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REVIEW

From the stem of the placental tree: trophoblast stem cells and their progeny

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ABSTRACT

Trophoblast stem cells (TSCs) retain the capacity to self-renew indefinitely and harbour the potential to differentiate into all trophoblast subtypes of the placenta. Recent studies have shown how signalling cascades integrate with transcription factor circuits to govern the fine balance between TSC self-renewal and differentiation. In addition, breakthroughs in reprogramming strategies have enabled the generation of TSCs from fibroblasts, opening up exciting new avenues that may allow the isolation of this stem cell type from other species, notably humans. Here, we review these recent advances in light of their importance for understanding placental pathologies and developing personalised medicine approaches for pregnancy complications.

KEY WORDS: Trophoblast, Stem cells, Placenta, Transcription factors, Epigenetics, Reprogramming

Introduction

The placenta is the organ that provides the interface between maternal and foetal bloodstreams and, as such, is the site of exchange of nutrients, oxygen, metabolites and other molecules between the mother and the offspring (Watson and Cross, 2005). Mammalian placentas are composite organs consisting of descendants of the trophoblast lineage and mesodermal cell types that, in the mouse, come together during embryonic development in a process called chorio-allantoic fusion (Fig. 1). Mesodermal derivatives form the umbilical cord and the foetal portion of the placental vasculature, whereas the trophoblast compartment gives rise to a variety of highly specialised placental cell types (Box 1; Fig. 2) that enable nutrient and gas exchange between the foetal and maternal blood circulations (Watson and Cross, 2005). The structural properties of these trophoblast cells, and their ability to modulate vascular, endocrine and immunological processes, facilitate and optimise the exchange of metabolites. Importantly, all of these specialised trophoblast cells can be derived from self-renewing, multipotent cells referred to as trophoblast stem cells (TSCs; Fig. 1), an *in vitro* model that has been successfully established and characterised in particular detail in the mouse (Tanaka et al., 1998).

TSCs represent an invaluable research tool, enabling investigations into the control of self-renewal and the identification of cues governing the differentiation of major trophoblast cell types. Despite the importance of this cell lineage for embryo implantation, developmental progression, long-term health and disease predisposition, our molecular understanding of TSCs has lagged far behind that of embryonic stem cells (ESCs). However, recent ground-breaking advances have substantially propelled our knowledge forward in terms of understanding the transcriptional regulation of mouse TSCs on the global scale, and the intersection between such transcriptional networks and signalling pathways as well as the epigenome. Although a seemingly equivalent trophoblast stem cell-like population exists in the early human placenta, it is still uncertain to what extent cell culture models reflect the identity and developmental plasticity of their placental counterparts. Nonetheless, the insights gained from studying mouse TSCs, as well as recent successes with the ability to reprogramme mouse embryonic fibroblasts (MEFs) into induced TSCs (iTSCs), are helping to pave the way towards isolating and propagating human trophoblast stem or progenitor cells. Achieving this goal would undoubtedly represent a major milestone in understanding early pregnancy complications, infertility and personalised medicine approaches in reproductive biology.

Here, we provide an overview of how the trophoblast lineage is established during development and how TSCs can be derived from developing embryos and differentiated *in vitro*. We discuss the signalling pathways and transcription factor networks that operate in TSCs, enabling them to either self-renew or direct their differentiation into specific trophoblast subtypes. Finally, we highlight recent advances in deriving iTSCs in the mouse and discuss the approaches that have been pursued in attempts to derive human TSCs or TSC-like populations.

Establishment of the trophoblast lineage

Specification of the trophoblast lineage occurs in the earliest cell fate decision event during preimplantation development (Fig. 1), when the trophectoderm (TE) and the inner cell mass (ICM) become segregated (Rossant and Tam, 2009; Artus and Hadjantonakis, 2012). Prior to this lineage establishment event, the embryo exists as a compacted morula that consists of apolar inner cells enclosed by polar outer cells. The polarity results from the apical domains of outer cells being enriched for components of the Par3-Par6 (Pard6a)-atypical protein kinase C (aPKC) cell polarity pathway. Similarly, there is a difference in cell-cell contacts, with outer cells having a ‘free’ surface, resulting in divergent distribution of adherens junctions between inner and outer cells (Artus and Hadjantonakis, 2012; Chazaud and Yamanaka, 2016). These differences in polarity and cell-cell adhesion lead to differential activation of the Hippo signalling pathway in outer versus inner blastomeres. In the inner cells, angiomotin (Amot) family proteins bind to the cell-cell adherens junctions as well as to the kinases neurofibromatosis 2 (Nf2) and large tumour suppressor 1 and 2 (Lats1/2). Lats1/2 phosphorylates Amot and this complex in turn phosphorylates Yes-associated protein 1 (Yap1), resulting in the cytoplasmic localisation of Yap1 (Cockburn et al., 2013; Hirate et al., 2013). Consequently, the activation of Hippo signalling in the inner blastomeres leads to initiation of ICM cell fate. In the outer cells, by

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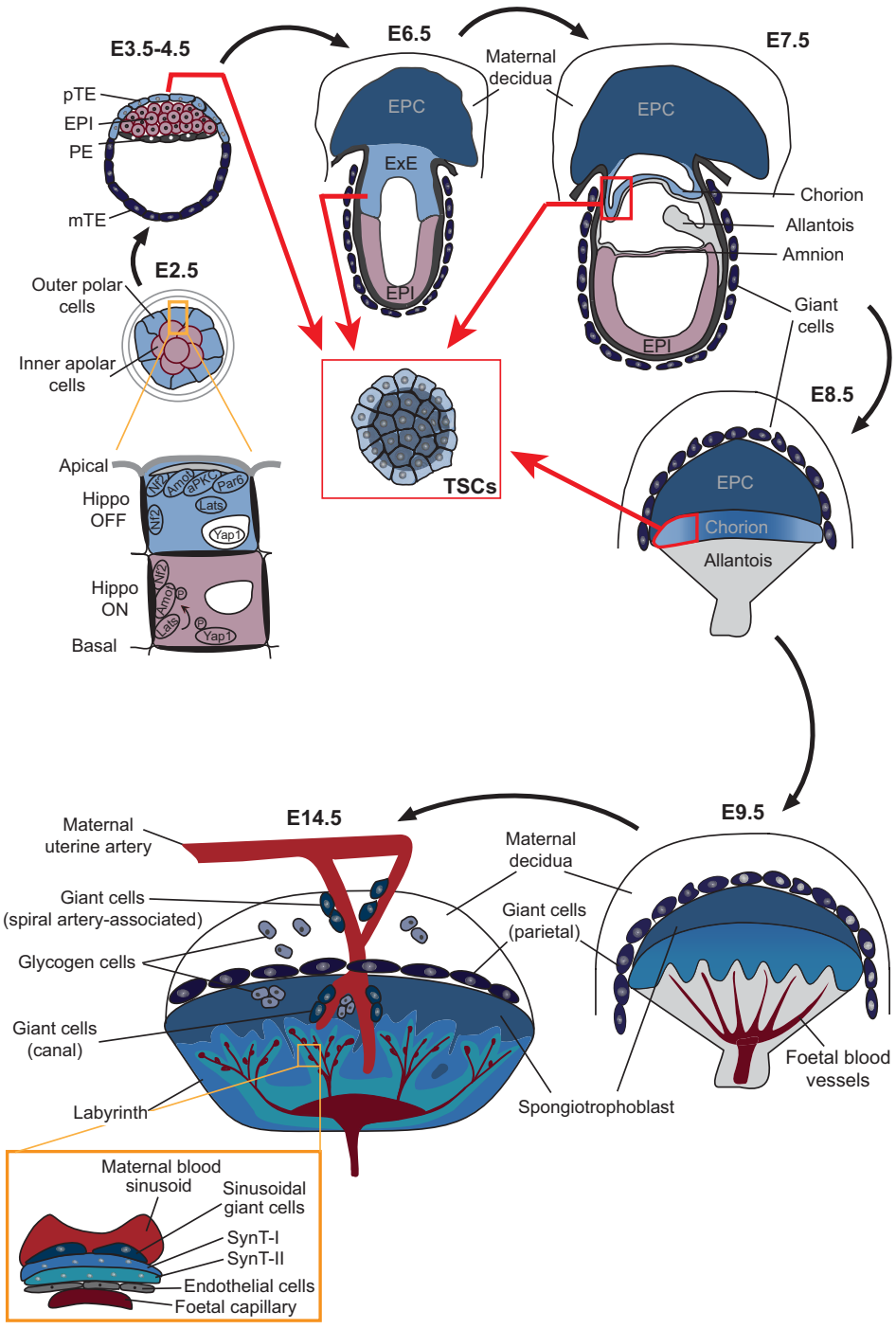

