

'From blood to blood': de-differentiation of hematopoietic progenitors to stem cells

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Recent studies have reported that fibroblasts or differentiated pluripotent cells can be reprogrammed with transcription factors (TFs) into cells with hematopoietic potential. A study published in *Cell* now suggests that committed blood precursors may provide a source for blood stem cell transplantation after reprogramming (Riddell *et al*, 2014). The authors report that a combination of eight TFs reprogram murine committed blood progenitors into long-term engrafting and serially transplantable hematopoietic stem cells with a molecular signature similar to their endogenous counterparts.

See also: **J Riddell *et al*** (April 2014)

Hematopoietic stem cells (HSCs) continuously generate blood by maintaining the balance between self-renewal and differentiation into all blood cell lineages. Transplantation of HSCs is a widely utilized cell therapy for a range of hematological disorders. However there are limitations as allogeneic transplantation depends on genetic matching between donors and recipients. Histoincompatibility differences, even in matched grafts, often result in the development of adverse clinical conditions such as graft-versus-host disease, graft rejection and infections. Therefore, alternative sources of patient-specific HSCs are needed for autologous stem cell therapy. Such alternatives can potentially come from *de novo* induction of HSCs from unrelated cell sources originating from the individual patient.

Different strategies have been employed to achieve this goal (Fig 1). Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) provide expandable and, for

iPSCs, patient-specific cell sources with which to accomplish this goal. A caveat is that these pluripotent cells have yet to be robustly differentiated into transplantable HSCs. The pathways to generate HSCs through endothelial-to-hematopoietic transition and the precise identity of definitive hemogenic precursors remain elusive. Over-expression of HoxB4 in mouse ESC-derived differentiating cells confers lympho-myeloid engraftment (Kyba *et al*, 2002). However, HoxB4 does not promote engraftment of human ESC-derived cells (Wang *et al*, 2005). The induction of pluripotent and alternative cell fates using combinatorial reprogramming technology has also set in motion efforts to directly or indirectly induce HSCs from fibroblasts. Expression of the pluripotency master transcription factor (TF) OCT4 in human fibroblasts in combination with instructive cytokines seems to increase the developmental plasticity of fibroblasts that results in the generation of hematopoietic progenitors (Szabo *et al*, 2010; Mitchell *et al*, 2014). More recently, the expression of Gata2, cFos, Gfi1b, and Etv6 was shown to be sufficient to program an endothelial-to-hematopoietic transition in mouse fibroblasts. This combination of TFs first induces endothelial-like cells with hemogenic potential characterized by the activation of a human CD34 reporter, the expression of Sca1 and Prominin1, and a global endothelial gene expression program. Upon additional culture, these cells generate hematopoietic cells with nascent HSC features very similar to HSCs isolated from the AGM and placenta. The induced hemogenic cells generate hematopoietic colonies after transgene silencing and short-term aggregation culture with mouse placenta (Pereira *et al*, 2013).

Riddell *et al* now report that a combination of eight TFs (Runx1t1, Hlf, Lmo2, Prdm5, Pbx1, Zfp37, Myc-n, and Meis1) combined with *in vivo* induction for 2 weeks can confer the capacity of long-term engraftment to otherwise committed blood progenitors (Riddell *et al*, 2014). Six of the TFs were identified by *in vivo* screening in donor hematopoietic cells in the peripheral blood, while two additional factors (nMyc and Meis1) were identified using *in vitro* colony assays. The resulting *in vivo* de-differentiated cells can generate all hematopoietic cell lineages, engraft mice in competitive transplantation assays, and also engraft secondary recipients. The authors also show that multi-lineage progenitor activity *in vitro* could be conferred to differentiated cells that repopulated the peripheral blood of primary recipients. Furthermore, using single-cell assays, the authors show that these cells express 151 genes involved in HSC biology in a similar pattern as endogenous HSCs. These results are exciting and show that differentiated blood progenitors can be pushed back up the 'Waddington landscape' to generate transplantable HSC. They also highlight that generating functional HSCs from unrelated cell types is an achievable goal.

The application of this technology to humans is dependent on the functional conservation of the eight TFs between mice and human. It is somewhat concerning that similar screening approaches in the human system using colony-forming and transplantation readouts have generated a completely different set of inductive TFs (ERG, SOX4, RORA, HOXA9, and MYB) (Doulatov *et al*, 2013). These authors used a strategy to re-specify lineage-restricted CD34⁺CD45⁺ myeloid progenitors derived from human

