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Human trophoblast stem cells: Real or not real?

Ching-Wen Chang [a, b], Mana M. Parast [a, b, *]

[a] Department of Pathology, University of California San Diego, La Jolla, CA, USA
[b] Sanford Consortium for Regenerative Medicine, University of California San Diego, La Jolla, CA, USA

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ABSTRACT

Abnormal trophoblast differentiation is the root cause of many placenta-based pregnancy complications, including preeclampsia and fetal growth restriction. Human trophoblast differentiation is difficult to study due to the lack of a stem cell model. Such a multipotent "trophoblast stem" (TS) cell, with the ability to differentiate into all trophoblast subtypes, has been derived from mouse blastocysts, but attempts to derive similar human cells have failed. We consider here several possibilities for the TS cell niche in the human placenta. Aside from discussion of such a niche in the pre-implantation blastocyst, we discuss evidence for these TS cells residing in the post-implantation villous cytotrophoblast layer, or even in the non-trophoblast portions, of the human placenta. It is our hope that recognition of the niche would lead to successful derivation and *in vitro* establishment of such cells, which could then be disseminated widely to the placental biology community for advancing the field. Availability of self-renewing human TS cells, whose gene expression and environment could be manipulated, will provide a platform, not just for the study of pathophysiology of placental disease, but also for the discovery of diagnostic biomarkers and therapeutic targets for common pregnancy complications.

1. Introduction

The concept of a stem cell niche was first suggested by Schofield in 1978 to describe the bone-marrow microenvironment of hematopoietic stem cells (HSCs) [1]. It was initially defined as the site of residence of stem cells in a particular anatomical tissue; however, over the past 30 years, this definition has evolved and now includes a particular microenvironment which protects stem cells, providing them with growth factors and extracellular matrix components to maintain their stemness. Knowledge of the niche has led to derivation of various types of stem cells, including embryonic stem cells (ESCs) and tissue-specific stem cells (such as HSCs, and mesenchymal stem cells/MSCs), all of which have proven useful as *in vitro* models for studying molecular mechanisms of lineage specification and organ development [2–4].

The human placenta has been dubbed the "least understood organ" [5]; unfortunately, this statement also applies to the stem cells which contribute to this organ, namely, trophoblast stem cells (TSCs) which give rise to the epithelial components of the placenta. Knowledge of the TSC niche in the polar trophectoderm of the mouse embryo led to their successful derivation almost 20 years ago [6]. However, isolation of similar cells from the human blastocyst has remained a challenge [7,8], due, at least in part, to the lack of knowledge regarding the TSC niche during early human development. Studies have pointed to both various gestational ages and various compartments, where such human TSC might exist [7–14]. In this review, we will discuss the various proposed niches for human TSC (Fig. 1), highlighting the many questions that remain and the areas which require further study.

2. Trophectoderm of the preimplantation blastocyst

Trophoblast lineage specification begins with the formation of the blastocyst, where inner cell mass (ICM), the precursor to all embryonic tissues as well as extraembryonic endoderm and mesoderm, separates from an outer layer of cells called trophectoderm (TE), which are thought to give rise to all trophoblast (extraembryonic ectoderm) subtypes after implantation [7]. TSC were first isolated from TE outgrowths of mouse blastocysts, using a combination of fibroblast growth factor 4 (FGF4) and mouse embryonic fibroblast (MEFs) [6]; the latter secrete TGF-beta/activin as the required active components, and thus can be replaced by media conditioned by the feeders [15]. Mouse TSCs are

* Corresponding author. Sanford Consortium for Regenerative Medicine, Department of Pathology, University of California San Diego, 9500 Gilman Drive, MC 0695, La Jolla, CA 92093, USA.
E-mail address: mparast@ucsd.edu (M.M. Parast).

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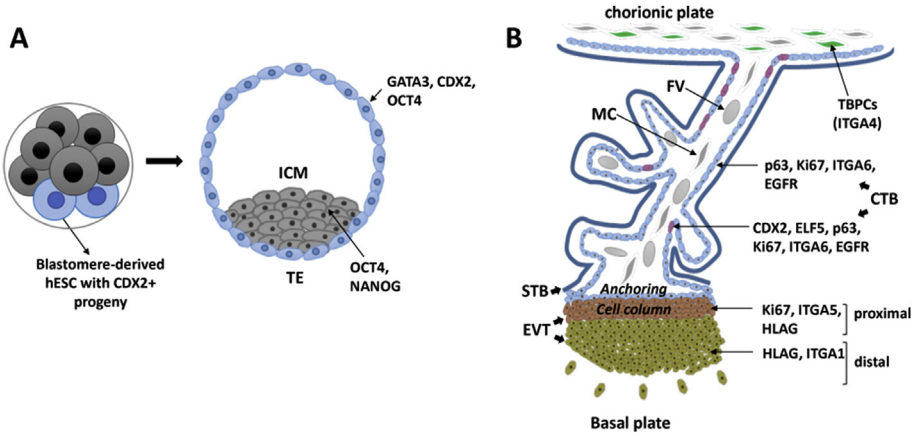

