Specifically, the authors found non-mutated wild-type BRAF in all cases of variant HCL, in all HCL cases expressing IGHV4-34, and surprisingly also in 21% of HCLc, disturbing the uniform picture of BRAF mutations in HCL.[2] In this series, BRAF mutations, which previously were described to be present in all cases of HCLc,[2] were detected in only 79% of HCLc. Xi et al propose that this genetic diversity supports the classification of variant and IGHV4-34$^+$ HCL cases as distinct disease subgroups with a pathogenesis different from HCLc, in terms of clinical presentation, immunophenotypic differences, and inferior responses to standard therapy.

BRAF is a member of the serine–threonine kinase RAF family, and participates in the mitogen-activated protein kinase (MAPK) signaling cascade downstream of RAS signaling proteins, transmitting survival and proliferation signals from cell surface receptors to the nucleus. BRAF mutations initially were discovered as oncogenic events in solid tumors, most notably in melanoma.[3] The mutated BRAF protein containing an amino acid switch at position 600 (V600E) results in the activation of BRAF kinase activity, causing constitutive downstream signaling and cell growth.

The recent initial report about BRAF mutations in HCL, based on whole-exome sequencing of HCL cells, caused great interest and excitement,[2] given that such a long-sought recurrent genetic lesion in HCL had not previously been identified. The high frequency of V600E BRAF mutations in HCLc and lack of such mutations in other B-cell malignancies indicates that this mutation represents a disease-defining mutation in HCL. The initial report about BRAF mutations in HCL based on 48 HCL samples has been extended and corroborated in larger cohorts of patients from different institutions, using allele-specific PCR assays.[4-6]

The findings by Xi et al overall confirm the importance and high prevalence of BRAF mutations in HCLc.[1] It is tempting to speculate that lack of BRAF mutations in a significant proportion of immunophenotype-defined HCLc cases (n = 11) could be related to the higher prevalence of HCLc patients who were on treatment or unresponsive to standard therapy in this series,[1] but as discussed by Xi et al, such HCLc cases with wild-type BRAF clinically do not appear to behave differently compared with BRAF-mutated cases.

Other potential developments of BRAF mutations in HCL are novel diagnostic possibilities and options for therapeutic targeting of BRAF, using BRAF inhibitors. Based on the data by Xi et al, BRAF mutational analysis can help distinguishing HCLc from HCL variant and IGHV4-34$^+$ cases on the molecular level, and therefore such analyses are likely to become part of the diagnostic armamentarium in HCL. The therapeutic potential of targeting BRAF mutations in HCL is more difficult to predict. In vitro responsiveness of BRAF-mutated HCL to the BRAF inhibitor PLX-4720[2] supports further development of BRAF inhibitors in HCL patients.

Despite the current excitement about BRAF in HCL, we need to keep in mind that the importance of BRAF mutations for HCL pathogenesis and disease progression remains ill-defined. The mere presence of V600E BRAF mutations does not indicate that this genetic lesion is critical for the disease process or that BRAF inhibitors will follow the successful path of BCR-ABL kinase inhibitors. The phase 3 experience with the BRAF inhibitor vemurafenib (PLX4032) in previously untreated melanoma demonstrates that this kinase inhibitor improves overall and progression-free survival in patients with BRAF V600E mutation compared with dacarbazine.[7] However, such BRAF inhibitor–induced remissions are typically short-lived because of development of resistance. This resistance to BRAF inhibitors is because of the ability of melanoma cells to flexibly switch their signaling programs among different RAF isoforms,[8] underscoring the complexity and oftentimes redundancy of cell signaling, and the ability of cancer cells to adapt and bypass certain signaling modules that are pharmacologically blocked. In-depth analyses into the function of BRAF in HCL, and clinical trials with BRAF inhibitors such as vemurafenib in HCL will help to better define the role of V600E BRAF mutations in HCL and will strengthen our understanding of the molecular pathogenesis of HCL.

