treatment with holotransferrin. In contrast, this report has clearly demonstrated that diferric transferrin, but not apotransferrin, causes an increase in hepcidin mRNA levels in freshly isolated murine primary hepatocytes.

However, it should be noted that the increase in hepcidin mRNA levels following the addition of holotransferrin to freshly isolated hepatocytes is relatively minor.

Hemojuvelin mutations result in juvenile hemochromatosis, which is indistinguishable from that caused by hepcidin mutations; furthermore, hemojuvelin has been shown to participate in the complex regulation of hepcidin. The authors therefore examined whether iron affects hepcidin production via a hemojuvelin-dependent pathway. Hemojuvelin exists both as a soluble and a cell-associated GPI-linked form, the latter being a BMP coreceptor that enhances hepcidin signaling in liver cells. As shown in the figure, BMP induction of hepcidin is enhanced via hemojuvelin in hepatocytes through a BMP/SMAD-dependent pathway. Its role as an enhancer may act to finely tune the BMP-mediated induction of hepcidin expression. Lin and colleagues have succeeded in identifying a component missing from previous in vitro studies: namely, the role of iron in the BMP/hemojuvelin/SMAD-mediated induction of hepcidin synthesis.

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GENE THERAPY

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RIS defines risk

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In this issue of *Blood*, Cattoglio and colleagues examine retroviral integration site (RIS) preferences in CD34$^+$ cells and conclude that gamma-retroviruses carry a higher risk for insertional mutation than lentiviruses.

To date, 4 out of 10 patients treated with Necker Hospital's gene-therapy protocol for X-linked severe combined immunodeficiency (X-SCID) have developed T-cell leukemia.[1] The mechanism that caused these serious complications was retroviral insertional mutagenesis. In every case, the gene-therapy vector (gamma-retrovirus–based) inserted and deregulated a nearby oncogene. Most remarkably, in 2 patients, the vector inserted and inappropriately activated the expression of a known T-cell oncogene, LIM-domain-only 2 (*LMO2*). In light of these complications, regulatory agencies expect improved understanding of the risk of this genotoxicity.[2]

So what is the probability of a retroviral vector inserting near an oncogene or tumor suppressor? Like so many things in biology, the answer is complicated and seems to depend on cell context. We now know that retroviruses have integration-site preferences. Studies in HeLa cells showed that gamma-retroviruses prefer to integrate near transcriptional start sites (26% of total sites analyzed) whereas lentiviruses prefer to insert within introns.[3] Now, Cattoglio and colleagues present similar findings in CD34$^+$ cells. They transduced CD34$^+$ cells with either gamma-retroviral or lentiviral vectors in vitro and harvested genomic DNA 1 to 12 days after infection. Integration sites were then cloned and mapped from this largely unselected population using standard methods. They analyzed 1030 gamma-retroviral integrations and 869 lentiviral integrations. As in the HeLa study, they found a gamma-retroviral bias for transcriptional start sites (29% of total) and for actively transcribed genes. Lentiviral integrations were also biased toward actively transcribed genes, but for intragenic rather than transcriptional start sites.

The most striking result, however, was the discovery of numerous recurrent integrations in this unselected population of cells. Cattoglio and colleagues show that 20% of the total gamma-retroviral integrations were in the same locus, whereas 12.5% of the lentiviral integrations were recurrent. Wu et al reported recurrent gamma-retroviral integrations in HeLa cells, but at a lower frequency (12%).[4] Additionally, Cattoglio and colleagues show that many of the recurrent gamma-retroviral integrations were cancer associated. In contrast, recurrent lentiviral integrations did not involve a statistically significant number of cancer-associated genes. Cattoglio and colleagues refer to these recurrent integrations as "hotspots," but it remains to be seen whether the exact same genomic regions will be targeted in other studies. These results also contrast with in vivo analyses of transduced cells in animal models in which recurrent integrations are infrequent, except in specific loci conferring selective growth advantage.[2] Furthermore, if hotspots in cancer-associated genes existed in murine hematopoietic stem cells, then one would anticipate a high frequency of leukemias arising from marrow transduced with empty vectors, which is a rare occurrence under standard conditions. An alternate interpretation is that the human genome is highly constrained for integration in cultured CD34$^+$ cells. In other words, there are some parts of the genome that are unavailable to gamma-retroviral integration. This raises the question of whether accessibility of the genome to integration can be altered by cell-culture conditions.

Investigation of these possibilities and additional RIS analyses will help define the risks of retroviral gene therapy.

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