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Review

Trophoblast stem cell derivation, cross-species comparison and use of nuclear transfer: New tools to study trophoblast growth and differentiation

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ABSTRACT

The trophoblast is a supportive tissue in mammals that plays key roles in embryonic patterning, foetal growth and nutrition. It shows an extensive growth up to the formation of the placenta. This growth is believed to be fed by trophoblast stem cells able to self-renew and to give rise to the differentiated derivatives present in the placenta. In this review, we summarize recent data on the molecular regulation of the trophoblast *in vivo* and *in vitro*. Most data have been obtained in the mouse, however, whenever relevant, we compare this model to other mammals. In ungulates, the growth of the trophoblast displays some striking features that make these species interesting alternative models for the study of trophoblast development. After the transfer of somatic nuclei into oocytes, studies in the mouse and the cow have both underlined that the trophoblast may be a direct target of reprogramming defects and that its growth seems specifically affected. We propose that the study of TS cells derived from nuclear transfer embryos may help to unravel some of the epigenetic abnormalities which occur therein.

Introduction

Trophoblast is an essential extra-embryonic tissue that arises during development of mammals. It supports embryonic patterning, foetal growth and nutrition. It gives rise to the foetal part of the placenta. Although being a temporary organ, disorders affecting the placenta may have long term effects (Godfrey, 2002). Trophoblast constitutes by itself an interesting cellular model due to its properties of extensive and fast growth, invasiveness, and cell migration. Intriguingly, it seems to be more easily affected than the embryo proper by the consequences of reprogramming of nuclear activity through nuclear transfer. Indeed, a recurrent phenotype displayed by clones in different mammalian species is placentomegaly. Is reprogramming "more difficult" in this tissue (Yang et al., 2007)?

In the mouse, trophoblast stem (TS) cells have been isolated *in vitro* from pre- and early post-implantation embryos (Tanaka et al., 1998). They can self-renew indefinitely in the presence of specific growth factors and in their absence readily differentiate into the different cell types present in the foetal part of the placenta.

In this review we will describe the development of the mouse trophoblast lineage during the early stages when it remains mostly undifferentiated and stem cells can be isolated: so from its origin up to the end of gastrulation. We will review the molecular regulation involved in the control of growth and differentiation of TS cells. Trophoblast growth in other species such as the ungulates displays specific characteristics that we will compare with the mouse. In some of these species, trophoblast cell lines have been isolated, the stem cell nature of which has been neither questioned nor demonstrated so far. This will be discussed here as an alternative hypothesis to understand trophoblast proliferation establishment and maintenance across mammals. At last we will emphasize the usefulness of mouse TS cell models to understand some placental growth disorders such as those found after nuclear transfer.

Specification of the trophoblast

The first visible differentiation event occurs at blastocyst stage in the mouse embryo, with the appearance of an epithelial sheet of cell (the trophoblast) surrounding a cavity and an inner cell mass (ICM) (for a review, see Yamanaka et al., 2006). The trophoblast is a multipotent tissue that will give rise to the few differentiated cell types in the foetal part of the placenta. The transcription factor Cdx2 is a key marker of the first lineage separation (Niwa et al., 2005). In absence of Cdx2, a blastocyst-like structure can initially form but soon degenerates. It indicates that although essential for maintenance of the trophoblast, Cdx2 may not be the first trigger of its initial formation. Indeed, recent studies suggest that cellular mechanisms such as polarization of the cells after asymmetric division in the morula play a triggering role and are initially independent of *Cdx2* expression (Dietrich and Hiiragi, 2007; Honda et al., 2008; Ralston and

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Rossant, 2008). Recently, a factor belonging to the TEAD family, Tead4, has been found to act at an earlier stage of trophoblast specification, but is not involved in the process of polarization (Yagi et al., 2007; Nishioka et al., 2008). Interestingly, this gene is only required during a narrow window of development, before implantation, and in its absence the trophoblast lineage does not appear. Its positioning in the complex model of trophoblast emergence and more specifically, its relationship with Cdx2 remains to be elucidated. Initially present in all cells of the morula, Cdx2 starts to be more concentrated in the nuclei of the outer cells than in the inner cells as epithelialisation progresses and blastocoel forms (Dietrich and Hiiragi, 2007; Strumpf et al., 2005). The transcription factor *Oct-4* is initially expressed in all cells of the morula and later become restricted to the ICM and then to the epiblast. Cdx2 and Oct-4 have been shown to reciprocally inhibit each other in embryonic stem cells in culture (Niwa et al., 2005; Smith et al., 2005). This mutual inhibition can be envisaged *in vivo* as a safety mechanism to lock the lineage segregation. So far, the order of events leading to the establishment of the first two lineages at blastocyst stage is not completely clear. Some actors are probably missing, that could make the link between the cellular and mechanistic processes of polarization, the blastocoel formation and the network of transcription factors that give the genetic identity of both lineages.

Apart from *Cdx2* and *Tead4*, another transcription factor encoding gene has been shown to be essential for the first steps of trophoblast development, *Eomes* (Table 1). This T-box transcription factor is required slightly later than Cdx2 in the embryo and although its expression is not initially dependant on Cdx2, the latter stimulates its expression (Niwa et al., 2005; Strumpf et al., 2005). In its absence, the blastocyst can be formed and maintained, but does not implant (Russ et al., 2000).

