cells into multi-nucleated syncytiotrophoblast cells, in chemically defined conditions. Gene expression profiling by bulk and single-cell RNA-seq, and immunocytochemical analysis confirmed robust and step-wise induction of genes associated with trophectoderm and placental development, such as GATA3, IGFBP3, KRT7, CDX2, followed by CGA, DAB2, TEAD3, DLX3, expressed in fused multi-nucleated cells. We then developed a chemically defined medium that supported stable and long-term self-renewal of cell lines established from both hESC- and hiPSC-differentiated TE cells while maintaining molecular features associated with trophoblast. These in vitro-generated proliferating trophoblast cells may prove ideal for modeling diseases of the placenta, drug screening, and cell-based therapies.

Key Points

- We established an efficient and scalable protocol to differentiate hPSCs into trophectodermal cells under chemically defined and xeno-free conditions.
- A subset of hPSC-derived TE cells spontaneously fuse into large multinucleated syncytiotrophoblast-like cells between days 6 and 10 (Fig. 1). The mono- and multinucleated TE cells display expression of a range of trophectodermal and trophoblast markers (Fig. 2).
- Bulk (Fig. 3) and single-cell RNA sequencing (Fig. 5) revealed upregulation of numerous trophoblast/placenta-associated genes (Fig. 4), e.g. GATA2/3, TFAP2A, H19, EPAS1, CGA, ERVFRD-1, CYP19A1, INSL4, HSD3B1 and others. Single-cell D3, D6, D9 samples integrated closely (R/Seurat) with external data containing trophectoderm cells from human peri-implantation stage embryos[1] (Fig. 5 middle) and close to the key cell types of the placental villi[2] (Fig. 5 bottom).
- We then developed culture conditions for a stable proliferation of these cells over more than 30 passages without loss of phenotype. The cells showed expression of trophoblast stem cell markers ELF5 and p63 (Fig. 6-7).

Controlled Differentiation of hPSC into Trophectoderm

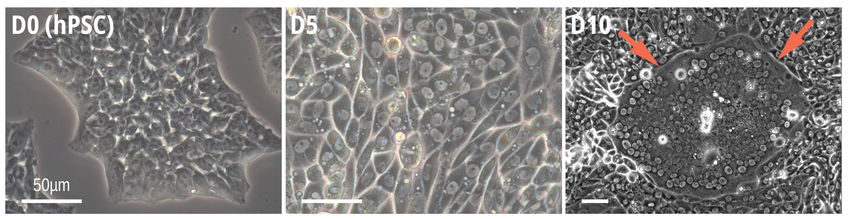


Figure 1. Morphological changes of hPSCs differentiating into TE. hPSCs (Day 0) cultured in E8 medium are differentiated step-wise into TE cells (Day 5) that fuse into multi-nucleated syncytiotrophoblast cells (Day 10). Efficient differentiation was achieved by using small molecules and recombinant proteins in the absence of genetic manipulation.

