The observation that targeting Atg7 reduced the cytoprotective effects of stromal cells is novel and has practical implications. For example, microenvironmental factors have been shown to protect leukemic cells, particularly leukemic stem cells, from chemotherapy. Although this phenomenon has generally been attributed to disruption of cytokine networks in the hypoxic microenvironment, the notion that such a process may be regulated by autophagy, and specifically Atg7 in AML, is new and could provide a more effective strategy for overcoming this problem.

Practically, targeting Atg7 will not be trivial, but the possibility does exist. For example, a ubiquitin E3 ligase inhibitor, the Nedd8-activating enzyme inhibitor pevonedistat, has recently shown promising activity in AML.[7] However, whether specifically inhibiting Atg7 E1 ligase activity will be sufficient to recapitulate the antileukemic effects of Atg7 knockdown (and disrupt its lipidation- and conjugation-related activities) remains to be determined. It should also be kept in mind that chemical versus genetic disruption of a protein can have disparate biological consequences.[8]

In summary, the study by Piya et al furnishes a cogent rationale for targeting autophagy in general, and more specifically, the autophagy-related protein Atg7 in AML. As with other targeted drugs, the ultimate role of Atg7 antagonists is likely to lie in combination with either conventional or novel agents, particularly those that elicit a cytoprotective autophagic response. Ongoing efforts will hopefully resolve these issues in the near future.

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

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● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Lozano et al, page 1282

Inherited CalDAG-GEFI deficiency

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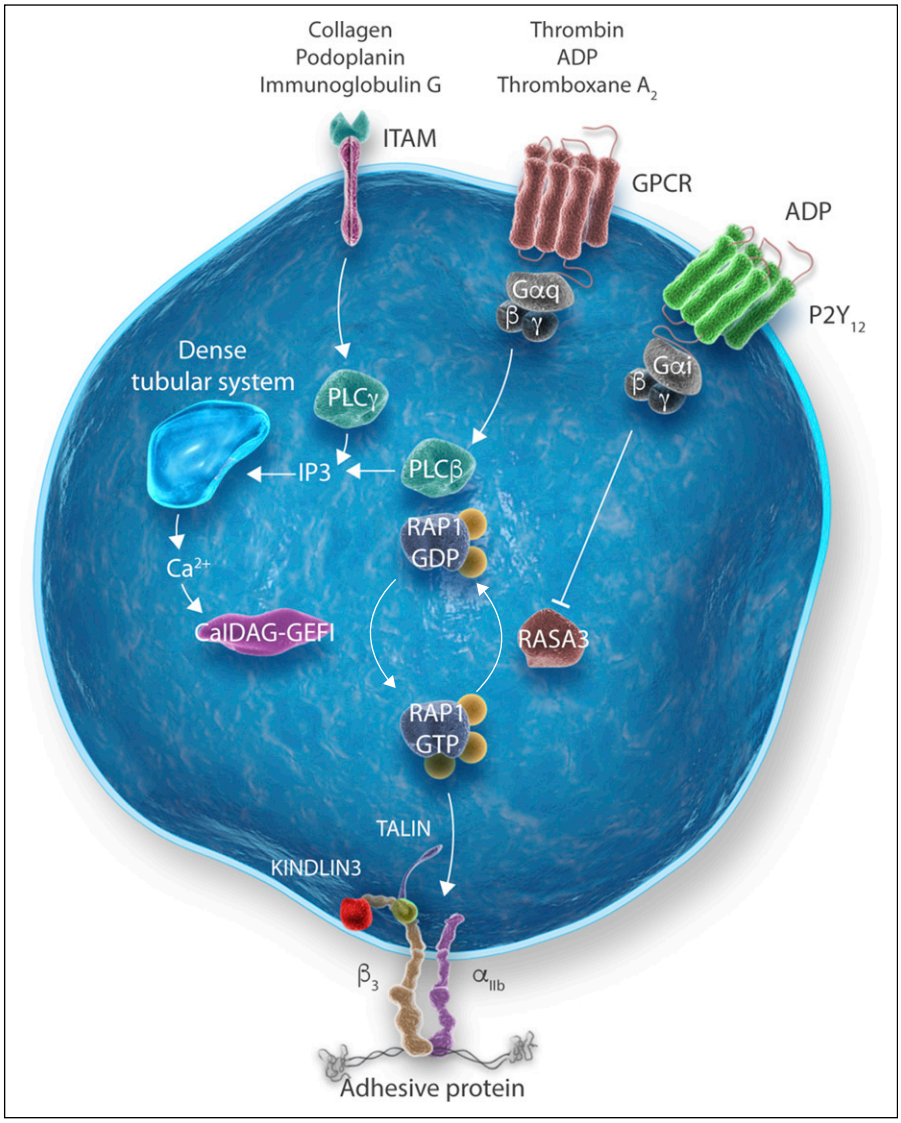
In this issue of *Blood*, Lozano et al describe 3 patients, belonging to 2 unrelated families, with severe inherited platelet dysfunction and bleeding diathesis, displaying 2 novel homozygous mutations in RAS guanyl-releasing protein-2 (*RASGRP2*), the gene encoding calcium- and DAG-regulated guanine exchange factor-1 (CalDAG-GEFI).[1]