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

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● ● ● PLATELETS & THROMBOPOIESIS

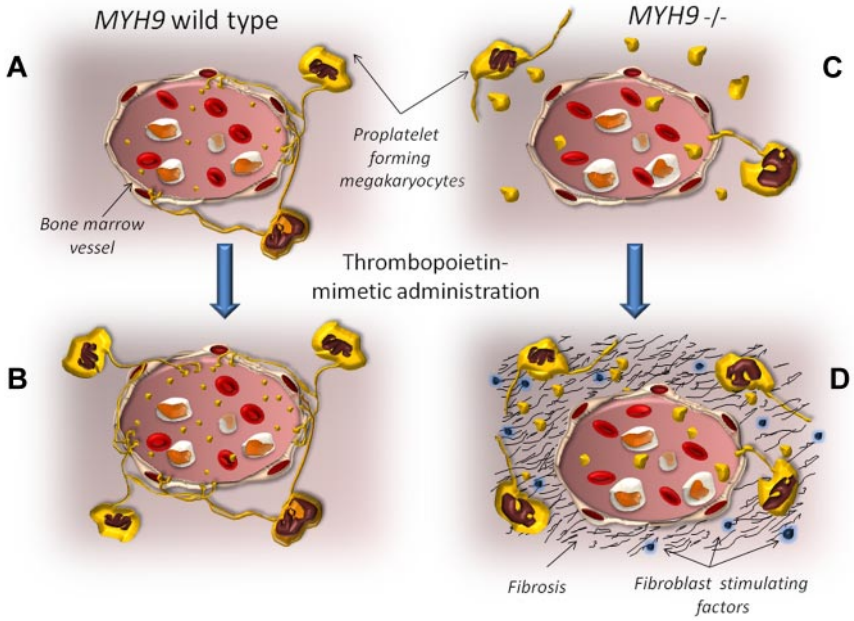
Comment on Léon et al, page 3333

Does "more" necessarily mean "better"?

Luciana Teofili and Luigi Maria Larocca UNIVERSITÀ CATTOLICA DEL SACRO CUORE

In this issue of *Blood*, Léon and colleagues describe the effects of romiplostim, a thrombopoietin (Tpo) mimetic peptide, in the mouse model of inherited platelet dysfunction because of mutation of the myosin 9 gene.[1]

The diagnosis of immune thrombocytopenia (ITP) may sometimes conceal more rare cases of inherited disorders of the platelet function, particularly in patients found to be resistant to steroids or splenectomy. On the whole, these last include a crowded list of dysfunctions, involving platelet surface constituents or intracellular components.[2] Because two Tpo receptor agonists, eltrombopag and romiplostin, have been approved for chronic ITP adult patients unresponsive to glucocorticoids, intravenous immunoglobulin, or splenectomy,[3] their potential use also in inherited thrombocytopenia is attractive.



In normal conditions, proplatelet-forming megakaryocytes release nascent platelet directly in the bloodstream (A). Thrombopoietic stimulating agents expand the megakaryocyte pool and increase platelet production and release (B). The *MYH9*$^{-/-}$ mice replicate the megakaryocyte and platelet abnormalities observed in patients with *MYH9*-related diseases. The proplatelet formation occurs distant from vessels, with consequent platelet release inside the bone marrow environment. Only a few giant platelets are released in the bloodstream (C). When romiplostin is administered to these animals, the *MYH9*$^{-/-}$ megakaryocyte pool expands, and it can be hypothesized that the release of giant platelets inside the bone marrow is boosted. As a result, several growth factors for fibroblasts are secreted from platelets into the environment (including transforming growth factor-β, basic fibroblast growth factor, and platelet-derived growth factor), inducing the progressive bone marrow fibrosis (D).

Léon et al report the effects of romiplostim in the mouse model of inherited platelet dysfunction because of mutation of the myosin 9 gene (*MYH9*).[1] So far, at least 45 mutations of *MYH9* have been described, accounting for an ensemble of autosomal-dominant inherited diseases, grouped as *MYH9*-related diseases (*MYH9*-RD), all characterized by the presence of thrombocytopenia with giant platelets and Döhle body–like inclusions within leukocytes.[4,5] These three alterations, also known as May-Hegglin anomaly, can variably associate with other phenotypic peculiarities, including presenile cataract, proteinuric nephropathy, and progressive sensorineural hearing loss.[4,5] Depending on the entity, the bleeding tendency is extremely variable, ranging from asymptomatic individuals to patients experiencing severe hemorrhages: in the latter, however, no therapy other than platelet transfusion is so far available.