Fig. 1. Placental development and TSC niches in mice. Embryonic and extra-embryonic cell fates start to be specified at the morula stage around E2.5. The morula consists of apolar inner cells enclosed by polar outer cells. This polarity results from accumulation of Par6/aPKC cell polarity components and differential distribution of adherens junctions (black thickenings) between inner and outer cells. The differences in polarity and cell-cell adhesion lead to activation of Hippo signalling in just the inner cells. As a result, by E3.5 the polar outer cells preferentially become trophectoderm (TE) and apolar inner cells predominantly give rise to the inner cell mass (ICM). Between E3.5 and E4.5, the ICM further differentiates into epiblast (EPI) and primitive endoderm (PE) lineages. The TE can be divided into polar TE (pTE), which is the region made up by TE cells in contact with the ICM/EPI, and mural TE (mTE), which surrounds the blastocoel. Continued proliferation of the pTE gives rise to the extra-embryonic ectoderm (ExE) by E6.5. ExE cells that move farther away from the embryo start to differentiate, forming the ectoplacental cone (EPC). By gastrulation, ExE cells in close proximity to the embryo form the chorion (at around E7.5), which then goes on to fuse with the mesoderm-derived allantois at E8.5 to form the early placenta. Between E9.5 and E14.5, the placenta develops further and grows in size, eventually giving rise to the mature placenta, which consists of three main layers: the labyrinth, the junctional zone consisting of spongiotrophoblast and glycogen cells, and a layer of parietal trophoblast giant cells (TGCs) bordering the maternally derived decidua. The exchange barrier in the labyrinth is made up of three trophoblast cell types (from the maternal to the foetal side) – a discontinuous layer of sinusoidal trophoblast giant cells, syncytiotrophoblast I (SynT-I), syncytiotrophoblast II (SynT-II) – and an endothelial cell layer of the foetal vasculature. Trophoblast stem cells (TSCs) can be derived from various stages of placental development (indicated by red arrows).

contrast, Hippo signalling is off and Amot is sequestered away from adherens junctions to the apical domain by components of the polarity pathway (Fig. 1). This prevents Amot binding to Lats1/2 and hence prevents Lats1/2 phosphorylation and the subsequent phosphorylation of Yap1 (Cockburn et al., 2013; Hirate et al., 2013). Unphosphorylated Yap1 translocates to the nucleus where it serves as a co-factor for the transcription factor TEA domain family member 4 (Tead4) to activate the expression of caudal type homeobox 2 (*Cdx2*) and thereby drive the establishment of TE cell fate (Nishioka et al., 2009).

In addition to the Hippo pathway, other signalling cascades and transcription factors regulate *Cdx2* expression in the early mouse embryo. For example, the Notch signalling effector Rbpj, together with Tead4, binds to the upstream enhancer of *Cdx2* and drives its expression in TE cells (Rayon et al., 2014). Similarly, binding of the transcription factor Tfap2c to an intronic enhancer is required for *Cdx2* activation and Hippo pathway suppression in outer blastomeres by maintenance of cell polarity via Pard6b (Cao et al., 2015). In summary, TE specification is determined by combinatorial inputs involving cell positioning, diverse signalling pathways and lineage-specific transcription factors.

The first cell lineages of the blastocyst give rise to distinct stem cell types

After the establishment of an early blastocyst consisting of the ICM and TE at embryonic day (E) 3.5 in mice, the next cell fate decision

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Box 1. Trophoblast cell types

After implantation, the sustained proliferation of polar trophectoderm (pTE) cells establishes the extra-embryonic ectoderm (ExE) and the ectoplacental cone (EPC). Cells at the outer margins of the EPC differentiate into secondary trophoblast giant cells (TGCs). These TGCs exhibit invasive properties and penetrate deeply into the maternal decidua, where they make contact with spiral arteries. The production of an array of pro-angiogenic and vasodilatory factors by TGCs ensures that the implantation site is exposed to an adequate supply of maternal blood and, hence, nutrients and oxygen. The chorion is formed at the time of gastrulation and goes on to fuse with the allantois to establish the 'blueprint' of the chorio-allantoic placenta. Patterning processes within the chorion and EPC then lead to the formation of various trophoblast cell types and placental layers (Simmons et al., 2008). For example, cells closest to the allantoic mesoderm start to invaginate into the chorionic ectoderm and fuse into two separate syncytiotrophoblast layers (SynT-I and -II) while undergoing a branching morphogenesis programme to form the elaborate vascular structure of the emerging placental labyrinth. Labyrinthine sinusoidal TGCs emerge from cells closely juxtaposed to SynT. The core of the EPC mostly differentiates into spongiotrophoblast and glycogen cells, whereas cells at the outer margins of the EPC differentiate into spiral artery-associated TGCs as well as into canal and parietal TGCs (Simmons et al., 2007). These differentiation processes establish the mature mouse placenta grossly composed of the labyrinth, the junctional zone (spongiotrophoblast, glycogen cells and parietal TGCs) and the maternal decidua.

gives rise to primitive endoderm (PE) and epiblast (EPI). Both of these lineages originate from cells of the ICM (Rossant and Tam, 2009; Artus and Hadjantonakis, 2012). Specification of PE versus EPI relies on the stochastic activation of fibroblast growth factor (Fgf)/extracellular signal regulated kinase (Erk; also known as MAP kinase) signalling in the ICM: Erk activation drives PE formation whereas its absence promotes EPI cell fate (Nichols et al., 2009; Yamanaka et al., 2010). At around E3.5, precursors of PE and EPI can already be distinguished within the ICM by the mutually exclusive expression of lineage markers such as GATA binding protein 6 (Gata6), which identifies PE precursors, and the homeobox factor Nanog, which marks EPI precursors (Chazaud et al., 2006). The precursor cells are subsequently sorted into their correct locations by a combination of active cell migration, positional induction and programmed cell death (Plusa et al., 2008). These processes are completed by around E4.5 giving rise to a late blastocyst that now consists of three lineages: EPI, PE and TE (Rossant and Tam, 2009; Artus and Hadjantonakis, 2012). During later stages of embryonic development, the EPI gives rise to the embryo itself whereas the PE and TE develop into extra-embryonic cell types of the yolk sac and placenta, respectively.

The detailed knowledge of these processes gained from studying the mouse model has facilitated the successful derivation of distinct types of stem cells from the early embryo, representative of each of the first three lineages. Thus, the EPI, PE and TE of mouse blastocysts can give rise to ESCs, extra-embryonic endoderm stem cells (XENs) and TSCs, respectively (Bradley et al., 1984; Beddington and Robertson, 1989; Tanaka et al., 1998; Kunath et al., 2005). Gene expression profiles, differentiation potential and the ability to contribute to mouse chimeras indicate that each of these stem cell types faithfully recapitulates properties of the original *in vivo* lineages. Similarly, their epigenomic landscapes reflect their developmental origin and define their identity and developmental plasticity (Hemberger et al., 2009; Senner et al., 2012).

