Gene Therapy Insertional Mutagenesis Insights

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In a milestone study describing the first "cure" of a genetic disease by retroviral gene therapy, 9 out of 10 infants born with X-linked severe combined immunodeficiency (SCID-X1, also known as γc or *IL2RG* deficiency) were successfully treated with autologous bone marrow stem cells infected ex vivo with an *IL2RG*-containing retrovirus (*1*, *2*). Unfortunately, almost 3 years after therapy was completed, two of the children developed T cell leukemia (*3*, *4*). Both patient leukemias contained an *IL2RG*-containing retrovirus integrated in the proximity of *LMO2*, a known human T cell oncogene (*5*, *6*), leading to aberrant transcription and expression of *LMO2* (*3*). It has been generally assumed that replication-defective virus-induced insertional mutagenesis would be extremely rare, so this high frequency raises serious concerns regarding the future of human gene therapy trials.

By searching our Mouse Retroviral Tagged Cancer Gene Database (http://RTCGD.ncifcrf.gov), which contains the sequences of more than 3000 retroviral integration sites cloned previously from mouse retrovirally induced hematopoietic tumors, we identified two leukemias with integrations at *Lmo2* and two leukemias with integrations at *Il2rg* (Fig. 1A). One of these leukemias (98-031) contains integrations at both *Lmo2* and *Il2rg* (Fig. 1A). These integrations are clonal (Fig. 1B), suggesting that they were acquired early during the establishment of the leukemia. The probability of finding a leukemia with clonal integrations at *Lmo2* and *Il2rg* by random chance is exceedingly small [supporting online material (SOM) Text], providing genetic evidence for cooperativity between *LMO2* and *IL2RG*. Leukemia 98-031 has a T cell phenotype and up-regulated *Lmo2* expression, a finding consistent with what is seen in SCID patient leukemias (*3*) (SOM Text).

Our results provide a genetic explanation for the high frequency of leukemia in these gene therapy trials. In transplant patients, *IL2RG* is expressed from the ubiquitous Moloney viral long terminal repeat (LTR). Although this was expected to be safe, our results suggest that retrovirally expressed *IL2RG* might be oncogenic due to some subtle effect on growth or differentiation of infected cells. IL2RG is a component of several cytokine receptors, and signaling through some of these receptors is known to enhance leukemogenesis (*7*). Overexpression of *IL2RG* was not observed in SCID patient leukemias or in mouse leukemia 98-031 (SOM Text); however, it remains possible that subtle effects on *IL2RG* expression or regulation by placement into a retroviral vector or after viral integration in a mouse leukemia is oncogenic in the absence of *IL2RG* up-regulation. Subsequently, a rare cell in which the oncogenic *IL2RG* virus integrates and deregulates a gene like *LMO2* that cooperates with it to induce leukemia is selected in transplant recipients and this rare cell expands to form the leukemia. Because all patients received millions of infected cells, it is likely that they all received cells containing a viral integration at *LMO2* (*3*), yet not all SCID patients developed leukemia. Therefore, additional cooperating mutations may be required for leukemia, a hypothesis consistent with the presence of other genomic rearrangements in these patient leukemias (*3*).

Our results bode well for future gene therapy trials. In most trials, the transplanted gene is unlikely to be oncogenic and occurrences of insertional mutagenesis will be low, as has been seen in trials conducted during the past several years. Only in rare cases where the transplanted gene is oncogenic in the context of a retrovirus will insertional mutagenesis represent a real problem.

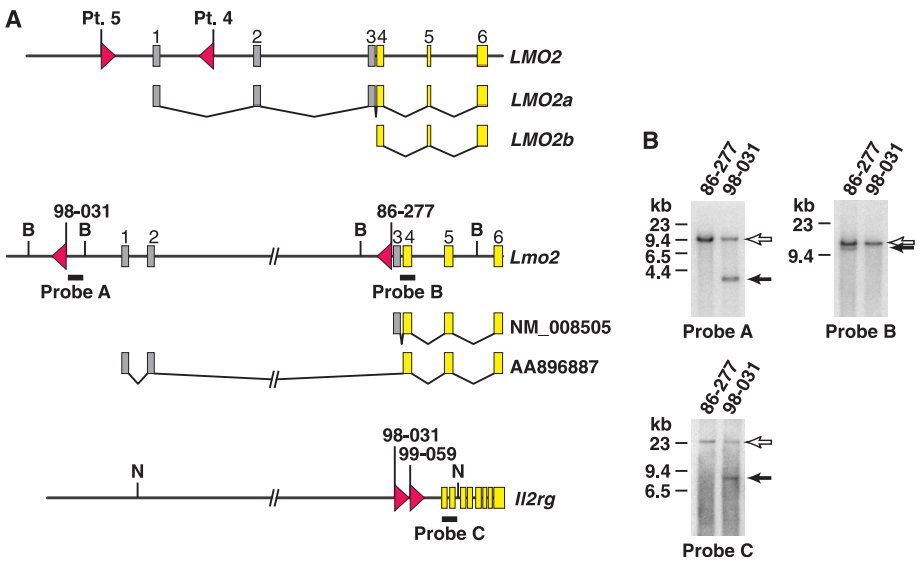

Fig. 1. Retroviral integrations in human SCID-X1 and mouse AKXD leukemias. (**A**) The human *LMO2* and mouse *Lmo2* and *Il2rg* intron and exon structure is shown above their transcripts. Retroviral integrations and their orientations are shown as red arrows along with tumor number or patient designation. Noncoding exons are in gray and coding exons in yellow; B and N designate site of restriction enzymes Bam HI and Nco I, respectively. Two *LMO2* transcripts are produced by alternate promoter usage: *LMO2a* is transcribed from a hematopoietic-specific promoter, and *LMO2b* is from a more widely used promoter. The murine *Lmo2* gene appears analogously regulated. Exons 1 and 2 are part of an expressed sequence tag (EST) cloned from the murine WEHI-3B monocytic cell line. The *Lmo2* EST (GenBank no. AA896887) was fully sequenced, and exons 1 and 2 are contiguous with coding exons 4 to 6 of the *Lmo2* Reference Sequence mRNA (NM_008505). (**B**) Bam HI- or Nco I-digested DNA from tumor lymph nodes was analyzed by Southern blot using the probes A and B (Bam HI) or probe C (Nco I) shown in (A). Open arrows, germline bands; closed arrows, rearranged bands.

References and Notes

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SOM Text
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