Generation of mature blood cells from pluripotent stem cells

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(Related Original Article on page 1651)

Transfusion of red blood cells and platelets is one of the most common life-saving procedures used in the clinic. Blood transfusions are needed to replace blood lost through traumatic injury and surgery. Transfusions are also used for the treatment of burn victims, recipients of organ transplants, and patients with leukemia, cancer, sickle cell diseases, thalassemia and thrombocytopenia. With new advances in medical treatment and an aging population, the demand for blood products continues to increase.[1] Currently, all blood components utilized for transfusion therapy are obtained through voluntary donation, which creates periodic shortages and concerns about disease transfer.

Stem cells are a logical alternative source for blood cells. Studies pioneered by Luc Douay's group demonstrated the feasibility of *ex vivo* production of red blood cells (RBC) from $CD34^+$ hematopoietic stem cells/progenitors isolated from cord blood, bone marrow or peripheral blood.[2] However, somatic hematopoietic stem cells are difficult to expand, which limits the possibility of using these cells for high scale industrial production of major blood components. Human embryonic stem cells (ESC) are pluripotent stem cells (PSC) capable of unlimited proliferation while maintaining the ability to form all the cells of the body, including blood cells.[3,4] Human ESC can be grown in unlimited numbers, thus their derivatives can also be produced in unlimited numbers. In 2006, Yamanaka's group demonstrated that mouse skin fibroblasts could be reprogrammed to a pluripotent ESC-like state via ectopic expression of four factors.[5] Soon after, induced PSC were obtained from human skin fibroblasts.[6,7] Like human ESC, human induced PSC proliferate indefinitely and can be efficiently differentiated into blood cells.[7-9] Since induced PSC can be generated from any individual without the ethical controversy associated with obtaining human ESC, they can be used to generate blood cells with phenotypic characteristics matching those of any living individual. Because human induced PSC were initially obtained using lentiviral vectors and reprogramming factors, some of which are known oncogenes, proviral integration was the most significant concern regarding translation of the use of these cells into the clinic. However this obstacle has been overcome in a short period and technologies allowing for the generation of transgene-free human induced PSC have already been developed.[8]

Recently, several groups have demonstrated the feasibility of RBC production from human ESC.[10,11] The work by Lapillonne *et al.*, published in this issue of the journal, shows that RBC can be produced from induced PSC on a large scale.[12] By differentiating induced PSC and human ESC, the authors were able to obtain up to 3.5×10^9 RBC from 10^6 PSC. As one pack of RBC concentrate contains approximately 2×10^{12} cells, only 10^9 PSC would be required to produce one unit of cells for transfusion. Given the huge expansion potential of PSC, these achievements bring the possibility of manufacturing blood cells closer to reality. However, in spite of these technological advances, the high costs associated with culturing and differentiating cells *in vitro* remain major challenges to the industrial production of RBC. Nevertheless, manufacturing RBC has several important advantages for clinical use. First, the continuity of blood supplies could be improved significantly; RBC of any blood group could be produced on demand, and an unlimited number of universal type (O Rh negative) RBC could be available for use in patients with acute hemorrhage with an unknown blood group. Manufactured blood products would also virtually eliminate the risk of viral, prion (the cause of fatal spongiform encephalitis), and bacterial transmission. RBC could be obtained without the presence of contaminating plasma, granulocytes and lymphocytes, significantly reducing the incidence of non-hemolytic transfusion reactions and graft-*versus*-host disease in immunocompromised patients. Younger RBC (neocytes) could also be produced, reducing the number of transfusions required for patients with chronic anemias, and therefore reducing haemosiderosis due to iron overload and its sequelae: diabetes, cardiac failure and death. Finally, genetically modified ESC or induced PSC with a rare blood group phenotype could be produced to generate RBC that fit specific clinical needs.

When patients lose blood they also need platelet transfusions. Platelets are extremely fragile and currently there is no clinically applicable method for their long-term storage. A total shelf-life of only 5 days translates into 3 days of potential use for hospitals after testing and shipping, so most blood banks have constant platelet shortages. Alloimmunization and development of refractoriness to platelet transfusions remain significant clinical problems, especially for patients with severe thrombocytopenia and bone marrow failure. The use of human PSC to produce platelets has the potential to reduce alloimmunization significantly by providing platelet products free of leukocytes and by manufacturing HLA-matched platelets on demand. Although the feasibility of megakaryocyte and platelet production from human ESC has been demonstrated,[13,14] the differentiation systems currently used require xenogenic feeder cells and are not robust enough to enable industrial production.

