Commentary

Stem Cell INVESTIGATION

Direct conversion of fibroblasts traces the way back to our first organ—the placenta

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Cell fate determination has always fascinated scientists predominantly embryologists and challenged them to decipher master genes or networks of transcriptional factors which together with cell signaling cascades act as key regulators for the cell lineage allocation. With the rediscovery of the pluripotent precursor cells in an adult body and the possibility to induce pluripotency in somatic cells (iPSCs), as sources for alternative therapeutic options in translational medicine, this field has attracted high interest in medical research and application (1). iPSC have several advantages over embryonic stem cells such as avoiding tumor formation and to circumvent ethical concerns. Meanwhile the understanding of pluripotency has broadened thanks to abundant studies on converting ESC and somatic cells into another cell fate using predominantly transfection of a bunch of lineage specific transcription factors. While many studies focused on the regulation of ESC conversion less is known about the hierarchically organized network of transcriptional factors which determine the trophoblast lineage for placenta development. The first and drastic lineage segregation to occur is the differentiation of the blastocyst into the inner cell mass (ICM) which give rise to the embryo and the trophectoderm (TE). The trophectodermal lineage establishes the placenta for a nutrition route to the fetus which guarantees the development the future embryonic life (2). After fertilization a series of cell divisions lead to eight seemingly identical cells, called blastomeres, which are defined as totipotent because of being capable to giving rise to both lineages either to ICM or TE. Once commitment to TE or ICM occurs, however, it is generally considered irreversible (3) and a stable genetic barrier between the embryonic and extraembryonic lineage is established.

Like embryonic stem cells (ESC) derived from the ICM of the blastocysts, trophoblast stem cells (TSC) can be established from the blastocysts or the postimplantation ectoplacental cone of the mouse characterized by self-renewal and the possibilities to differentiate into the trophoblast subpopulations of placental tissue with the support of special culture treatment (4). With the help of a number of targeted deletions in early mouse embryos a set of transcription factors, including *Cdx2*, *Eomes*, *Elf5*, *Ets2*, *Gata3*, *Tcfap2c*, *Esrrb*, *Sox2* and *Tead4*, have been identified to be critical for the establishment and/or maintenance of the multipotent state of TSCs with *Tead4* on the top of this hierarchically ordered network (5).

Several attempts have been made to overcome the established cell lineages from ESC into TSC by converting ESC directly with a cocktail of transcription factors mentioned above, but these iTSCs stop at an intermediate state and are not completely transformed into a stable TS cell phenotype and differ in respect to incomplete reprogramming and a distinct epigenetic memory. In a previous study Cambuli *et al*. (6) could give evidence that this incomplete reprogramming is due to a group of TSC genes, so called gate keeper genes, such as *Elf5*, *Tead4* and *Hand1* which did not change their methylation profile after conversion. To achieve their function, these lineage genes need to be hypomethylated, but even the experimentally induced rapid demethylation approaches failed because of a rapid re-methylation in the culture medium. The authors suspected that the epigenetic memory is retained as ES cell memory and represent the key to stabilize the barrier between this two cell lineages. Thus not only the

appropriate set of transcription factors but the epigenetic marks which are laid down during lineage allocation must be deleted for complete reprogramming. The present commentary focus on the study of Kubaczka *et al*. recently published in *Cell Stem Cell*, 2015 (7) which further corroborated and extended these findings by using a bright strategy. Instead using ESC, the group of Schorle started from mouse embryonic fibroblasts (MEFs) and postnatal tail tip fibroblast and converted these somatic cells into TSC. This direct conversion approach from somatic cells without a pluripotent intermediate state has been already successfully performed by Tanabe *et al*. transforming fibroblasts into neurons (8) and recently by Benchetrit *et al*. (9) into iTSC.

Kubaczka *et al*. (7) started their strategy by transforming MEFs cultured in TS cell medium using a transfection cocktail from 12 candidate genes for trophoblast conversion and induced these genes for 10 days. This approach resulted in iTSC colonies expressing Cdx2, Elf5, Eomes and Tfap2c, protein and mRNA, in a similar level as TSCs combined with the disappearance of the fibroblast specific *Fsp1* and the lack of ESC markers such as *Pou5f1(Oct4)* and *Nanog*.

