This report is of interest because it identifies Hippo pathway dysregulation for the first time in lymphoma. The Hippo pathway was originally discovered in Drosophila through its regulation of body and organ size by inhibiting cell proliferation and promoting apoptosis. Its role in cancer is increasingly being recognized with key components of the pathway acting as both oncogenes and tumor suppressors. Hartmann et al provide 2 lines of evidence to support a role for Hippo in MCL pathogenesis. This is an exciting development because it identifies for the first time the Hippo pathway as a tumor suppressor genes contributing to lymphoma tumorigenesis. Decreased expression of Hippo members MOBKL2A, MOBKL2B, and LATS2 was associated with inferior survival. Second, loss of the genomic regions where these 3 genes are located was observed in almost 40% of MCL cases. MOBKL2A, MOBKL2B are homologues of the MOBI gene that interacts with LATS in inhibition of YAP, a potent growth promoter. Evidence is growing to support the function of YAP as an oncogene as well as a tumor-suppressor function for its inhibitory upstream Hippo pathway components (see Figure 2 for summary of Hippo pathway). Thus, the findings of decreased expression of some of the Hippo members paves the way for further investigation of the Hippo pathway in lymphogenesis. Is it perturbed in other B-cell lymphomas? This would be an interesting question to answer as well as raising the distinct possibility of a new therapeutic target, potentially with broad application.

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**Another Link to STAT activation**

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Aberrant JAK–STAT activation characterizes human myeloproliferative neoplasms. The study by Oh and colleagues in this issue of Blood identifies STAT activation through loss of negative feedback by novel mutations of the adapter protein Lnk.

Human myeloproliferative neoplasms (MPNs) result from dysregulated cytokine signaling. Further understanding of the key signaling nodes and relevant driver mutations in these disorders is biologically and clinically important. The best example of this targeted approach has been the treatment of chronic myelogenous leukemia (CML). The Philadelphia chromosome translocation t(9;22) product BCR–ABL was identified in the 1980s in CML cells. The aberrant protein phosphorylation due to the activated kinase activity of the BCR–ABL fusion protein led to remarkably successful new drugs targeting the kinase domain. The patients lacking BCR-ABL were essentially without a genetic explanation for many years but this is starting to change quickly. In these disorders, one common feature has been cytokine-independent proliferation due to constitutive activation of the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signaling pathway (see figure). Significant advances in genetic analysis beyond metaphase...
cytogenetics such as single nucleotide polymorphism arrays and high-throughput sequencing have led to rapid identification of additional genetic lesions for human MPNs. Identification of the V617F mutation in the pseudokinase domain of JAK2 in 2005\(^2\)-\(^4\) was rapidly followed by identification of mutation W515L in the myeloproliferative leukemia (MPL) receptor in 2006,\(^3\) and JAK2 exon 12 mutations in 2007.\(^6\) These activating mutations were found in humans, validated in cell lines, and shown to recapitulate the disease and pathophysiology in mouse retroviral and transgenic models, thus setting the standard for characterization of newly identified disease-associated mutations.

The reciprocal of increased activation of tyrosine kinases is decreased negative feedback regulation. Although descriptions of changes in methylation status of suppressor of cytokine signaling 2 (SOCS2) and SOCS3 have been observed, no mutations in these genes have yet been reported. Recent reports of frequent mutations in c-Cbl highlight the essential role of ubiquitin-mediated degradation of signaling components in the control of myeloproliferation.\(^3,^6\) c-Cbl mutations are more complex than simple loss of function, because they still maintain adapter protein function and can facilitate signaling in a positive manner.\(^3\) In the current study by Oh et al.,\(^1\) another link to activation of JAK-STAT is reported which is of immediate clinical interest. In mouse models it has been known that the inhibitory adapter protein Lnk is associated with erythropoietin and thrombopoietin signaling and is required for their down-modulation, and Lnk can bind to wild-type and mutant JAK2 and modulate growth. However, no mutations in Lnk had been described.

From a total of 33 JAK2\(^{V617F}\)-negative MPN samples, Oh and colleagues found 2 patients, 1 with primary myelofibrosis and 1 with essential thrombocytopenia with whole or partially inactivating mutations in Lnk. These mutations were cloned and tested biochemically in transfected BaF3 cells and shown to exhibit JAK/STAT activation with high levels of STAT3 and STAT5 activation. Interestingly, Lnk mutations did not evoke cytokine-independent growth but rather stimulated thrombopoietin and granulocyte colony-stimulating factor–induced growth. The authors did not explore the very interesting but more subtle differences between the mutants and it will be important to move deeper into understanding the biochemical consequences of these mutations on Lnk localization. The broader prevalence in a larger clinical sample size will also be needed to determine whether these mutations could occur in polycythemia vera alone or alongside JAK2 or MPL mutations and whether they are associated with additional subtypes and overlap syndromes proceeding to leukemic transformation.

A notable feature of the study is application of the sophisticated intracellular phospho-flow cytometry method to identify a unique STAT3/STAT5 double-positive population. It will be interesting to know how broadly this population occurs in other types of MPN and whether the phospho-STAT3/5 double-positive population confers greater diagnostic or prognostic power than phospho-STAT3 or phospho-STAT5 alone. The response of the STAT3/5 population to JAK inhibition suggests potential therapeutic opportunity at least for controlling proliferative and survival signaling.