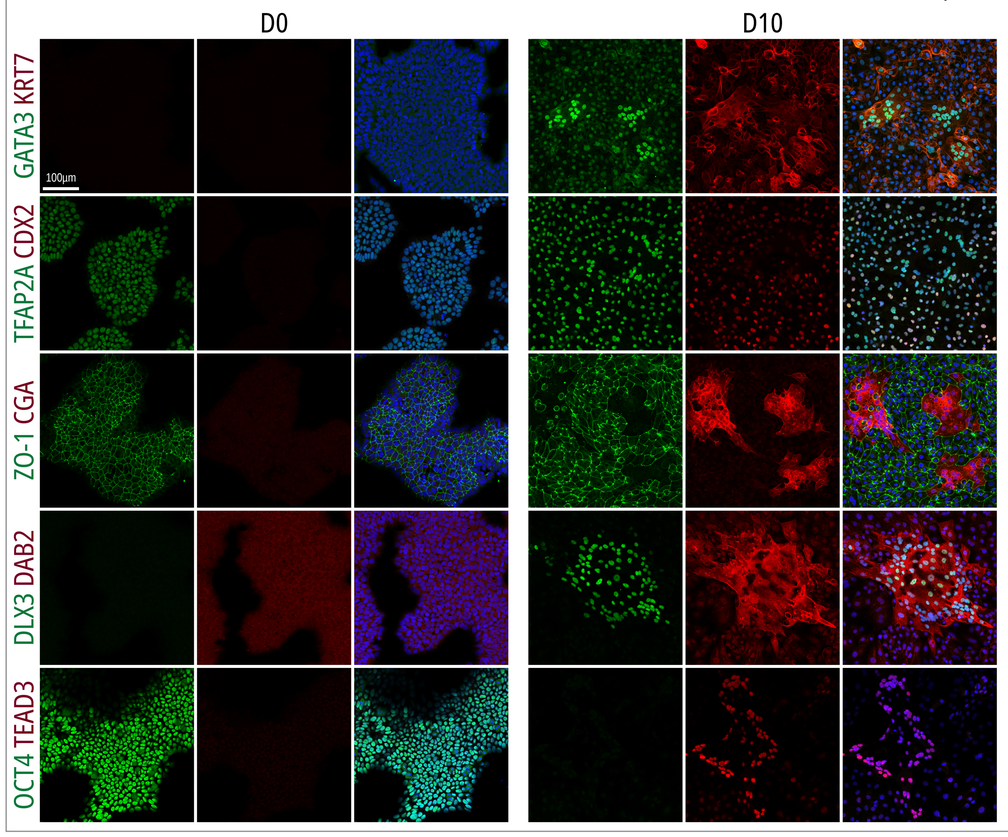


Figure 2. Immunofluorescence showing expression of specific markers of TE and trophoblast. Comparison of pluripotent stem cells (Day 0) and differentiated cells (Day 10) indicate expression of GATA3, KRT7, CDX2, CGA, DLX3, DAB2 and TEAD3 by differentiated cells. Pluripotency-associated transcription factor OCT4 was only expressed in Day 0 cells. TFAP2A and ZO-1 were expressed at both developmental stages but immunoreactivities appeared stronger in differentiated cells, consistent with their epithelial properties.

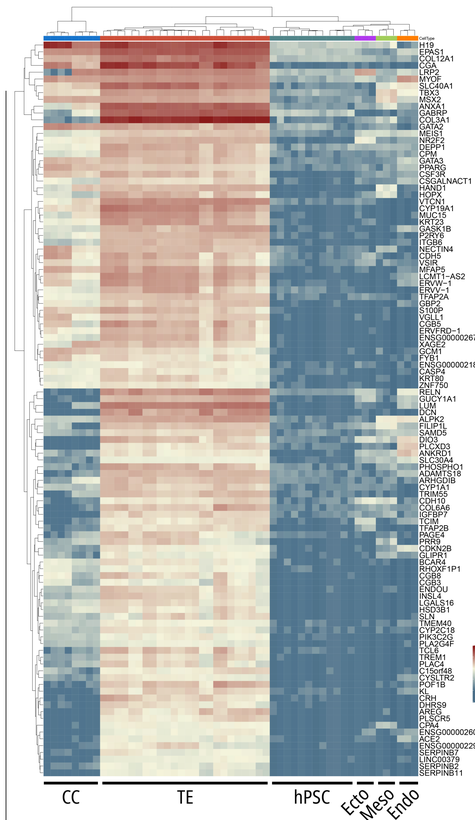


Figure 3. RNA-seq. Heatmap of 108 top differentially expressed genes between induced TE and hPSC (three different hPSC lines). CC – choriocarcinoma lines JEG-3, BeWo. Ecto/Meso/Endo – ectoderm, mesoderm and endoderm differentiation.

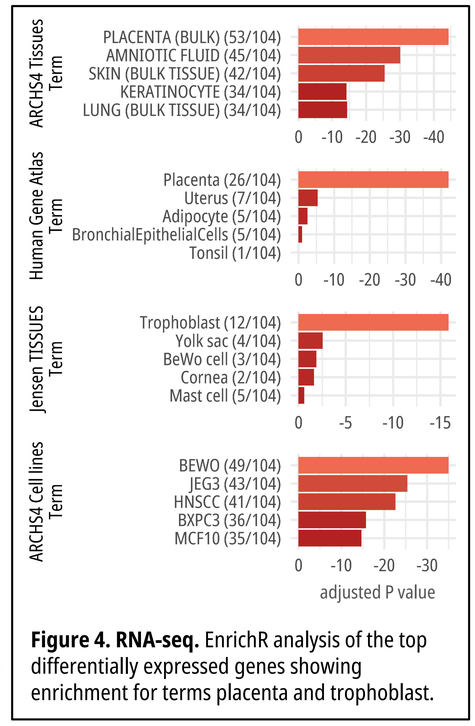


Figure 4. RNA-seq. EnrichR analysis of the top differentially expressed genes showing enrichment for terms placenta and trophoblast.