Derivation and culture of mouse TSCs

The existence of a stem cell population within the early trophoblast compartment had been suggested by early transplantation and chimerisation experiments (Rossant et al., 1978). Building on these insights from mouse embryology, the derivation of stable TSCs was

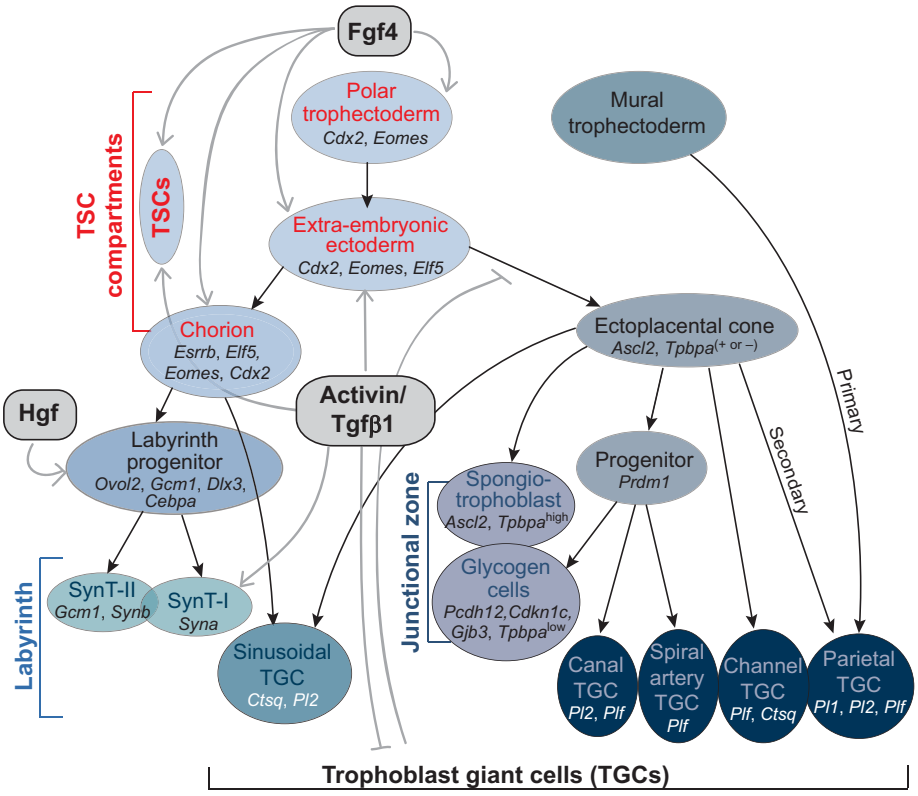

Fig. 2. Signalling pathways involved in trophoblast cell differentiation. An overview of the cell fate transitions involved during trophoblast lineage specification is shown, highlighting the growth factors that influence each of the various trophoblast cell types as well as the key marker genes that are expressed in each cell type. Fgf4 is required for the development of polar trophectoderm, extra-embryonic ectoderm and chorion, and drives TSC self-renewal. Activin/ Tgfβ1 stimulates extra-embryonic ectoderm and TSCs as well as the differentiation of syncytiotrophoblast (SynT), while inhibiting the formation of spongiotrophoblast and trophoblast giant cells (TGCs). TGCs secrete activin and thereby act in an autocrine feedback loop to inhibit TSC differentiation. Hepatocyte growth factor (Hgf) is required for labyrinth progenitors. Note that family members of the trophoblast giant cell-expressed placental lactogen genes are also known under the following gene symbols: *Pl1=Prl3d1*, *Pl2=Prl3b1* and *Plf=Prl2c2*.

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pioneered by the Rossant lab in 1998, first from the extra-embryonic ectoderm (ExE) of E6.5 mouse conceptuses, and then also from E3.5 blastocysts. TSCs from both stages (referred to as $TS_{6.5}$ and $TS_{3.5}$) were indistinguishable; they could both indefinitely proliferate in culture, and their derivation efficiencies were very high: 64% for $TS_{3.5}$ and 44% for $TS_{6.5}$ (Tanaka et al., 1998). It is intriguing to note that, indeed, TSCs can be derived from embryos up until E8.5 from cells within particular niches of the trophoblast compartment that seemingly retain a developmental plasticity akin to that of the blastocyst's TE (Tanaka et al., 1998; Uy et al., 2002). This is in contrast to ESCs, which cannot be derived from stages later than the blastocyst. These TSC niches are found in the ExE of early post-implantation conceptuses and in the chorionic ectoderm after gastrulation (Fig. 1).

Upon culture in appropriate conditions, TSCs indefinitely self-renew and retain multipotency, i.e. the ability to differentiate into all specialised trophoblast cell types of the placenta, such as syncytiotrophoblast (SynT), spongiotrophoblast and the various types of trophoblast giant cells (TGCs) that are found at the foeto-maternal interface (Fig. 1; Box 1). Thus, all tested markers that are expressed in specific trophoblast cell types *in vivo* are also expressed in TSCs upon differentiation, although the temporal progression of their upregulation may differ for the various trophoblast subtypes.

Originally, TSCs were derived and cultured in a MEF-conditioned medium containing 20% serum and supplemented with Fgf4 (Tanaka et al., 1998). Although this formula ensures the robust growth of TSCs and preserves their ability to colonise the placenta in chimeric mice, the poorly characterised composition of serum and the batch-to-batch variability of serum and MEFs urged researchers to establish better-defined culture conditions. Initial advances demonstrated that the conditioned medium can be replaced by a component of the transforming growth factor beta superfamily, such as Tgfβ1 or activin (Erlebacher et al., 2004). However, the first fully chemically defined and standardised TSC media was developed by Kubaczka et al. and named TX (Kubaczka et al., 2014). It consists of DMEM/F12 basal medium supplemented with ten ingredients including Tgfβ1, Fgf4, heparin and insulin. In contrast to standard TSC conditions, in which cells are grown on bare plastic, culture in TX media requires the coating of tissue culture surfaces with extracellular basement membrane components such as Matrigel or Synthemax (Kubaczka et al., 2014). In another report, a distinct media formulation containing Fgf2, activin A and the small molecules XAV939 (a canonical Wnt inhibitor) and Y27632 (Rho-associated kinase p160ROCK inhibitor) in chemically defined medium (CDM/FAXY), used together with a fibronectin coating, was shown to be sufficient for TSC derivation and maintenance (Ohinata and Tsukiyama, 2014). In both cases, the chemically defined media supports the full differentiation repertoire of TSCs and retains their capacity to contribute to mouse chimeric placentas (Kubaczka et al., 2014; Ohinata and Tsukiyama, 2014). These improved culture methods provide major advances as they now offer well-defined systems that can be used to address fundamental questions in TSC biology.

Differentiation potential of TSCs

Although TSCs serve as an inexhaustible source of undifferentiated, multipotent stem cells that retain the plasticity to differentiate into all trophoblast cell types in chimeric placentas, it has proven to be difficult to unleash this full differentiation potential *in vitro* (Tanaka et al., 1998; Niwa et al., 2005; Adachi et al., 2013; Kubaczka et al., 2014, 2015; Benchetrit et al., 2015). The most common method of inducing TSC differentiation involves withdrawal of the essential growth factors Fgf and Tgfβ1/activin (or conditioned medium). Yet under these conditions the major differentiation route of TSCs is towards TGCs, whereas SynT is comparatively under-represented in differentiated TSC cultures (Fig. 3).

