Placenta 34, Supplement A, Trophoblast Research, Vol. 27 (2013) S56–S61

Contents lists available at SciVerse ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Review: Trophoblast differentiation from human embryonic stem cells

T.G. Golos [a,b,c,*], M. Giakoumopoulos [a,c], B. Gerami-Naini [a,1]

[a] Wisconsin National Primate Research Center, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA
[b] Dept. of Comparative Biosciences, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA
[c] School of Veterinary Medicine, and Obstetrics and Gynecology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA

ARTICLE INFO

Article history:
Accepted 22 November 2012

Keywords:
Embryonic stem cells
Trophoblast
Embryoid body

ABSTRACT

The human embryo is not a feasible experimental system for the detailed study of implantation and early placentation, so surrogate systems have been sought for investigating the determination of the trophectoderm lineage, its differentiation into trophoblasts of the early implantation site, and subsequently the morphogenesis of the definitive placenta. An alternative to the use of embryos for studying early placental development was revealed by work with human embryonic stem cells (hESC), demonstrating BMP2/4-stimulated trophoblast differentiation, and spontaneous formation from embryoid bodies (EBs). These cells display a trophoblastic transcriptome, as well as a placental protein and steroid hormone secretory profile, and invasive and chemotactic behavior resembling human placental trophoblasts. With EB-derived trophoblasts, two-dimensional and three-dimensional paradigms and other modifications of the culture environment, including extracellular matrix and aggregation with placental fibroblasts, impact on trophoblast differentiation. Recent studies have questioned the identity of the trophoblasts directed by BMP treatment of hESC, and careful attention to culture conditions is needed to interpret different results among research groups. Although the precise placental counterpart of the hESC-derived trophoblast remains unclear, hESC-derived trophoblasts remain an intriguing platform for modeling early implantation.

1. Introduction

The specification of the trophoblast lineage and the subsequent formation of the placenta are among the earliest differentiation events to take place in mammalian development. *In vivo,* trophectoderm formation is heralded by the onset of compaction at the morula stage, which occurs approximately 4–5 days after fertilization, although molecular events are likely to be put into motion well before this event [1,2]. Following blastocyst formation, the pathway of subsequent embryo development, implantation, and placentation and placental morphogenesis diverges significantly among mammals, encompassing filamentous embryonic elongation, as in ruminants, intrauterine embryonic diapause, as in carnivores, and invasive blastocyst intrusion and invasion into the receptive decidua, as in the initiation of human pregnancy [3]. For the modeling of human placentation, model systems have been developed ranging from immortal cell lines from placental trophoblast-derived tumors or targeted immortalization, to human placental explant tissue, to isolation of individual cells, all with the goal of developing approaches to interrogate this most important time in our development. Recent excellent reviews have broadly covered the range of options and resources in this area and the reader is referred to those papers for a more comprehensive perspective [4–6]. The current review is focused on the use of human embryonic stem cells to model these earliest stages of our development. We will first present a historical perspective on the recognition that nonhuman primate and then human embryonic stem cells provided an opportunity to study trophoblast differentiation. The majority of this review will then present the model for hESC-derived trophoblast differentiation using a specific paradigm, the formation of embryoid bodies, as the platform for these endeavors. Finally, recent developments in the field which have called into question one of the models for BMP4-driven trophoblast differentiation will be discussed, and directions for future research will be proposed.

2. Primate ESC differentiate to trophoblasts

In 1995 Thomson et al. [7] reported the derivation of ES-like cells from rhesus monkey preimplantation blastocysts derived

* Corresponding author. Wisconsin National Primate Research Center, University of Wisconsin, 1223 Capitol Court, Madison, WI 53715-1299, USA. Tel.: +1 608 263 3567; fax: +1 608 263 3524.
E-mail address: golos@primate.wisc.edu (T.G. Golos).
[1] Current address: Dept. of Oral Pathology, Tufts University School of Dental Medicine, Boston, MA, USA.

0143-4004/$ – see front matter © 2012 Published by IFPA and Elsevier Ltd.
http://dx.doi.org/10.1016/j.placenta.2012.11.019

from natural mating and nonsurgically flushed from the uterus. These cells had key characteristics of ESC, including the expression of surface markers characteristic of pluripotent cells, and the ability to form structures derived from all three embryonic lineages when teratomas were formed following injection into immunodeficient mice. It was also determined that upon spontaneous differentiation in culture, there was evidence of trophoblast differentiation, including secretion of chorionic gonadotropin (CG), and expression of the CG alpha and beta subunit mRNAs [7].

This demonstrated a novel attribute of these cells, in that mouse ESC do not possess the ability to spontaneously form trophoblasts in the absence of exogenous genetic manipulation. The significance of this observation was reinforced when the Thomson lab [8] reported the derivation of hESC, and commented that trophoblast differentiation was evidenced again by the secretion of hCG into the culture medium upon spontaneous differentiation. Surprisingly, however, our lab has not been able to demonstrate the production of trophoblast-specific protein expression in teratomas from either rhesus monkey or human ESC (unpublished data) and perhaps this is due to some limitation of the murine environment, or an undefined inhibitory effect thereof, on trophoblast differentiation.

