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Breaking the first lineage barrier – many roads to trophoblast stem cell fate

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ABSTRACT

Recently, direct cell fate conversion attempts between the embryonic and extra-embryonic lineage gained new momentum. Two concomitant publications were published, describing the successful generation of transgene-independent, self-renewing trophoblast stem cells (TSCs) from murine fibroblasts. Cells were faithfully converted, displaying high similarity to blastocyst or extraembryonic ectoderm derived TSCs. Here, we summarize and compare published attempts aiming at the direct induction of trophoblast-fate from either mouse embryonic stem cells or fibroblasts.

1. Introduction

In mice, cell fate segregation takes place shortly after fertilization when outer cells of the morula become specialized towards prospective trophoblast fate. This decision is orchestrated by several cues including cell polarity and position, cell-cell signaling and differential expression of transcription factors (TFs) [1]. Once cell fate segregation is completed at the blastocyst stage, cells are kept in their respective lineage through genetic and epigenetic signatures that reinforce transcriptional programs, collectively referred to as the lineage barrier [2,3]. In this review, we focus on induced cell fate changes from different somatic cell types into extraembryonic trophoblast stem cells (TSCs) and the experimental manipulations attempting to overcome this particular lineage barrier.

2. Stem cells of the blastocyst

An early mouse blastocyst is comprised of two cell lineages (Fig. 1). The trophectoderm (TE) envelops the pluripotent cells of the Inner Cell Mass (ICM), which later give rise to all germ layers. The multipotent cells of the polar TE, which are in close proximity to the ICM, continue to proliferate and later develop into the embryonic part of the placenta. Prior to implantation, the second cell fate decision takes place, when the ICM segregates into the pluripotent epiblast and the primitive endoderm [4]. Under appropriate culture conditions, blastocyst stage embryos give rise to three stem cell entities, which faithfully recapitulate the respective fate of the tissues in vivo; the pluripotent embryonic stem cells (ESCs) are the in vitro equivalent of the ICM/Epiblast [5] and two extraembryonic stem cell types, the primitive endoderm derived (XEN) cells [6] and extraembryonic ectoderm derived TSCs [7]. Stem cells of all three lineages self renew, i.e. they can be cultured indefinitely in vitro and retain their fate specific developmental potential indicated by their potential to chimerize the respective tissue upon reintroduction into blastocysts [6–8]. Each of the three stem cell types is characterized by a unique DNA methylation profile [3] and differs in the genome wide location of active and repressive histone marks [9] further locking the cells in their respective fate.

3. ESC ←→ TSC conversions

Induced pluripotent stem cells (iPSCs) can be generated by forced expression of four TFs, Oct4, Sox2, Klf4 and cMyc (OSKM) from all somatic germ layers [10,11]. Interestingly, not only cells of the somatic lineage have been shown susceptible to reprogramming towards pluripotency, but also TSCs, indicating that experimentally the lineage barrier can be overcome [12,13]. While Kuckenberg et al., found that expression of all four factors is necessary to induce pluripotency in TSCs, Wu et al., succeeded with

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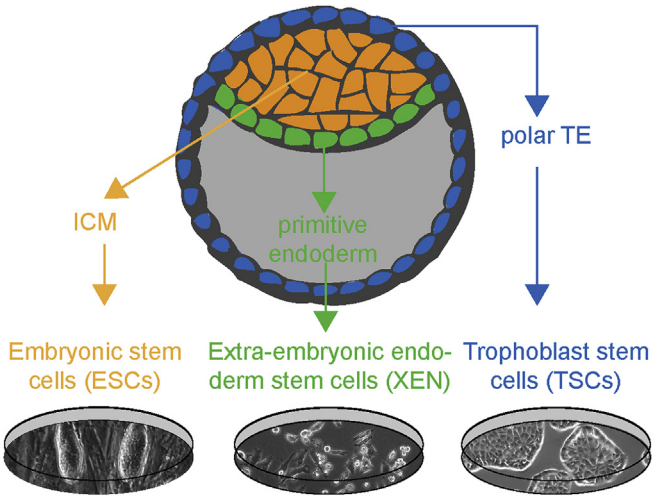

Fig. 1. Blastocyst-derived stem cell lines. The pluripotent Inner Cell Mass (ICM), depicted in orange, gives rise to embryonic stem cells. Primitive endoderm (green), lining the ICM, can be cultured as XEN cells. Cells of the trophectoderm (TE, blue) are enveloping both ICM and primitive endoderm. Polar TE cells adjacent to the ICM can be cultured as trophoblast tem cells (TSCs).

Oct4 overexpression alone, albeit at a 50 fold reduced efficiency compared to the four factor approach [12,13]. Of note, terminally differentiated trophoblast giant cells transduced with OSKM were not able to give rise to iPSC colonies [12], indicating that post-mitotic polyploid cells are refractory to cell fate conversion. The frequency of iPSC induction from TSCs is reportedly 1–2 orders of magnitude lower, compared with standard murine embryonic fibroblast to iPSC approaches, however, different means of efficiency calculation hamper the comparison between experimental approaches and this statement should be regarded with caution.

