and that the cells respond to changes in pH and 2,3-diphosphoglyerate. 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.[2] 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. ■

REFERENCES

1. Qiu C, Olivier EN, Velho M, et al. Globin switches in yolk sac-like primitive and fetal-like definitive red blood cells produced from human embryonic stem cells. Blood. 2008;111:2400-2408.
2. Giarratana MC, Kobari L, Lapillonne H, et al. Ex vivo generation of fully mature human red blood cells from hematopoietic stem cells. Nat Biotechnol. 2005;23:69-74.

HEMATOPOIESIS & STEM CELLS

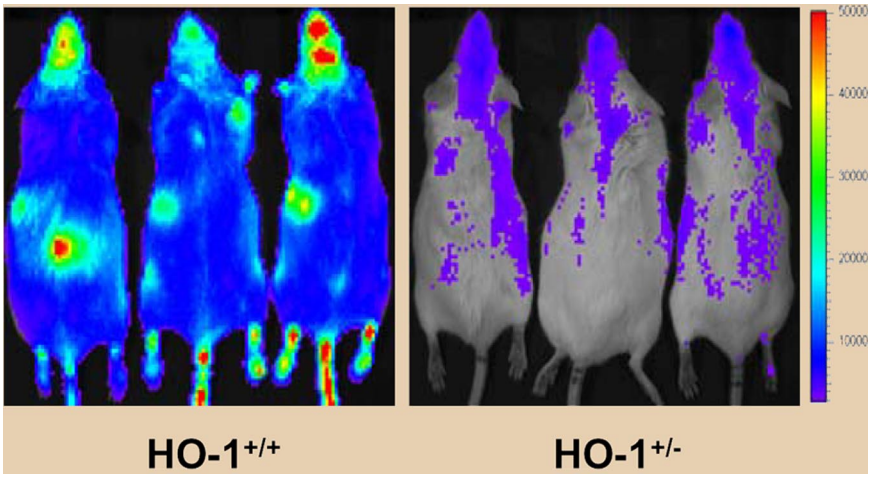
Comment on Cao et al, page 4494

HO-1 extends to stem cells

Richard J. Cornall UNIVERSITY OF OXFORD

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.

Heme 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 election transfer. Heme can also be highly toxic due to its redox potential, and therefore, its levels are tightly regulated 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 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.[1] In contrast, heme-oxygenase 2 (HO-2) is a constitutive isoform, which is expressed under homeostatic conditions.



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

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$^{-/-}$), which show a high level of embryonic lethality and anemia,[2] and in a child with HO-1 deficiency, who had severe growth retardation, hemolytic anemia, coagulopathy, and early atherosclerosis.[3] In comparison, HO-2–deficient mice survive longer and breed normally but experience long-term effects of chronic hypoxia.[4]

Cao et al now report that hematopoietic stem cells (HSCs) or progenitor cells from heterozygous HO-1–deficient mice (HO-1$^{+/-}$) 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$^{+/-}$ 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,[5] p21,[6] and Gfi-1,[7] 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

conditions of stress. The findings imply that HO-1 may play an important part in an evolutionarily conserved mechanism that balances proliferative capacity, stem cell function, and the response to environmental stress.

Another interesting implication of these results in HO-$1^{+/-}$ mice is that a similar and small quantitative difference in HO-1 gene expression during stress may have profound effects on cell function in humans. The human HO-1 gene promoter is characterized by a (GT)n repeat with common length polymorphisms affecting gene expression that have been associated with susceptibility to a wide variety of diseases.[8] Thus, long GT repeat polymorphisms that cause lower expression of HO-1 are associated with emphysema, atherosclerosis, and stroke while shorter repeats are associated with increased susceptibility to cerebral malaria, some cancers, and miscarriages but have better liver and kidney transplant survival. The findings of Cao et al indicate that it will now be important to discover if and when these polymorphisms affect stem cell function in solid organs or in bone marrow, for example, after irradiation. The fact that several commonly used drugs (aspirin, statins, rapamycin and cyclosporine) induce or repress HO-1 heightens the importance of these results.