The *MYH9* gene encodes for the isoform A of nonmuscle myosin of class II, one of the myosin superfamily motor protein members. After the hydrolysis of ATP at the catalytic sites, the myosin-II interacts with actin filaments and regulates the cytoskeleton in all eukaryotic cells. Indeed, myosin-II drives several processes requiring energy and motion, such as cell migration, cell adhesion, and differentiation or tissue morphogenesis. In general, most cell types possess more than one single isoform, but cells from granulocytic and megakaryocytic lineages express uniquely the myosin-IIA isoform; myosin-IIA is also abundantly, though not exclusively, expressed in kidney, eye, and cochlea, all organs frequently involved in *MYH9*-RD.

The pathogenesis of thrombocytopenia in *MYH9*-RD is not fully understood. Many evidences suggest that, in these patients, megakaryocytic migration within the bone marrow and proplatelet ability formation are deeply disturbed. Seemingly, myosin-IIA is a key regulator of proplatelet formation, the physiologic mechanism by which megakaryocytes stretch out branching processes along marrow sinusoids and release nascent platelets into the bloodstream. In particular, myosin-IIA inhibits proplatelet formation triggered by type I-collagen, thus preventing the platelet release at a distance from sinusoid vessels. Actually, megakaryocytes isolated from patients with *MYH9*-RD formed proplatelets in vitro even in adhesion to collagen type I, suggesting an ectopic platelet release. Indeed, it is conceivable that *MYH9* mutations result in abnormal platelet production inside the bone marrow, with few giant platelets released into the bloodstream.[5]

Because *MYH9* knockout mice have an early embryonic mortality, Léon and colleagues have used the loxP/Cre recombinase strategy to specifically ablate the MYH9 expression into the megakaryocytic lineage.[6] Like *MYH9*-mutated patients, *MYH9*$^{-/-}$ mice show thrombocytopenia, large and immature platelets, and impaired platelet contractile activity. Léon et al have found that romiplostin administration heightens the platelet count in *MYH9*$^{-/-}$ mice significantly less than in wild-type controls, despite the greater increase of bone marrow megakaryocytes in the former. Moreover, under romiplostin administration, *MYH9*$^{-/-}$ platelet express low levels of glycoprotein VI and glycoprotein Ib-IX-V complexes. Finally, romiplostin administration causes in *MYH9*$^{-/-}$ mice a progressive increase of reticulin fibers in the bone marrow, not observed in wild-type controls.[1]

All these findings suggest that romiplostin, by expanding the *MYH9*$^{-/-}$ megakaryocyte pool, emphasizes the platelet release inside bone marrow, likewise amplifying the secretion of growth factors inducing fibrosis such as platelet-derived growth factor, transforming growth factor-β, and basic fibroblast growth factor. In turn, the increased fiber deposition further stimulates the propletelet formation by megakaryocyes and, in the meanwhile, induces them to release metalloproteases, thus explaining the increased cleavage of adhesive glycoprotein complexes[7] (see figure). The megakaryocytic lineage is crucial in the induction of bone marrow fibrosis and chronic stimulation of megakaryocytopoiesis is a well-known system to induce marrow fibrosis in animal models.[8]

We have recently reported that patients with thrombocytosis because of the inherited *MPL*Ser505Asn activating mutation have not only a significant risk of thrombosis, but also evolve to bone marrow fibrosis. In these patients there is an unequivocal association between fibrosis and aging, with progressive increase of reticulin fibers, which in some middle-age and in most elderly patients appears diffuse and surrounded by focal bundles of collagen.[9] The problem of marrow fibrosis in ITP patients undergoing Tpo mimetic therapy is truly felt, so that monitoring of cell

counts and the peripheral blood smear is recommended. If new morphologic abnormalities or cytopenias are noted or if there is a loss of response to treatment, a bone marrow biopsy with staining for reticulin and collagen should be performed.[10]

A recent Italian trial has first reported that patients with *MYH9* mutations significantly benefit from 6-week eltrombopag treatment, with increase of platelet count and disappearance of bleeding tendency in most of them.[11] Whereas the experiences gathered so far in ITP patients indicate that Tpo-mimetic-induced fibrosis is reversible if drug therapy is discontinued, the irreversibility of marrow fibrosis associated with *MPL*Ser505Asn mutation provides ground for reflection. In addition, data presented here by Léon and colleagues cast doubt that, under prolonged Tpo stimulation, patients with thrombocytopenia sustained by ineffective megakaryocytopoiesis would be predisposed to this complication.