The reciprocal case of ESC to TSC conversion is also prevented by the lineage barrier, since under normal conditions ESCs do not spontaneously differentiate into cells of the extraembryonic lineages [8]. But, under experimental conditions, following genetic manipulation, ESCs can be forced to differentiate into TSCs and these approaches have been a valuable tool to identify key factors or antagonists of the TE/TSC TF circuitry (Fig. 2). Fig. 3 and Table 1 summarize the attempts, which succeeded in overcoming the lineage barrier between ESCs and TSCs, although with major differences in terms of completeness of this conversion.

The first study on this topic underpinned the importance of the pluripotency factor Oct4 for ESC fate determination through counteracting trophoblast fate. It was shown that conditional reduction in Oct4 levels leads to a loss of pluripotency. Further, after 3–5 days a prominent change in morphology, with colonies forming monolayers of epithelial cells resembling TSCs, occurs [14].

Consequently, overexpression of the Oct4 antagonist, the TF *Caudal-related homeobox 2* (*Cdx2*) is able to induce trophoblast morphology and upregulation of trophoblast markers [15]. When *Eomesodermin* (*Eomes*), a factor downstream of Cdx2, is overexpressed, differentiation towards TE/TSC fate is induced with the same efficiency, making both Cdx2 and Eomes strong candidates for key regulators of TE formation [15]. It had been reported that Tead4 acts upstream of Cdx2 during preimplantation leading to the initiation of TE formation [16]. Thus, expression of Tead4 in ESCs is able to transactivate Cdx2, followed by induction of trophoblast fate [17,18]. Activation of Cdx2 by other means, such as activation of the Ras–mitogen-activated protein kinase (MAPK) signaling, yields analogous results [19]. Conditional overexpression of another key factor of the TE/TSC transcription factor network, the TF *AP-2γ* (Tfap2c), also induces TSC-like fate, even in the absence of Cdx2 [20]. Interestingly, these experiments revealed that Cdx2 and Tfap2c are both required to induce expression of Elf5, a member of the Ets superfamily of TFs [20]. However, the precise hierarchy of both factors has not been fully established yet since Cdx2's role as a master regulator acting upstream of Tfap2c has been challenged [21]. In contrast, the role of Elf5, being further downstream in the TF cascade has been extensively studied and is of particular interest. The differential expression of Elf5 between ESCs and TSCs is regulated via DNA methylation. When Elf5 itself is overexpressed in ESCs, it also triggers conversion into TE-fate, however, cells fail to maintain the undifferentiated TSC state and differentiate into post-mitotic trophoblast derivatives [22]. The same is observed upon overexpression of GATA-binding factor 3 (Gata3) [23]. Taken together, these experiments helped to unravel the transcription factor network required to induce and maintain TE/TSC fate and revealed subtle differences in the tested factors. While most of such TSC-like cells obtained by overexpression of a single factor contributed to placental tissue, the claim that stable TSC fate has been induced was subject to controversy [24]. There was an urgent need to systematically compare transgenic approaches of ESC to TSC conversion, since not all previous studies addressed the following questions: i.) How stable are the induced cell types ii.) How closely do they resemble bona fide TSCs?

Cambuli et al. characterized the above-mentioned manipulations in ESCs, i.e. downregulation of Oct4, overexpression of Cdx2 and conditional Erk1/2 activation as well as a combination of the latter. Interestingly, in depth epigenetic analyses demonstrated that those ESCs did not fully convert into TSCs. Instead, they retained an epigenetic memory of the cell fate of origin. The group identified nine additional loci, which display differences in methylation between ESCs compared to TSCs. In addition to the already known Elf5 locus, they form a critical epigenetic signature of the TSC lineage. Elf5 and the newly identified genes harbor a differentially methylated region in their respective promoters. They are hypomethylated and expressed in TSCs but hypermethylated and repressed in cells of the somatic lineage [24,25]. These signature loci remained hypermethylated even if ESCs were cultured in conditions promoting the naïve state of pluripotency (i.e. known to cause global DNA demethylation) prior to TSC induction approaches [24,26]. Interestingly, since then, it has been shown that TSC-like cells can be induced from naïve but not primed pluripotent ESCs [27]. Importantly, although the TSC-like cells have been found to be only partially converted, they contributed exclusively to placental tissue upon blastocyst injection. This suggests that placental chimerization, which serves as gold standard for proving TSC-potential, needs to be reevaluated, since the in vivo environment seems to aid in completing the conversion process [24]. So while, the data of Cambuli et al., demonstrated incomplete conversion, a recent report using a modified CRISPR/Cas9 tool to mediate activation of Cdx2 expression in ESC, appears to have resulted in stable conversion into TSCs. The resulting cells, named cviTSCs displayed epithelial cell morphology, Elf5 expression level and Elf5 promoter methylation suggestive of complete TSC induction [28]. Interestingly, all analyzed cviTSC lines displayed higher Elf5 transcript levels compared to a control TSC line. This is in contrast to previous studies, where expression level of endogenous trophoblast factors did not reach levels of bona fide TSCs [24,28]. Although these experiments are promising, two aspects have to be regarded with caution. First, most clones of cviTSC retain residual expression of the pluripotency factors Nanog and Oct4. Furthermore, the ability of cviTSC to contribute to placental tissue is rather low, and cross sections indicate that most of the cells of cviTSC origin are giant cells [28]. Therefore, future experiments are required to reveal whether these shortcomings are due to the limited duration of

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