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

REFERENCES

1. Kitamuro T, Takahashi K, Ogawa K, et al. Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells. J Biol Chem. 2003;278:9125-9133.
2. Poss KD, Tonegawa S. Heme oxygenase 1 is required for mammalian iron reutilization. Proc Natl Acad Sci U S A. 1997;94:10919-10924.
3. Kawashima A, Oda Y, Yachie A, et al. Heme oxygenase-1 deficiency: the first autopsy case. Hum Pathol. 2002;33:125-130.
4. Poss KD, Thomas MJ, Ebralidze AK, et al. Hippocampal long-term potentiation is normal in heme oxygenase-2 mutant mice. Neuron. 1995;15:867-873.
5. Nijnik A, Woodbine L, Marchetti C, et al. DNA repair is limiting for haematopoietic stem cells during ageing. Nature. 2007;447:686-690.
6. Cheng T, Rodrigues N, Shen H, et al. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science. 2000;287:1804-1808.
7. Hock H, Hamblen MJ, Rooke HM, et al. Gfi-1 restricts proliferation and preserves functional integrity of haematopoietic stem cells. Nature. 2004;431:1002-1007.
8. Exner M, Minar E, Wagner O, et al. The role of heme oxygenase-1 promoter polymorphisms in human disease. Free Radic Biol Med. 2004;37:1097-1104.

NEOPLASIA

Comment on Zebedin et al, page 4655

The dilemma of anticancer therapy: tumor-specific versus immune effects

Laurence Zitvogel and Guido Kroemer INSTITUT GUSTAVE ROUSSY

The PI3Kδ isoform is required for both optimal growth of Abelson-transformed leukemia cells and antileukemic NK-cell effectors. Therefore, the simultaneous inhibition of PI3Kδ in tumor and host cells results in therapeutic failure.

Deregulation of the phosphoinositide 3-kinase (PI3K) pathway can occur by activating mutations in growth factor receptors or in the PIK3CA locus coding for PI3Kα, by loss of function of the lipid phosphatase PTEN, by up-regulation of protein kinase B (PKB/Akt), or by the impairment of the tuberous sclerosis complex (TSC1/2). All these events stimulate cancer growth and proliferation and have thus prompted a major interest in therapeutic inhibition of the PI3K pathway. One particular PI3K isoform, PI3Kδ, is selectively expressed in leukocytes and, hence, might constitute a pharmacologic focus for the "targeted" treatment of hematological malignancies.

In an elegant study published in the present issue of *Blood*, Zebedin and colleagues explore the effect of the PI3Kδ knockout on Abelson virus (cAbl)–induced leukemia in mice. For this, PI3Kδ was either removed from the transformed cells themselves, or from the host environment into which transformed cells were injected. These experiments reveal a formidable contradiction. PI3Kδ deficiency in leukemic cells retarded tumor progression while PI3Kδ deficiency in nonleukemic host cells accelerated the fatal course of leukemia. Intriguingly, the simultaneous removal of PI3Kδ from leukemic cells and the host had no effect on leukemic progression at all. This latter result suggests that complete and selective pharmacologic PI3Kδ inhibition (something that obviously would affect both tumor and host cells) would have no therapeutic benefit on PI3Kδ-overexpressing Bcr/abl-positive human leukemias.

Zebedin et al also show that PI3K$\delta^{-/-}$ mice exhibit an accelerated development of cancers that are usually controlled by natural killer (NK) cells, such as Abelson-transformed cells, Eμ-myc–induced B-cell lymphoma, EL4 thymoma, and B16 melanoma. These PI3Kδ effects were also found on a *RAG2*$^{-/-}$ background, indicating that they must involve other immune effectors besides B or T lymphocytes. Indeed, PI3K$\delta^{-/-}$ NK cells poorly lysed leukemic cells, correlating with a general defect in exocytosis that affects both degranulation and IFNγ secretion. Although formal proof that accelerated tumor progression must be attributed to this NK defect is elusive, these results make it highly plausible that PI3Kδ-dependent effectors of the innate immune system play a major role in tumor control.

The results by Zebedin et al contribute to a general debate on the mechanisms through which anticancer chemotherapeutics (fail to) act. Accumulating evidence indicates that radiotherapy and some chemotherapeutic agents, in particular anthracyclines, can trigger specific immune responses that result either from immunogenic cancer cell death or from immunostimulatory off-target effects. This anticancer immune response then helps to eliminate residual cancer cells (that failed to be killed by chemotherapy) or maintain micrometastases (or perhaps cancer stem cells) in a stage of dormancy.[1]

Ideally, anticancer chemotherapeutics should induce a cellular stress response and/or immunogenic cancer cell death that trigger an effective immune response. Genotoxic agents induce NKG2D ligands through an ATM-dependent and Chk1-dependent DNA damage pathway.[2] Such NKG2D ligands on the surface of tumor