The pluripotency of hESC was further strengthened in 2002 by Xu et al. [9] who demonstrated that remarkably, when hESC were treated with BMP4 or related agonists, there was consistent differentiation to a uniform population of cells with an epithelial morphology, and upon analysis by microarray, the population of transcription factors, secreted proteins and other key markers supported the conclusion that the cells were trophoblasts. This was corroborated by the secretion of hCG, as well as progesterone (P4) and estradiol-17β (E2), the combination of which is a unique characteristic of primate trophoblasts. Although it remains unclear whether BMPs play a role in *in vivo* trophoblast differentiation [10], this model has been adopted by a number of researchers, and has been a valuable and reliable paradigm for the induction of trophoblast differentiation from pluripotent hESC, including definition of the ontogeny of gene expression in these cells [11], demonstration of the role of "physiologically low" oxygen culture conditions in the balance between pluripotency and differentiation [12], and comparison of BMP-induced trophoblasts with human embryonic trophectoderm [13]. There have been comprehensive reviews of the cell biology of the BMP response [6,14,15] so we will only return to this model later to discuss recent controversial findings.

3. An alternative model for trophoblast differentiation: embryoid body formation

Reasoning that pluripotent hESC could serve as embryonic surrogates, our lab investigated whether the formation of embryoid bodies (EBs) by suspension culture of undifferentiated hESC colonies could support trophoblast differentiation. EBs from human and primate ESC will undergo differentiation to all three germ layers, although not in an organized way reflecting *in vivo* embryonic development [7,8]. We first simply addressed whether there was evidence of trophoblast formation in suspension-cultured EBs. This was confirmed by the detection of consistent (although modest) secretion of hCG, P4 and E2 into the culture medium within 48 h after initiation of suspension culture [16]. Immunohistochemistry (IHC) confirmed the formation of cytokeratin- and hCG-positive cells, typically at the periphery of the suspension EBs [16].

Recognizing that the trophoblast-like cells on the surface of the EBs might be able to be recovered for more detailed analysis upon adherent culture of the suspension structures, we found that within several weeks of allowing the EBs to adhere to the culture surface, there was amplification of hCG secretion, in association with outgrowth of cells with a trophoblast morphology [16]. Further evidence supporting a trophoblast identity of these cells included their expression of hCG and cytokeratin 8/18 as detected by flow cytometric staining of a monocellular suspension of these "outgrowths", and their ability to respond to 8-bromo-cAMP with an increase in hCG production, a classic hallmark of trophoblast identity [17] (data in preparation, Gerami-Naini et al.). Nonetheless, hormone secretion declined in culture with time [16].

Additional paradigms were implemented in efforts to further define the differentiation potential of the ESC-derived trophoblasts. First, we devised a three-dimensional culture system, with the hypothesis that the presence of an extracellular matrix (Matrigel) will support an implantation-like environment, and foster the formation of villous structures, recapitulating the formation of the placenta by the implanting embryo. We were able to show dramatic effects of this three-dimensional environment. Cells detached from the surface of the EBs when embedded into Matrigel [16], and hormone secretion by trophoblastic outgrowths was substantially higher, and sustained for longer periods of time, in the 3-dimensional vs. 2-dimensional environment [18]. We have not pursued the mechanisms or regulation of this outgrowth into the Matrigel, and this is an area that deserves further investigation, in comparison with mechanisms identified in other *in vitro* trophoblast systems [19] and hypothesized to be important for *in vivo* implantation. In addition, the 2D vs. 3D cellular signaling pathways which control hormone secretion are likely to be very important, and we attempted to implement a rigid vs. flexible collagen gel paradigm to study this question [20]. However, extensive digestion of the collagen gel matrix by (presumably) secreted matrix metalloproteinases prevented the use of this platform (Giakoumopoulos and Golos, unpublished).

4. Further characterization of the 2-dimensional trophoblast outgrowths

Further characterization has been ongoing for the cells which arise as 2-dimensional outgrowths from adherent embryoid bodies. First, we evaluated whether Matrigel itself, or specific extracellular matrix components thereof, was promoting the differentiation of trophoblasts in the 2-dimensional paradigm. Fig. 1 illustrates that culture on a variety of purified extracellular matrix proteins (e.g., fibronectin, laminin, collagen IV) which comprise important elements of Matrigel all were sufficient at supporting the development of hormonal secretory capability not significantly different

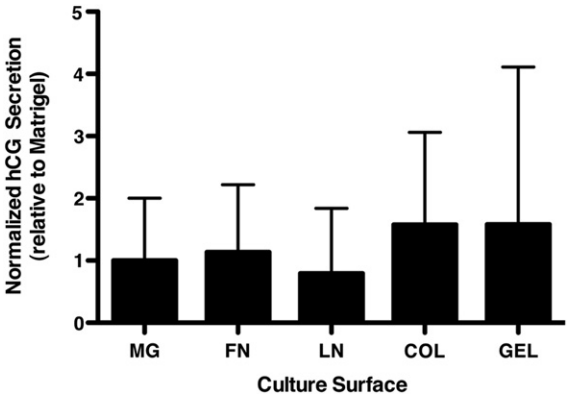

Fig. 1. Effect of extracellular matrix growth surface on secretion of hCG by EB-derived trophoblast outgrowths. Representative data from three experiments is shown at 10 days post-plating. Secretion of hCG was determined by radioimmunoassay, and was normalized to metabolic activity determined by WST-8 assay (Dojindo Molecular Technologies, Rockville, MD).