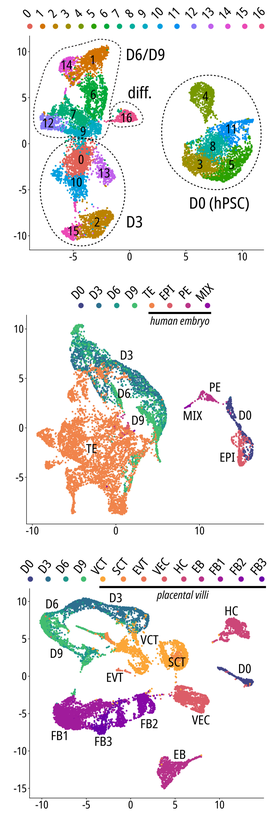


Figure 5. Time-course single-cell RNA-seq (10X Genomics) of TE differentiation. UMAP plot showing identified clusters, "diff." – differentiated subpopulation with syncytial marker expression (top). Seurat integration with external datasets: human embryo[1] (middle) and placental villi[2] (bottom). Embryo: EPI – epiblast, PE – primitive endoderm, TE – trophectoderm; Placenta: VCT – villous cytotrophoblast, EVT – extravillous trophoblast, SCT – syncytiotrophoblast, FB – fibroblasts, VEC – vascular endothelial cells, HC – Hofbauer cells, EB - erythroblasts

Establishment and Long-Term Expansion of hPSC-derived Trophoblast Stem Cells

Figure 6. Morphology, long-term proliferation and expression of p63 by trophoblast stem cells.

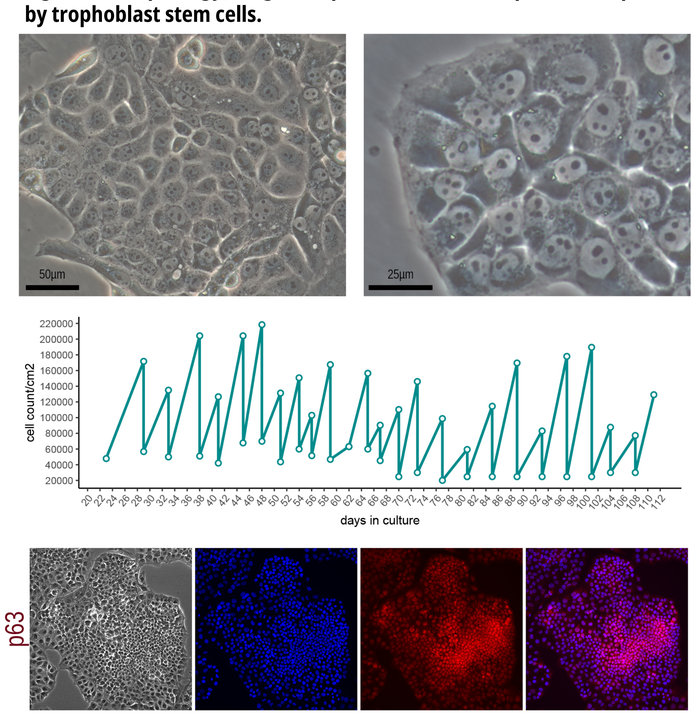
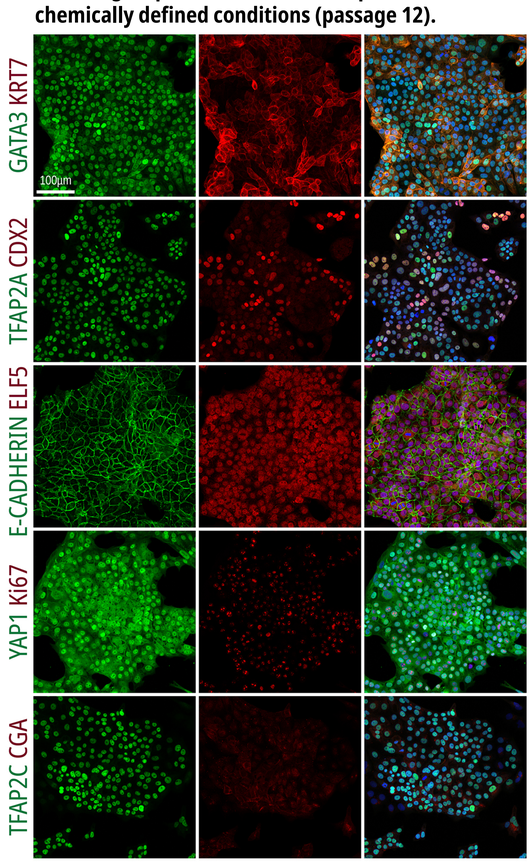


Figure 7. Immunofluorescence analysis of self-renewing trophoblast stem cells expanded under chemically defined conditions (passage 12).



References:

1. *Zhou, F. et al. Reconstituting the transcriptome and DNA methylome landscapes of human implantation. Nature 572, 660–664 (2019).*

2. *Suryawanshi, H. et al. A single-cell survey of the human first-trimester placenta and decidua. Sci. Adv. 4, eaau4788 (2018)*

Funding: NIH Common Fund; NCATS Intramural Research