Mouse and human blastocyst-derived stem cells: vive les differences

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ABSTRACT

Lessons learned from conserved vertebrate developmental pathways have catalyzed rapid advances in pluripotent stem cell differentiation towards therapeutically relevant cell types. The most highly conserved phases of development are associated with the early patterning of the body plan—the so-called phylotypic stage. Both prior to and after this stage there is much more divergence across species. Developmental differences between human and mouse at the blastocyst and early post-implantation stages might help explain the differences among the different stem cell lines derived from these embryos. A better understanding of these early stages of human development will aid our ability to generate and manipulate human stem cells and their derivatives.

Introduction

Differentiation protocols designed to generate specialized cell types or even mini-organs from human pluripotent stem cells are becoming increasingly sophisticated. These approaches offer hope for future cell-based therapies for many degenerative diseases and devastating injuries. They also provide new tools for drug discovery and toxicology, and open up opportunities to study stages of human development not accessible by any other means. Developmental biologists have been able to show that the mechanisms of germ layer formation at gastrulation, anterior-posterior body axis patterning, somite formation, limb development and formation of organ anlage are fundamentally conserved across vertebrate evolution. Knowledge of such pathways has been translated into stepwise differentiation protocols to take embryonic stem cells (ESCs) from pluripotency to formation of a variety of fetal-type specialized cells, without direct knowledge of the relevant stages of human development in vivo.

However, when it comes to the next key phases of maturation of these fetal progenitors into fully functional adult cells, there are still too few cases in which this has been successfully achieved. This is still a real challenge for the field [see, for example, the discussion on generating lung cells elsewhere in this issue (Snoeck et al., 2015)]. In part, this reflects the fact that these stages tend to show more variation among species. Thus, studying maturation of cell types in the mouse or in mouse ESCs might not adequately inform human stem cell differentiation. More direct analysis of human fetal and postnatal physiology may provide some more clues to help in the quest for the perfect human stem cell differentiation protocol.

It should come as no surprise to developmental biologists that the early phases of tissue differentiation from ESCs seem to be relatively conserved, whereas final maturation pathways vary across species. This typifies the concept of the phylotypic phase of development (Richardson, 1995), in which action of conserved developmental genes, including the Hox genes, leads to the fundamental patterning of the body axis and organ rudiments. Prior to and after this stage of development, much more variation in morphology and transcriptional profiles are seen across vertebrate evolution—the so-called ‘hourglass model’ of development (Duboule, 1994; Raff, 1996). This model also predicts that there will be potential differences among ESCs from different mammalian species, as ESCs are derived from the blastocyst stage, before the phylotypic stage. Indeed, there is considerable variation even in the capacity to derive ESCs across different mammalian species and, in the two species most well characterized, the mouse and the human, there are clear differences between the properties of ES or induced pluripotent stem (iPS) cell lines derived under standard conditions.

In this Spotlight article, I argue that these differences might reflect the fact that ESCs arise from a stage of development that is mostly concerned with making extra-embryonic cell types that play no permanent role in the formation of the fetus itself, but are undergoing highly divergent selection pressures related to variation in implantation and placentation across mammalian species.

Blastocyst: conservation and divergence of lineage functions

Formation of the blastocyst represents the first lineage segregation event during embryonic development of every eutherian mammal. Morphologically, all blastocysts contain three distinct cell types: (1) an outer monolayer of polarized cells, the trophoderm (TE), enclosing the inner cell mass (ICM), which consists of (2) a layer of primitive endoderm (PE) covering (3) a compact group of cells, the epiblast (EPI) (Fig. 1). Extensive studies in the mouse have shown that these lineages are specified to their future fates by the late blastocyst stage [reviewed by Rossant and Tam (2009)]. The EPI cells are the pluripotent cells of the blastocyst producing all germ layers of the fetus itself, whereas the TE gives rise to the trophoblast layers of the placenta and the PE largely gives rise to the endoderm of the yolk sacs. Although experimental lineage analysis is not possible in humans, it seems likely that these general lineage relationships also hold true in the human blastocyst.

Mechanistically, local FGF/ERK signaling levels are crucial for the development of all three lineages and their derived stem cells in mouse (Lanner and Rossant, 2010). FGF4 is produced by a subset of cells in the early ICM, under the control of the pluripotency factor partners, OCT4 and SOX2 (Yuan et al., 1995), and signals to adjacent cells within the ICM, directing them towards the PE pathway. Blocking FGF/ERK signaling or enhancing FGF action during ICM development can transform all ICM cells to EPI or PE, respectively (Nichols et al., 2009; Yamanaka et al., 2010). The same FGF signal from ICM cells also signals to overlying TE cells and promotes their proliferation and self-renewal (Goldin and...
totipotency is only lost just prior to implantation (De Paepe et al., 2003). This heavy reliance on the FGF pathway is a very parsimonious use of a single signal from a single cell type to promote lineage specification and proliferation at the blastocyst stage in the mouse.

