Inherited thrombocytopenias: the beat goes on

A. Koneti Rao and Natthapol Songdej

In this issue of Blood, Bottega et al document mutations in ACTN1, which encodes the cytoskeletal protein α-actin 1, in 10 of 239 consecutive probands with an inherited thrombocytopenia—making ACTN1 an important cause of familial thrombocytopenia.

Way back in 1909, Richard May, and in 1945, Robert Hegglin independently described a clinical entity characterized by inclusions in white cells, large platelets, and thrombocytopenia, an association that was subsequently named the May–Hegglin anomaly. However, it was only at the turn of the century that the genetic basis of this entity was established as a mutation in MYH9, the gene encoding for the non–muscle myosin heavy chain IIA. This amalgamated several syndromes with eponyms (Sebastian, Fechtner, ANKYD26, GFI1B, and PRKCAG) under 1 rubric of thrombocytopenia with absent radii, HOX411 in congenital thrombocytopenia with radioulnar synostosis, and ANKYD26, GFI1B, and PRKCAG. To date, about 2 dozen genes have been linked based means of manipulating the rate of platelet production.

Microtubule mobility during the process of proplatelet elongation was tracked in β1-tubulin-Dendra2–expressing primary mouse megakaryocytes using FLAC microscopy, which enables spots to be marked along the proplatelet shaft by irreversibly photoconverting Dendra2 from green to red with a 405-nm laser. FRAP microscopy was used to further analyze the kinetics of elongation of released proplatelets expressing β1-tubulin-Dendra2. A 488-nm laser was used to bleach a 1.5-μm spot and fluorescence recovery rates determined as an indicator of microtubule sliding. Incomplete recovery in the bleached region was most likely due to immobile microtubules. Similar findings were obtained using human umbilical cord blood–derived megakaryocytes, demonstrating that this is a common mechanism, and adding credence to mouse megakaryocytes as a model for resolving the mechanism of platelet production in humans.

Three structurally distinct compounds were used to inhibit dynein in this study, namely, sodium orthovanadate (Na3VO4), erthyro-9-(2-hydroxy-3-nonyl)-adenine (EHNA), and ciliobrevin D, the last being the newest and most selective dynein inhibitor of the 3. Na3VO4 and EHNA have known off-target effects, the former inhibiting protein–tyrosine phosphatases and the latter inhibiting phosphodiesterases, in addition to dynein ATPase activity. However, the fact that all 3 compounds had similar inhibitory effects on proplatelet elongation suggests they act through the same mechanism. In contrast, inhibition of tubulin polymerization by nocodazole had no effect on proplatelet elongation, as previously reported, and taxol, which stabilizes microtubules, inhibited microtubule sliding by an as yet unknown mechanism.

Findings from this study fill an important gap in our knowledge of the mechanism driving platelet formation. It unifies previous in vitro findings by Hartwig and Italiano, and in vivo findings by Junt et al and Pertuy et al, demonstrating that large fragments of megakaryocytes enter the sinusoidal lumen where they further subfragment into platelets in the circulation. Thus, the terminal stages of platelet production are actively taking place within the blood, where platelets adopt their final shape and size, rather than immediately at the point of entry, as is sometimes depicted in schematic representations. All the while, the dynein is driving microtubule sliding and proplatelet elongation.

The application of quantitative fluorescence microscopy techniques to address a fundamental question of hematology makes this study a must-read. It also highlights key unanswered questions, including: how shear force gets converted into biochemical signals that increase dynein function and proplatelet formation, how this mechanism is suppressed in the bone marrow, how microtubule cross-linking and twisting influence the direction of net microtubule movement, and how platelets are ultimately released from the tips of proplatelet shafts in the circulation. Watch this space.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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to inherited thrombocytopenias. To this rapidly moving field, the present article by Bottega et al\(^1\) adds evidence that ACTN1 mutations are an important mechanism.

In 2013, Kunishima et al\(^7\) reported 6 Japanese pedigrees with an autosomal dominant macrotrombocytopenia, mild bleeding symptoms, and mutations in ACTN1, the gene encoding α-actinin 1 and a member of the actin-cross-linking protein superfamily that participates in cytoskeletal organization. α-Actinins exist as antiparallel dimers and have 2 functional domains—an N-terminal actin-binding domain and a C-terminal calmodulin-like domain. Platelets and megakaryocytes express mainly α-actinin 1. These authors found ACTN1 mutations in 6 of 13 pedigrees with autosomal dominant macrotrombocytopenia, all in the ACTN1 functional domains. Moreover, they showed that expression of the ACTN1 variants in Chinese hamster ovary cells or in mouse fetal liver–derived megakaryocytes caused disruption of the normal actin-based cytoskeleton structure; in mouse megakaryocytes, there was a reduction in proplatelet tips, consistent with impaired platelet production. Of note, ACTN1 mutations accounted for 3.5% of their patients with autosomal dominant macrotrombocytopenia and were the fourth most common cause.

Also in 2013, Gueguen et al\(^8\) reported a missense mutation in ACTN1 in a large French kindred of 55 members, of whom 26 had autosomal dominant macrotrombocytopenia. The mutation in the actin-binding domain of α-actinin 1 segregated with macrotrombocytopenia and studies in COS-7 cells corroborated a disruption of the actin cytoskeleton by the mutation. Interestingly, in the French pedigree and in 5 of 6 Japanese pedigrees,\(^7\) the ACTN1 mutations were cytosine guanine dinucleotide mutations; the cytosine guanine dinucleotide site is a common mutational hot spot in the human genome.