Fig. 1. Schematic diagram demonstrating potential trophoblast stem cell niches in the human placenta at various gestational stages. (A) In the preimplantation stage, embryonic stem cells (hESC) lines have been derived from early blastocysts, which appear to show totipotency (the ability to differentiate into endoderm, mesoderm, ectoderm, and trophectoderm, the latter defined as CDX2$^+$ cells). In the blastocyst-proper, two different cell lineages are observed: trophectoderm (TE) and inner cells mass (ICM). Unlike mouse TE, in the human blastocyst, CDX2 expression initially overlaps with OCT4 in TE, where GATA3 is also expressed. (B) In the post-implantation placenta, potential trophoblast progenitor cell populations include cytotrophoblast subpopulations, such as those which co-express p63, CDX2, and ELF5, as well as ITGA4$^+$ cells in the chorionic mesenchyme. Proximal cell column trophoblast are proliferative, but deemed as committed precursors to the invasive extravillous trophoblast lineage. TBPC, trophoblast progenitor cell; CTB, cytotrophoblast; STB, syncytiotrophoblast; EVT extravillous cytotrophoblast; MC, mesenchymal cell; FV, fetal vessel.

characterized by expression of transcription factors, CDX2, ELF5, and EOMES, all of which are required for maintenance of this lineage *in vivo* [11,12]. In the absence of feeders and FGF4, the cells lose the above markers, and begin to differentiate [6]. Recent studies of human embryos, however, have shown that, of the above factors, only CDX2 is specifically expressed in the pre-implantation TE [9,14]. In addition, unlike the mouse, human TE initially co-expressed OCT4 along with CDX2, with OCT4 being confined to the ICM only in later stage blastocysts (~6 days post fertilization) [14]. More recently, Blakeley et al. have taken these findings further using single-cell RNAseq, showing that key mouse TE-associated genes, including *Elf5*, *Eomes* and *Id2,* are completely absent in human TE; conversely, genes highly expressed in human TE, including *CLDN10*, *TRIML1* and *PLAC8,* were absent in mouse TE [9]. Interestingly, of these, CLDN10, a tight junction component, may participate in TSC niche formation, as establishment of apicobasal cell polarity is a critical step in TE formation in mice [16,17]; also, a related family member, CLDN11, is required for establishment of the spermatogonial stem cell niche in mice [18]. Further study is needed to unravel functions of these genes in human TE, with respect to both trophoblast lineage specification and/or TSC maintenance.

To further study early lineage commitment in the human embryo, two groups recently recapitulated this process *in vitro*, showing that early embryonic development can take place in the absence of any maternal tissues [19,20]. They observed the blastocysts to always attach on the side of the polar TE, the area where trophoblast is nearest the inner cell mass; this is distinct from the same process in mouse, where mural TE initiate attachment [21]. At this stage in the human embryo, TE was best defined by nuclear expression of GATA3, although a variable amount of CDX2 expression was also present. Following attachment, the embryo flattened and the GATA3$^+$ TE acquired strong filamentous CK7 staining, followed, after a few days, by appearance of multinucleated cells and induction of human chorionic gonadotropin-beta (hCGβ) expression in the expanding TE. Taken together, these results indicate that polar TE is critical during implantation and that trophoblast differentiation, at least in these early stages, can be induced within the embryo itself, without input from maternal tissues [19,20].

Recently, derivation of embryonic stem cell (hESC) lines have been reported from single blastomeres of 8-to-10-cell human embryos; these hESC lines appear to have a unique gene expression and DNA methylation profile, indicating a higher competence toward trophoblast differentiation [22]. However, due to the early nature of the embryonic stage used for their derivation, they cannot be widely distributed for study [22]. Attempts to derive TSC from human blastocyst-stage embryos, based on culture conditions used for mouse TSC derivation or variations thereof, have been unsuccessful [23]. A comparative study of FGF receptors has recently revealed that FGFR2, the main FGF receptor expressed in the mouse blastocyst, is not expressed in the human blastocyst. Interestingly, and similar to ELF5, FGFR2 does appear to be expressed in the post-implantation cytotrophoblast [23]. Based on these latter observations, it has been proposed that, more likely, the human TSC niche may reside in the post-implantation placenta [23].

3. Postimplantation chorionic villi

Following implantation, placental villous development rapidly progresses from invagination of proliferating cytotrophoblast (primary villi), to invasion of these structures by mesenchymal cells (secondary villi), and subsequent formation of primitive fetal blood vessels within them (tertiary villi) by the fourth week of gestation [24,25]. At this stage, two distinct trophoblast subpopulations are observed: a proliferative mononuclear cytotrophoblast (CTB) shell which is immediately adjacent to the villous mesenchyme, and a multinucleated syncytiotrophoblast (STB) which abuts the maternal vascular space (Fig. 1). Continuous proliferation of the CTB shell at points of contact with the uterine decidua leads to formation of a third trophoblast subpopulation, the invasive extravillous trophoblast (EVT) [24]. In these regions of anchoring columns, there is a progression of differentiation from a proliferative villous CTB, to a proliferative “proximal column” EVT, to a non-proliferative “distal column” EVT, finally into fully differentiated invasive EVT (Fig. 1). The latter include at least two subtypes, based on their localization: interstitial EVT which invade decidua singly or in groups, and endovascular EVT, which invade and remodel maternal spiral arterioles [24,25]. Along this differentiation pathway, markers of CTB (p63, EGFR) are lost, and those of EVT (HLAG, MelCAM) are gained [24–26]. During this differentiation, an elegant integrin switching is also observed, with loss of ITGA6, and gain of ITGA5 and ITGA1 [27–29].

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