Although the present study stopped short of functionally testing the Lnk mutations in a mouse model, the biochemical data suggest that Lnk mutations in vivo could behave much like those of the Lnk\(^{-/-}\) mouse model that has been well characterized by several groups. Further study could provide unique insights into the signals required for pathogenesis and the role of Lnk in myelofibrosis. This interesting brief report provides additional diagnostic markers for human MPNs and again highlights the downstream activation of STAT3 in these disorders. A similar scenario has emerged in juvenile myelomonocytic leukemia where mutations in RAS, SHP-2, NF1, and c-CBL result in activated STAT5. Although a variety of signaling pathways including ERK and AKT are activated by these mutations, thus far the degree of phospho-STAT5 as determined by intracellular flow cytometry stands out as an attractive biomarker for patient prognosis.\(^10\) Additional interrogation of the JAK-STAT pathway in hematologic malignancies promises to reveal additional new insights into leukemogenesis and new approaches for targeted therapy.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

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Comment on Ren et al, page 869

A Munc in the platelet granule works

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The membrane fusion regulator Munc13-4 facilitates calcium–stimulated release of cytolytic and inflammatory mediators from lymphocytes and granulocytes. In this issue of Blood, Ren et al reveal a similar requirement for Munc13-4 in secretion from activated platelets and provide new insights into a human genetic disease.1

Munc13-4 stimulates fusion of platelet granules with the plasma membrane to release granule contents. (Top) Presecretory phase in which secretory lysosomes, platelet α-granules or dense granules, or other LRO is “docked” at the plasma membrane. Fusion requires engagement of a vSNARE (red) on the granule membrane with a tSNARE complex (green) on the plasma membrane. Munc13-4 (orange) stimulates this engagement in a calcium-dependent manner. Upon cell activation and calcium influx (bottom), the membranes fuse, releasing the contents of the granule to the extracellular space. Highlighted are examples of LRO cargoes that are known to be regulated by Munc13-4.

When platelets are stimulated, they release a variety of soluble mediators from 3 different types of intracellular storage compartments: α-granules, dense granules, and lysosomes. To release their contents to the extracellular space, the membrane of each compartment must fuse with the plasma membrane, a process that is driven by cognate interactions between soluble N-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) family proteins. Formation of a 4-helix bundle by cytoplasmic coiled-coil domains of a tSNARE complex on the plasma membrane and a vSNARE on the granule membrane liberates the energy required to drive membrane fusion.2 Similar SNARE-dependent fusion events mediate intracellular membrane trafficking between all secretory and endosomal compartments, but granule release must be more tightly regulated so that it only occurs after calcium influx induced by platelet activation. The SNAREs themselves are not calcium-responsive; rather, regulatory proteins must ensure that granules accumulate at plasma membrane fusion sites and that appropriate SNARE complexes form at the right time. Identifying these regulatory proteins is of paramount importance both for understanding how to manipulate platelet granule release for therapeutic means and for identifying genetic abnormalities that might underlie bleeding disorders. Ren et al identified Munc13-4 as one such SNARE regulator in platelets.1

Munc13-4 is a member of a small family of proteins that regulate SNARE-dependent fusion at the plasma membrane. The gene encoding Munc13-4 is mutated in familial hemophagocytic lymphohistiocytosis type 3 (FHL3),3 a disease in which patients suffer from uncontrolled inflammation, lymphoproliferation, and neuronal abnormalities. The hematologic symptoms of FHL3 reflect the failure of several hematopoietic cell types to secrete contents from storage compartments—referred to collectively as lysosome-related organelles (LROs)—in response to agonists. The affected LROs include cytolytic granules of cytotoxic T cells and natural killer cells,3,4 azurophilic granules of basophils and mast cells,6 and secondary and tertiary granules of neutrophils6; lethality in FHL3 reflects the loss of Munc13-4 function in cytolytic granule release and consequently of cytotoxic T and natural killer cell function. Cytolytic granules in Munc13-4−/− deficient T cells accumulate properly at the plasma membrane but do not release their contents,1 suggesting that Munc13-4 participates in activating calcium-induced LRO fusion with the plasma membrane. Munc13 family members bind in vitro to tSNAREs (eg, see Guan et al), suggesting that they prime fusion by either facilitating formation of 4-helix SNARE bundles or by “tethering” LROs to the plasma membrane to allow SNARE complexes to form (see figure).

Ren et al built upon earlier findings by Shirakawa and colleagues6 that platelets express Munc13-4 and that addition of recombinant Munc13-4 to permeabilized platelets enhances calcium-induced release of a dense granule cargo, serotonin. To test whether Munc13-4 is required for platelet granule release, Ren et al turned to the Unc13dJinx mouse model of FHL3, in which the Unc13d gene encoding Munc13-4 is inactivated. The key finding is that after stimulation with thrombin, Unc13dJinx Munc13-4−/− deficient platelets secrete dramatically less platelet factor-4 (from α-granules) and beta-hexosaminidase (from lysosomes) than wild-type platelets and fail completely to secrete serotonin (from dense granules). This secretion defect correlated with 2 signs that granules failed to fuse with the plasma membrane—a loss of thrombin-stimulated surface expression of α-granule and lysosomal membrane proteins, and retention of intracellular granules by electron microscopy—but was not a consequence of ineffective signaling, because Unc13dJinx platelets responded to thrombin stimulation appropriately by calcium influx, tyrosine phosphorylation, and integrin activation. Using a beautiful in vitro reconstitution system, addition of recombinant full-length Munc13-4 to permeabilized Unc13dJinx platelets fully restored secretion from all 3 granule types. A truncated