The current article by Bottega et al\(^1\) strengthens the association of ACTN1 mutations with inherited thrombocytopenias. The authors had previously identified a causative mutation in 111 of 239 consecutive probands with inherited thrombocytopenias. Here they report missense mutations in ACTN1 in 10 of the remaining 128 index cases (4.2% of total cohort) through studies using whole exome sequencing in 7 subjects and Sanger sequencing in 121. Eight were novel variants affecting highly conserved amino acid residues in ACTN1. These patients had mild thrombocytopenia (mean platelet count, 103 × 10\(^3\) ± 26 × 10\(^3\)/L in 31 subjects) with increased mean platelet volume (12.6 ± 1.7 fL; 50 controls: 10.1 ± 1.4 fL) and mild to no bleeding manifestations, although 4 of 18 subjects undergoing surgery had increased blood loss. Of note, 3 individuals from one family developed leukemia; the association of ACTN1 mutations with myeloid malignancies needs to be clarified. The platelet aggregation responses were normal in the patients. These observations were complemented by studies in fibroblasts showing altered actin cytoskeleton by the ACTN1 mutations. Overall, these 3 studies\(^17,8\) provide convincing evidence to link ACTN1 mutations with inherited thrombocytopenias and reflect on the relative high prevalence in such patients.

A better understanding of inherited thrombocytopenias is important in clinical practice, beyond predisposition to bleeding, which is highly variable and often mild. First, many of these patients are first recognized in their adulthood,\(^4\) and in the absence of a family history of thrombocytopenia, there is the risk of misdiagnosis as immune thrombocytopenia and resulting unnecessary therapy. Second, some gene mutations have prognostic implications, such as the association with myeloid malignancies with RUNX1 and ANKRD26 mutations or worsening renal function or hearing loss with MYH9 mutations. Not all patients with MYH9 mutations demonstrate inclusions in neutrophils on the routine peripheral smear, and additional studies are needed. Last, there may be therapeutic implications; for example, the role of eltrombopag in patients with MYH9 mutations.\(^5\)

From a mechanistic perspective, inherited thrombocytopenias involve mutations in genes with diverse functions, although for many the specific function in megakaryocytes/platelets remains unclear. The genes include genes coding for transcription factors (RUNX1, FLI1, GATA1, GFI1B, HOXAI), thrombopoietin receptor (MPL), cytoskeletal proteins and related signaling (MYH9, TUBB1, ACTN1, FN1, WASP), surface membrane glycoproteins and related signaling (GPIBA, GPIBB, GPIX, ITG2B, ITGB3), proteins involved in vesicle transport (VBE4,1L2), exon-junction complex involved in RNA processing (RBM8A,4), and cyclic adenosine monophosphate–dependent protein kinase (PRKACG).\(^3-6\) Mutations in the ANKRD26 5’ untranslated region result in loss of RUNX1 and FLI1 binding and in ANKRD26 silencing.\(^9\) Thus, different genes likely affect distinct mechanisms in megakaryocytes and platelets.

With new insights come new questions. The currently identified mutations explain the genetic basis in about half of patients, even at specialized centers. What do the others have? What are the unrecognized clinical implications (such as predisposition to malignancies) of specific mutations? What measures can be used to raise platelet counts on a temporary basis besides platelet transfusions?

Patients with inherited thrombocytopenias constitute an untapped reservoir of information into the basic biology of the cells involved. Studies are needed to identify the impact of the mutations on specific processes in megakaryocytes and platelets. In some entities, there is strong evidence for associated platelet dysfunction, as in patients with RUNX1 mutations.\(^10\)

Recent advances in the area of inherited thrombocytopenias are impressive. The available state-of-the-art technologies will continue unraveling new causes and yield insights into megakaryocyte and platelet biology. The beat goes on—and the tempo is high.

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THROMBOSIS & HEMOSTASIS

Comment on Laurent et al, page 881

PI3Kβ inhibition: all that glitters is not gold

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In this issue of Blood, Laurent et al demonstrate that phosphatidylinositol 3-kinase β (PI3Kβ) activity is essential for thrombus stability at a high shear rate, highlighting a potential risk of embolization upon PI3Kβ inhibition.1

The stringent need to improve current antithrombotic therapies urges the identification of novel targets for antiplatelet treatments. The elucidation of the molecular mechanisms and critical players of platelet activation represents a mandatory approach to identify suitable intracellular effectors able to control specific signaling pathways. In the last 10 years, a growing volume of evidence has pointed to the β isoform of PI3Kβ as the most promising novel target for antithrombotic drugs,2 and PI3Kβ selective inhibitors have revealed a remarkable ability to prevent platelet activation and thrombus formation without affecting hemostasis.3 The study by Laurent et al documents a previously unrecognized function for platelet PI3Kβ in thrombus stabilization at a high shear rate and thus provides an important warning that therapeutic inhibition of PI3Kβ may increase the risk of embolization and secondary ischemic events in the microcirculation.

PI3Kβ belongs to the class I PI3K family, enzymes able to produce phosphatidylinositol 3,4,5-trisphosphate in the living cell.4 This membrane-anchored second messenger initiates intracellular signaling pathways by recruiting pleckstrin homology domain–containing proteins, including the protein kinases PDK1 and Akt. PDK1 phosphorylates Akt on Thr308, priming the subsequent phosphorylation on Ser473 required for full activation. Akt propagates PI3K-initiated signals by phosphorylating many downstream substrates, including glycogen synthase kinase 3 (GSK3), to initiate pathways whose precise contribution to platelet activation is still largely unclear.

Platelets express all the 4 different isoforms of class I PI3Ks (α, β, δ, and γ), but it is well documented that PI3Kβ, but not other class I isoforms, is essential for platelet activation by collagen,5-7 is a critical regulator of integrin αIIbβ3 outside-in signaling,8-9 and...
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