

hematopoiesis? As multiple genes are expressed from trisomic chromosomes,<sup>6</sup> it is possible that cooperation among several Hsa21's is responsible for cT21 effects, as has also been suggested for explanation of other phenotypes of DS.<sup>7</sup>

Similarly to the leukemias of DS, sporadic childhood leukemias are initiated in utero. Somatic structural or numerical chromosomal aberrations acquired in fetal hematopoietic progenitors initiate the growth of a preleukemic clone.<sup>8</sup> Thus, the relevance of studies, such as the 2 reported by Tunstall-Pedoe and Chou and their colleagues, extends beyond DS. They are important for the general understanding of how normal fetal hematopoietic development is diverted toward leukemia.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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ciently induce hESC differentiation into adult red blood cells.

Lu and colleagues report in this issue of *Blood* that they have been able to produce large amounts of red blood cells from hESCs using a procedure that involves a combination of embryoid body formation in the presence of a cell-permeable recombinant HoxB4 transcription factor, amplification in liquid culture of hematopoietic and erythroid precursors, and final maturation and enucleation in the presence of feeder layer.

Several major issues must be resolved before the manufacture of red blood cells can become a reality. The first challenge that must be met is the development of a highly scalable and economically viable production method because the number of cells that is present in each unit of blood is considerable. Lu et al have produced over 10 billion cells, an unprecedented number for the field. In addition, several of the steps in their protocol should be scalable. This is an important achievement that provides a proof of principle that industrial production might one day be achievable. However, the number of cells necessary to cover even a small fraction of transfusion needs is several orders of magnitude higher than what has been achieved to date because a 220-mL unit of packed red blood cells contains about 2 trillion cells. Therefore, considerable biologic engineering remains to be done to reach the necessary scale at a reasonable cost. The final cost of red cell manufacturing will be highly dependent on the purity level and on the amount of testing that will be acceptable to the regulatory agencies.

A second challenge is to produce functionally normal cells. Three major types of red blood cells are produced during development that differ by morphology, size, and gene expression profiles, and by the type of hemoglobin produced. All current protocols to produce red cells from hESCs, including the one described by Lu et al, yield cells with an embryonic or fetal phenotype that are normally produced early in gestation.<sup>1</sup> Whether such cells could be used for transfusion is unclear but seems unlikely, given the multiple differences that exist between adult and fetal or embryonic red cells. Coaxing hESCs into adult red cells therefore remains to be solved.

In addition to being at an appropriate developmental age, the cells obtained from hESCs should be fully functional. Lu et al show that the oxygen equilibrium curves of the hESC-derived cells are comparable to normal red blood cells

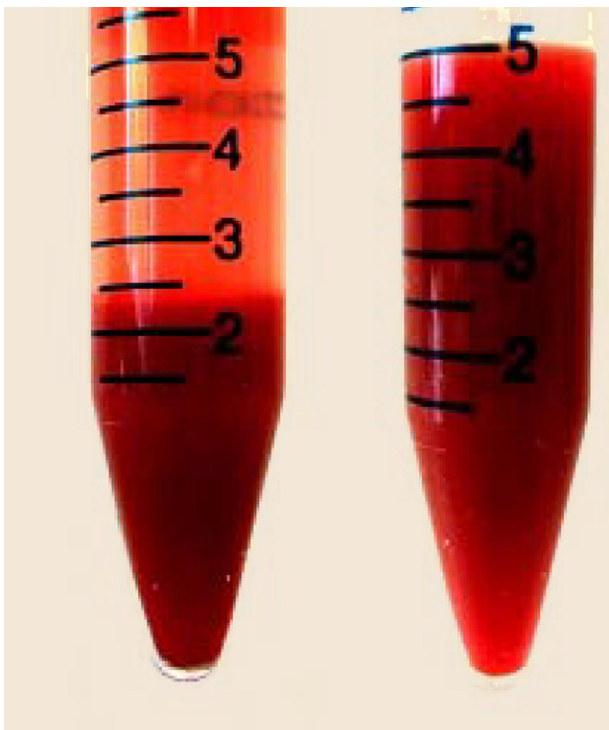
## ● ● ● HEMATOPOIESIS & STEM CELLS

Comment on Lu et al, page 4475

# Toward the manufacture of red blood cells?

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In this issue of *Blood*, Lu and colleagues describe a new method to differentiate human embryonic stem cells into large amounts of mature red blood cells.



**In vitro produced human embryonic stem cell-derived red blood cells. See the complete figure in the article beginning on page 4475.**

**A**lthough the red blood cell supply is currently very safe, a donorless industrial process to manufacture red blood cells for transfusion is desirable. This would be beneficial because periodic shortages develop for rare blood groups and because the risk of contamination by new or emerging pathogens cannot be completely eliminated. Human embryonic stem cells (hESCs) or induced pluripotent stem cells can grow undifferentiated for long periods of time in vitro and are therefore a potentially unlimited source of cells from which red blood cells could be produced in large amounts if a method could be developed to effi-

and that the cells respond to changes in pH and 2,3-diphosphoglycerate. This is encouraging evidence that red blood cells produced from hESCs in vitro are relatively normal and in agreement with work done with hematopoietic stem cells.<sup>2</sup> However, major questions, such as the half-life and the immunogenicity of these cells, must be addressed. Procedures to eliminate undifferentiated cells that could be tumorigenic must also be developed.

