Reconstructing blood from induced pluripotent stem cells
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Abstract

The direct reprogramming of human somatic cells to induced pluripotent stem cells (iPSCs) offers exciting prospects for disease modelling and regenerative medicine. Several recent reports support the feasibility of generating various blood cell types from iPSCs through in vitro-directed differentiation. However, the derivation of hematopoietic stem cells (HSCs) capable of long-term reconstitution of all hematopoietic lineages appears to be more challenging. These hurdles notwithstanding, cell engineering strategies aiming to correct genetic defects at the stem cell level are already emerging. Robust methodologies for the generation of definitive human HSCs conferring high-level, multilineage, long-term, hematopoietic reconstitution thus are direly needed before the therapeutic potential and safety of iPSC-derived cell products can be thoroughly investigated.

Introduction and context

In 2006, Takahashi and Yamanaka [1] reported the direct reprogramming of mouse fibroblasts to pluripotent stem cells, which were termed induced pluripotent stem cells (iPSCs). This scientific breakthrough was achieved through overexpression of the four transcription factors OCT4, SOX2, KLF4, and c-MYC [1]. The successful reprogramming of human somatic cells reported the following year [2-4] opened new frontiers for disease modelling and regenerative medicine. Since then, several methodologies, using viral, non-viral, or chemical approaches, have been devised to establish iPSCs from various mouse and human somatic cell types [5]. These include mouse cells in all stages of the hematopoietic hierarchy and human progenitor cells from cord blood, bone marrow, and peripheral blood [6-9]. It is now widely accepted that mouse and human iPSCs possess morphological, molecular, and developmental attributes that closely resemble those of blastocyst-derived embryonic stem cells (ESCs), although their global gene expression patterns and epigenetic state may not be identical [5,10]. Directed differentiation protocols already in use in ESCs were promptly applied to iPSCs, yielding numerous cell types, including neurons, cardiomyocytes, adipocytes, and endothelial and hematopoietic cells.

However, the generation of human hematopoietic stem cells (HSCs) conferring multilineage, long-term, hematopoietic reconstitution remains elusive. HSCs originate in early embryonic development, after which they expand and self-renew in successive anatomical locations. Hematopoiesis is initiated with the specification of a subset of mesodermal cells known as hemangioblasts, which yield both endothelial and hematopoietic progeny. The first hemangioblasts arise in the primitive streak of the embryo and then migrate to the yolk sac, where they form blood islands [11]. The ensuing first wave of hematopoiesis predominantly yields erythroid cells, which express embryonic and fetal globins and macrophages, but not lymphoid cells. The immediate precursors of definitive HSCs are arterial endothelial cells [12-15], which become HSCs capable of long-term multilineage repopulation of adult hosts in the dorsal aorta of the aorta-gonad-mesonephros (AGM) region and the chorioallantoic vessels of the placenta. These early CD34+, c-kit+, CD41+ HSCs next migrate to the placenta and mostly to the fetal liver, where their
numbers further expand. Around birth, the main site of hematopoiesis shifts from liver to bone marrow, from which most blood cells originate throughout the organism’s adult life span.

Recent advances
In vitro generation of blood cell types from ESCs and iPSCs
Hematopoietic lineage specification from pluripotent cells is obtained in one of two general approaches, using either embryoid body formation in the presence of hematopoietic cytokines or co-culture with stromal cell lines such as the OP9 stroma cell line. Several blood cell types have been successfully generated from murine or human ESCs. These include mouse and human B- and T-lineage cells, megakaryocytes, and erythroid cells. The latter have revealed that human ESC-derived erythropoiesis closely mimics primitive erythropoiesis, characterized by the expression of the embryonic $\epsilon$ and $\zeta$ and the fetal $\alpha$ and $\gamma$ globins [18,19]. The analysis of globin gene expression in erythrocytes derived from human iPSCs likewise revealed a pattern of primitive erythropoiesis [20].

