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Lucky 13

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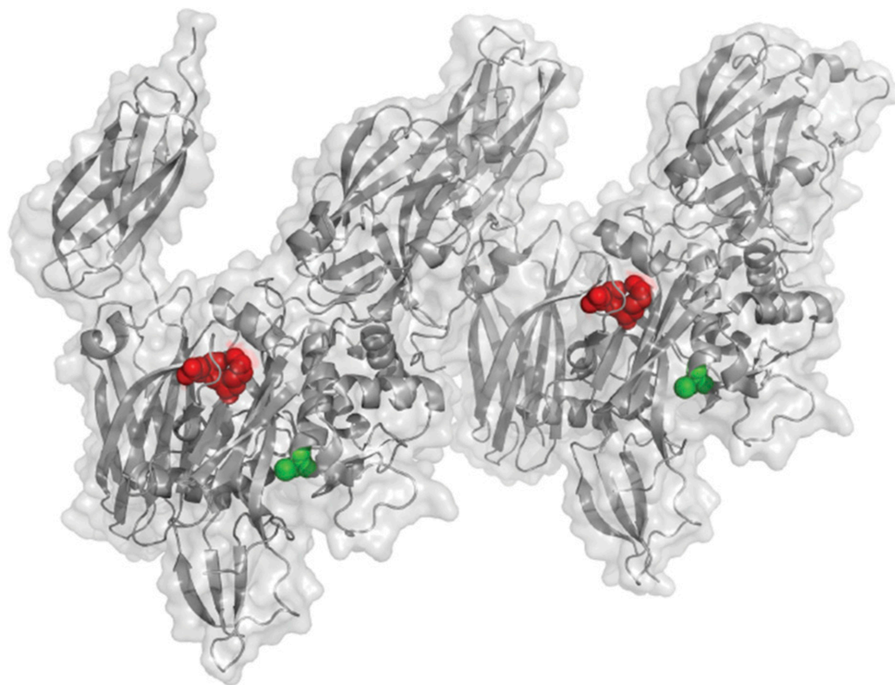
In this issue of *Blood*, Hur et al[1] hit the jackpot with their discovery that plasmin inactivates activated blood coagulation factor XIIIa (FXIIIa), but not zymogen factor XIII (FXIII), and that FXIIIa is inactivated during clot lysis, but not during clot formation. Their study resolves years of conflicting results regarding the inactivation of FXIIIa by plasmin.

FXIII, known previously as "fibrin stabilizing factor" and "Laki-Lóránd factor," is unique among all the coagulation factors.[2] In addition to its distinction of having the last Roman numeral of the numbered coagulation factors and being the last to be activated, it is the only zymogen transglutaminase in the blood coagulation cascade. Most of the other named coagulation factors are either serine protease zymogens or zymogen cofactors, and none of them have transglutaminase activity. FXIII circulates in 2 forms, a plasma form and a platelet form.[3] The plasma form is a heterotetramer of 320 kDa that consists of 2 83-kDa A subunits and 2 77-kDa B subunits that circulates at concentrations of about 10 μg/mL. The A subunits are the transglutaminase zymogens. In contrast, the platelet form of FXIII consists only of dimeric A subunits and is stored in platelet α granules until the platelets are activated.

FXIII zymogen is activated via a single proteolytic cleavage by thrombin at the R37-G38 bond, releasing a 4-kDa activation peptide and converting FXIII into FXIIIa.[4] In the plasma form, the B subunits subsequently dissociate, resulting in an activated A_2 dimer, termed A_2*. In platelet FXIII, the A_2 dimer is activated by thrombin after its release from the platelet.



This projection, based on a previously solved structure of the activated FXIIIa A_2 dimer,[8] shows the K468-Q469 plasmin cleavage site in red and its proximity to the active-site cysteine in green. This site is cleaved only in activated FXIIIa, not FXIII zymogen, and this cleavage event inactivates FXIIIa. See Figure 2B in the article by Hur et al that begins on page 2329.

In both forms, the active A_2* dimer covalently crosslinks specific substrate proteins, particularly fibrin.[5] This crosslinking is achieved by an unusual reaction in which glutamine side chains in substrate proteins are crosslinked with closely apposed lysine side chains that have the appropriate spatial geometry.[2] The glutamine and lysine side chains can either be in the same protein or in separate proteins, resulting in covalent isopeptide bonds consisting of γ-glutamyl-ε-lysine crosslinks. For example, fibrin strands can be covalently crosslinked together, resulting in fibrinolytic resistance. In addition, the fibrinolytic inhibitor α_2-antiplasmin can be covalently crosslinked to fibrin, which also results in fibrinolytic resistance.[6] The well-known D-dimers that are used clinically to assess a variety of coagulation pathophysiologies have their origin in the FXIIIa-mediated crosslinking of fibrin strands. FXIII is required for optimal fibrin strength and fibrinolytic resistance; deficiencies can result in a lifelong bleeding diathesis, impaired wound healing, and chronic miscarriage.[3]