Inherited platelet function disorders (PFDs) are associated with increased risk of mucocutaneous bleeding of various levels of severity. They may be classified based on abnormalities of platelet components that share common characteristics[2]: (1) platelet receptors for adhesive proteins; (2) platelet receptors for soluble agonists; (3) platelet granules; (4) signal transduction pathways; and (5) procoagulant phospholipids. Less well-characterized PFDs are grouped in a sixth category of miscellaneous abnormalities.[2] The increasing understanding of the biochemical pathways regulating platelet activation allows the identification of novel PFDs in the category of abnormalities of signal transduction pathways. In 2014, Canault et al described 3 siblings from consanguineous parents, with lifelong bleeding diathesis and PFDs associated with inherited dysfunctional CalDAG-GEFI, the exchange factor that plays a fundamental role in Ca^{2+}-dependent activation of Ras-proximate-1 (Rap1) in platelets.[3]

Rap1 is a small guanosine triphosphatase (GTPase) belonging to the RAS (rat sarcoma) family. Like other RAP proteins, it is a switch that cycles between a guanosine diphosphate (GDP)–bound inactive form and a guanosine triphosphate (GTP)–bound active form.[4] Guanine nucleotide exchanging factors are responsible for Rap1 activation, whereas GTPase-activating proteins (GAPs) deactivate Rap1 by hydrolyzing GTP to GDP.[5] The GTP-bound form of Rap1 regulates the activation of integrins, including $\alpha_{IIb}\beta_3$ in platelets, which, in its activated form, binds adhesive proteins to enable platelet aggregation.[5] When platelets are stimulated by agonists that bind to G-protein–coupled receptors (GPCRs) of the Gq type, or to immunoreceptor tyrosine-based activation motif (ITAM)–coupled receptors, the phospholipase C (PLC) β or γ isoform is activated and forms inositol 1,4,5-trisphosphate (IP3) (see figure). IP3 releases Ca^{2+} from stores, which causes rapid CalDAG-GEFI–dependent Rap1 activation, followed by $\alpha_{IIb}\beta_3$ activation, binding of adhesive proteins, and platelet aggregation.[5] The process is regulated by RASA3, the GAP that switches Rap1-GTP back to Rap-GDP in platelets, prohibiting sustained signaling.[5] Interestingly, $P2Y_{12}$, the GPCR of the Gi type that binds adenosine 5′-diphosphate (ADP), inactivates RASA3, allowing sustained Rap1 signaling and full platelet aggregation.[5] This explains why $P2Y_{12}$-deficient platelets undergo a normal initial wave of aggregation, followed by rapid deaggregation when they are stimulated by ADP, even at very high concentrations.[6]

Considering the importance of CalDAG-GEFI in triggering platelet aggregation, it can be predicted that its deficiency or loss of function is associated with abnormalities of platelet aggregation. Indeed, CalDAG-GEFI