Granulocyte transfusions are used to treat severe neutropenia in patients undergoing myelotoxic therapies. Granulocytes have a very short shelf-life (24 hours) and have to be HLA-matched to prevent alloimmunization. As an alternative approach, myeloid progenitors with the potential to differentiate into mature myelomonocytic cells can be generated from stem cells and used to improve immune function in patients with bone marrow failure. Recent studies demonstrated that $Lin^-CD34^+CD43^+CD45^+$ multipotent myeloid progenitors could be generated from human ESC and induced PSC, expanded with granulocyte-monocyte colony-stimulating factor, and differentiated, with high efficiency, into almost all types of mature myelomonocytic cells, includ-

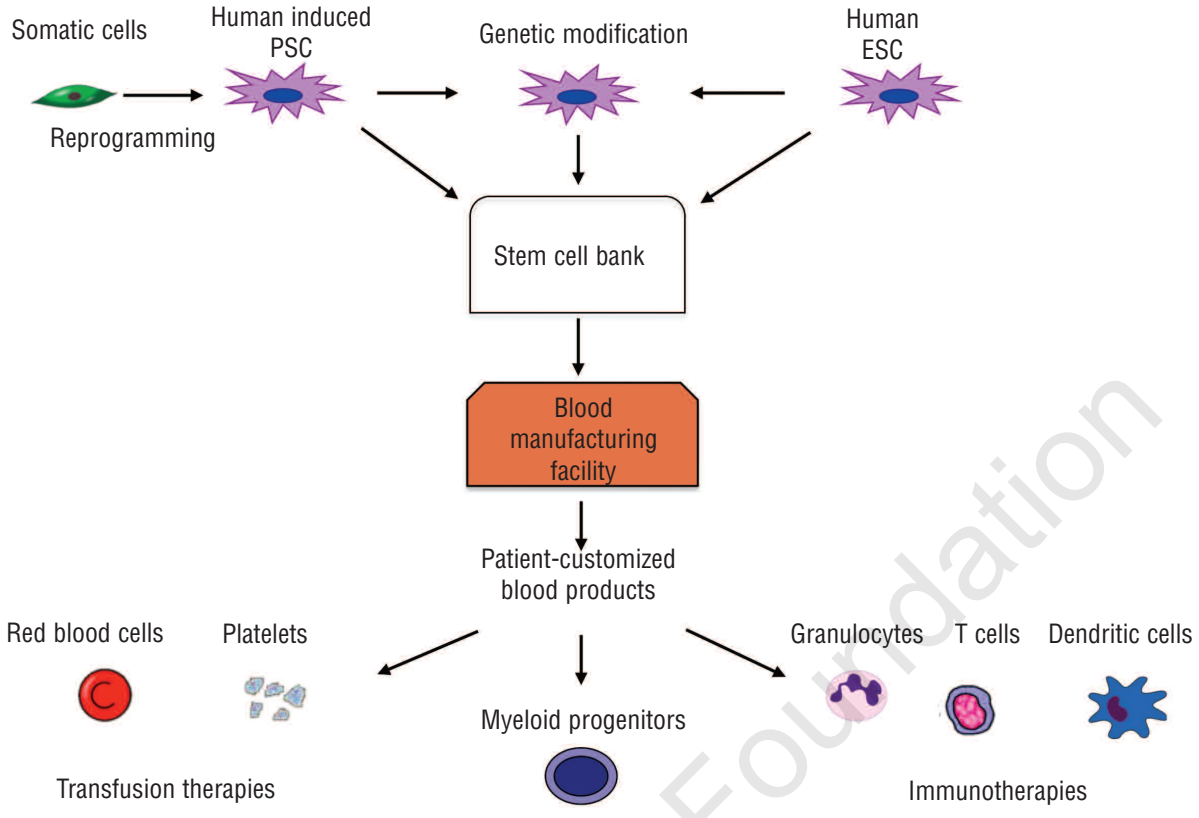

Figure 1. The use of human induced pluripotent stem cells (PSC) and human embryonic stem cells for manufacturing blood products. Human PSC lines with the most commonly used RBC and platelet genotypes/phenotypes can be obtained and used for continuous manufacturing of blood products. To meet specific clinical needs (*e.g.*, transfusion of alloimmunized patients, patients with rare blood groups), cells with unique genotypes/phenotypes can be obtained from a stem cell bank to manufacture blood products on demand. In addition, human PSC can be genetically modified to produce RBC for drug delivery or platelets for targeting specific pathways in the coagulation cascade to treat bleeding disorders or coagulopathies. Human PSC-derived hematopoietic progenitors can be used to treat bone marrow failure due to chemotherapy-induced myelotoxicity. Human PSC also provide an opportunity to manufacture dendritic cell-based vaccines for cancer immunotherapy with a broad variety of MHC genotypes/phenotypes. By using human PSC genetically engineered to express tumor-specific T-cell receptor, cytotoxic T cells can be produced to target malignant tumors.