Some clues regarding how this bias between TGC and SynT differentiation is controlled came from studies demonstrating that activin, in the absence of Fgf, promotes the differentiation of TSCs into SynT (Natale et al., 2009). Similarly, reduction of histone deacetylase (Hdac) activity (either by Hdac inhibition or by ablation of the hypoxia-inducible factor Hif) skews TSC differentiation towards a chorionic trophoblast and SynT cell fate (Maltepe et al., 2005). SynT cells are essential for the establishment of the maternal-foetal exchange surface, ensuring the adequate supply of nutrients and gases to the embryo. SynT formation involves cell-cell fusion, which is enabled by expression of the pro-fusogenic syncytin genes *Syna* and *Synb* (Dupressoir et al., 2005, 2011). Other factors, such as the transcription factors Hand1 and Mdfi, preferentially act in the TGC differentiation pathway (Kraut et al., 1998; Hughes et al., 2004). Expression levels of the cell surface protein Plet1 were also found to introduce a bias in TSC differentiation; $Plet1^{high}$ cells are prone to differentiate towards TGCs whereas $Plet1^{low/null}$ cells preferentially form SynT (Murray et al., 2016). Moreover, TSCs grown in the absence of Fgf/conditioned medium for 3 days separate, without further manipulation, into $Plet1^{high}$ and $Plet1^{low}$

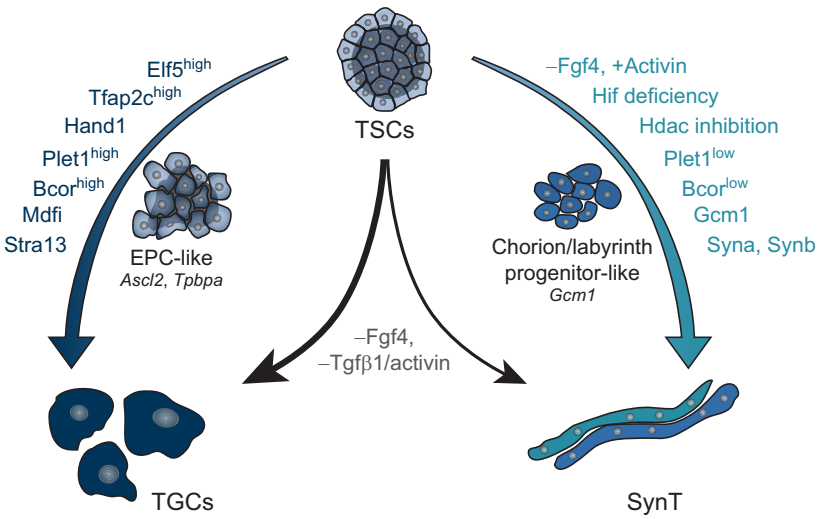

Fig. 3. Directing TSC differentiation. The self-renewal of TSCs relies on Fgf and Tgfβ1/activin signalling. In the absence of these growth factors, TSCs differentiate along two major trajectories: predominantly towards trophoblast giant cells (TGCs) or, to an apparently lesser extent, towards syncytiotrophoblast (SynT). Various conditions, examples of which are indicated, can bias differentiation to promote selectively either the TGC or the SynT path. TSCs differentiating *in vitro* to TGCs and SynT pass through temporary states reminiscent of those of *in vivo* EPC (EPC-like) and chorionic or labyrinthine progenitor (chorion/labyrinth progenitor-like) cells, respectively.

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cells that can be conveniently isolated by flow cytometry and may well represent progenitor pools of both populations (Fig. 3). Although these various systems provide promising leads, to date we lack defined protocols that allow for the directed, homogeneous differentiation of TSCs into defined placental progenitors and specific trophoblast cell types. Indeed, the establishment of such protocols remains one of the main challenges of the field.

Placental progenitors *in vivo* and *in vitro*

Despite the exhaustion of stem cell potential after E8.5, the mouse placenta continues to grow nearly until the end of term (Adamson et al., 2002; Uy et al., 2002; Simmons and Cross, 2005). This suggests that placental expansion after this time point relies on proliferative precursor cells, and opens up the exciting possibility that, 'downstream' of TSCs, trophoblast lineage-restricted progenitor cells can be isolated and perhaps propagated. To date, two different types of such potential progenitor cells have been identified. Ueno et al. (2013) isolated Epcam^{high}, hepatocyte growth factor-dependent, multipotent precursors of the labyrinth layer that retain the capacity to differentiate into all three major cell types of this compartment, i.e. the SynT layers I and II (SynT-I, SynT-II) as well as sinusoidal TGCs (sTGCs) that form a discontinuous interface to the maternal blood circulation (Figs 1 and 2). In addition, Mould et al. (2012) identified a population of PR domain containing 1 (Prdm1; also known as Blimp1)-positive, proliferative precursors in the spongiotrophoblast layer that give rise to particular TGC subtypes, namely those associated with spiral arteries and maternal blood canals, as well as to glycogen cells (Fig. 2). These studies provide major advances in our understanding of the hierarchy of specific stem and progenitor cells orchestrating placental development. Unfortunately, however, so far neither of these progenitors can be maintained in culture, hampering their detailed analysis. It remains to be seen whether improved media supplementation with particular growth factors or small molecules can recapitulate conditions that allow the establishment and maintenance of such novel, defined *in vitro* cell models with lineage-restricted potential.

Signalling pathways required for TSC self-renewal

As discussed above, the derivation and maintenance of TSCs relies on the presence of Fgf4 and Tgfβ1/activin in the media (Tanaka et al., 1998; Erlebacher et al., 2004). As such, a number of recent studies have aimed to examine exactly how these growth factors function to control the self-renewal and differentiation of TSCs (Fig. 2).

During early embryonic development, the ICM and EPI serve as a source of Fgf4 for developing TE/trophoblast cells, which express the compatible receptor Fgfr2c, ensuring continued proliferation and coordinated development. Accordingly, deletions of various components of the Fgf pathway cause severe trophoblast and/or placental phenotypes and interfere with the derivation of TSCs from such mutant embryos. These studies identified the Fgf/Raf/Mek/Erk branch as being predominantly active during early trophoblast development (Arman et al., 1998; Saba-El-Leil et al., 2003; Bissonauth et al., 2006; Ralston and Rossant, 2006; Yang et al., 2006).

The vital role of Fgf/Erk signalling in TSCs has recently been further dissected using pharmacological inhibitors in time-course experiments (Adachi et al., 2013; Latos et al., 2015a). Treatment of TSCs or transdifferentiated TS-like cells with either the Fgf receptor inhibitor PD173074 or the mitogen-activated protein kinase kinase (Mek) inhibitor PD0325901 resulted in downregulation of TSC markers (*Cdx2*, *Esrrb*, *Sox2*, *Eomes*, *Elf5*) and upregulation of differentiation markers (*Cdkn1c*, *Ascl2*). RNA sequencing analysis after 3 and 24 h of exposure to PD0325901 revealed that *Esrrb* and *Sox2* were amongst the fastest and most profoundly downregulated genes (Latos et al., 2015a), identifying them as primary targets and direct mediators of Fgf signalling in TSCs (Fig. 4). This notion is further supported by the finding that combined forced expression of *Sox2* and *Esrrb* supports Fgf-independent self-renewal of TSCs (Adachi et al., 2013). Even after a 4-week culture period in the absence of Fgf, Sox2/Esrrb-overexpressing TSCs retained chimerisation potential and readily contributed to all cell types of the mouse placenta following blastocyst injection. This experiment highlights the capacity of Esrrb and Sox2 to substitute for Fgf, hence pinpointing the importance of these two transcription factors in transmitting the Fgf growth factor signal to sustain TSC self-renewal (Adachi et al., 2013).

