Different approaches to reactivate fetal γ-globin gene expression through alteration of chromatin looping. (A) Reactivation of fetal γ-globin transcription through the introduction of an artificial transcription factor that tether’s the γ-globin promoter to the LCR as previously described.6 (B) Reactivation of fetal γ-globin transcription through pharmacologic inhibition of H3K9 methyltransferases as described in Krivega et al. Professional illustration by Patrick Lane, ScEYEence Studios.

suggesting that pharmacologic inhibition of G9a could be used to counteract fetal γ-globin silencing.

To determine the phase of erythropoiesis at which inhibition of G9a is most efficient to increase levels of HbF, the authors used a 3-stage ex vivo differentiation system with human CD34+ hematopoietic progenitors from adult donors. They demonstrate that inhibition of G9a/GLP methyltransferase activity with the small molecule inhibitor UNC06389 leads to a pronounced increase in HbF (up to 30% of total hemoglobin) when applied at the time of erythropoietin-mediated induction of erythroid differentiation. This effect is mediated through upregulation of fetal γ-globin and downregulation of adult β-globin expression. At the molecular level, the authors show that the drug leads to a locus-wide decrease in H3K9me2, which is accompanied by complex changes in G9a binding (ie, increased binding at the fetal promoter, decreased binding at the adult promoter, and no change at the LCR). Similarly, they observed a shift in binding of the looping factor LDB1 from the adult to the fetal gene promoters. Finally, they show that the fetal γ-globin gene relocates to achieve closer proximity to the LCR.1

Taken together, these results establish G9a as a major player in the maintenance of γ-globin silencing in adult erythroid cells. Furthermore, it suggests a mechanism whereby the G9a-mediated H3K9me2 mark on the γ-globin promoter prevents spatial proximity with the LCR through inhibiting binding of the “looping factor” LDB1. Testing this model will require additional experiments to determine the sequential order of events following H3K9 methyltransferase inhibition.

Finally, a surprising finding is that G9a remains bound to the β-globin locus upon drug treatment, despite a widespread loss of the H3K9me2 mark. This result is at odds with the current model of G9a spreading on chromatin through its ankyrin domain-mediated recognition of H3K9me2. However, it is consistent with the absence of a visible phenotype in knock-in mice carrying a mutant form of G9a that is unable to bind to H3K9me2.10 Although these results converge to suggest that the interaction of G9a with its substrate does not play a dominant role in the maintenance of G9a binding to chromatin in vivo, we cannot exclude the possibility that the interaction with H3K9me2 is important for the initial establishment of G9a binding (ie, spreading). A candidate factor for retaining G9a binding to the β-globin locus in the absence of H3K9me2 is G9a-heterodimerization partner GLP, whose H3K9me1 binding activity appears to play a dominant role in vivo.10 In that regard, it will be interesting to analyze GLP binding and H3K9me1 enrichment on the β-globin locus upon inhibition of H3K9 methyltransferase activity.

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

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THROMBOSIS AND HEMOSTASIS

Comment on Mammadova-Bach et al, page 683

Polymerized fibrin activates glycoprotein VI

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In this issue of Blood, Mammadova-Bach et al show that polymerized fibrin binds to the platelet receptor glycoprotein VI (GPVI), amplifying thrombin generation and enhancing thrombus growth.1

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A large body of evidence shows that GPVI, a 58-kDa transmembrane protein in platelets, is the major receptor for collagen, and its interaction with type I and III fibrillar collagens leads to stable platelet adhesion and thrombus growth. Platelet adhesion is initiated at the site of vascular injury by the interaction of von Willebrand factor with the GPIb-V-IX complex on the platelet surface, which captures circulating platelets in high fluid shear to the site of injury. Transient platelet adhesion facilitates engagement of α2β1 and GPVI with exposed collagen in the wound site and these interactions lead to stable platelet adhesion. Interaction of GPVI with collagen activates the adherent platelets, triggers secretion, exposes procoagulant phospholipid on the platelet surface, and recruits more circulating platelets to the thrombus.

Despite these important functions of GPVI in initiating primary hemostasis, individuals with congenital GPVI deficiency only show very mild bleeding tendency, which is explained in part by the presence and compensatory function of other collagen receptors on platelets. Consistent with the mild phenotype observed in humans, GPVI knockout mice also show only slightly prolonged bleeding times but no overt bleeding. Surprisingly, GPVI-deficient mice are protected from experimental thrombosis induced by either mechanical arterial injury or exposure to ferric chloride or in a model of induced thrombosis. Furthermore, GPVI deficiency is also protective of acute thrombosis induced by atherosclerotic plaque rupture in ApoE-deficient mice. These protective effects are ascribed to reduced thrombus growth and thrombin generation. These unexpected results also question the role of collagen as an agonist for GPVI because there is no fibrillar collagen in a growing thrombus. If not fibrillar collagen, what is the physiologic agonist for GPVI in a growing thrombus? Despite this gap in knowledge, beneficial antithrombotic effects and low risk of bleeding suggest that inhibiting GPVI would be a promising approach in treating thrombosis. Thus, reagents that target the collagen binding site in GPVI and anti-GPVI antibodies have been developed and these reagents show efficacy in treating thrombosis in clinical studies. However, the long-sought-after physiologic agonist for GPVI has not been identified.

In this issue, Mammadova-Bach et al provide evidence that the physiologic ligand for GPVI in the platelet thrombus is polymerized fibrin. The GPVI-fibrin interaction promotes thrombus growth by 2 reciprocal amplification loops (see figure): (1) by exposing procoagulant phospholipid on the platelet surface, enhancing assembly of the tenase and prothrombinase complexes, promoting thrombin generation, and increasing fibrin deposition; and (2) by triggering platelet secretion and recruiting additional circulating platelets to the growing thrombus. Using a purified system of washed platelets and coagulation factors, these investigators show that thrombin generation is dependent on platelets and is significantly inhibited by GPVI blockade. Using normal and afibrinogenemic plasma, they show thrombin generation depends on fibrinogen, and is inhibited by the Gly-Pro-Arg-Pro peptide, a specific inhibitor of fibrin polymerization. Additional experiments confirm that GPVI binds only polymerized fibrin and that the antibody Fab 9O12 used in GPVI blockade also inhibits the GPVI-fibrin interaction. Using normal and GPVI-deficient platelets, and GPVI-blocking antibodies, these investigators further show that the GPVI-fibrin interaction leads to platelet spreading and stable activation, accompanied by phosphatidyserine exposure and recruitment of circulating platelets to the fibrin–platelet clot. The ability of GPVI to bind polymerized fibrin and not fibrinogen at high wall-shear rates distinguishes this interaction from that of integrin αIIbβ3 with fibrinogen. Simultaneous blockade of αIIbβ3 with abciximab and GPVI with Fab 9O12 reduces thrombin generation additively, suggesting that both of these receptors contribute to thrombus growth but by independent and complementary mechanisms. By identifying polymerized fibrin as the long-sought-after physiologic ligand for GPVI, these investigators establish a novel mechanism for the function of GPVI. These new insights show GPVI at the intersection between primary hemostasis and the coagulation cascade, and provide the basis for the design of safe and effective antithrombotic drugs.

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Polymerized fibrin activates glycoprotein VI

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