The authors improved their experimental approach elegantly and narrowed down their candidate genes by selecting clones which resembled most likely TSC but had only four integrated candidate genes, *Eomes*, *Tfap2c*, *Ets2* and *Gata3*.These four transcription factors were able to induce endogenous *Cdx2* and *Elf5* in iTSC. Moreover, these clones re-expressed epigenetically regulated gatekeeper genes such as *Tead4*, *Cdx2*, *Eomes Tfap2c*, *Gata3*, *ETs2 and Elf5* in a pattern indistinguishable from non-induced TSC. This gene independency was confirmed by gene array expression profiles which reveal that iTSC and control TS-EGFP shared the gene clustering pattern in great quantities but was distinct from the parental MEFs. In addition, these clones regain self-renewal capacity similar to parental TSC. The use of a previously published new serum free defined TSC culture medium (10) seem to improve this conversion and iTSCs gained gene stability for 40–50 passages. A further reduction of the four transcription factors, however, did not induce reprogramming of MEFs into iTSC which indicated that a set of interacting transcription factors is needed for TSC induction already at the beginning of lineage allocation. Using the same experimental approach they successfully converted fibroblasts from the tail of newborn mice with less pluripotency state compared to MEFs, but failed to generate stable iTSC from ESCs.

The appropriate functionality of these iTSCs was tested according to well established tests: iTCSs were able to differentiate along their lineages in vitro as indicated by marker gene profiles, they contributed to the placenta when injected into blastocysts and formed the typical transient tumors with hemorrhagic lesions under the skin of nude mice (11). These iTSC tumors gave proof that the iTSC were able to differentiate into a specific type of giant cells, the spiral arteries lining trophoblast (SpA-TGC), which is able to invade into host vessels.

This study of Kubaczka *et al*. (7) gave further evidence that *Cdx2* does not set up the TE lineage but has the role to keep the undifferentiated state of TSC by suppressing *Oct4*. In fact *Tfaf2c* and *Eomes* bind to *Tead4*, *Elf5* and *Hand1* promoter which put them both into the upper hierarchical position. Moreover, unexpectedly *Elf5* does not belong to the four lineage determiners but instead *Ets2*.

The authors asked further, why this trans-differentiation into TSC is more effective starting from somatic cells than from ESC and suggested that the recovery from the epigenetic signature is one of the most important mechanisms. Kubaczka *et al*. (7) found that the global methylation pattern in all iTSC clones resembled each other but distinguishes from parental MEF pattern. However to note, MEFs were generally less methylated than ESCs and epigenetic signature of MEFS was closer to iTSC than to ESC. Moreover, all MEF derived iTSC display a more authentic epigenetic signature when compared to ESC based reprogrammed TSCs.

In MEFs derived iTSC all important lineage genes which are repressed by methylation in ESC were demethylated but *Oct4* remained unchanged. Moreover, hypomethylated CG islands in MEFs, which are enriched in Homebobox genes linked to the embryonic lineage, were re-methylated in TSC. Thus the resetting of the epigenetic memory seems to be more uncomplicated from MEFS into iTSC than from ESC into iTSC.

These new strategies may offer important advantages with respect to a direct conversion of somatic cells as an excellent tool to detect the appropriate hierarchical organization of the molecular network for trophoblast lineage differentiation as the first embryonic organ for establishing and to maintain pregnancy. Furthermore it gives the chance to decipher the most important methylation signatures to keep the gate between TE and ICM.

Because placental insufficiency is the most common identifiable cause of intrauterine growth restriction, this study represents a knowledge base to establish human iTSC, which would provide a tool to unravel the mechanisms

of human placental pathogenesis in a trophoblast *in vitro* system.

While it is possible to establish human embryonic stem cells, reports on stable human TSCs are hampered by the problem to compare them with appropriate controls and test systems to prove convincingly their trophoblast character.

To date TS like cells have been already derived from conventional hESCs (12), from human blastomeres ESC (13) or from human fibroblastic iPSC (14) but nothing is reported of long term gene independency as well as of a conversion of the complete methylation signature or the methylations status of defined key genes such as *Elf5*.

It is worthwhile now to re-investigate the possibility to establish stable hTSC by the use of different human fibroblast such as human foreskin fibroblasts, or placental fibroblast with the strategy described by Kubaczka *et al*. (7). In particular, it has been recently shown that mesenchymal stem cells (MSC) from bone marrow-and adipose tissue-derived MSCs represented an optimal stem cell source for tissue engineering and regenerative medicine (15).

To summarize the discovery presented in the study of Kubaczka *et al*. (7) demonstrated that a small combination of TSC specific transcription factors can directly re-program somatic cells, here fibroblasts, and recover not only the appropriate genetic but also the epigenetic signature of TSC. These findings support the view that these lineage barriers can be overcome easier than assumed when the optimal somatic cell in combination with the appropriate set of transcription factors is employed.

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Footnote

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