

Regulators of Human Stem Cell Proliferation and Engraftment

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Abstract

Transplantable human hematopoietic stem cells can be routinely detected by their ability to engraft sublethally irradiated immunodeficient mice with both lymphoid and myeloid progeny for periods of at least 6-8 weeks [1]. These cells include subsets with both short-term and longterm reconstituting activity, the former being responsible for most of the human cells seen in fully immunodeficient mice (eg, NOD/SCID- β_2 microglobulin^{-/-} mice) in the first 8 weeks and the latter being responsible for the lower levels of engraftment seen after the same period of time in less compromised (eg, NOD/SCID) mice [2]. Interestingly, human cells with short- and longterm hematopoietic reconstituting potential show further differences in the regulation of their ability to engraft when they are activated into cycle short-term reconstituting cells being unaffected whereas longterm reconstituting cells lose their transplantability when they transit S/G₂/M [2,3]. A closely related population of primitive human hematopoietic cells (referred to as longterm culture-initiating cells or LTC-ICs) are detected by virtue of their ability to generate intermediate types of progenitor cells for at least 5 weeks when co-cultured with stromal cell feeder layers, the intermediate progenitors being those that produce colonies of granulocytes and macrophages and/or erythroblasts in 2-week semi-solid cultures [4]. The proliferative status of these various stages of primitive human hematopoietic cells is regulated by their exposure to distinct types of both positively acting (mitogenic) and negatively acting (cytostatic) cytokines. We have recently discovered that stromal-derived growth factor 1 (SDF-1) belongs to a group of chemokines that act as inhibitors of primitive human progenitor cycling and is unique in its ability to force the entry of human LTC-ICs into G₀. SDF-1 can similarly act on proliferating human cells with longterm hematopoietic reconstituting ability in NOD/SCID mice. These cells can be shown to be actively proliferating at early times post-transplant in primary NOD/SCID mice by their high sensitivity to treatment with 5-fluorouracil *in vivo* or with high specific activity ³H-thymidine *in vitro* as revealed when they are subsequently assayed in secondary NOD/SCID mice. Prior SDF-1 treatment in the primary mice abrogates this sensitivity. Importantly, this exposure to SDF-1 *in vivo* also causes a marked increase in the number of reconstituting cells that can be detected in the secondary mice even without exposure to a cycle-active drug - consistent with the restoration of engraftment potential by cycling longterm reconstituting stem cells forced to re-enter G₀. A similar, albeit less marked effect has also been achieved by SDF-1 treatment of human stem cells stimulated to proliferate *in vitro*. These findings demonstrate that cell cycle activation is a major but reversible constraint to the use of proliferating stem cells for transplantation therapies and suggest a novel approach to overcoming this limitation.

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 2. Glimm H, et al. Previously undetected human hematopoietic cell populations with short-term repopulating activity selectively engraft NOD/SCID- β_2 microglobulin-null mice. *J Clin Invest* 2001;107:199-206.
 3. Glimm H, et al. Human hematopoietic stem cells stimulated to proliferate *in vitro* lose engraftment potential during their S/G₂/M transit and do not reenter G₀. *Blood* 2000;96:4185-4193.
 4. Sutherland HJ, et al. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. *Proc Natl Acad Sci USA* 1990;87:3584-3588.
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