Myeloid disorders arise in Dnmt3a-null marrow

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In this issue of Blood, Celik et al1 and Mayle et al2 report in 2 separate studies that loss of Dnmt3a in hematopoietic stem cells (HSCs) using an Mx-cre inducible knockout model causes disturbed blood cell formation.

Both studies report that irradiated mice transplanted with Dnmt3a-null HSCs died within 1 year of hematologic malignancies. The malignancies of which the mice died represented myeloid abnormalities that are mostly consistent with myelodysplastic syndromes. A low percentage of the animals died with acute leukemia. Sequencing experiments revealed that the leukemia cells in these animals, in contrast to the mice that showed preleukemic disease, had acquired additional mutations, frequently in genes encoding signaling molecules involved in proliferation. Both teams propose that the Dnmt3a-null mouse model and the myriad of hematologic malignancies observed are representative of hematopoietic disorders found in humans carrying DNMT3A mutations.3-5 The hypothesis that mutant DNMT3A acts as a dominant negative of DNMT3A by healthy bone marrow cells is a likely explanation for this finding. Acute myeloid leukemia (AML) in humans is a clonal disease, and the early appearance of DNMT3A mutations in hematopoietic progenitor or stem cells in humans can be viewed as a competitive situation as well. The primitive cells that are initially transformed by mutant DNMT3A mutations reside in a marrow that consists of sufficient healthy HSCs that do not carry the mutation. However, with time, leukemias can arise in those individuals. The investigators propose that the Dnmt3a-null HSCs resemble the DNMT3A mutation as found in primary AMLs. Therefore, one could also argue that in the Dnmt3a-null competitive transplantation setting, leukemias may arise, but that the in vivo experiments did not take long enough and additional serial transplantations may be required to obtain leukemias in those mice, notwithstanding the presence of healthy HSCs in the marrow. No matter the outcome of such studies, the 2 reports published here, together with the previous Nature Genetics study, emphasize the importance of applying complementary in vivo approaches to obtain a more complete understanding of mechanisms of transformation.

Importantly, the studies as carried out in these 2 papers allowed the investigators to study the in vivo effects of Dnmt3a loss of function as a single event and in combination with additional mutations in the Dnmt3-null HSCs. In fact, the investigators suggest that a major role for Dnmt3a is “to balance proliferation and differentiation to maintain the hematopoietic progenitor populations.”1 Moreover, knockout of Dnmt3a in HSCs is the onset of preleukemia in those animals, which, as the result of additionally acquired mutations, may transform into AML or acute lymphoblastic leukemia. These cooperating mutations seem to occur frequently in genes that play a role in the signaling cascades that regulate proliferation, eg, tyrosine kinase or Ras genes. Leukemias appeared with high incidence as the result of cooperation between Dnmt3a loss of function and mutations in either NRas or c-Kit. The fact that comparable mutations were found in patients with myeloid malignancies with DNMT3A mutations is another reason for the investigators to believe that Dnmt3a-null HSCs may resemble human DNMT3A mutant transformed cells and that the model can be applied to unravel the mechanism of transformation in humans.

Because Dnmt3a encodes a DNA methyltransferase, altered DNA methylation patterns are predicted in the genome of the transformed HSCs of Dnmt3a knockout mice or in human disease with DNMT3A mutations. The presence of disturbed methylation patterns, as reported by Mayle et al2, points to such effects. No common methylation profiles were found among the distinct diseases, but rather specific methylation patterns were apparent that discriminated the different malignancy types. The absence of the DNA methyltransferase activity encoded by Dnmt3a most likely plays an essential role in the generation of these defective epigenomic patterns in these malignancies. It is unclear, however, whether these different patterns are relevant for the development of the malignancies and involve genes that play a role in transformation. We are only at the beginning of understanding the function of DNMT3A on DNA methylation and how defects in this enzyme are involved in epigenomic alterations and the development of hematological malignancies. Mouse models as reported here are important to increase our understanding...
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Enhancing BCR signals at the cell membrane