Regulation of Rap1 activation by CalDAG-GEFI and RASA3. Agonists binding to GPCRs of the Gq type or to ITAM-coupled receptors activate the PLC β or γ isoform to form IP3, which releases Ca^{2+} from stores. Ca^{2+} causes rapid CalDAG-GEFI–dependent Rap1 activation, which, through the cooperation of talin and kindlin-3, promotes the binding of adhesive proteins to $\alpha_{IIb}\beta_3$, enabling platelet aggregation. The process is regulated by RASA3, which hydrolyzes Rap1-GTP to inactive Rap-GDP. $P2Y_{12}$, the GPCR of the Gi type that binds ADP, inactivates RASA3, allowing sustained Rap1 signaling and full platelet aggregation. Professional illustration created by Somersault18:24.

knockout (KO) mice displayed severe abnormalities of platelet aggregation induced by various agonists, with the exception of high concentrations of thrombin.[7]

A platelet phenotype similar to that of CalDAG-GEFI KO mice was observed in CalDAG-GEFI–deficient patients described by Canault et al[3] and by Lozano et al: platelet aggregation was defective, except when induced by high concentrations of collagen or of a thrombin receptor-activating peptide. These data are compatible with the presence in human platelets of alternative pathways for integrin activation that do not require CalDAG-GEFI.[8] All patients had moderate/severe mucocutaneous bleeding episodes, which occasionally caused iron-deficiency anemia and required red blood cell and/or platelet transfusions. Interestingly, the severity of the bleeding diathesis seemed to decrease in adult life.[1,3]

An important difference is that, although the patients described by Canault et al did not display abnormal integrin-dependent leukocyte function,[3] β2 integrin activation was impaired in stimulated neutrophils from the patients described by Lozano et al.[1] Deficiency of CalDAG-GEFI is expected to affect leukocyte function, in addition to platelet function, because Rap1 activation is also important for leukocyte integrin activation.[4] As a matter of fact, neutrophils from CalDAG-GEFI–deficient mice failed to adhere firmly to stimulated venules and to migrate into sites of inflammation.[9] One possible explanation for the discrepancies between Canault's patients and KO mice is that, compared with the total CalDAG-GEFI deficiency in KO mice, structural domains important for leukocyte functions could be spared by the patients' homozygous c.G742T mutation of the *RASGRP2* gene, which was associated not with defective expression of the protein but with a glycine-to-tryptophan substitution at position 248 (p.Gly248Trp), which impaired the catalytic activity of CalDAG-GEFI.[3] This hypothetical explanation is corroborated by the findings by Lozano et al. One of their patients, a 9-year-old child of Chinese origin, displayed a homozygous mutation (c.C1142CT) in exon 10 of *RASGRP2*, which led to a p.Ser381Phe substitution in the catalytic domain of CalDAG-GEFI: the mutation was associated with severely reduced expression of an unstable protein with reduced enzymatic activity. In 2 Spanish siblings from the second family described by Lozano et al, a nonsense homozygous variation (c.C337CT) (p.Arg113X) in exon 5 of *RASGRP2* was identified, which is expected to truncate CalDAG-GEFI synthesis. Therefore, the expression of CalDAG-GEFI was severely impaired in the patients described by Lozano et al, different from the patients described by Canault et al, whose platelets expressed normal levels of the protein. Further studies are needed to test whether this diversity is indeed responsible for the different phenotypes.

Despite the different results of in vitro experiments of leukocyte function, none of the patients displayed overt immune defects or susceptibility to bacterial infections, suggesting that alternative pathways of integrin activation in leukocytes compensate for CalDAG-GEFI deficiency. When the final common steps of integrin activation are defective, more generalized severe and clinically relevant abnormalities of platelet and leukocyte functions ensue. For instance, patients with leukocyte adhesion deficiency III (LADIII) display severe generalized defect of platelet aggregation (comparable to that of

patients with Glanzmann thrombasthenia, caused by αIIbβ3 deficiency[2]), severe bleeding diathesis, and frequent infections.[10] LADIII is associated with mutations in *FERMT3*, which encodes kindlin-3, which plays an essential role in integrin activation in humans.[4,10]

In conclusion, the study by Lozano et al describes additional patients with bleeding diathesis and inherited platelet function defect associated with CalDAG-GEFI defects, adding information on the structure-function relationship of the molecule and giving insights into the broad spectrum of abnormalities associated with severely reduced expression of the protein in humans.

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