● ● ● **NEOPLASIA**

What gene have I ID'ed?

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Retroviral insertional mutagenesis screens have identified of dozens of potential leukemia/lymphoma genes in mice and rats. Sauvageau and colleagues suggest that proviral insertions may affect the expression of multiple nearby genes in leukemia cells, and that the genes affected may be cell-type dependent.

Hundreds of articles have been published that describe the induction of leukemia or lymphoma in rodents by chronic infection with murine leukemia viruses (MuLV). MuLV induce malignancy by acting as insertional mutagens, activating proto-oncogenes or inactivating tumor suppressor genes.[1] Non-random clusters of proviral insertion sites, called common sites of proviral insertion (CIS), represent selection for the rare insertions that can alter leukemia genes, causing disease. Many important leukemia/lymphoma genes, including many involved in human cancer, have been identified in this way.[1] Nevertheless, the field has never formally agreed on what criteria should be adopted to identify the cancer gene(s) at a given CIS, so a variety of circumstantial data are collected to find the most promising candidates to pursue functionally. A more systematic approach is warranted.

In the report from Sauvageau et al, the authors used low-density Taqman arrays to perform qRT-PCR on the CIS-associated genes within 50 kilobase pairs (kb) of the median integration site for 20 CIS to determine how many and which genes are affected by proviral insertions at CIS. This is the first time large-scale expression analysis around CIS has been undertaken in such a way. Sauvageau et al suggest that several nearby and some more distant genes are usu-ally affected at each CIS. Furthermore, for the same CIS a different set of genes seems to be up-regulated depending on the lineage of the transformed cell (see figure).

This paper indicates layers of unexpected complexity in retroviral mutagenesis experiments. Ideally, one would have complete saturation of all proviral insertion sites and gene expression microarray data so that one could cluster like cases and deduce altered transcriptional networks resulting from changes in the expression of specific target genes at CIS. However, complicating these analyses are the choice of controls for comparisons of gene expression levels. What is the appropriate noncancer cell type to use? Should phenotypically similar cancers from the same screen, but lacking a proviral insertion at this CIS, be used? Both approaches have faults. Appropriate normal cells for comparison are hard to identify. Expression may be high for a gene in a control leukemia that lacks a proviral insertion at the relevant CIS, because that gene is altered as a result of another insertion mutation in the same pathway. Indeed, Sauvageau et al find this may often be the case. Finally, the field has never adopted standard criteria to identify statistically significant CIS, making results from different screens hard to compare.[2] These complexities indicate that care must be taken in interpreting the results of retrovirus-based insertional mutagenesis screens.

Sauvageau et al performed a sensitized cancer screen in which some mice infected with MuLV were carrying hypomorphic *Eed* genes.[3] Their work verifies that *Eed* has tumor suppressor activity, emphasizing the
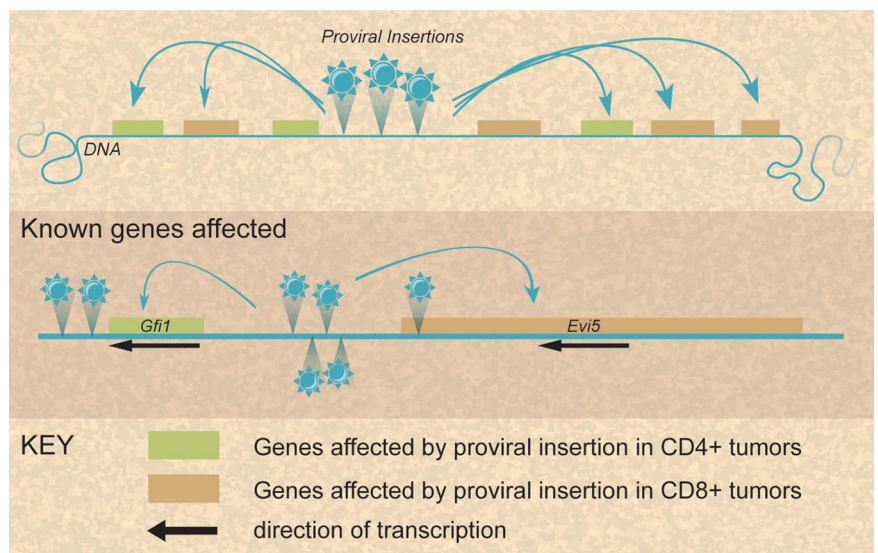
Modes of leukemia gene alteration at complex loci after proviral insertion. Illustration by Debra Tyler.

role of polycomb complexes in controlling self renewal and cancer. Interestingly, no specific MuLV-induced mutations were uncovered that correlated with *Eed* genotype, suggesting that its loss generally predisposes to leukemogenesis, without altering the subsequent genetic pathways chosen. It remains to be seen whether most such sensitized screens will result in the identification of specific cooperating genes or whether most predisposing mutations will only influence the latency/susceptibility to MuLV induced cancer but not the genetic pathway along which the leukemia will evolve.

