

Insight into Hematopoiesis from Primate and Human Gene Transfer Studies

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Abstract

The curative potential of oncoretroviral gene transfer was demonstrated in the correction of SCID-X1 disease (Calvanazzo-Calvo et al., 2000) and ADA-SCID (Bordignon et al., 2002). We assume that a sustained polyclonal contribution of ex vivo manipulated stem and progenitor cells is a key feature for successful therapeutic treatment. To investigate this hypothesis, we performed in vivo clonality analysis in peripheral blood samples from 3 SCID-X1 patients compared with samples from 2 ADA-SCID patients, where full restoration could not be achieved (Kohn et al., 1998). The random integration of retroviral vectors creates genomic/proviral fusion sequences that uniquely label each affected cell and its clonal progeny. High resolution integration site analysis (LAM-PCR) showed that multiple clones have formed transgene-corrected lymphoid hematopoiesis in the SCID-X1 gene therapy trial. The further tracing of identical integration sites in sorted myeloid colonies and lymphoid cell samples demonstrated that similar to the primate model, pluripotent hematopoietic stem or progenitor cells have been tagged by ex vivo transduction. In contrary, transgene corrected lymphoid hematopoiesis in the two ADA-SCID patients analyzed has been mono- to oligoclonal for more than 8 years after transplantation. Single clones continue to contribute from approximately 25% up to 100% of genetically corrected lymphocytes, indicating that clonal selection has affected the ability of providing a genetically corrected polyclonal T-cell repertoire in this study. Because determining the number and activity of corrected cell clones can demonstrate limitations of their therapeutic potential that the quantification of vector cDNA fails to detect, this observation has major implications for the design and analysis of ongoing and future gene transfer trials. Counting and tracing of hematopoietic progenitor and stem cells *in vivo* is possible, yields decisive and surprising information on the biology of hematopoiesis and should be a component of every preclinical and clinical gene transfer study using integrating vector systems.