Derivation of HSCs from ECSs and iPSCs
The generation of self-renewing multipotent HSCs from ESCs or iPSCs appears to be challenging. Several early reports illustrated the difficulty of producing HSCs capable of reconstituting adult, irradiated recipients [21,22]. Intravenous injection of murine or human ESC- and iPSC-derived hematopoietic cells resulted in little or no engraftment [23-28]. More recent studies have yielded more encouraging results. Ledran et al. [29] reported that co-culture of human ESCs on primary AGM stroma induced HSCs capable of primary and secondary hematopoietic engraftment into nonobese diabetic/severe combined immunodeficiency disease Il2rg-/- (NOG) mice (0.11-16.26%). Furthermore, culture of human ESCs and iPSCs with medium conditioned by HepG2 cells, a human hepatocarcinoma cell line, was shown to enhance the generation of mesodermal derivatives, including hematopoietic cells, with the ability to repopulate sublethally irradiated NOG mice for almost 1 year [30].

Efforts to engineer functional adult HSCs from ESCs or iPSCs
Attempts to engineer definitive hematopoiesis have so far largely focused on HOX family members (particularly HOXB4 and HOXA10), which are transcription factors involved in the formation or maintenance of HSCs [11]. Ectopic expression of HOXB4 in mouse ESCs has been reported to give rise to HSC-like cells capable of long-term repopulation [31,32]. However, the in vivo reconstitution obtained is strongly biased toward myeloid cells, hardly yielding lymphoid cells [32,33], while continuous HOXB4 expression poses oncogenic risks [34]. Cdx genes were also shown to promote the specification of hematopoietic progenitors in mouse ESCs [35]. However, these strategies have not been successful toward the generation of engraftable HSCs from human ESCs [25], highlighting the difficulty in translating mouse ESC-based studies into human pluripotent stem cell engineering.

Several investigators have observed that globin switching occurs in cultured human ESC-derived erythroid progeny in a time-dependent fashion, although the underlying mechanisms are not understood [18,19,36]. Fetal hematopoietic cells transplanted into adult sheep were previously shown to switch over time [37]. It is therefore possible that a prolonged in vitro culture or in vivo maturation is needed for the generation of developmentally mature erythroid progeny.

Implications for clinical practice
The advent of iPSCs holds great promise for regenerative medicine. iPSC-based research is poised to enable a watershed of knowledge on human cell development as well as provide critical tools for disease modelling and in vitro drug screening. The potential for developing novel cell therapies is equally tantalizing but still uncertain at this time. Reprogramming technologies offer the prospect of generating blood cell types on a patient-specific basis. Thus, red blood cells could be generated for individuals with severe congenital anemias or polytransfused subjects who require a unique blood cell type. A repertoire of naïve T lymphocytes could be generated for subjects with acquired or congenital deficiencies or aging subjects afflicted by immunosenescence. However, the methods for generating such cell types are still in their infancy and marred with considerable biological, safety, and economic uncertainties. A holy grail for this field is to generate HSCs, which would open new doors for both disease correction and regenerative medicine. Two recent proof-of-principle studies highlighted the potential of combined gene and cell therapy with autologous iPSCs to treat Fanconi anemia and sickle cell disease [26,33]. Robust protocols for the directed differentiation of human iPSCs to all hematopoietic cells will provide valuable tools for modelling hematopoiesis and hematological disorders, as exemplified in a recent study on polycythemia vera [9]. However, as reviewed above, there remain significant obstacles to the generation of adult HSCs capable of long-term, pan-lineage, hematopoietic reconstitution. Further studies are also required to assess the hematopoietic potential of human iPSCs in comparison with that of ESCs [9,38-40]. Thus,
the well-deserved excitement about iPSCs must be tempered with patience as a considerably larger body of basic investigation is needed to unravel the biological and therapeutic potential as well as the safety profile of iPSC-derived hematopoietic cell products.

Abbreviations
AGM, aorta-gonad-mesonephros; ESC, embryonic stem cell; HSC, hematopoietic stem cell; iPSC, induced pluripotent stem cell; NOG, nonobese diabetic/severe combined immunodeficiency disease Il2rgnull.

Competing interests
The authors declare that they have no competing interests.

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