

Stem Cell Gene Therapy

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The ability to transfer genes into repopulating hematopoietic stem cells *ex vivo* and to achieve regulated transgene expression in specific lineages following transplantation and hematopoietic reconstitution would create many therapeutic opportunities. Although the initial use of murine oncoretroviral vectors to transfer genes into primitive murine hematopoietic cells was reported twenty years ago, translation of this approach to clinical application has been slow and has required considerable effort. Despite progress being achieved in the murine system with correction of single gene defects in murine models of human immunodeficiencies and chronic granulomatous disease, the much lower efficiency of gene transfer into human stem cells has hampered potential success. The necessity for high level oncoretroviral vector gene transfer to achieve therapeutic benefit was circumvented in two recent clinical trials designed to cure severe combined immunodeficiency due to a deficiency of the common γ -chain of the lymphoid cytokine receptor (X-SCID) or adenosine deaminase. In these trials, a potent selective repopulating advantage of the gene corrected lymphoid cells resulted in therapeutically relevant numbers of functional lymphocytes. However, for other hematological disorders such as the β -thalassemia, sickle cell disease, and chronic granulomatous disease, a repopulation advantage of genetically modified cells is not enjoyed, emphasizing the need for improved human stem cell gene transfer methodology.

Recent and ongoing work with HIV-based lentiviral vectors offers the promise of improved human hematopoietic stem cell gene transfer that will be required in order to correct some hematopoietic disorders.

Despite the theoretical advantages and the encouraging data from *in vitro* culture systems and xenotransplantation studies of transduced human cells using immunodeficient mouse assays, the use of lentiviral vectors based on HIV-1 to transduce reconstituting stem cells in nonhuman primate models has given disappointing results. The proportion of genetically modified cells in peripheral blood has generally been relatively low in the majority of animals and in some cases such cells disappeared over time. We have evaluated the hypothesis that this low level of transduction of non-human primate hematopoietic stem cells reflects a well-described post-entry block, mediated at least in part by TRIM 5- α , that has been noted for transduction of various Old World monkey cells with HIV-1 virus or vectors. Therefore, we developed an SIV-based vector system analogous to our previously described HIV-1-based system. We found that SIV vector particles were far more efficient at transducing rhesus CD34⁺ cells and the progenitors within that population than were HIV vector particles. Importantly, SIV vector particles efficiently transduced rhesus hematopoietic repopulating cells as assessed in the autologous transplantation model. Three animals have been transplanted with autologous, peripheral blood mobilized CD34⁺ cells transduced with an SIV vector encoding GFP. Greater than six months post-transplantation, each animal demonstrates efficient gene transfer into their repopulating cells, with marking levels ranging between 5-25% in peripheral blood leukocytes. This SIV-based vector using the rhesus macaque autologous stem cell transplantation system will now allow modeling of human stem cell-targeted HIV-based lentiviral vector gene

transfer.

Despite these encouraging data, a cautionary stance for stem cell based gene therapy has been adopted since two of the children with X-SCID who underwent gene therapy later developed lymphoid leukemia. The lymphoid transformation appears to have been at least partially due to activation of the LMO-2 proto-oncogene by the retroviral vector LTR, underscoring the importance of vector design on the potential to activate genes near integration sites. Recent data also suggest that both oncoretroviral and lentiviral vectors favor integration into genomic locations in and/or near active genes. Although the development of the lymphoid leukemia in the X-SCID children appears to have resulted from a confluence of several factors, some likely disease specific, attention to evaluating the potential genotoxicity of therapeutic vectors seems both warranted and prudent. We have thus begun studies to determine whether integrated, self-inactivating (SIN) globin lentiviral vectors, which lack a viral LTR, have the potential to cause transcriptional activation akin to that seen in the X-SCID patients. To test the likelihood of the activation of integrated vectors by interactions with genomic sequences surrounding the integration site, we initially compared the mobilization rate of an integrated SIN globin lentiviral vector containing β -globin locus enhancer elements with that of a SIN lentiviral vector containing the MSCV U3 LTR region. 293T cells were transduced to a high vector copy number with the SIN globin vector or a SIN lentiviral vector containing the LTR element, followed by transfection of viral packaging genes to rescue aberrantly transcribed vector genomes. Although both vectors had a comparable transducing titer (globin $2 \times 10^7 \pm 4 \times 10^6$ TU/ml, n=4 vs. MSCV $4 \times 10^7 \pm 2 \times 10^6$ TU/ml, n=9), the globin vector had a “mobilized” titer 60-fold less than that of the MSCV LTR vector (p<0.0001). We estimate that 1 in 300,000 globin vector integration events were mobilized, compared to a frequency of 1 in 12,000 with the MSCV vector, consistent with the possibility of less transcriptional activation attributable to the globin vector.

To directly ascertain whether an integrated globin vector could influence endogenous transcriptional activity in developing primary erythroid cells, clonal spleen colony erythroblasts containing a single globin vector insertion were derived from transduced bone marrow cells of β -thalassemic mice. The transcriptional profile of 21 single-copy globin vector clones and 15 untransduced, control clones was determined using the Affymetrix Mouse 430A microarray (representing ~15,000 genes). Expression of 4500-6000 genes was observed in all samples. Using a 2-fold difference in the level of expression as a cutoff, only 0.02% of all the genes among the 21 clones had potentially different expression compared to the control clones, well below the expected 2% rate of random differences (Genomics 83:321, 2004). Ligation-mediated PCR was then used to clone the sequences of the vector-genomic DNA junctions, allowing identification of vector insertion locations using the NCBI database. Six clones had 16 genes, including N-ras that were located within 100kb of the vector insertion site and were represented on the array. Of those genes that were expressed, only 2 had a 2.0-2.8-fold difference in signal value between the clone and the controls. Evaluation of these potential changes by real time RT-PCR has confirmed a change in expression for one of these genes. However, both microarray and real time RT-PCR demonstrated that expression of N-ras was unchanged. These data suggest that integrated SIN globin lentiviral vectors have a low rate of altering transcriptional activity in target cells and therefore may be inherently less genotoxic than vectors containing a viral LTR.

In summary, we believe that our SIV vector system represents a significant advance to evaluate gene transfer in a nonhuman primate model. This now allows modeling of human stem cell-targeted HIV-based lentiviral vector gene transfer using a rhesus macaque autologous stem cell transplantation system. Further study and focused attention on the elements of vector design which may influence expression of genes surrounding integration sites along with the careful tracking of clonal

populations in which the integrated vector genome is near a proto-oncogene will be critical in evaluating the potential safety of future gene therapy approaches for the treatment of human genetic diseases.