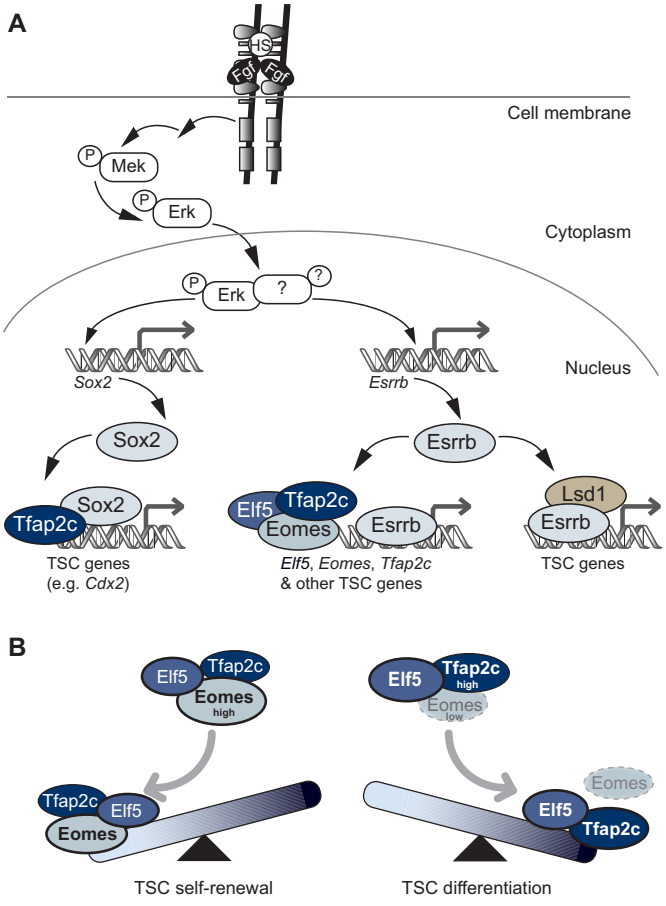

Fig. 4. Fgf signalling drives transcription factor networks in mouse TSCs. (A) The binding of Fgf4 to its receptor, in trophoblast the Fgfr2c isoform and aided by heparan sulphate (HS) chains, triggers a phosphorylation cascade resulting in Mek and Erk kinase activation. Phosphorylated Erk, possibly together with an unknown factor, induces the expression of *Sox2* and *Esrrb*. The resultant Sox2 protein forms a complex with Tfap2c that binds to and regulates a cohort of TSC-specific genes (e.g. *Cdx2*). Similarly, Esrrb controls the expression of key TSC genes including those encoding important transcription factors, such as *Elf5* and *Eomes*. In turn Elf5, Eomes and Tfap2c form a complex that promotes TSC self-renewal. (B) In the presence of high amounts of Eomes, the Eomes-Elf5-Tfap2c troika powers the undifferentiated state. The onset of differentiation is demarcated by an abrupt decline in Eomes levels, combined with a proportional increase in Tfap2c and Elf5 levels; in this context, the Elf5-Tfap2c complex shifts its genomic occupancy, now binding to early differentiation-associated genes and driving their expression, thereby promoting exit from the stem cell state.

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Although these data collectively provided a fairly detailed picture of the Fgf signalling cascade in TSCs (Fig. 4), much less is known about the second growth factor requirement – the Tgfβ superfamily component. As mentioned above, early experiments showed that Tgfβ1 or activin A can substitute for conditioned medium (Erlebacher et al., 2004) during both the derivation and maintenance of TSCs. Later studies attempted to tease apart the differential actions of Tgfβ1 and activin A, while also showing that TSCs do not respond directly to Nodal (Natale et al., 2009). Activin A in the presence of Fgf inhibits TSC differentiation. Intriguingly, activin A (but not its receptors) is expressed by TGCs, thus pointing to a self-regulatory mechanism in which activin A acts in a paracrine manner to balance the stem cell pool versus the extent of TGC differentiation (Fig. 2). In the absence of an Fgf signal, the continued presence of activin but not Tgfβ1 prolongs the expression of SynT markers while significantly delaying the expression of spongiotrophoblast and TGC markers (Figs 2 and 3) (Natale et al., 2009). These results suggest that activin rather than Tgfβ1 (or Nodal) acts directly on TSCs, influencing both TSC maintenance and cell fate depending on whether the cells are also exposed to Fgf (Guzman-Ayala et al., 2004; Natale et al., 2009). The precise downstream targets of activin (or Tgfβ1) following activation of the canonical Smad signalling cascade are difficult to disentangle. In human ESCs, it has been shown that the key trophoblast transcription factor eomesodermin (*EOMES*) is directly bound and regulated by SMAD2/3 and thus positively affected by activin (Teo et al., 2011). In mouse TSCs, activin promotes *Eomes* expression indirectly by suppressing the Bcl6 corepressor (Bcor), a negative regulator of *Eomes*. As a mediator of activin signalling, Bcor is also required for proper TGC and spongiotrophoblast differentiation (Zhu et al., 2015). Together, these findings suggest that the combined action of both Fgf and Tgfβ signalling promotes the expression of a core network of transcription factors that is essential for TSC self-renewal.

Transcription factor networks operating in TSCs

One of the most compelling aspects of trophoblast specification and TSC establishment is that none of the transcription factors studied so far is strictly specific to the trophoblast lineage. For example, in addition to being expressed in TSCs, both Sox2 and Esrrb are readily expressed in ESCs, where they regulate self-renewal and pluripotency (Martello and Smith, 2014). Yet in striking contrast to the situation observed in TSCs, the expression of Sox2 and Esrrb in ESCs is not driven by Fgf/Mek signalling (Martello et al., 2012). Furthermore, chromatin immunoprecipitation analysis of the binding sites of these two transcription factors revealed only a partial and highly limited overlap between TSCs and ESCs, indicating that both Sox2 and Esrrb regulate different sets of genes in these two stem cell types (Adachi et al., 2013). For instance, Sox2 binds to the POU domain pluripotency gene *Oct4* (*Pou5f1*) only in ESCs whereas in TSCs it binds to the trophoblast marker gene *Cdx2* (Adachi et al., 2013). Similarly, binding of Esrrb to the key TSC locus *Elf5* (E74-like factor 5) was exclusively observed in TSCs but not in ESCs (Latos et al., 2015a). Insights into the context-dependent genomic occupancy profile of Sox2 came from the sequence analysis of Sox2-bound regions and revealed enrichment for the conserved DNA-binding motif of Tfap2c, one of the core TSC transcription factors. It was further demonstrated that in TSCs, Tfap2c interacts with Sox2 at the protein level, thereby recruiting it to TSC-specific regions (Fig. 4) (Adachi et al., 2013).

Despite detailed mass spectrometry analyses of Esrrb protein interactors, an analogous cell type-specific association with lineage-defining transcription factors was not identified for this factor. However, the protein interactome of Esrrb differs profoundly between ESCs and TSCs, and it is most likely that the cell type-specific composition of Esrrb protein complexes determines the genomic binding pattern of Esrrb. As such, Esrrb has been shown to directly bind to and regulate several important TSC transcription factor encoding loci including *Eomes* and *Elf5*, thus providing new insights into stem cell type-specific gene regulation (Fig. 4) (Latos et al., 2015a). Taken together, these studies shed new light on the functional versatility of transcription factors and the context-dependent wiring that allows them to drive distinct transcriptional networks in different stem cell types.