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In this issue of Blood, Lu et al demonstrate the requirement of membrane localization for proper functioning of the human germinal center–associated lymphoma (HGAL) gene product in enhancing signals generated by engagement of the antigen receptor on germinal center B cells.1

HGAL, also known as germinal center–expressed transcript 2, was first identified in profiling studies as an interleukin (IL)–4–inducible gene whose expression in cases of diffuse large B-cell lymphoma (DLBCL) predicts for longer overall survival.2,3 As the name suggests, the protein product of HGAL is expressed in normal B cells undergoing the germinal center (GC) reaction and is typically observed in B-cell malignancies that originate within GCs such as follicular lymphomas, Burkitt’s lymphomas, and DLBCLs. A mouse homolog of HGAL, known as M17, is identified, but targeted disruption of its expression has only revealed that the presence of this protein is dispensable for the GC reaction.4 However, recent work by the Lossos laboratory,5 including the paper in this issue,1 has brought clarity to the role of HGAL in GC B cells and has also revealed directions for further research.

HGAL is already demonstrated by this group as an enhancer of antigen receptor (B-cell receptor [BCR]) signaling in GC B cells,1 and the paper by Lu et al is a biochemical study showing that this signal enhancing function of HGAL is crucially dependent on myristoylation, palmitoylation, and lipid raft localization. Thus, in resting B cells, a proportion of HGAL resides in lipid rafts together with the src family kinase Lyn (see panel A). Syk may associate with HGAL in these rafts, but such an association is likely nonproductive in terms of Syk kinase activity. BCR is, for the most part, also largely located outside these rafts. This all changes during BCR engagement (see panel B); BCR is recruited to lipid rafts where immunotyrosine activation motifs (ITAMs) within CD79a and b become phosphorylated by Lyn. This, in turn, attracts and activates Syk, which, through coassociation with HGAL (possibly by interacting with phospho-tyrosines within HGAL), has heightened kinase activity leading to enhanced induction of the BCR signaling pathway. Active Syk then catalyzes a phosphorylation reaction that rapidly sends HGAL out of the lipid raft/BCR signalsome and to the proteasome for destruction. Importantly, expressed cytosolic HGAL (ie, a mutant that can neither be myristoylated nor palmitoylated) does not associate with Syk, nor does it enhance BCR signaling. However, this does not mean cytosolic HGAL is without function. Ectopic expression of this HGAL mutant blocks cell migration in response to IL-6 and stromal cell–derived factor 1 (SDF-1), presumably through its described role in activating RhoA signaling and influencing actin/myosin dynamics.6,7

We can now dissect the function of HGAL and divide its role in mediating enhancement of BCR signaling from that in regulating cell migration. This ability potentially gives insight into why expression of HGAL is associated with good outcome in DLBCLs. BCR plays an important role in the pathogenesis of GC-derived lymphomas, and the signal enhancing effect of HGAL may provide growth signals to the malignant cells. Why HGAL expression is linked to good outcome in DLBCL is likely because of the role it plays in retaining malignant cells within the GC environment by keeping their migration in check.6,7 This notion is supported by demonstration that transgenic expression of HGAL in B cells leads to enlargement of Peyer’s patches,8 an organ where B lymphocytes require functional CXCR4 for egress.8 Considering that ectopic expression of HGAL inhibits cell migration to SDF-1 (CXCL12, the ligand of CXCR4), it is highly probable that the enlarged Peyer’s patches in HDAC-transgenic mice are due to restricted egress of B cells. Moreover, earlier findings from this group showing that miR-155 targets HGAL expression suggest that high expression of this micro-RNA in some cases of DLBCL may contribute to malignant cell dissemination and aggressive tumor behavior.9

A chief question now is whether HGAL works in concert with BCR signaling to block malignant cell migration. Critically, cytosolic HGAL is more efficient than wild-type HGAL in blocking cell migration. Conceivably, in BCR-stimulated cells, the efficiency with...
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