The first transfusions in modern times occurred more than 100 years ago, 70 years before the first transplantations because red blood cells are among the simplest cells present in the body. They are easy to harvest and store, do not express the HLA antigens, and do not have a nucleus. They are therefore less immunogenic than most cells and cannot

cause tumors. Many of these same characteristics make them an attractive translational target for the hESC field. Importantly, most of the difficulties that are highlighted above apply to the manufacture of other cell types that have therapeutic potential. Therefore, the development of a procedure to manufacture red blood cells from hESCs would pave the way to the production of other cell types.

*Conflict-of-interest disclosure:* The author declares no competing financial interests. ■

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and is increased in response to oxidative stress, hypoxia, heavy metals, and several inflammatory cytokines. HO-1 also helps to maintain the steady-state level of heme through a feedback loop in which heme binds and inhibits a HO-1 transcriptional repressor, Bach1.<sup>1</sup> In contrast, heme-oxygenase 2 (HO-2) is a constitutive isoform, which is expressed under homeostatic conditions.

Studies over the last 2 decades have shown that the heme-oxygenases have an important protective role in many aspects of normal physiology. This is revealed in HO-1-deficient mice (HO-1<sup>-/-</sup>), which show a high level of embryonic lethality and anemia,<sup>2</sup> and in a child with HO-1 deficiency, who had severe growth retardation, hemolytic anemia, coagulopathy, and early atherosclerosis.<sup>3</sup> In comparison, HO-2-deficient mice survive longer and breed normally but experience long-term effects of chronic hypoxia.<sup>4</sup>

Cao et al now report that hematopoietic stem cells (HSCs) or progenitor cells from heterozygous HO-1-deficient mice (HO-1<sup>+/-</sup>) show increased proliferation and recovery of hematopoietic lineages after stress due to 5-FU treatment, transplantation, or a combination of phlebotomy and heme challenge. However, HO-1<sup>+/-</sup> HSCs have a reduced capacity to rescue lethally irradiated mice or to serially repopulate irradiated recipients. This suggests that HO-1 normally limits the proliferation and differentiation of hematopoietic progenitors during stress and that the failure of this mechanism can lead to premature exhaustion of the HSC pool. It will be interesting to discover if similar effects occur during aging.

A similar proliferative exhaustion of stem cells has been previously reported for mice deficient in Lig4,<sup>5</sup> p21,<sup>6</sup> and Gfi-1,<sup>7</sup> when proliferation occurs in response to DNA damage or dysregulation of cell cycle control. Reduced HO-1-dependent breakdown of heme, and the absence of the antioxidant, antiproliferative and antiapoptotic effects of its metabolites might therefore affect HSC function in similar ways. In this case, however, the authors suggest that the most likely cause of increased proliferation during stress is reduced CO-dependent activation of p38MAPK pathway, leading to low levels of p21 in the rapidly dividing cells. They suggest that loss of one allele of HO-1 may be sufficient to maintain the steady-state metabolism of heme but insufficient under

#### ● ● ● HEMATOPOIESIS & STEM CELLS

Comment on Cao et al, page 4494

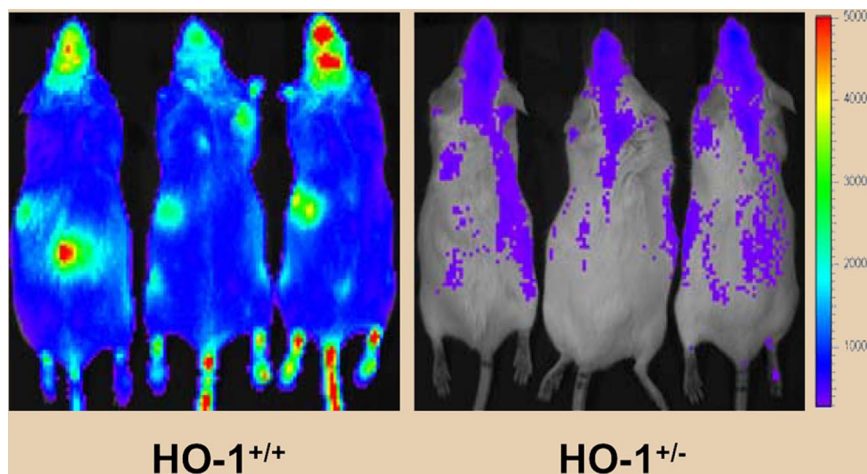
## HO-1 extends to stem cells

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In this issue of *Blood*, Cao and colleagues show that HO-1 plays a key role in the maintenance of HSCs by limiting the stress-induced proliferation of progenitors and the exhaustion of HSC populations.

**H**eme is an essential molecule in a wide variety of biological processes, including the transport and detection of oxygen and other diatomic gases and several forms of catalysis that depend on electron transfer. Heme can also be highly toxic due to its redox potential, and therefore, its levels are tightly regu-

lated in all cells. The degradation of heme is an important part of this regulation, and it is carried out by 2 ubiquitously expressed heme-oxygenases that convert the molecule into carbon monoxide (CO), iron, and biliverdin (which is later converted to bilirubin). Heme-oxygenase 1 (HO-1) is the inducible isoform



**Ineffectiveness of primitive HO-1<sup>+/-</sup> bone marrow cells.** In vivo bioluminescence imaging shows the reduced capacity of luciferase expressing bone marrow from HO<sup>+/-</sup> mice to reconstitute hematopoietic cells in lethally irradiated recipients. See the complete figure in the article beginning on page 4494.