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Human Embryonic Stem Cells Assemble and Fulfill Their Developmental Destiny

How do we study the endocrine cell differentiation of the placenta? The accepted course of action dictates that you use primary trophoblast cell cultures derived from human placentas. This is accomplished using methods to isolate cytotrophoblasts that possess the potential to spontaneously differentiate into endocrinologically active syncytial trophoblast within a prescribed period of time (1). Depending upon the nature of the scientific question, you may use cell lines established from human choriocarcinomas (*e.g.* Jeg3, Jar, or BeWo cells). If you are more adventurous, you could opt for trophoblast stem cell populations derived from the mouse or rat (2, 3). Each model system has its merits and limitations. Golos and Thomson and their colleagues (4, 5) have discovered intriguing new experimental approaches for studying the endocrine cell differentiation of human trophoblast cells. These investigators have developed strategies for differentiating human embryonic stem (ES) cells into trophoblast cells capable of synthesizing and secreting placental hormones. Under routine culture conditions, human ES cells exhibit a modest capacity to spontaneously differentiate along the trophoblast lineage (6). Two methods have now been established to promote trophoblast cell differentiation from human ES cells: 1) treatment with bone morphogenetic protein (BMP) family members (4); and 2) aggregation and long-term culture on or within Matrigel, as described in this issue of *Endocrinology* (5). These two experimental manipulations lead to the *in vitro* formation of syncytial trophoblast, and their corresponding production of chorionic gonadotropin (CG), progesterone, and estradiol-17β, mimicking endocrine features of human trophoblast cells developing *in situ*.

To place these exciting new investigative tools into the appropriate context, it is necessary that we delve into the biology of ES cells. First of all, it is important to appreciate that an ES cell is an experimental outcome of creating culture conditions for *ex vivo* propagation of stem cells isolated from blastocysts. Although blastocyst-derived cell populations from the human and mouse have been called ES cells they are not equivalent. Human and mouse ES cells differ in their derivation, the mechanisms they use for self-renewal, and their developmental potential, especially regarding their propensity to form cells of the trophoblast lineage (Fig. 1; Refs. 7 and 8). Human ES cells can differentiate into structures represented by each of the embryonic germ layers, as well as extraembryonic endoderm and trophoblast (6). In contrast, two types of stem cells have been isolated from the mouse blastocyst: 1) ES cells and 2) trophoblast stem (TS) cells. Mouse ES cells most closely correspond to the postimplantation epiblast, are pluripotent and capable of reconstituting all cell types of the body (7–9), whereas the developmental potential of TS cells is restricted to the trophoblast lineage (3, 7). Culture conditions for establishing human TS cells have not been reported. Unlike human ES cells, mouse ES cells do not routinely exhibit a capacity for trophoblast cell differentiation. This species difference is not an entirely new concept. Hints of a species difference between human and mouse stem cells first emerged during investigations with embryonal carcinoma cells over two decades ago (10, 11). Embryonal carcinoma cells are stem cells with an embryonic phenotype and are derived from teratocarcinomas. Human embryonal carcinoma cells can differentiate into trophoblast cells, a characteristic not usually shared with mouse embryonal carcinoma cells.

A few exceptions exist to this view of the developmental potential of mouse ES cells. Oct4 is a POU family transcription factor associated with maintenance of stem cell status. Forced down-regulation of Oct4 in mouse ES cells yields spontaneous formation of trophoblast cells (12, 13). Such observations are consistent with the findings that Oct4 actively suppresses CG subunit gene expression (14, 15) and support the notion that Oct4 is a pivotal inhibitor of trophoblast development (13, 16). Unexpectedly, recent studies have demonstrated that mouse ES cells in long-term culture (>43 d) can form blastocyst-like structures and express trophoblast-specific genes (17). Long-term mouse ES cell cultures may allow for reprogramming of the developmental potential of the stem cells. There is also a suggestion that some mouse ES cell lines may harbor a minute population of so-called “pre-TS cells” (18). The pre-TS cell is hypothesized to be a precursor of TS cells and ultimately the trophoblast lineage. Deficiency in the nuclear enzyme poly(ADP-ribose) polymerase-1 results in spontaneous differentiation of a small proportion of mouse ES cells into trophoblast derivatives, possibly through promoting survival and differentiation of the pre-TS cells (18). Thus, under a few special circumstances it appears that mouse ES cell populations can serve as progenitors for trophoblast cells.

In contrast to mouse ES cells, human ES cells can be efficiently directed to differentiate into trophoblast cells. Exposure of human ES cells to BMP family members results in the morphologic and endocrine differentiation toward a trophoblast cell phenotype (4). The nature of the culture conditions is critical for BMP responsiveness. Monolayer cultures of human ES cells plated on Matrigel and exposed to mouse embryonic fibroblast conditioned medium (MEF-CM) and basic fibroblast growth factor (FGF2) respond to BMPs by differentiating into trophoblast within a few days. Alternatively, cells dissociated from human ES cell aggregates called embryoid bodies (see below) and cultured on fibronectin respond differently to BMPs; instead genes representative of

Abbreviations: BMP, Bone morphogenetic protein; CG, chorionic gonadotropin; ES, embryonic stem; FGF2, basic fibroblast growth factor; MEF-CM, mouse embryonic fibroblast conditioned medium; TS, trophoblast stem.

