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Research Highlight
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Successful derivation of human trophoblast stem cells†

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The placenta is essential for fetal development during gestation. Defects in the placenta may have detrimental effects on both the mother and the fetus. How placenta forms successfully to ensure a healthy pregnancy and why placentation inadequacy is associated with malignant pregnancy outcome are not well understood.

The placenta contains diverse subpopulations of trophoblasts, which are all derived from the trophectoderm of the blastocyst. In 1998, Janet Rossant's lab firstly derived mouse trophoblast stem cells (mTSCs) from blastocysts and the extraembryonic ectoderm (ExE) of postimplantation mouse embryos [1]. The stemness of mTSCs was maintained by fibroblast growth factor 4 (FGF4), and these cells contributed exclusively to the trophoblast lineages in chimeras (Figure 1A). By similar strategies, TSCs were also derived from rhesus monkey, bovine and porcine blastocysts [2]. Weizhi Ji's lab obtained rabbit TSC-like cells by culturing rabbit embryonic stem cells (ESCs) in the presence of bone morphogenetic protein 4 (BMP4) [3]. TSC lines have been served as a powerful tool for the study of trophoblast differentiation and placental development.

Establishment of human TSCs is crucial due to the ethical issue of studying human placentation in vivo. Investigators attempted to isolate equivalent populations from human trophectoderm as mTSCs, but colonies were all quickly differentiated. In 2011, Susan J. Fisher's lab identified chorion as a source of continuously self-replicating trophoblast progenitor cells (TBPCs) [4]. They subsequently isolated TBPCs from chorion and maintained these cells at a proliferative state in a medium containing FGF and an inhibitor of activin/nodal signaling. Four years later, this lab derived human TSCs (hTSCs) from UCSFB6, human ESCs derived from single related blastomeres [5], and these cells exhibited high levels of mRNAs encoding trophoblast markers caudal type homeobox 2 (CDX2), cytokeratin-7, and human chorionic gonadotropin. Like TBPCs, UCSFB6-derived cells also expressed glial cells missing homolog 1, high mobility group AT-hook 2, gata binding protein 3, and growth and differentiation factor 15, and had the ability to form the natural trophoblast cell types of the human placenta. However, both TBPCs and UCSFB6-derived cells exhibited mesenchymal morphologies, and most of the human TSCs criteria should be further tested. An alternative way to obtain TSCs is to treat human ESCs or induced pluripotent stem cells (iPSCs) with BMP4 [6]. However, these TSCs are not the real TSCs although they showed some characteristics of native TSCs.

Now, a recent paper published in *Cell Stem Cell* by Takahiro Arima's lab reported the derivation of hTSCs from blastocysts and early placenta [7]. In this study, Okea et al. initially analyzed the transcriptomes of primary trophoblast cells to infer how cytotrophoblast (CT) cells maintained an undifferentiated state in vivo and found that CT cells could be maintained under conditions similar to those of epithelial stem cells such as hair follicle stem cells. They then cultured CT cells under optimized culture conditions containing numerous inhibitors and growth factors known to enhance in vitro proliferation of various epithelial stem cells. They found that a culture medium containing CHIR99021 (a Wnt activator), epithelial growth factor (EGF), transforming growth factor beta (TGF-β) inhibitors, valproic acid (a histone deacetylase [HDAC] inhibitor), and Y27632 (a Rho-associated protein kinase [ROCK] inhibitor) could maintain CT cells for at least 5 months and eventually obtained TS^{CT}. These TS^{CT} cells could differentiate into extravillous trophoblast (EVT)-like cells when cultured in medium containing decidua-derived NRG1, Matrigel, and TGF-β inhibitor A83-01 and into syncytiotrophoblast (ST)-like cells after forskolin treatment. They then randomly selected three clonal lines and confirmed that individual TS^{CT} cell could differentiate into both EVT- and ST-like cells. Using a similar method, they derived TSCs from human blastocysts (TS^{blast}), and the differentiation potential of these cells was also confirmed. Both TS^{CT} and TS^{blast} cells can proliferate for >150 doublings. Transcriptome and DNA methylome profiling showed that TS^{CT} and TS^{blast} cells had gene expression profiles similar to those of corresponding primary cells. The placenta-specific DNA methylome was largely maintained in