There is still incomplete information on the molecular and cellular events of human blastocyst formation, compared with the mouse, but what is known suggests that there are differences in timing and potentially in mechanisms of lineage formation and function [reviewed by De Paepe et al. (2014)]. The blastocyst stage of development looks superficially similar between mouse and human (Fig. 1), but the human blastocyst undergoes at least one more round of cell divisions before implanting in the uterus. Concomitant with this extended free-living phase prior to implantation, it seems that lineage commitment is delayed when compared with the mouse. Expression of the lineage-specific transcription factors known to be associated with cell fate specification in the mouse blastocyst begins later in human than in mouse. For example, the expression of CDX2, a key transcription factor required for mouse TE specification, only begins after blastocyst formation in humans, and overlaps in expression with the pluripotency factor OCT4, which is not restricted to the ICM until just prior to implantation (Niakan and Eggan, 2013). Recent studies have shown that both inside and outside cells from fully expanded human blastocysts can separately reconstitute a blastocyst, and that totipotency is only lost just prior to implantation (De Paepe et al., 2013). Thus, the morphological events of formation of a polarized outer epithelium, a blastocoel cavity and an ICM occur prior to actual lineage specification. In the mouse, lineage restriction is complete by the mid-blastocyst stage (Rossant and Tam, 2009), and blastocyst formation can begin in the absence of lineage commitment in embryos mutant for genes such as Cdx2 (Strumpf et al., 2005) and components of the HIPPO signaling pathway (Cockburn et al., 2013; Hirate et al., 2013; Nishioka et al., 2009). However, the time between first formation of the blastocyst and implantation is shorter in the mouse, perhaps explaining earlier segregation of gene expression and cell fate.

Interestingly, the mechanisms of lineage specification in the human embryo seem to differ significantly from the mouse: the ICM is not sensitive to inhibition of FGF signaling in the same manner as in the mouse. When human pre-implantation embryos were grown in the presence of ERK inhibitors to the blastocyst stage, there was no apparent effect on the formation of the PE (Kuijk et al., 2012; Roode et al., 2012; Van der Jeught et al., 2013), as would have been predicted from the mouse, suggesting that the formation of EPI versus PE is not under the tight control of FGF levels in the human. In addition, levels of FGF receptors are low in the human TE, which does not proliferate in response to FGF (Kunath et al., 2014). Does this reflect a general downplaying of the importance of this pathway in the human? More understanding of the relative availability and roles of different signaling pathways in the two embryos is still needed.

Implantation and placental variation between mouse and human

The timing and invasiveness of implantation of the mammalian blastocyst varies remarkably across species, from the relatively early, deep implantation seen in mouse and human to the very late, superficial implantation seen in pigs, cows and horses. But even between mouse and human, there are differences. In the mouse, the implantation site is surrounded by a rapid expansion of the uterine stroma, the decidua, but the TE cells themselves do not invade into the stroma. Rather, the TE overlying the ICM proliferates in response to FGF to form the solid structure of the extra-embryonic ectoderm—the stem cells for the later placenta (Fig. 1A). In humans, the first TE of the blastocyst is highly invasive, and only later are there proliferative cores of trophoblast cells in the chorionic villi that might act as stem cells for the developing placenta (Fig. 1B). This might explain why the human TE is unresponsive to FGF-promoted cell proliferation.

The relative importance of the yolk sac in mouse and human might also play a role in apparent differences in the regulation of primitive endoderm versus epiblast formation. In the mouse, the visceral yolk sac plays a crucial role as the major interchange between the fetus and mother before the placenta is established. In humans, the few data available suggest that the yolk sac is rather vestigial and that the early invasive trophoblast plays a stronger nutritive role. These early functional differences could drive different pathways of TE and PE differentiation between mouse and human that could also lead to differences in the properties of the remaining pluripotent cells by default.

