

Multipotent human cells expand indefinitely

Our ability to dissect out the mechanisms of stem cell self-renewal and differentiation is enhanced by *in vitro* models for differentiation down multiple lineages. This is one of the primary reasons that embryonic stem cells are so important. But as debate rages regarding use of human embryonic stem cells, work on other human stem cell populations with high plasticity continues. Reyes and colleagues (page 2615) show that non-hematopoietic primary bone marrow cells, obtained from human donors ages 2-50 and grown *in vitro* for over a year, maintain the ability to differentiate *in vitro* into multiple cell types. Depending on the culture conditions, the cells differentiate into uniform populations of myocytes, osteoblasts, chondrocytes, adipocytes, or endothelial cells, all of which constitute mesenchymal tissues. The authors therefore call these cells mesodermal progenitor cells (MPCs). MPCs can also form a stromal layer that supports long-term hematopoietic cell survival. Previously, it was unknown whether the same marrow-derived cells could both support hematopoiesis and form mesenchymal tissues. Of note, the authors did not identify conditions in which MPCs differentiate into hematopoietic cells, also of mesenchymal origin.

These are novel and important findings because they indicate for the first time that multipotent human primary cells can be cultured and expanded indefinitely while maintaining their plasticity. This difference from mesenchymal stem cells described previously (Pittenger et al, *Science*. 1999;284:143-147; Phinney et al, *J Cell Biochem*. 1999;72:570-585) may be due in part to growing the cells on fibronectin-coated plates at low density. Potential clinical uses of MPCs expanded *in vitro* include autologous transplantation into growing or healing tissues or use as a stromal support layer for hematopoietic cells. MPCs could also poten-

tially be used for allogeneic transplantation, as they have neither HLA-Dr nor HLA1 surface expression and therefore may elude immune rejection. Also, MPCs can be infected with retroviral vectors and could be used in gene therapy approaches. Perhaps most critical for basic science applications, dissection of the molecular mechanisms of stem cell self-renewal and cell differentiation can be performed using these cells.

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Globin vectors: control without the locus

Hereditary hemoglobinopathies have long been favorites of gene therapists, and a genetic cure would be a wonderful extension of the decades of research spent understanding the molecular biology of hemoglobin gene expression. The most realistic approaches rely on retroviral vectors, which have the potential to integrate in hematopoietic stem cells, albeit at disappointingly low frequencies in primates. In order to achieve high-level, erythroid-specific expression, scientists have turned to the β -globin locus for transcriptional control elements, especially globin promoters in combination with parts of the locus control region (LCR) and intron sequences. The low vector titers and frequent rearrangements caused by these elements highlight the difficulties associated with including sequences that are normally not transcribed, or are now transcribed in reverse orientation, in an RNA vector. Despite these problems, globin gene therapists have stubbornly persisted, and recent results show that optimized cassettes including β -globin LCR, promoter, and intron sequences can be incorporated into a lentiviral vector (instead of a conventional murine leukemia virus vector) and lead to potentially therapeutic hemoglobin protein

levels in mice receiving transplants (May et al, *Nature*. 2000;406:82-86).

An alternative approach to globin vector design is to abandon problematic regulatory elements from the β -globin locus and achieve high-level, erythroid-specific expression some other way. Bodine and colleagues showed that a double-copy murine leukemia virus vector containing the promoter for the red cell membrane protein ankyrin produced a respectable 8% γ -globin transgene mRNA expression level (relative to endogenous α -globin) in mice receiving transplants (Sabatino et al, *Proc Natl Acad Sci U S A*. 2000;97:13294-13299). Here, Moreau-Gaudry and colleagues (page 2664) extend these findings by combining nonglobin, erythroid-specific promoters such as ankyrin with different erythroid enhancers and a viral posttranscriptional regulatory element in a self-inactivating lentiviral vector. Using a green fluorescent protein transgene, they find that the vectors can be produced at high titers, that they are stable, and that erythroid-specific expression can be obtained in a majority of erythroid cells in mice receiving transplants. A similar vector with a γ -globin transgene produced 11% to 28% mRNA expression levels (relative to endogenous α -globin) in MEL cells. Unfortunately, the globin vectors were not tested in transplantation experiments; so it is not clear if the same expression levels can be achieved *in vivo*. Still, these experiments demonstrate that the problems of including β -globin locus regulatory elements in retroviral vectors can be avoided, and there is no reason to believe that the optimal vector design has been achieved yet. The next test will be to see whether these vectors can surpass the impressive performance of lentiviral vectors based on the β -globin locus and produce therapeutic protein levels after transplantation.

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