ing neutrophils, eosinophils, dendritic cells, Langerhans' cells, macrophages, and osteoclasts.[15] The methodology described already enables the production of more than 10^6 $CD34^+CD45^+$ highly proliferative myeloid progenitors from 10^6 human PSC. Although the potential of human PSC-derived myeloid progenitors to restore myeloid cell production *in vivo* remains to be evaluated in animal models, these cells can already be used to screen the myelotoxicity of chemotherapeutic drugs. Despite the relative ease with which human PSC can be differentiated into erythromyeloid cells, generation of human PSC-derived lymphoid cells remains a significant challenge. Kaufman's group demonstrated successful differentiation of human ESC into natural killer cells.[16] T cells were obtained from human ESC using DLL1-expressing OP9 stromal cells or by transplanting differentiating human ESC together with human thymic tissue into immunocompromised mice.[17,18] While the potential of human ESC to generate B cells has been shown,[19,20] significant improvements in differentiation conditions are needed to achieve efficient production of B cells from human PSC.

Advances in the identification of reprogramming factors, the generation of human induced PSC, and their differentiation to blood cells provide a unique opportunity for the development of novel technologies for manufacturing patient-customized blood products. Current studies clearly demonstrate the feasibility of massive *ex vivo* production of erythromyeloid cells from human PSC. The next key steps toward clinical translation of human PSC-based therapies for blood diseases include development of completely defined, animal product-free conditions for the PSC differentiation; bioreactor based-technology for further scaling up of cell production; and evaluation of the therapeutic potential and safety of human PSC-derived blood cells in animal models.

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Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

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IDH1 and *IDH2* mutations in myeloid neoplasms – Novel paradigms and clinical implications

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doi:10.3324/haematol.2010.030015

(Related Original Articles on pages 1668 and 1754 and related Letter on page 1797)

In the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues[1] myeloid neoplasms include myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS), myelodysplastic/myeloproliferative neoplasms (MDS/MPN), and acute myeloid leukemia (AML). In the last few years there have been major advances in our understanding of the molecular bases of these disorders, and molecular genetic data are increasingly being used for diagnosis, risk assessment and definition of treatment strategies.[2,3] These data now include information on mutations in the *IDH1* and *IDH2* genes.

IDH1 and *IDH2* encode the enzymes isocitrate dehydrogenase 1 and 2, respectively. The essential information about these genes and their products is reported in Table 1.

Somatic mutations of IDH1 *and* IDH2 *in malignant gliomas*

In 2008, though a genome-wide analysis Parsons *et al.*[4] identified somatic mutations at codon 132 of *IDH1* in approximately 12% of patients with glioblastoma multiforme, the most common and fatal type of brain cancer. In a subsequent study, these authors detected somatic mutations that affected amino acid 132 of *IDH1* in more than 70% of gliomas.[5] In most cases, arginine 132 was mutated to histidine (R132H). Some tumors without mutations in *IDH1* had mutations affecting the analogous amino acid (R172) of the *IDH2* gene, strongly indicating a role of mutations in the $NADP^+$-dependent isocitrate dehydrogenase genes in the pathogenesis of these malignancies. Overall, brain tumors with *IDH1* or *IDH2* mutations represented a distinctive subgroup of low-grade and secondary gliomas with a better outcome compared to that of tumors with wild-type *IDH* genes.

A causal relationship between acquired error in cellular metabolism and malignant transformation

Since a single copy of the gene – *IDH1* or *IDH2* – is mutated in human gliomas, Dang and co-workers[6] hypothesized that the mutations do not result in a simple loss of function. They did an elegant study to determine the impact of the *IDH1* (R132H) mutation on cellular metabolism, and showed that it resulted in the production of 2-hydroxyglutarate.[6] Since overproduction of this metabolite is associated with a high risk of brain tumors in patients with inborn metabolic errors, the authors concluded that the accumulation of excess 2-hydroxyglutarate *in vivo* contributes to the formation and malignant progression of gliomas, establishing a link between abnormal metabolism and malignancy.[7] The altered metabolic pathway associat-