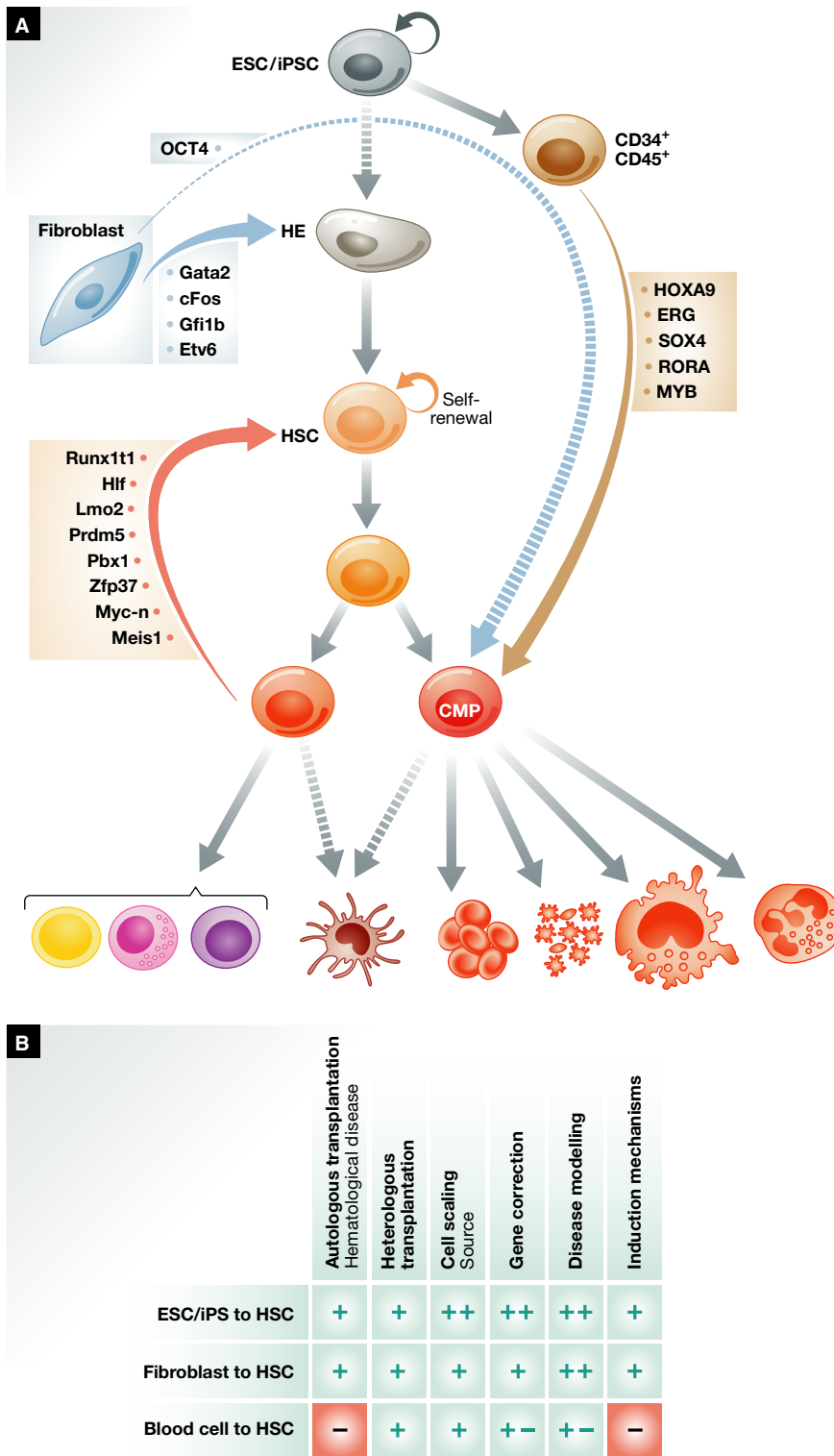


Figure 1. Strategies to induce hematopoietic stem cells.

(A) Strategies for the induction of hematopoietic stem cells (HSCs) and hemogenic endothelial cells (HE) from fibroblasts (Szabo *et al*, 2010; Pereira *et al*, 2013), embryonic/induced pluripotent stem cells (ESC/iPSC) (Doulatov *et al*, 2013), or committed blood progenitors (Riddell *et al*, 2014). The transcription factors used for each induction are highlighted. (B) Advantages and disadvantages of fibroblasts, ESC/iPSCs, and blood cell types as a cell source for inducing HSCs. The disadvantages of starting with blood cells are highlighted.

ESC/iPSC and showed that this combination of TFs confers short-term engraftment with myeloid and mature adult beta-globin producing erythroid cells. In the case of Riddell *et al*, it would be also of interest to address the efficiency of reprogramming and to know whether these factors would work with cells from outside the hematopoietic system. There exists a considerable disadvantage to starting with hematopoietic progenitor cells compared to differentiating ESCs/iPSCs or programming fibroblasts. In patients with hematopoietic disorders caused by congenital or acquired mutations in the stem/progenitor hematopoietic pool, the mutations will be carried over to the differentiated progeny, limiting the use of these cells for autologous transplantation (Fig 1). For therapeutic applications, the ‘blood to blood’ approach will be more limited when compared to an ‘unrelated cell type to blood’.

To date, the developed culture methods using cellular and non-cellular substrates can only sustain transplantable HSCs in culture for a limited period of time and are not comparable to the robust protocols that can expand and maintain pluripotent stem cells. The employment of an *in vivo* reprogramming approach as reported by Riddell *et al* might circumvent this problem by providing the correct niche for maturation of generated cells. Similar approaches have proven successful in other systems such as the induction of mouse cardiomyocytes (Qian *et al*, 2012) and *in vivo* induction of pluripotent cells (Ohnishi *et al*, 2014). A drawback is that an exclusive *in vivo* induction hinders an understanding of the reprogramming process (Fig 1). In addition, in the light of the recent findings on the induction of pluripotent cells (Ohnishi *et al*, 2014), by performing reprogramming *in vivo*, there is high risk that partially or completely reprogrammed cells that have undergone transformation may acquire aggressive cancerous activity.

For the future, we need to develop assays to walk the fine line between ‘reprogramming’ and ‘transformation’ to correctly program ‘bona fide’ HSCs. Given that six of the eight Riddell *et al* TFs are proto-oncogenes, it will be necessary to carefully address the normal versus malignant nature of these de-differentiated cells in addition to the functional properties of the mature blood cell types derived from them. Indeed, deletion of *Pax5* in B-cell progenitors is sufficient to confer multi-lineage potential but results

in aggressive lymphomas *in vivo* (Cobaleda *et al.*, 2007). Generating and expanding human patient-specific HSCs *in vitro* may be the 'Holy Grail' of regenerative stem cell biology. The recently published strategies and the article by Riddell *et al* take us a step closer to this goal.

Conflict of interest

The authors declare that they have no conflict of interest.

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