Finally, another important difference between mouse and human is that only the mouse blastocyst can enter diapause—a state of ‘suspended animation’ that occurs naturally during lactational delay or artificially by removal of the ovaries. This process is dependent on the LIF signaling pathway (Nichols et al., 2001), which also enhances ESC self-renewal. Diapause embryos contain all three cell types of the blastocyst and are held in this state until activated by a hormonal signal from the mother.
**Deriving stem cell lines from the early embryo**

In the mouse, it has proven possible to isolate permanent self-renewing stem cell lines from all three lineages of the blastocyst. These cell lines retain the lineage restriction shown in the embryo itself, as assessed by chimera formation. Mouse ESCs derive from the EPI cells of the ICM (Boroviak et al., 2014), whereas XEN cells derive from the PE (Kunath et al., 2005) and trophoblast stem (TS) cells derive from the TE (Tanaka et al., 1998) (Fig. 2).

The culture conditions required for derivation and self-renewal of these cell lines can be related back to the signaling pathways involved in lineage specification and maintenance in the embryo itself (Fig. 2). Consistent with the EPI-promoting effect of FGF/ERK inhibition in the embryo, derivation of stable naïve ESCs from mouse embryos is promoted by inhibition of FGF/ERK signaling, along with inhibition of GSK3 and activation of the LIF/Jak/Stat pathway [2iLIF conditions, see Ying et al. (2008)] (Fig. 2). In the presence of LIF alone, mouse ESCs show dynamic heterogeneity of cell states (Chambers et al., 2007; Hayashi et al., 2008; Toyoooka et al., 2008), because endogenous FGF produced by undifferentiated cells is constantly driving other cells away from the naïve state. Consistent with this, ESCs that are heterozygous for *Oct4*, the pluripotency factor that regulates FGF4 production, are actually more stable than wild-type cells (Karwacki-Neisius et al., 2013), and FGF4-mutant ESCs are resistant to differentiation in vitro (Kunath et al., 2007). Whereas mouse ESCs thrive under conditions of FGF/ERK inhibition, derivation of XEN cells and maintenance of TS cells actually requires active FGF/ERK signaling, as predicted from the embryo itself (Fig. 2).

In humans, permanent TS and XEN lines have not yet been reported. Consistent with the absence of early proliferation of the TE in the human blastocyst, TS cells cannot be derived directly from human embryos under the conditions used in the mouse (Kunath et al., 2014) (Fig. 2B). XEN-like cells have been reported to be produced following overexpression of SOX7 in human ESCs (hESCs) (Séguin et al., 2008), but no-one has reported direct derivation from the blastocyst. hESCs were derived first in 1998 (Thomson et al., 1998) and were shown to require FGF and activin for self-renewal in culture, conditions that are antithetical to the maintenance of mouse ESCs (Ying et al., 2008) (Fig. 2). Does this relate to differences in blastocyst development and the function of the early lineages in the two species? Many studies have suggested that hESCs more closely resemble mouse EpiSCs, which are pluripotent stem cell lines derived from post-implantation epiblast under FGF/activin conditions (Brons et al., 2007; Tesar et al., 2007) (Fig. 2). hESCs are most successfully derived from the ICM of very late human blastocysts (Chen et al., 2009). At this point, the EPI cells of the ICM might be more similar to post-implantation epiblast, given that TE development is delayed and implantation is different from the mouse.

It has always been anecdotally agreed that the existence of blastocyst diapause must be related in some way to the relative ease of derivation of ESCs from the mouse compared with any other mammalian species. Recent attempts to derive hESCs with properties more similar to mouse naïve ESCs are still somewhat contradictory and controversial (Chan et al., 2013; Gafni et al., 2013; Takashima et al., 2014; Theunissen et al., 2014; Ware et al., 2014). However, the stability of the naïve pluripotency network in the mouse does not seem to be easily duplicated in human cells. This might relate back to the diapause issue – activation of a regulatory network that can hold cells in a pluripotent state against their natural tendency to differentiate might be more important for species that undergo diapause. In human embryos, pluripotent cells are always rapidly transiting to differentiation and might never normally activate the stable naïve state. Analysis of gene expression data has suggested that human and mouse naïve-type cells resemble their respective Blastocysts more closely than each other (Huang et al., 2014). Thus, we start to realize that it might not be possible to identify the exactly same pluripotent stem cell state in mouse and human embryos – so vive les differences!

**Implications for replicating developmental pathways from human pluripotent cells**

Despite the differences between mouse and human ES and iPS cells, the fundamental conservation of lineage specification and later developmental pathways still ensures that we can learn much from translating mouse findings into the human system. However, we need to be aware that the starting route to pluripotency might differ between the two species, with potential implications for the next phases of differentiation. Further understanding of the different routes towards pluripotency and towards escape from pluripotency in both human and mouse are still needed if we are to learn how to control differentiation pathways relevant to understanding human biology and treating disease.

**Competing interests**
The author declares no competing financial interests.

**References**


