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The results of Zhang and colleagues provide further support for the notion that gene therapy might be used for the induction of immune tolerance to clotting factors. However, this study also poses several questions that remain to be addressed, including whether certain anatomic sites of transgene expression preferentially result in tolerance induction,[5] and why results using a similar neonatal retroviral delivery protocol for factor VIII resulted in a 50% incidence of inhibitor development.[6]

Despite repeated challenges to the progress of gene therapy over the past few years, preclinical progress in the treatment of hemophilia using this therapeutic modality continues to be very encouraging. There remains optimism that this will eventually be converted to clinical success.

—David Lillicrap
Queen's University

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MLL—a gene of interest

The mixed lineage leukemia gene, *MLL*, has been a gene of interest to clinical and basic scientists alike for more than a decade. This gene, located in chromosome band 11q23, is frequently rearranged by translocations, and less often by inversions, in de novo acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) of infants, children, and adults, as well as secondary AML after treatment with topoisomerase II inhibitors. *MLL* is remarkable in that it is involved in rearrangements with more than 40 different partners. It has also been shown to undergo internal rearrangement by partial tandem duplication in a subset of adults with AML with normal cytogenetics or trisomy 11.[1] *MLL*-rearranged leukemias are often aggressive, and they generally carry a worse prognosis than their *MLL*-unrearranged counterparts. Thus, from a clinical perspective, *MLL*-rearranged leukemias are both intriguing and challenging.

MLL is also fascinating from a molecular biology standpoint. Originally identified by cloning human chromosome band 11q23 breakpoints, *MLL* was shown to be the mammalian homolog of *Drosophila trithorax*. Under normal circumstances, *MLL* encodes a histone methyltransferase that has been reported to assemble a supercomplex of proteins involved in transcriptional regulation.[2] As a result of chromosomal rearrangements in acute leukemias, MLL is fused in-frame with a wide variety of nuclear and cytoplasmic proteins, and it undergoes oncogenic activation by multiple mechanisms, including acquisition of transcriptional effector domains (following fusion with nuclear transcription factors, cofactors, or chromatin remodeling proteins) and dimerization.[3,4] Perhaps the best understood targets of normal and chimeric MLL proteins are the clustered homeobox or *HOX* genes[5]; however, gene expression analyses suggest that a number of other potentially important target genes exist in *MLL*-rearranged leukemias. Investigations aimed at unraveling the complex and varied molecular mechanisms underlying *MLL*-rearranged leukemias, with the goal of identifying potential therapeutic targets, are ongoing.

With the application of fluorescence-based cytogenetic technologies, a new subgroup of patients with myeloid malignancies, overrepresentation of band 11q23, and increased MLL copy number has recently been identified. In this issue of *Blood*, Poppe and colleagues (page 229) present novel data suggesting an etiologic role for *MLL* gain of function in AML and myelodysplastic syndromes (MDSs) with amplification, as opposed to rearrangement, of 11q23. Selected for the study were 31 cytogenetically well-characterized patient samples, and one cell line, with 11q23 gain. Fluorescence in situ hybridization (FISH) was performed to define the copy numbers of *MLL*, *DDX6*, *FLI1*, and *ETS1* across a 10-megabase (Mb) region of the 11q23 amplicon; all 4 genes were retained in all cases. Based upon the number of copies of *MLL* and the results of routine cytogenetic analyses, patients were divided into 3 groups with low (3-5 copies), intermediate (5-10 copies), and high (> 10 copies) levels of 11q23 gains. Expression levels of 6 selected 11q23 oncogenes (*MLL*, *DDX6*, *CBL*, *ETS1*, *PLZF*, and *FLI1)* were then compared with 11q23 genomic status, and highly significant expression differences were consistently identified only for *MLL* and *DDX6*. Functionality of the *MLL* transcript was indirectly demonstrated by up-regulation of *HOXA9* gene expression in samples with high-level *MLL* amplification. Expression of 5 mixed lineage leukemia-associated genes, *PROML1*, *ADAM10*, *NKG2D*, *ITPA*, and *MEIS1*, was also strongly up-regulated in samples displaying high-level 11q23 amplification. Finally, the investigators showed significantly increased *MLL* expression in a separate unselected series of MDS samples without *MLL* rearrangement. Taken together, the data indicate that *MLL* is a key target of 11q23 overrepresentation, *MLL* overexpression results in gain of *MLL* function, and the transcriptional events associated with *MLL* amplification are similar to those seen with *MLL* rearrangement. This is the first report of the functional consequences of 11q23 overrepresentation in myeloid malignancies, and it suggests that patients with these diseases might also benefit from therapy targeted for *MLL*-rearranged acute leukemias.