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

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Zamolodchikov and Strickland, page 3342

Aβ and C(lot), but not D(egradation)

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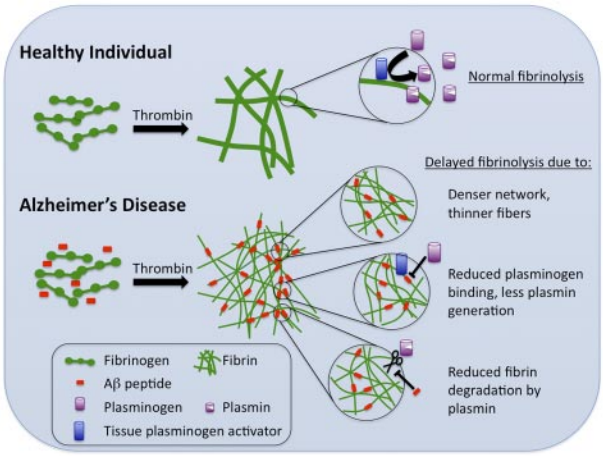
In this issue of *Blood*, Zamolodchikov and Strickland examine the association of β-amyloid (Aβ) peptide with fibrin(ogen).[1] These findings shed new light on the relationship between Alzheimer disease and cardiovascular disease, as well as on a novel biochemical mechanism regulating clot stability and dissolution.



The mechanisms by which Aβ peptide decreases tissue plasminogen activator/plasmin(ogen)-mediated fibrinolysis.

Alzheimer disease (AD) is a neurodegenerative disorder with still undefined etiology. The prevailing hypothesis that suggests Aβ peptide deposition and accumulation into plaques promotes AD is giving way to a more complex picture that also includes vascular dysfunction. Observations that cardiovascular disease (CVD; stroke, cardiac disease, and atherosclerosis) is an established risk factor for AD (reviewed in de la Torre[2]) lend credence to the hypothesis that these diseases share a common etiology.

Indeed, recent work has identified a likely candidate at the nexus of these disease processes: fibrinogen. Fibrinogen is the protein substrate of the multifaceted procoagulant and proinflammatory enzyme thrombin. Fibrin, the product of thrombin's proteolytic cleavage of fibrinogen, provides biophysical and biochemical support to blood clots, and subsequent degradation of fibrin by plasmin is an essential step in wound healing. Abnormalities in fibrin structure and/or dissolution are correlated with bleeding in hemophilia, as well as a host of thrombotic disorders, including myocardial infarction, ischemic stroke, and venous thromboembolism (reviewed in Wolberg[3]). Previous in vitro and in vivo studies from the Strickland laboratory have shown that Aβ mediates the formation of clots that have altered structure and increased resistance to fibrinolysis.[4,5] The co-existence of these 2 features is consistent with prior studies showing that fibrin network structure and fiber diameter directly regulate the rate of fibrinolysis; plasma clots with a dense network of fibers—like those that form in the presence of Aβ—are dissolved at a slower rate than those with an open (coarse) network of fibers.[6] Thus, the mechanism correlating Aβ-induced formation of abnormal fibrin structure with delayed fibrinolysis seemed straightforward, and the story could have ended there.

However, Zamolodchikov and Strickland have now taken this investigation one step further. While confirming that Aβ mediates the formation of clots with abnormal structure and resistance to fibrinolysis, they made the intriguing discovery that the abnormal resistance of these clots to fibrinolysis occurs though mechanisms that are independent of the fiber structure.[1] Using an elegant combination of turbidity measurements, protein binding assays, and confocal microscopy, the authors show that by binding to the fibrin