MEDICINE

Murine Leukemia Induced by Retroviral Gene Marking

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Somatic gene transfer is a promising therapeutic strategy, but it may also evoke new types of side effects related to genetic damage or transgene activity. Retroviral vectors, the best tool currently available for stable genetic modification, integrate at random positions in the cellular genome. The risk of mutagenesis of cellular sequences promoting a malignant phenotype is about 10^{-7} per insertion (*1*). As tumor development requires further genetic lesions, a single copy of an otherwise innocuous transgene is not expected to produce severe side effects. Nevertheless, we observed leukemia induction in an animal model of retroviral gene marking.

Using a replication-defective vector (*2*), we introduced the clinically used dLNGFR marker gene (*3*) into murine bone marrow (BM) cells before transplantation into irradiated [10 grays (Gy)] C57Bl/6J mice ($n = 5$). No hematopoietic alterations were observed within 28 weeks. Pooled BM cells were transplanted into secondary irradiated (10 Gy) recipients ($n = 10$). All 10 developed hematopoietic disorders within 22 weeks. One animal showed extramedullary hematopoiesis with islands of blasts, three had preleukemia with reduction of splenic white pulp and elevated blast counts, and six succumbed to overt acute myeloid leukemia (AML) analogous to human AML M5 phenotype (monocytoid). Sublethally irradiated (7.5 Gy) tertiary recipients ($n = 8$) developed lethal AML M5 within 4 months. Vectors encoding other marker proteins did not lead to a similar disease in control animals ($n > 70$). Further controls excluded transgene sequence alterations, the presence of replicating retroviruses, or activation of endogenous retroviral sequences.

All diseased mice carried the same leukemic clone with a single vector copy integrated into the murine gene Evi1 (ecotropic viral integration site–1) (Fig. 1A). This was revealed by sequencing of the cellular 5′ flanking sequence from secondary recipients (S1, S2, and S7) after cloning by polymerase chain reaction (PCR) (*4*) and confirmed in one primary and all other secondary recipients (Fig. 1B). Leukemic cells expressed Evi1 RNA (Fig. 1C), initiated from both long terminal repeats (LTR) (*5*).

Activation of the transcription factor Evi1 may contribute to human preleukemia and AML, but it is not sufficient to induce AML in mice nor is it associated with AML subtype M5 (*6*). The uniform disease identity suggested a cooperating event common to all subclones, possibly involving the transgene product. dLNGFR was introduced as a biologically inert cell-surface tag derived from p75NTR, the low-affinity receptor for neurotrophins (*3*). However, the p75NTR cytoplasmic domain, which is deleted in dLNGFR, has a proapoptotic function (*7*). Both p75NTR and dLNGFR may associate with each of the three different tyrosine kinase receptors (Trk) for neurotrophins (*7*, *8*). A construct similar to dLNGFR transformed fibroblasts in the presence of TrkA and the neurotrophin nerve growth factor (NGF) (*8*). Neurotrophins and Trk receptors, but not p75NTR, are expressed in several hematopoietic cell types, and deregulated Trk activity may lead to AML in mice and humans (*9*). Leukemic cells of tertiary mice expressed both TrkA (Fig. 1C) and dLNGFR (Fig. 1D) and responded to NGF for proliferation in vitro (*5*).

These data strongly suggest that transforming loops were initiated by the combination of insertional oncogene activation with signal interference evoked by the transgene product. Well-designed animal models and multicenter efforts will be required for systematic risk assessment of side effects related to transgene insertion and expression, especially when targeting long-lived stem cells.

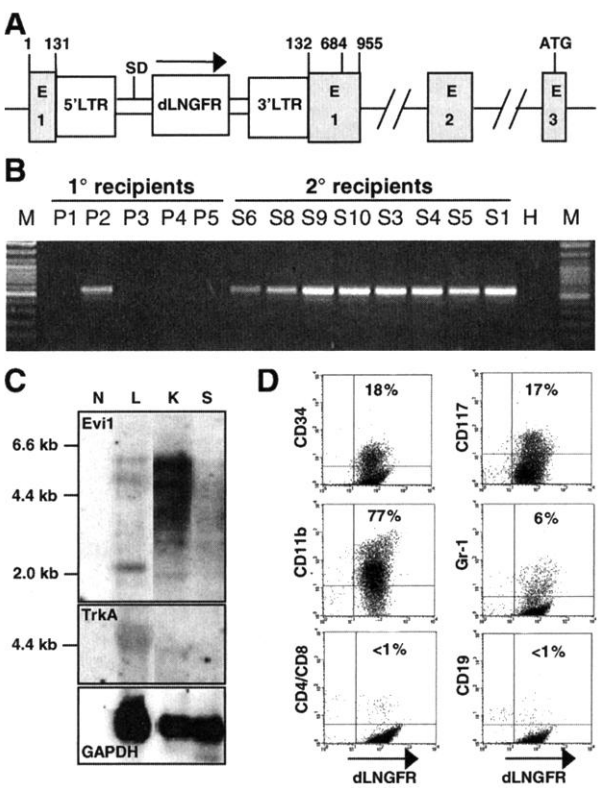

Fig. 1. Insertional activation of Evi1 and transgene expression. (**A**) Location and orientation (arrow) of the retroviral vector inserted into the Evi1 gene. SD, retroviral splice donor. The first three exons, E1, E2, and E3 (the first coding exon), are indicated by gray boxes, and nucleotide positions of E1 are indicated by numbers (GenBank accession number M64494). Position 684 refers to the insertion site of an ecotropic retrovirus reported by Morishita *et al.* (*5*, *10*). (**B**) PCR identifying the vector's 3′ flanking sequence in the leukemic clone, confirming its origin in primary recipient P2. H, healthy control. (**C**) Northern blot analysis for Evi1 and TrkA transcripts in leukemic cells (L), normal murine splenocytes (S), and kidney cells (K). N, negative control. GAPDH, RNA load. (**D**) Flow cytometry of leukemic cells demonstrated expression of dLNGFR and of lineage antigens CD11b, Gr1, CD34, and CD117, but no expression of CD4, CD8, and CD19.

References and Notes

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