An education for stem cells

Haematopoietic stem cells give rise to all blood lineages, and their production *in vitro* has been a long sought goal of stem-cell biology. Two groups now achieve this feat through different means. See Articles p.XXX & p.XXX

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The entire blood system can be restored from a single haematopoietic stem cell (HSC). These self-renewing cells reside in the bone marrow, where their proliferation and differentiation are tightly regulated to supply the blood cells for an entire lifetime. The high regenerative capacity of HSCs makes them central to life-saving bone-marrow transplants, but also makes them prone to acquire leukaemia-initiating genetic mutations. The ability to manufacture HSCs in the laboratory therefore holds enormous promise for cell therapy, drug screening and studies of leukaemia development. The first HSCs in the embryo are thought to develop from specialized 'haemogenic' endothelial cells that line the walls of a blood vessel called the dorsal aorta[1, 2]. The precise molecular mechanisms behind this intriguing conversion remain to be defined, but are widely believed to be important for successful *in vitro* generation of HSCs[3]. Two studies[4,5] online in *Nature* took this supposition as the basis for the development of new protocols for successful *in vitro* HSC production.

In the first study, Sugimura *et al.* started with human pluripotent stem cells (hPSCs), which can give rise to any cell type in the body. The authors adapted a previous protocol to derive haemogenic endothelial cells from hPSCs. They then identified a subset of 7 transcription factors (*ERG*, *HOXA5*, *HOXA9*, *HOXA10*, *LCOR*, *RUNX1*, *SPI1*) capable of inducing the hPSC-derived endothelial cells to become immature HSCs. In the second study, Lis *et al.* directly converted adult mouse endothelial cells to immature HSCs through overexpression of a panel of four transcription factors (*FosB*, *Gfi1*, *Runx1* and *Spi1*). This second strategy opens up the exciting prospect that HSCs could eventually be directly induced from a person's own tissues.

The development of HSCs has previously[6] been likened to progression from a maternity ward to a finishing school, with the former being the place where HSCs are born, and the latter the place where they mature in response to external signals. This analogy seems fitting for the current papers (Fig. 1). Programming by transcription factors was required for HSC birth in both cases. However, extracellular cues were essential for subsequent maturation.

Sugimura *et al.* provided these 'finishing school' cues by transplanting their HSCs into the bone marrow of adult mice. By contrast, Lis and colleagues grew their cells on a layer of foetal endothelial cells, which released as-yet-undefined factors that support HSC development, before transplantation into mice. HSCs generated by either protocol could engraft into serially transplanted recipients and gave rise to all blood-cell lineages — a major step forward from previous methods.

The dorsal aorta is believed to act as both the maternity ward and the finishing school for HSCs during normal embryonic development[7]. The layer of endothelial cells used by Lis *et al.* captures some aspects of this native setting. However, by injecting their cells directly into adult mouse bone marrow to achieve HSC maturation, Sugimura *et al.* instead exposed the cells to signals from the niche in which adult HSCs normally reside. Perhaps the finishing-school site for Sugimura and colleagues' cells is the perivascular HSC niche — a region close to blood vessels that is partly made up of endothelial cells. There is accumulating evidence[8] that this niche is involved in the maintenance of adult HSCs. It is therefore plausible that there is more flexibility than previously thought in the type of environment that can support maturation of HSCs, and that one of the main requirement for HSC maturation is an endothelial wall.

When asked why they should be given public money, developmental biologists have long argued that a better understanding of normal developmental processes will be crucial for generating authentic cell types for cell-based therapies and drug screening. The current papers provide excellent examples of this, because their protocols borrow features from embryonic mechanisms. Both studies used endothelial cells as starting material, in analogy to HSC development in the embryo, and the transcription-factor cocktails that they used included factors such as Runx1 and Spi1, that are known[9–11] to regulate the birth of the first HSCs during embryonic development *in vivo*.

Despite of the excitement that the current papers will generate, some limitations are noteworthy. Only Lis and colleagues addressed the potential of the programmed HSCs to

become cancerous, finding no evidence of leukaemia for up to 20 months after engraftment. Longer follow-up will be required — especially for the human cells produced by Sugimura and co-workers. Future investigations are also likely to focus on how the transcription-factor cocktail is introduced. The current studies used genetic vectors derived from retroviruses that integrate into random sites in the host-cell genome. Expression of the transcription factors is then driven by a drug called doxycycline that can be administered as needed. 'Cleaner' genome-engineering strategies, such as CrispR–Cas9 technology that can direct insertion of sequences into specific sites in the genome, are advancing rapidly. These techniques could eliminate concerns that retroviral integration might cause the unwanted activation of cancer-causing genes[12], or that expression of the transcription factors might not be completely extinguished under inducible, doxycyline-based systems. This can be a concern, given that many of the transcription factors used in the current studies have also been implicated in leukaemogenesis.

Another caveat is that less than one in a thousand cells of the engineered HSC population engrafted over the long-term in either study. Although this was sufficient to repopulate the blood, further studies will be required to fully define the molecular make-up of HSCs produced *in vitro*. This is particularly pressing given that both studies suggest that, although the programmed cells are similar to HSCs, they are not identical.

HSCs are defined by their ability to engraft long term and give rise to all blood-cell lineages. This is arguably the most stringent assay of any adult stem cell. The fact that cells fulfilling these criteria have been produced both from hPSCs and adult mouse endothelial tissue not only opens up exciting opportunities in the haematopoiesis field, but also represents a milestone for the wider stem-cell community. Although further studies are needed, the long journey to translate the promise of stem-cell research into direct patient benefit may just have become a little shorter.

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Figure 1 | Production of haematopoietic stem cells (HSCs) *in vitro*. Two groups report different protocols to produce HSCs, from endothelial cells, which line blood vessels. Sugimura *et al.*[1] started with human pluripotent stem cells (hESCs), and used a previous protocol to induce them to differentiate into specialized endothelial cells. Lis

et al.[2] isolated adult endothelial cells from mice. The groups used overlapping cocktails of transcription factors, including Runx1 and Spi1, to generate HSCs — a step that can be thought of as a maternity ward, where HSCs are born. Immature HSCs must receive as-yet-unknown extracellular signals in a ‘finishing school’ environment for maturation. Sugimura *et al.* achieved this by transplanting their cells into the bone marrow of adult mice. Lis *et al.* grew their cells on a layer of embryonic endothelial cells.