Given that Sox2 and Esrrb can replace Fgf signalling and regulate crucial trophoblast genes, they seem to be on top of the transcription factor hierarchy that operates in TSCs. However, many other transcription factors are essential for TSC derivation and maintenance, including Cdx2, Eomes, Elf5, Ets2, Tfap2c, Gata3, Tead4 and others (Yamamoto et al., 1998; Russ et al., 2000; Auman et al., 2002; Werling and Schorle, 2002; Donnison et al., 2005; Strumpf et al., 2005; Nishioka et al., 2009; Ralston et al., 2010). Analyses of mouse mutants ablated for the genes that encode these factors suggest that they occupy relatively different positions in the hierarchy of early trophoblast specification and development. For instance, whereas Cdx2 is essential for the maintenance of TE integrity at the blastocyst stage, *Eomes*-deficient embryos arrest only after implantation, suggesting that Eomes becomes essential at a slightly later stage (Russ et al., 2000; Strumpf et al., 2005). *Ets2*-, *Elf5*- and *Tfap2c*-null embryos show a lethal phenotype that manifests even later as these embryos implant but fail to form the ExE, whereas *Esrrb* knockouts lack chorionic and other diploid trophoblast cell types (Luo et al., 1997; Yamamoto et al., 1998; Auman et al., 2002; Werling and Schorle, 2002; Donnison et al., 2005). Additional insights into the mutual relationships between these transcription factors have been provided by gain-of-function experiments in ESCs, which result in the induction of transdifferentiation into trophoblast-like cells (TLCs) to varying extents. For example, *Cdx2*- and to a lesser degree *Eomes*-driven TLCs show a certain capacity for self-renewal. By contrast, *Tfap2c*-induced TLCs can be maintained for several passages but exhibit strongly enhanced rates of differentiation into post-mitotic trophoblast cell types, which is also observed in TLCs induced by *Elf5*-overexpression (Niwa et al., 2005; Ng et al., 2008; Kuckenberg et al., 2010; Cambuli et al., 2014). These experiments demonstrate the divergent capacities of these transcription factors to induce trophoblast gene expression and sustain proliferative potential.

The importance of transcription factor stoichiometry in TSCs

The experiments discussed above demonstrate that the sheer presence of specific transcription factors is not sufficient for lineage specification and the self-renewing capacity of TSCs. Indeed, studies in ESCs have revealed that defined levels of transcription factor expression, and their stoichiometry in relation to each other, are crucially important for self-renewal, differentiation and reprogramming (Niwa et al., 2000; Carey et al., 2011; Karwacki-Neisius et al., 2013; Radzisheuskaya et al., 2013). Similar findings were recently reported in TSCs, in which the precise amount of the transcription factor Elf5 controls the balance between self-renewal and differentiation (Latos et al., 2015b). Thus, the manipulation of *Elf5* levels by either depletion or overexpression results in precocious TSC differentiation both *in vivo* and *in vitro*.

The first evidence for such an abundance-dependent function came from the analysis of expression patterns in early embryos.

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Indeed, at E6.5, *Elf5*, *Eomes* and *Tfap2c* are jointly present in the ExE, i.e. the presumptive TSC niche. However, outside of this compartment where cells start to differentiate, *Eomes* is downregulated, whereas *Elf5* and *Tfap2c* are upregulated. These differential dynamics are equally preserved *in vitro*. Intriguingly, this loss of co-expression is observed despite the fact that these transcription factors can mutually reinforce each other's expression. How can this apparent paradox be explained? Insights into this question have been provided by protein immunoprecipitation and mass spectrometry analyses that revealed a stoichiometry-dependent interaction between Elf5, Eomes and Tfap2c (Latos et al., 2015b). Thus, the undifferentiated stem cell state is characterised by the formation of a ternary complex driven by preferential binding of Elf5 to Eomes, in the presence of low amounts of Tfap2c. Perhaps through the mutual transcriptional reinforcement, Tfap2c levels increase (and exceed those of Eomes) at the onset of differentiation, thereby making it Elf5's favoured interactor. This change in preferred binding partners results in a redistribution of transcription factor complexes to different target sites. In undifferentiated TSCs, the ternary Eomes-Elf5-Tfap2c complex binds to and drives the expression of TSC-associated genes, whereas the Elf5-Tfap2c complex induces the expression of genes promoting differentiation (Fig. 4). Overall, this study provided the first insights into a stoichiometry-sensitive transcription factor network operating at the interface of TSC self-renewal and differentiation (Latos et al., 2015b).

Reprogramming into iTSCs

The identity of most cell types is defined by the specific repertoire (and stoichiometry) of transcription factors they express. Therefore, manipulation of key transcription factors can lead to profound cell fate changes. In ground-breaking experiments, Takahashi and Yamanaka demonstrated that the forced expression of only four transcription factors (Sox2, Oct4, Myc, Klf4) in MEFs can induce their reprogramming into induced pluripotent stem cells (iPSCs) that could differentiate into the three embryonic germ layers (Takahashi and Yamanaka, 2006). Since then, numerous transcription factor expression manipulations have been employed to generate a vast diversity of functional cell types. Among these have been several approaches to overcome the very first lineage decision to and from a TSC fate (Fig. 5).

Forced expression of the four Yamanaka factors in TSCs results in their conversion to iPSCs. The successful reprogramming of these cells was confirmed on the transcriptional and epigenetic level, and by their differentiation and chimerisation potential (Kuckenberg et al., 2011). However, the TSC-to-iPSC reprogramming efficiency is low at 0.0017%, compared with 0.171% when using MEFs as starting material, suggesting that the embryonic-trophoblast lineage barrier is difficult to overcome. Interestingly, conversion in the opposite direction – from ESCs to TSCs – has proven to be even more challenging, and bona fide iTSCs have not yet been generated via this approach. A number of studies have reported that the expression of various single TSC transcription factors in ESCs, including Tead4, Cdx2, Eomes, Elf5, Tfap2c and Gata3, results in different degrees of transdifferentiation into TLCs (Niwa et al., 2005; Ng et al., 2008; Nishioka et al., 2009; Kuckenberg et al., 2010; Ralston et al., 2010). Similar results were obtained with forced downregulation of Oct4 or activation of the Erk pathway (Niwa et al., 2000; Lu et al., 2008). However, detailed epigenomic, transcriptomic and cell surface marker analyses of such inducible Cdx2 (iCdx2), iOct4-KO and iErk systems revealed that, although transdifferentiation is initiated, lineage conversion remains

