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Pluripotent cell states and unexpected fates.

Martin Pera
The Jackson Laboratory, martin.pera@jax.org

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Pluripotent cell states and unexpected fates

Martin F. Pera[1,*]
[1]The Jackson Laboratory, Bar Harbor, ME 04660, USA
*Correspondence: martin.pera@jax.org
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Pluripotent stem cells provide a powerful model for the study of human development and its disorders. Recent studies, including two in this issue of Stem Cell Reports, raise important questions concerning the developmental potential of human pluripotent stem cells, and how the behavior of these cells *in vitro* mirrors normal embryogenesis.

Human pluripotent stem cells (hPSCs) grown under standard culture conditions are often considered to equate developmentally to mouse primed epiblast stem cells, representing a state of pluripotency similar to the late epiblast of the peri-gastrulation embryo. It is therefore somewhat surprising that many reports have confirmed that these archetypal hPSCs are able to give rise to amnion and primordial germ cells, since these are lineages thought to arise from the post-implantation epiblast very soon after implantation (review, Pera and Rossant, 2021). A cohort of recent publications, including two in this issue of Stem Cell Reports (Soncin et al., 2022; Seetharam et al., 2022), raises further fascinating questions concerning the developmental potential of archetypal hPSCs.

In the mouse, the first three cell lineages of the mammalian conceptus—trophoblast, epiblast, and primitive endoderm—segregate prior to implantation, and there is no evidence that the epiblast contributes to these lineages thereafter. In the human embryo, the precise timing of trophoblast and primitive endoderm specification have been subject to some discussion, but, as in the mouse, it has been widely held that the two extraembryonic lineages are set aside before implantation. Largely for these reasons, the finding (originally reported by Ren-He Xu in James Thomson's laboratory nearly 20 years ago; Xu et al., 2002) that bone morphogenetic protein (BMP) 4 treatment of archetypal hPSC can drive their differentiation into trophoblast-like cells has long been controversial. In fact, some recent studies have suggested that gene expression in the BMP-treated archetypal hPSC cells resembles that of amnion, rather than trophoblast (Guo et al., 2021; Io et al., 2021). However, two reports in this issue, and several other published reports and pre-prints, collectively argue that conventional hPSCs indeed can differentiate into trophoblast cells.

Seetharam et al. (2022) surveyed RNA sequencing (RNA-seq) data from three published BMP differentiation studies and compared them using a placenta database and data from blastocyst and early embryo cultures. They performed principal component analysis across a range of experimental models of trophoblast and amnion cells, and carried out cluster analysis on single-cell RNA-seq (scRNA-seq) studies of hPSC differentiation. The conclusions of this study were that the BMP4 protocol commonly used by most authors indeed yields trophectoderm with a bias toward syncytiotrophoblast formation. The authors also noted similarities in gene expression between early trophoblast and amnion. Soncin et al. (2022) use a modified BMP4-based protocol to produce trophoblast stem cells (TSCs) with properties very similar to those of TSCs derived directly from embryos, or from naive pluripotent stem cells. These TSCs could be differentiated into syncytiocytotrophoblast or extravillous trophoblast, and produced trophoblast tumors when engrafted into immuno-deprived animals. scRNA-seq analysis suggested a direct pathway from the primed pluripotent state through a trophoblast-like stage to trophoblast stem cell, with no naive cell-like intermediate.

Other recent studies support the concept that archetypal hPSCs can differentiate to the trophoblast lineage. Wei et al. (2021) showed that BMP4 treatment could enhance generation of TSCs from primed hPSCs. These TSCs could also form extravillous cytotrophoblasts and syncytiotrophoblasts. The authors documented a distinct epigenetic status of TSCs. Their data showed some overlap in amnion and trophoblast gene expression, but ruled out the possibility that their cells were amnion. Jang et al. (2022) also documented development of trophoblast stem cell-like cells capable of differentiation into functional extravillous trophoblast and syncytiotrophoblast from archetypal hPSCs in the presence of BMP4. Ohguishi and Eiraku (BioRxiv, https://doi.org/10.1101/2021.06.28.450118) showed that simply inhibiting activin and fibroblast growth factor (FGF) signaling could drive conversion of trophoblast stem cell-like cells from primed hPSCs, through an amnion-like intermediate. The cells produced syncytia with gene expression consistent with syncytiotrophoblasts. The authors speculated that amnion might be a source of syncytiotrophoblasts in primate development.

The likely source of amnion in the primate post-implantation embryo has recently been examined by

Rostovskaya et al. (2022) who surveyed transcriptomic data from scRNA-seq experiments performed on embryos, and followed this analysis up with experiments on hPSCs in culture. These authors identified two stages in primate amniogenesis, the first originating from what the authors called a partially primed state, which proceeded through a trophoblast-like intermediate to yield amnion cells. These "first-wave" amnion cells could participate in amniotic cavity morphogenesis. The second wave of amniogenesis was shown to arise from what might be regarded as a more conventional primed state of pluripotency, one that differentiates toward amnion through a stage resembling embryonic ectoderm. This wave of amniogenesis was not accompanied by cavitation. The authors contend that this second wave accounts for the development of amnion from conventional primed hPSC cultures. This interpretation is somewhat difficult to reconcile with reports of amniotic cavity formation by archetypal hPSCs (Shao et al., 2017), but the finding of a trophoblast-like intermediate in early amnion formation further highlights the close relationship between intermediate cell states of both lineages.

How then should one interpret these recent studies, along with the previous work showing clearly that archetypal hPSC cultures can differentiate into primordial germ cells and, under some conditions, into cells resembling primitive endoderm? It is possible that cells similar to the partially primed cells described by Rostovskaya et al. (2022), equating to early post-implantation epiblast, actually exist as a minority population in archetypal hPSC cultures (review, Pera and Rossant, 2021). Such cells might retain some features of naive pluripotent stem cells (which can form trophoblast and extraembryonic endoderm), while having also undergone capacitation to enable their differentiation into amnion, the germline, and somatic lineages. It is also possible that the primate epiblast displays considerably more developmental plasticity than its murine counterpart, and retains the ability to feed into extraembryonic and germ layer lineages well past the early post-implantation stage. Current studies certainly suggest a close relationship between amnion and trophoblast lineages, and it may be that epiblast trajectories between the two are subject to enhanced plasticity under certain conditions *in vitro* and during development in the embryo. These possibilities are not mutually exclusive. Unraveling the lineage pathways and fate decisions that primate epiblast-like cells follow *in vitro* is absolutely critical for human embryo modeling and its application to the study of early development and its disorders. These recent studies pose exciting challenges for future experiments to address.

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