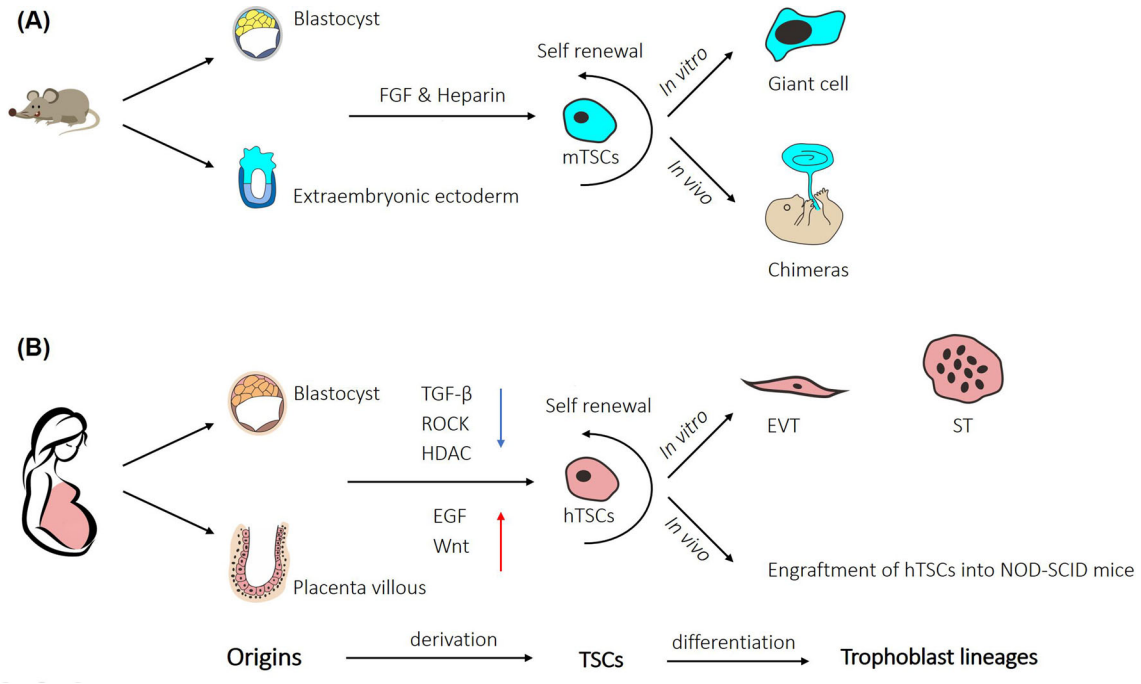

Figure 1. Schematic illustration of the procedure for deriving mTSCs and hTSCs. (A) mTSCs are derived from blastocyst and ExE in the presence of FGF4 and heparin, and have the capacity to proliferate and differentiate into giant cells in vitro and contribute to normal development in chimeras. (B) hTSCs are derived from first-trimester villous and blastocyst by inhibiting (blue arrow online and dark grey in print) TGF-β, HDAC, and ROCK and activating (red arrow online and dark grey in print) Wnt and EGF. hTSCs have the ability to differentiation into EVT and ST and to mimic trophoblast invasion feature when injected into NOD-SCID mice. This figure is available in color at *Biology of Reproduction* online.

TS^{CT} and TS^{blast} cells. When injected subcutaneously into nonobese diabetic (NOD) severe combined immunodeficiency (SCID) mice, these cells exhibited some key features of trophoblast invasion during implantation (Figure 1B). Of note, Caudal Type Homeobox 2 (*CDX2*), T-box Protein Eomesodermin (*EOMES*), Estrogen Related Receptor Beta (*ESRRB*), and Sex Determining Region Y-Box 2 (*SOX2*), which encode transcription factors required for mTSCs self-renewal, were poorly expressed in human CT, TS^{CT}, and TS^{blast} cells. Activation of Wnt and EGF and inhibition of TGF-β, HDAC, and ROCK were important to derive TS^{CT} and TS^{blast} cells, while activation of FGF and TGF-β and inhibition of Wnt and ROCK were critical for the derivation of mTSCs. FGFR2c-mediated FGF signaling appeared dispensable for the proliferation of human trophoblast cells.

Although further in vivo and in vitro studies are needed to determine the differentiation potential of the TS^{CT} and TS^{blast} cells into various types of EVT cells and to identify CT subpopulations and their specific markers which are informative to clarify the source of TS^{CT} cells, the generation of TS^{CT} and TS^{blast} cell lines indeed provides a very useful model for the study of human placenta. It will also help us understand the pathogenesis of developmental disorders with trophoblast defects, such as miscarriage, preeclampsia, and intrauterine growth restriction.

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