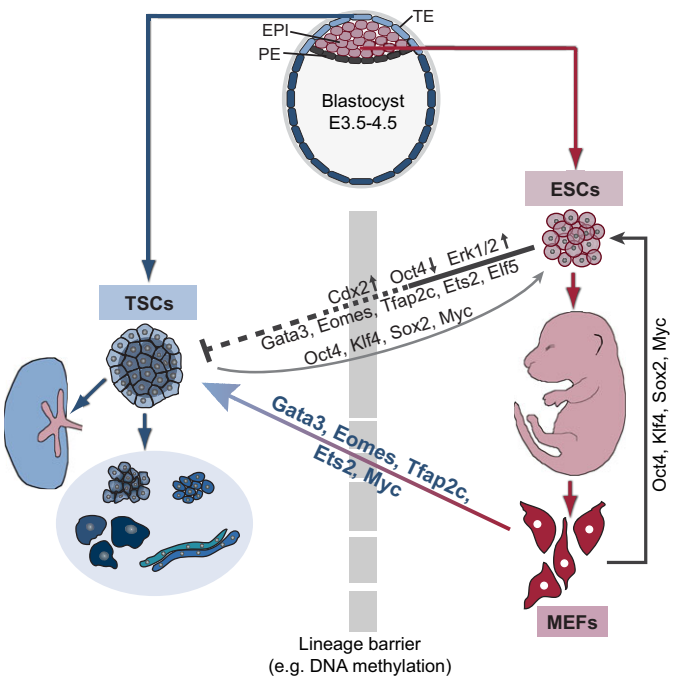

Fig. 5. Reprogramming cells to a trophoblast fate. ESCs and TSCs can be derived from blastocyst stage mouse embryos. Studies suggest that, from around this stage in development onwards, tight epigenetic lineage barriers (imposed, for example, by DNA methylation) are put in place to keep the embryonic and trophoblast lineages strictly apart (grey bar). Reprogramming approaches from and to TSCs thus require this first incisive lineage-separating barrier to be overcome. Indeed, TSCs can be reprogrammed into ESC-like cells using the Yamanaka factors Oct4, Klf4, Sox2 and Myc, albeit at a lower efficiency than when using mouse embryonic fibroblasts (MEFs) as starting material. Conversely, attempts to reprogramme ESCs into TSCs via the manipulation of various transcription factors or signalling pathways result in only partially converted trophoblast-like cells. However, an apparently complete conversion of MEFs into induced TSCs can be achieved by overexpressing three core transcription factors (Gata3, Eomes and Tfap2c), with optional addition of Ets2 and Myc, in MEFs. The finding that MEFs but not ESCs can be reprogrammed towards TSCs by this approach can be explained by the seemingly 'lower' epigenetic barrier between MEFs and TSCs. Thus, a cohort of key loci that are, like *Elf5*, highly methylated in ESCs but hypomethylated in TSCs, exhibit lower methylation levels in MEFs than in ESCs. This difference likely creates an advantage to iTSC generation towards TSCs as it reduces the extent to which epigenetic reprogramming has to occur.

incomplete and an epigenetic memory of the ESC origin is retained in all models tested (Cambuli et al., 2014). Moreover, the proliferative and self-renewing capacity of the resulting TLCs is difficult to retain, likely a consequence of a suboptimal balance of the transcription factors expressed and/or activated in the process.

These studies urged caution for the use of such TLCs in functional studies, owing to their limited comparability to bona fide TSCs. However, two recent studies brought a breakthrough to the TSC reprogramming concept, using MEFs instead of ESCs as the starting material. To identify the relevant transcription factors, the authors recapitulated the Yamanaka approach (Takahashi and Yamanaka, 2006; Benchetrit et al., 2015; Kubaczka et al., 2015) and screened in total a battery of 14 transcription factors with known functions in trophoblast: Cdx2, Eomes, Elf5, Ets2, Tead4, Tfap2c, Tfap2a, Id2, Gata3, Hand1, Esrrb, Dppa1, Myc and Utf1. These studies demonstrated that the forced expression of three (Gata3, Eomes and Tfap2c; GET) or four (GET+Ets2) transcription factors in MEFs is sufficient to fully reprogramme them into iTSCs (Benchetrit et al., 2015; Kubaczka et al., 2015). As far as the

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requirement of Ets2 is concerned, it is worth noting that this discrepancy may arise from different doxycycline induction times (20 days without versus 10 days with Ets2) between the two reports. The importance of Ets2 for the reprogramming process is further indicated by its high frequency of integration in iTSC clones in the four-factor approach. Both groups consistently reported an efficiency of the MEF-to-iTSC reprogramming process of around 0.03%, as calculated by the number of transdifferentiated colonies per number of cells plated. Expression of *Myc* along with the GET factors improved reprogramming efficiency by about twofold (Benchetrit et al., 2015). Extensive gene expression analysis revealed that the expression patterns of these iTSCs are highly similar to those of bona fide TSCs. Similarly, epigenomic characterisation showed that DNA methylation status, the distribution of histone variant H2A.X, and the acetylation of histone H3 lysine 27 (H3K27ac) are consistent and highly similar between iTSCs and TSCs. Of special interest was the methylation status of *Elf5*, a gene that is hypermethylated and repressed in ESCs and MEFs but hypomethylated and actively expressed in TSCs. Both studies confirmed that this important locus was hypomethylated and thus had undergone complete epigenetic reprogramming in iTSCs. Expression data further showed that iTSCs were able to differentiate *in vitro* along the trophoblast pathway and formed haemorrhagic lesions in teratomas, as expected from trophoblast. Most importantly, when injected into blastocysts, iTSCs were capable of colonising all layers of chimeric placentas (Benchetrit et al., 2015; Kubaczka et al., 2015). Taken together, these data convincingly demonstrate the generation of true iTSCs, providing considerable progress and improvement in reprogramming strategies compared with previous TLC attempts.

The concept of using MEFs and not ESCs as starting material for reprogramming towards the trophoblast lineage may seem counter-intuitive at first, in particular when considering the significantly higher developmental plasticity of ESCs. Interestingly, however, overexpression of the GET+Ets2 transcription factor cocktail in ESCs did not lead to the acquisition of iTSC identity (Kubaczka et al., 2015). The resulting cells rather resembled incompletely converted TLCs, underpinning the notion that the ESC-TSC barrier may be more difficult to overcome than the boundary between MEFs and TSCs, at least under these reprogramming conditions (Kubaczka et al., 2015). Consistent with this view, cells never pass through an intermediate pluripotent stage (i.e. an ESC state) during the MEF-to-iTSC reprogramming process, as demonstrated by the lack of expression of the pluripotency markers Oct4 and Nanog. A vital explanation for the apparent paradox that MEFs are more amenable to iTSC reprogramming than ESCs was provided by Kubaczka et al., who showed that MEFs exhibit a DNA methylation profile that is intermediate to that of ESCs and TSCs. This affected in particular a cohort of loci that are, like *Elf5*, hypermethylated in ESCs but hypomethylated in TSCs and thus need to lose methylation during the reprogramming process. These loci collectively exhibited lower methylation levels in MEFs than in ESCs. This feature probably constitutes an important beneficial factor that enables the seemingly complete conversion process into iTSCs by reducing the extent to which epigenetic reprogramming has to occur (Kubaczka et al., 2015).

The quest for human TSCs as a model for human trophoblast development

The successful reprogramming of MEFs to iTSCs has provided a great advance, particularly in aiding the quest for human TSCs, which arguably remains the biggest challenge of the trophoblast field. The derivation of human iTSCs (hiTSCs) could provide the long-sought *in vitro* model, analogous to hiPSCs, for investigating the mechanisms driving human trophoblast development and disease, in particular during early stages of development when placental tissue is not readily available or amenable to study. It would also offer new insights into personalised medicine, via the generation of patient-derived hiTSCs. We anticipate that the efforts of many laboratories will now focus on determining the cocktail of transcription factors and the media composition that will yield reprogrammed hiTSCs. However, the reprogramming conditions required for hiTSCs will most likely differ to some extent from those used in the mouse, reflecting developmental differences between both species.

The trophoblast subtype repertoire in humans is, for example, seemingly more restricted than that of the mouse, or at least our common view and knowledge of it is. Its principal components consist of a population of villous cytotrophoblast (VCT) cells that either go on to fuse into syncytiotrophoblast (ST) or proliferate to form cell columns along which well-defined differentiation into invasive extravillous trophoblast (EVT) cells occurs. It is these EVTs that invade into the maternal decidua, trigger profound remodelling of the maternal vasculature, and promote blood flow towards the developing placenta. By contrast, the ST layer is responsible for all nutrient and gas exchange between the maternal and foetal blood circulations (Knöfler and Pollheimer, 2013). As such, the VCT layer is commonly seen as a precursor cell population that may retain bi-potential differentiation properties towards either ST or EVT. Perhaps consistent with this view, it has been demonstrated on first trimester placentas that the VCT layer is proliferative and contains a small fraction of ELF5/CDX2 double-positive cells, which may represent a trophoblast stem or progenitor cell population *in vivo* (Hemberger et al., 2010). However, although first trimester human trophoblast can be isolated, the crux has been that it very rapidly loses its proliferative capacity and readily differentiates in culture. Despite many attempts, the derivation of a self-renewing, multipotent hTSC population from first trimester trophoblast has so far remained unsuccessful.