—Diane C. Arthur
National Cancer Institute

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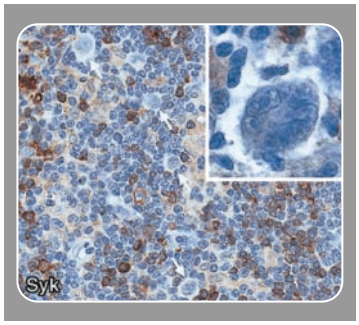
Hidden identity of Hodgkin/Reed-Sternberg cells

The identity of Hodgkin/Reed-Sternberg (H/RS) cells has been a subject of intense investigation. Only recently has there been conclusive evidence that H/RS cells are derived from germinal center B cells in the large majority of Hodgkin lymphomas (HL).[1] However, H/RS cells usually do not express membrane markers characteristic of B cells. The study by Marafioti and colleagues (page 188) sheds light on the hidden B-cell identity of H/RS cells. They found that these cells lack several intracellular signaling molecules found in normal B cells and most B-cell lymphomas. Specifically, the protein spleen tyrosine kinase (Syk), the B-cell linker protein (BLNK), and phospholipsase γ2 (PLC-γ2) were consistently absent from H/RS cells in classical HL. In contrast, lymphocytic and/or histiocytic (L&H) variants of H/RS cells in all cases of nodular lymphocyte predominance HL (NLPHL) expressed these B-cell–specific signaling molecules. This finding is in agreement with the known biologic and clinical differences between classical HL and NLPHL. In NLPHL, L&H variants of H/RS cells retain many of the characteristics of normal germinal B cells, including ongoing hypermutation of immunoglobulin (Ig) genes.

The current study not only enhances our understanding of the biology of HL, but can also prove to be of diagnostic value. The different expression patterns of B-cell signaling molecules could facilitate the sometimes difficult morphologic distinction of NLPHL from lymphocyte-rich classical HL, which has a clinical behavior more like other types of classical HL.[2] Lyn kinase, another B-cell signaling molecule, was absent in 75% of classical HL and in a



similar proportion (15 of 19) of NLPHL. It will be interesting to learn whether this finding will help to distinguish NLPHL from progressively transformed germinal centers, a clinically benign condition that often precedes, follows, or coexists with NLPHL.

The study by Marafioti et al revealed heterogeneous expression of Lyn and Fyn Src kinases in classical HL. Other studies have shown selective loss of B-cell transcription factors. It would be interesting to learn whether this heterogeneity is associated with the variable presence of Epstein-Barr virus detected in H/RS cells in 40% to 50% of classical HL.[3] The possible association of Epstein-Barr virus with intracellular signaling molecules is not addressed in the current study. Correlations of heterogeneous activity of intracellular signaling molecules with responses to therapy and patient outcome are possible avenues of future research.

Now that Marafioti et al have shown that NLPHL expresses some signaling components used by the B-antigen cell receptor, one would like to know whether these pathways are active. Their expression could simply be a reflection of a particular transcriptional pathway, and the function of these proteins might be unnecessary for the growth of NLPHL cells. On the other hand, the presence of these signal transduction molecules could mean that their function is necessary to develop NLPHL. In order for Syk to activate PLCγ2, it is likely that the cells would also need to express a Tec family protein tyrosine kinase, which in normal B cells is Bruton tyrosine kinase (Btk). One would like to know whether Btk, or another Tec kinase, is expressed and activated in NLPHL. There are several ways that the roles of Btk, Syk, PLCγ2, and BLNK could be investigated in NLPHL. Phosphospecific antibodies that detect activated proteins could be used, although not all of these antibodies work well for immunohistochemistry. Cell lines derived from NLPHL[4] could also be studied with phosphospecific antibodies and inhibitors of Syk and PLCγ2 and perhaps siRNA to inhibit protein expression. If these proteins are necessary to develop NLPHL, then drugs that inhibit Syk, Btk, or PLCγ2 could be of therapeutic benefit.

—Marshall E. Kadin and Christopher Carpenter
Harvard Medical School

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