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Comment on Kirsammer et al, page 767

Of mice and Down syndrome

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Analyzing hematopoiesis in the Ts65Dn mouse, which is trisomic for many orthologs of human chromosome 21 genes, may shed light on leukemogenesis in Down syndrome, as demonstrated by Kirsammer and colleagues in this issue.

Children with Down syndrome (DS) have a 10- to 20-fold higher risk of developing acute leukemia than children without Down syndrome and a 500-fold greater incidence of acute megakaryocytic leukemia (AMkL), highlighting a unique predisposition to develop a specific leukemia subtype. In addition, a small proportion of Down syndrome neonates are born with a variant of AMkL, the transient myeloproliferative disorder (TMD), which can resolve spontaneously, though approximately 20% of these infants will subsequently develop AMkL.

A seminal finding, initially reported from the laboratory of Dr John Crispino and subsequently confirmed by other groups, described acquired somatic mutations in exon 2 of the transcription factor gene *GATA1* (localized to Xp11.23) with nearly 100% penetrance in DS TMD and AMkL cases.[1-3] Sequence alterations in the region encoding the N-terminal activation domain of GATA1 include insertions, deletions, missense, nonsense, and splice-site mutations at the exon 2/intron boundary, resulting in the synthesis of a short-form GATA1 (GATA1s; 40-kDa) protein that exhibits altered transactivation capacity compared with the 50-kDa wild-type protein. GATA1 mutations are believed to represent early or initiating "genetic hits" in a multistep process of leukemogenesis in Down syndrome that can begin prenatally.[4]

A new study from the Crispino lab in this issue of *Blood* continues to contribute to our understanding of the biology of leukemia in children with Down syndrome. Using the Ts65Dn strain of mice, which displays several of the classical features of Down syndrome, including heart defects, cognitive deficits, and craniofacial dysmorphology, Kirsammer and colleagues characterized hematopoiesis in the mice with a series of comprehensive studies. The Ts65Dn mice have trisomy of the distal region of mouse chromosome 16q, estimated to be representative of 94 human chromosome 21-localized genes from the Down syndrome critical region. Among their observations, Kirsammer et al found that the mice had red blood cell macrocytosis (frequently observed in healthy individuals with Down syndrome) and developed thrombocytosis, megakaryocyte hyperplasia, dysplastic megakaryocyte morphology, and myelofibrosis. Interestingly, GATA1 mutations were not detected in the mice, nor did the mice develop leukemia.

The studies in the Ts65Dn mice suggest that the abnormal hematopoiesis in the mice is linked to overexpression of one or more of the orthologs of human chromosome 21 genes, and this background may prime hematopoietic cells for the development of leukemia. A candidate chromosome 21-localized gene, *AML1* (*RUNX1*), which is linked to the biology of acute leukemias in children and adults, did not appear to be linked to the myelofibrosis and megakaryocyte hyperplasia in the mice.

Narrowing down the field of candidate genes that include the analysis of the oncogene transcription factors ETS2 and ERG, which are also localized to the Down syndrome critical region, is a logical extension of the current studies. We still do not know the linkage of chromosome 21-localized genes and the generation of the GATA1 mutations and whether additional cooperating gene mutations are required. The role of miRNAs, which are down-regulated in megakaryocytic differentiation of CD34+ hematopoietic progenitors and up-regulated in AMkL cell lines including the chromosome 21-localized miRNA, miR-99a, requires further analysis, as another important clue in Down syndrome leukemogenesis.[5]

Ultimately, studies may identify one or more chromosome 21–localized genes linked to the generation of GATA1 mutations and the development of AMkL and discover whether these genes may also be linked to the extremely high event-free survival rates (> 80%) of Down syndrome AMkL patients.

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