Another key factor that has hampered approaches to derive hTSC or hTSC-like populations is that the fundamental biology of early human trophoblast is still fairly poorly understood. While some elements of the growth factor environment and transcriptional networks underpinning self-renewal are shared with the mouse, others are divergent (Blakeley et al., 2015), highlighting the need for thorough molecular investigations of early human trophoblast. The lack of such detailed knowledge has impeded the propagation of hTSCs to date. Indeed, the first strategy to derive hTSCs has been by using preimplantation embryos. Such attempts were made soon after the establishment of murine TSCs but revealed profound differences in the signalling and hence growth factor requirements, as human blastocysts do not express the FGF receptor and therefore do not seem to rely on an FGF signal (Kunath et al., 2014). Interestingly, however, robust expression of FGFR2 was observed at a later stage, in the VCT layer at 5 weeks of gestation but was lost by 10 weeks of gestation, suggesting that FGF-dependent trophoblast expansion may occur later in human development and that VCT may serve as a source of FGF-dependent hTSCs (Hemberger et al., 2010; Kunath et al., 2014). Given the fact that key TSC transcription factors like CDX2, ELF5 and TFAP2C are expressed in the VCT compartment (Hemberger et al., 2010; Kuckenberg et al., 2012), it is tempting to speculate that important aspects of the FGF-dependent transcriptional networks are conserved in mouse and human. These data warrant further detailed studies of the mouse as a model, alongside early human trophoblast, to advance our knowledge base and assist the derivation of hTSCs.

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Alternative approaches have focused on a novel method of culturing human embryos *in vitro* over the early post-implantation period (Deglincerti et al., 2016; Shahbazi et al., 2016) and single-cell expression studies of human blastocysts (Blakeley et al., 2015). Indeed, new insights into hTSCs were recently provided by the analysis of cell lines originating from single blastomeres of 8- or 12-cell human embryos (Zdravkovic et al., 2015). In comparison to standard blastocyst-derived hESCs, such cell lines exhibit partial trophoblast competence, as judged by the upregulation of trophoblast-related genes and on the basis of their DNA methylation profiles. Furthermore, when differentiated into embryoid bodies, these blastomere-derived lines are at first CDX2 positive and subsequently express markers typical for trophoblast including cytokeratin 7 (KRT7), chorionic gonadotropin, GCM1 and GATA3 (Zdravkovic et al., 2015). Although the extent to which these cell lines faithfully recapitulate the full repertoire expected from bona fide trophoblast requires further investigation, this study opens up the exciting possibility that the derivation of hTSCs might be feasible from very early stage embryos, when cell fate decisions have not yet been fully implemented.

While the quest for deriving true hTSCs continues, a number of transformed cell lines have been generated from human placental material (King et al., 2000) and have been used to gain insights into human placental development. Owing to their diverse origins and transformed states, however, these cell lines provide heterogeneous models that only partially mimic trophoblast identity and/or behaviour. In attempts to establish a cell culture model that reliably exhibits features of bona fide human trophoblast, other approaches have been pursued, most notably those involving the use of hECSs. hESCs treated with the bone morphogenetic protein BMP4 acquire at least some features resembling human trophoblast (Xu et al., 2002). Various refinements of culture conditions have been made, although the true identity of the resulting cells remains debated (Bernardo et al., 2011; Amita et al., 2013; Roberts et al., 2014; Horii et al., 2016). A fundamental problem in this context is the tremendous difficulty in confirming the trophoblast identity of *in vitro* derived or converted cell types in humans. To overcome this difficulty, a set of defined criteria has recently been proposed (Lee et al., 2016). These criteria encompass a set of hallmarks that are typical for primary first trimester trophoblast, and aim to facilitate the identification of true trophoblast in culture models. The criteria include a subset of protein markers (TFAP2C, KRT7, GATA3), HLA class I profile, the methylation and expression status of *ELF5*, and the expression of microRNAs (miRNAs) from a miRNA cluster on chromosome 19. It is important to note that each of these criteria is not exclusive to trophoblast; rather these parameters need to be used in combination. For example, expression of the chromosome 19 miRNA cluster is not trophoblast specific as this region is also transcribed in hESCs, albeit at lower levels (Lee et al., 2016). For many contexts in which the trophoblast identity of cells is in question, the methylation status of the *ELF5* promoter seems to provide a reliable readout and serves as a good indicator of human trophoblast. As in the mouse, human *ELF5* is hypomethylated and active in trophoblast cells but hypermethylated and repressed in hECSs and placental fibroblasts (Ng et al., 2008; Hemberger et al., 2010; Lee et al., 2016). However, other epithelial cell types also express *ELF5* and thus the lack of *ELF5* methylation alone is not strictly indicative of trophoblast. Moreover, the absence of DNA methylation is often necessary but not sufficient for transcriptional activation, indicating that the analysis of *ELF5* methylation status should be combined with expression analysis using first trimester trophoblast as reference. Indeed, based on the defined criteria proposed (Lee et al., 2016), BMP4-treated hESCs, which are currently one of the most widely used *in vitro* models, exhibit only some characteristics of human trophoblast. Despite a drop in DNA methylation (from >80% to 30%), *ELF5* expression remains very low in these cells, transcript levels of the miRNA cluster are much lower than expected from bona fide trophoblast, and their HLA I expression pattern does not reflect that of primary trophoblast (Lee et al., 2016). Furthermore, a comparison of ST obtained from hESCs and from term placenta revealed that, despite overall gene expression similarities, they are sufficiently different to be considered two different cell types (Soares and Vivian, 2016; Yabe et al., 2016). Thus, although these trophoblast-resembling cells may serve as a useful tool for some functional analyses, some caution is necessary in their use and in data interpretation, and hESC-derived trophoblast-like cells should not per se be regarded as identical to primary placental trophoblast.

Conclusions

The derivation of mouse TSCs and the establishment of culture conditions promoting their sustained self-renewal have provided investigators with an invaluable research tool, enabling a vast array of functional experiments that have propelled our understanding of how trophoblast development is regulated (Tanaka et al., 1998). TSCs offer an inexhaustible source of non-transformed trophoblast cells for biochemical analysis. Indeed, they have been used in a number of recent studies to investigate transcriptional networks, to characterise epigenetic signatures, and to examine protein interactomes, providing a great deal of new insights into the biology of early trophoblast (Kidder and Palmer, 2010; Rugg-Gunn et al., 2010; Senner et al., 2012; Chuong et al., 2013; Latos et al., 2015a,b). TSCs also serve as a good model system for genetic manipulations including short-hairpin RNA mediated knockdowns and a range of CRISPR/Cas9-mediated alterations such as gene knockouts and knock-ins, and they offer a well-defined tool for either genetic or chemical compound screening (Odiatis and Georgiades, 2010; Latos et al., 2015a,b). In addition, as they retain the ability to differentiate into various trophoblast cell types both *in vitro* and *in vivo*, TSCs can be used to investigate the mechanisms driving cell fate decisions.

Given the diverse applications of mouse TSCs mentioned above, the derivation of hTSCs or hiTSCS would be a major milestone and would open many avenues into human trophoblast research. We anticipate that future efforts will focus on deciphering the mechanisms driving TSC specification and directed, cell type-specific trophoblast differentiation. These endeavours will involve optimising the culture conditions for known placental stem and progenitor cells and deriving novel self-renewing trophoblast progenitor sub-populations. This could be accomplished by establishing placental organoids that rely on intrinsic signals driving self-organisation coupled to self-renewal (Rai and Cross, 2015). This strategy has proved to be successful for a number of mouse and human organoid systems (Huch and Koo, 2015). Furthermore, as the placenta is a composite organ, it would be interesting to co-culture and thereby study the interactions between its trophoblast and mesoderm-derived components, as well as with surrounding cell types of the decidualised endometrium.

Competing interests

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