Maintenance of the trophoblast identity and more specifically, of its proliferation ability requires additional transcription factors (Table

Table 1
Characteristics of genes expressed in the mouse trophoblast lineage and importance for TS cell derivation

Gene name	Expression pattern in embryo and trophoblast lineage	Phenotype of mutant embryos	TS derivation from mutant	References
Tead4	Starts at 2-cell stage, after implantation is restricted to trophoblast lineages	Die at 3.5 dpc, no blastocyst formation	No	(Hattori et al., 2007; Nishioka et al., 2008)
Cdx2	Polar and mural TE at 3.5 dpc, proximal ExE	No TE determination	No (from blastocyst)	(Beck et al., 1995; Niwa et al., 2005)
Eomes	TE, proximal ExE and chorion, posterior epiblast and primitive streak	Arrest at blastocyst stage, no implantation	No (from blastocyst)	(Ciruna and Rossant, 1999; Strumpf et al., 2005)
Elf5	ExE at 5.5 dpc and after	Loss of ExE at E5.5	No (from blastocyst)	(Donnison et al., 2005)
Sox2	ICM, epiblast, ExE, chorion	Die before 6.0 dpc, loss of epiblast and Exe	No (from blastocyst)	(Avilion, 2003)
Foxd3	ICM and epiblast, a subset of cells in ExE, primary and secondary TGC	Die at 6.5 dpc, loss of epiblast, expansion but precocious differentiation of Exe	No (from blastocyst and ExE)	(Tompers et al., 2005)
Ets2	Restricted to TE from 5.0 to 6.75 dpc, then in primitive streak after 7.75 dpc	Die at 8.0 dpc, loss of ExE, smaller EPC	No	(Georgiades and Rossant, 2006; Yamamoto et al., 1998)
Esrrb	ExE at 5.5 dpc, chorion at 7.5 dpc	Die at 10.5 dpc, reduced proliferation of trophoblast, no placental development	No (from blastocyst)	(Luo et al., 1997; Tremblay et al., 2001)
Dll1	ExE at 6.5 dpc	Die at 10 dpc, abnormal placenta development	Yes but abnormal differentiation	(Papadaki et al., 2007)
Fgf signaling pathway				
Fgf4	ICM, epiblast	Die before 5.5 dpc just after implantation	ND	(Feldman et al., 1995; Goldin and Papaioannou, 2003)
Fgfr2	Blastocyst, then restricted to ExE	Die at 4.5 dpc just after implantation	ND	(Arman et al., 1998; Goldin and Papaioannou, 2003)
Frs2	Polar and mural TE, ExE	Die at 8.5 dpc, defect in A-P polarity	No (from blastocyst and ExE)	(Gotoh et al., 2005)
Ptpn11 (Shp2)	Ubiquitous	ICM death, reduced number of TGC	No	(Yamanaka et al., 2006)
Erk2	Ubiquitous, but P-Erk2 is present in EPC and ExE	Die at 8.0 dpc, no ExE and EPC	No (from blastocyst and ExE)	(Corson et al., 2003; Saba-El-Leil et al., 2003)
Tgfb signaling pathway				
Nodal	ICM and epiblast, then posterior epiblast	Die at 7.5 dpc, no primitive streak, defect in A-P polarity, defect in ExE molecular patterning (see text)	ND	(Brennan et al., 2001; Guzman-Ayala et al., 2004; Takaoka et al., 2006; Varlet et al., 1997)
Activin A	Decidua	ND	ND	(Chen et al., 2006; Crossley et al., 1995)
Furin and Pace 4	ExE	Double mutant: defect in primitive streak formation and A-P polarity	ND	(Beck et al., 2002; Guzman-Ayala et al., 2004)
Smad2	Ubiquitous (P-Smad2 throughout the embryo at 5.5 dpc–8.5 dpc)	Die at 8.5 dpc, size reduction, defect in ExE, defect in gastrulation and visceral endoderm patterning	ND	(Brennan et al., 2001; de Sousa Lopes et al., 2003; Weinstein et al., 1998)
Wnt3	Posterior epiblast at 5 dpc then primitive streak	Die at 8 dpc, no primitive streak	ND	(Ben-Haim et al., 2006; Liu, 1999)
Bmp4	At 3.5 dpc in ICM and polar TE, at 6.5 restricted to ExE	Die before 9.5 dpc, size reduction, impaired mesoderm formation and patterning of anterior visceral endoderm	ND	(Goldman et al., 2006; Soares et al., 2005; Winnier et al., 1995)
Acvr1B (ALK4)	In epiblast and ExE between 5.5 dpc and 7.5 5 dpc	Die between 8.5 and 9.5 dpc, ExE and epiblast intertwined and disorganised, defect in visceral endoderm	ND	(Gu, 1998; Chang et al., 2002; Erlebacher et al., 2004)
AcvR2B (ActRIIB)	In epiblast and ExE since 6.0 dpc	Post-natal lethality: cardiac and intestine defects	ND	(Feijen et al., 1994; Oh and Li, 1997; Song et al., 1999; Chang et al., 2002; Erlebacher et al., 2004)

Index: AP – Antero-Posterior; EPC – Ecto Placental Cone; ExE – Extra-Embryonic Ectoderm; dpc – day post-coitum; ND – not determined; TGC – Trophoblast Giant Cells; TE – trophectoderm.