The regulation of activated FXIIIa has remained an enigma for many years.[7] Unlike the activated serine protease coagulation factors, which are typically inactivated by serpins such as antithrombin, there are no accessory proteins that combine with FXIIIa to inactivate its active site cysteine. Previous studies had suggested that plasmin, which is activated during fibrinolysis, inactivates FXIIIa by proteolysis, somewhat reminiscent of factors Va and VIIIa inactivation by activated protein C. The study by Hur et al[1] clarifies many aspects of this proteolytic inactivation of FXIIIa by plasmin. Their findings show that plasmin cleaves the K468-Q469 bond in active FXIIIa, but not in the FXIII zymogen (see figure[8]). This cleavage event inactivates FXIIIa, preventing further crosslinking of its substrates. Additional cleavage sites were also observed, but their resulting peptide fragments were detected 8 to 38 times less frequently than the K468-Q469 cleavage. These results have clinical implications in the use of tissue plasminogen activator (tPA) for thrombolysis during heart attack and stroke. The authors propose that inactivation of FXIIIa by plasmin due to tPA

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administration may contribute to the hemorrhagic side effects of tPA. It is tempting to speculate that administration of FXIII[9] might decrease tPA-induced hemorrhage by restoring FXIIIa that has been inactivated by plasmin, but further studies are necessary to test this hypothesis.

Conflict-of-interest disclosure: Oregon Health & Science University and D.H.F. have a significant interest in Gamma Therapeutics, a company that may have a commercial interest in the results of this research and technology. This potential institutional and individual conflict of interest has been reviewed and managed by Oregon Health & Science University. ■

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● ● ● THROMBOSIS AND HEMOSTASIS

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Free hemoglobin: a boost to platelet thrombi

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In this issue of *Blood*, Da et al report that, in a high shear stress model, free hemoglobin (Hb) binding to von Willebrand factor (VWF) elicits the formation of platelet aggregates on surfaces coated with fibrinogen. This defines a novel paradigm for understanding the development of thrombosis in mechanical circulatory support devices.[1]

Mechanical circulatory support devices may contribute to the development of thrombosis not only by contact of their artificial surfaces with blood but also through hydrodynamic forces. This high shear stress induces the transition of VWF from a globular state to an extended chain conformation,[2] potentially facilitating its interaction with platelets. Hydrodynamic forces may also indirectly contribute to the development of thrombosis through the destruction of red blood cells. Hemolysis is indeed often associated with platelet activation[3] and several mechanisms involving free Hb may account for this, including direct platelet activation by Hb, the production of reactive oxygen species, and the scavenging of nitric oxide.[4,5]

Using in vitro conditions representative of those occurring on the surface of extracorporeal membrane oxygenation devices, Da et al[1] now demonstrate that free Hb binds directly to VWF, thereby increasing the affinity of the VWF A1 domain for the glycoprotein Ib (GPIb) receptor on the surface of platelets (see figure). The increased interaction between platelets and VWF then allows a massive binding of platelets to insolubilized fibrinogen or to components of the extracellular matrix such as collagen.[1] This qualitative and quantitative alteration of platelet binding and aggregation induced by free Hb may contribute to the development of thrombi in circulatory devices despite strong anticoagulation with heparin.

The role of VWF in thrombosis in mechanical circulatory support devices may appear paradoxical. Indeed, VWF is secreted by endothelial cells into plasma as a large multimeric glycoprotein and the size of the multimers determines their ability to mediate platelet aggregation. However, in mechanical circulatory support devices, the hydrodynamic forces induce the loss of the high molecular weight multimers of VWF, a so-called acquired von Willebrand syndrome, predisposing patients to bleeding complications.[6,7] It remains to be determined whether the interaction of free Hb with low and intermediate molecular weight multimers increases VWF activity, which might reduce the risk of bleeding yet promote the development of thrombosis in circulatory support devices. Alternatively, the interaction of free Hb with VWF may contribute to the disappearance of the largest multimers trapped in platelet aggregates.

The study of Da et al was carried out under experimental conditions representative of the circulation in mechanical circulatory support devices. However, it is tempting to speculate that the modulation of platelet interaction with VWF that is mediated by free Hb may also play a role in other clinical situations. Microangiopathic hemolytic anemia of diverse origins is characterized by thrombosis in small vessels, which results in the destruction of red blood cells and increased free Hb levels. In such diseases, free Hb may thus contribute to a vicious circle, accelerating the formation of platelet aggregates and the subsequent clinical thrombotic and hemorrhagic complications. Such a mechanism may play an even more important role in cases of thrombotic thrombocytopenic purpura, a disease caused by a defect in the cleavage of ultralarge VWF molecules by the VWF protease a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13).[8] It has been previously reported that free Hb can inhibit the cleavage of ultralarge VWF strings by ADAMTS13,[9] so it will be interesting to determine whether free Hb also significantly

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