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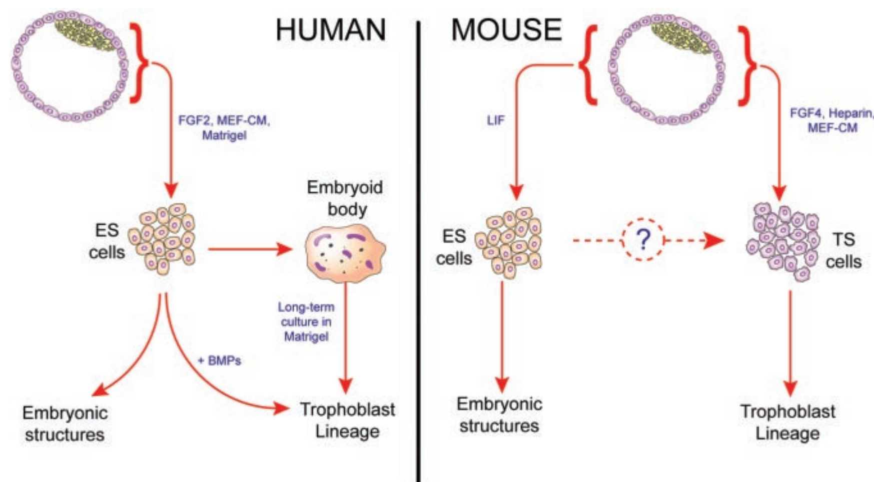

FIG. 1. The developmental potential of human *vs.* mouse blastocyst-derived cell populations. ES cell populations have been established from human blastocysts in the presence of FGF2 and unidentified factors derived from MEF-CM. The cells have the capacity to differentiate into embryonic structures and into trophoblast. The latter differentiation pathway can be promoted by addition of BMPs or after aggregation into embryoid bodies and long-term culture on or within Matrigel in the absence of self renewal factors. In contrast, two types of cell populations have been derived from mouse blastocysts: ES cells and TS cells. Mouse ES cell propagation is facilitated by leukemia inhibitory factor (LIF). Mouse ES cells most closely resemble epiblast and have the potential of differentiating into all embryonic structures but not usually into trophoblast. Expansion of TS cells is promoted by exposure to FGF4, heparin, and factors produced by MEFs. Mouse TS cells contribute to the multilineage trophoblast of the placenta. Surprising recent observations indicate that cells within long-term mouse ES cell cultures (>43 d) may be reprogrammed and acquire the capacity to contribute to the trophoblast lineage.

each of the embryonic germ layers are activated (19). Gerami-Naini and Dovzhenko and their colleagues (5) also generated embryoid bodies from human ES cells. Embryoid body formation leads to a complex interplay of cell-to-cell signaling and is a well-established method to induce differentiation of stem cells. Human ES cell-derived embryoid bodies were inserted into Matrigel rafts and cultured in the absence of self-renewal factors (FGF2, MEF-CM) for up to 8 wk. Dramatic increases in CG and steroid hormone production began after 3 wk of culture and were maximal by 5–6 wk of culture. The 3-wk latency to overt trophoblast cell differentiation is unexplained but suggestive that the embryoid bodies proceeded through systematic programming events before the generation of signals required for trophoblast cell differentiation. The impact of long-term culture of human and mouse ES cells on trophoblast cell development is an intriguing coincidence. It is compelling to propose that the programming events may have included the elaboration of a BMP family member; however, it is also possible that the culture conditions used by Gerami-Naini and Dovzhenko and their co-workers (5) were as much selective for the trophoblast lineage as they may have been instructive. Interestingly, a common element in the two protocols resulting in pronounced trophoblast cell differentiation from human ES cells was the inclusion of Matrigel, a complex extracellular matrix rich in laminin and other regulatory molecules.

These two methods for manipulating human ES cells offer considerable promise for understanding mechanisms controlling the growth and differentiation of the human trophoblast lineage. The possibilities are fascinating. Can these cells be stimulated to differentiate into the various human trophoblast cell lineages, including acquisition of phenotypes consistent with both villous and extravillous trophoblast? Can the cells be used therapeutically for the treatment of pregnancy-related disorders such as preeclampsia, which is associated with failures in trophoblast? Thomson and Golos and their colleagues have established a proof of principle. If conditions can be devised to direct trophoblast cell differentiation, then the elaboration of strategies to instruct and select for differentiation along other cell lineages should be imminent.

A few final thoughts are warranted. First, it is important to realize that the derivation of trophoblast cells, discussed above, has been demonstrated in only a limited subset of human ES cells. Is the ability to differentiate along the trophoblast cell lineage a general rule for all human ES cells or is it unique to those generated on mouse feeder layers by Thomson and his colleagues? The derivation and biology of mouse ES cells is influenced by the genetics of the donor blastocyst (8). Thus, it may not be a surprise if the derivation and developmental potential of human ES cells is similarly impacted by genetic variation. Addressing such an important issue would be greatly assisted by an increase in the access to new stem cell populations from human embryos. Correspondingly, additional insight may be gained by studying the potential of other stem cell populations (adult) to be reprogrammed toward a trophoblast phenotype. Finally, most of our knowledge of blastocyst-derived cell populations is restricted to the mouse and human. The significance of the species difference will be better appreciated once strategies for the *ex vivo* propagation of blastocyst-derived cells from a more diverse collection of species have been achieved.

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