and selectin ligand expression but has hematopoietic defects in receptor activation and adhesive functions that are attributed to the lack of expression of kindlin-3. Kindlin-3 forms a ternary complex with the cytoplasmic tail of β3-integrin along with talin. The LAD-III defect has provided solid evidence that focal clusters of bound integrins effectively mechanotransduce outside-in signals that are critical to both stable adhesion and migration necessary to navigate the final steps of immigration.

In this issue of Blood, Jakob et al report that HPK1 participates during inside-out and outside-in signaling of neutrophil recruitment during acute inflammation.1 Whereas HPK1 has previously been reported to participate in signaling lymphocyte functions, Jakob et al have provided the first data to reveal its function during chemokine induction of neutrophil arrest, adhesion strengthening, pseudopod formation, and cell migration via high-affinity lymphocyte function-associated antigen 1. Using HPK1-deﬁcient mice, Jakob et al observed a significant defect in the neutrophils’ capacity to efﬁciently arrest and migrate along and across inﬂamed endothelium, analogous to the kindlin-3 defect in LAD-III. One caveat in this comparison is that HPK1 appears to speciﬁcally modulate LFA-1 and not Mac-1 function. A particularly exciting component of this study was the finding that HPK1 formed a complex with the adaptor mammalian actin-binding protein 1, previously shown by this same group to reinforce neutrophil adhesion-dependent functions.4 These observations begin to shed light on how tension transmitted through focal clusters of LFA-1 serve to reinforce its linkage to the cortical cytoskeleton via outside-in signaling. Still unknown is the relationship between HPK1, mammalian actin-binding protein 1, kindlin-3, and talin, all of which are enriched on the cytoplasmic domain of CD18 and function to stabilize high-affinity LFA-1. An intriguing possibility is that enrichment of these adaptors facilitates assembly of a cytoskeletal linkage critical to efﬁcient neutrophil mechanotaxis, or directional sensing using shear stress. Such a process would involve signal transduction modulated by focal clusters of high-affinity LFA-1 bound at sites enriched in dimeric intercellular adhesion molecule 1 near endothelial junctions.5 Future studies should shed light on the precise nature of force-facilitated integrin activation and signaling. One proposed scenario is that LFA-1 functions as a traction sensor by converting tensile bond forces to conformational changes that in turn initiate dynamic recruitment of these linking molecules to the cytodomain of the integrin.6 HPK1 is now established to function as a clutch that engages active mechanotactic crawling that guides a neutrophil efﬁciently to the site of inﬂammation.

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

PLATELETS & THROMBOPOIESIS

Comment on Mazharian et al, page 4205

SHPing in different directions in platelet production

Jorge Di Paola1 1UNIVERSITY OF COLORADO

In this issue of Blood, Mazharian and colleagues characterize Shp1 and Shp2 conditional knockout (KO) murine models, underscoring the role of these phosphatases not only on platelet function but also on megakaryocyte development and platelet counts and size.1

Protein tyrosine phosphatases (PTPs) are a superfamily of enzymes that, along with protein tyrosine kinases, act in a coordinate manner to allow phosphorylation and dephosphorylation of proteins, therefore regulating intracellular signaling. Two members of this family, the ubiquitously expressed Src homology 2 domain tyrosine phosphatases SHP-1 and SHP-2, are involved in a vast array of cellular functions through their role in tyrosine phosphorylation of intracellular proteins. From cell development to growth and differentiation, several reports have characterized the involvement of these non-transmembrane PTPs in cell signaling pathways for hormones, growth factors, and cytokines and their potential implication in disease states such as autoimmunity, diabetes, and cancer.2

Both SHP-1 and SHP-2 have been implicated in platelet activation responses such as adhesion and aggregation, particularly by interacting with ITAM-containing receptors and integrins. Nevertheless, despite sharing similar signature sequences, their role appears to differ substantially, with SHP-1 being mostly a negative regulator of intracellular signaling and SHP-2 facilitating signaling via the Ras-mitogen-activated protein kinase pathway.3,4

Human SHP-1 and SHP-2 are encoded by PTPN6 and PTPN11, respectively, with highly conserved orthologs in mice. Complete murine nulls for both PTPs have been developed and they either result in death within a few weeks after birth (Shp-1) or exhibit embryonic lethality (Shp-2). Although these models have provided

REFERENCES


useful scientific information, the inability to study older mice represents a major limitation for studying the role of these molecules in hematopoiesis.\(^{1}\)

To solve this issue, the authors made use of mouse genetics by developing megakaryocytic-/platelet-specific Ptpn6, Ptpn11, or Ptpn6/Ptpn11 double conditional KO mice and thoroughly studied thrombopoiesis and platelet function. This combination of transgenic mice allowed the investigators to draw a signaling map (albeit rudimentary) of the effects of these PTPs on megakaryocyte development and function and platelet activation responses. Overall and perhaps not surprisingly, they found that the disruption of Shp-1 and Shp-2 expression had different phenotypic effects, indicating that both molecules may exert their effect through distinctive mechanisms.

Although the Shp-1 conditional KO showed normal platelet counts and size, platelets from these mice exhibited an abnormal response to collagen due to decreased expression of glycoprotein VI, a major signaling collagen receptor. On the other hand, the Shp-2 conditional KO showed low platelet counts and thrombocytopenia, a finding likely attributed to defective thrombopoiesis that was perhaps due to decreased integrin and Mpl (thrombopoietin receptor) signaling and increased platelet clearance. As with many other platelet KO murine models, neither Shp-1 nor Shp-2 showed increased bleeding upon injury. Interestingly, the Shp-1/Shp-2 double KO mice expressed a much more severe phenotype, indicating that these 2 molecules are intrinsically involved in the proliferation, survival, and normal function of megakaryocytes.

The authors then compared the extreme phenotype of the Shp-1/Shp-2 double KO with the G6b-B KO mouse. G6b-B is an ITIM-containing receptor that is tyrosine phosphorylated and closely interacts with Shp-1 and Shp-2. The G6b-B KO mice also exhibit bleeding and macrothrombocytopenia (see figure).\(^{6}\)

Although the platelet phenotype of the G6b-B mice is strikingly similar to the Shp-1/Shp-2 double KO, megakaryocytes in the G6b-B mice show normal development and intact Mpl signaling, which is fundamentally affected in the Shp-1/Shp-2 double KO. This finding is extremely relevant for the further dissection of these pathways and their critical involvement in megakaryopoiesis.

Understanding the SHP-1 and SHP-2 roles in platelet formation and function could have significant clinical implications. Remarkably, gain of function mutations in PTPN11 that cause up-regulation of SHP-2 cause Noonan syndrome,\(^{7}\) which is an autosomal dominant disorder characterized by short stature, dysmorphic facial features, and heart disease. Several reports have shown an association of this syndrome with bruising and bleeding with alterations of coagulation but also with platelet functional defects or thrombocytopenia. Practicing hematologists are often consulted on this issue, but the etiology of the platelet dysfunction or abnormal counts remains elusive.\(^{8}\)

Additionally, somatic mutations of this gene account for one-third of nonsyndromic juvenile myelomonocytic leukemia cases.\(^{9}\) These 2 clinical examples underscore the importance of PTPs in human hematopoietic disorders.

This important study helps to set up the basis of our understanding not only of the basic molecular mechanism that regulates these pathways in megakaryopoiesis and platelet function but also of the potential laboratory and clinical implications that these alterations have for hematological diseases.

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

References


SHPing in different directions in platelet production

Jorge Di Paola