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Fibrinogen and factor XIII in venous thrombosis and thrombus stability

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Abstract

As the third most common vascular disease, venous thromboembolism is associated with significant mortality and morbidity. Pathogenesis underlying venous thrombosis is still not fully understood. Accumulating data suggest fibrin network structure and factor XIII-mediated crosslinking are major determinants of venous thrombus mass, composition, and stability. Understanding the cellular and molecular mechanisms mediating fibrin(ogen) and factor XIII production and function and their ability to influence venous thrombogenesis and resolution may inspire new anticoagulant strategies that target these proteins to reduce or prevent venous thrombosis in certain at-risk patients. This article summarizes fibrinogen and factor XIII biology and current knowledge of their function during venous thromboembolism.

Graphical Abstract

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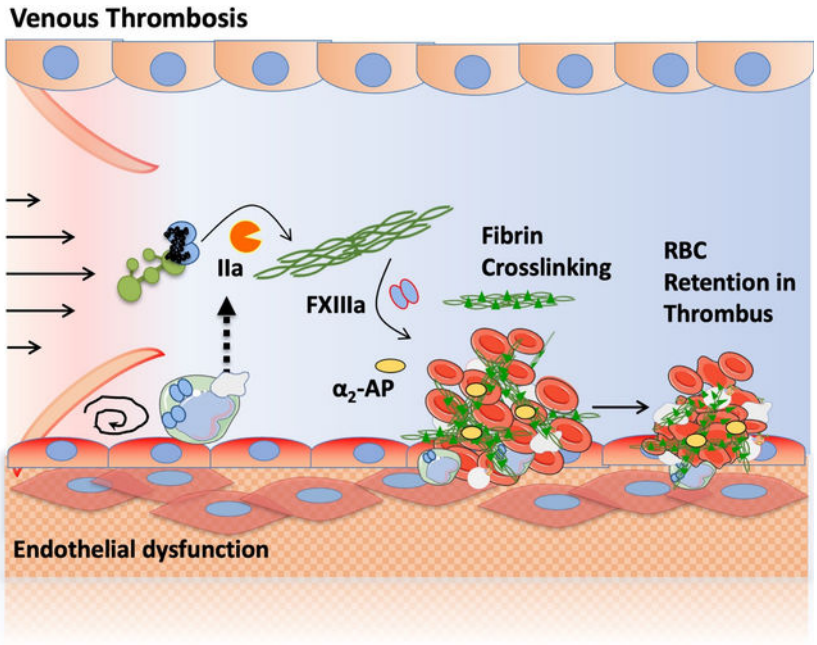

Keywords

Fibrinogen; factor XIII; venous thrombosis; red blood cell; hemostasis

INTRODUCTION

Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and/or pulmonary embolism (PE), affects 1–2 individuals per 1000 each year globally, with relatively higher incidence in North America and Europe than in Asia.[1,2] VTE is the third most common vascular disease after acute myocardial infarction and stroke, and is associated with high mortality and morbidity.[3,4] Up to half of patients with a DVT develop post-thrombotic syndrome and up to 4% patients with a PE develop chronic thromboembolic pulmonary hypertension, which reduce quality of life and result in a substantial economic health-care burden.[5–8]

Several classes of anticoagulants are currently used to prevent VTE or reduce thrombus extension, including indirect thrombin/factor [F] Xa inhibitors, vitamin K antagonist, and direct thrombin/FXa inhibitors. Heparin binds to antithrombin and accelerates the antithrombin-dependent inactivation of several coagulation proteases (e.g., thrombin, FXa, and FIXa).[9] The oral vitamin K antagonist warfarin, first approved in the 1950s, is used for treatment or secondary prevention of VTE and stroke in patients with atrial fibrillation.[10] Warfarin blocks the vitamin K epoxide reductase and therefore, formation of vitamin K_1 and vitamin KH_2, which are essential for γ-carboxylation of vitamin K-dependent proteins including FVII, FIX, FX, and prothrombin, and anticoagulant proteins C and S. Several small molecule inhibitors arrived on the market in the early 21st century; these direct oral anticoagulants inhibit FXa or thrombin and have been increasingly used for prevention or treatment of VTE and stroke because of their superior benefit-to-risk ratio compared to

heparin or warfarin.[11] Clinical observations of individuals with FXII or FXI deficiency and experience with animal models of these deficiencies have sparked interest in FXII or FXI(a) inhibition as potentially safer anticoagulation strategies, and several FXI(a) inhibitors are in clinical development.[12–16] By reducing the production or activity of procoagulant proteins, all of these anticoagulants can reduce thrombus extension and venous thrombosis recurrence. However, these drugs are also associated with bleeding risk, likely because they each reduce thrombin-mediated fibrin formation. This limitation has fueled a continued search for new effective drugs with improved safety profiles.

Factor XIII (FXIII) functions in the final step of the coagulation cascade, where its activated form FXIIIa catalyzes the formation of crosslinks within fibrin fibers to stabilize the clot. The use of new mouse models and development of novel technologies, including intravital microscopy to visualize blood cells, fibrin, and FXIII during thrombus formation in vivo, have revealed newly appreciated roles of fibrinogen and fibrin (collectively “fibrin[ogen]”) and FXIIIa-mediated crosslinking in venous thrombus structure, stability, composition, and mass. These findings suggest fibrin(ogen) and FXIII(a) might be effective targets for reducing venous thrombosis in certain situations.

FIBRIN(OGEN) AND FXIII IN HEMOSTASIS

Fibrin(ogen)

Fibrinogen structure and function.—Fibrinogen is one of the most abundant plasma proteins (2–4 mg/mL, 6–12 μM) and the most abundant circulating coagulation protein. Fibrinogen is expressed constitutively, but its expression can be upregulated 2–3-fold above baseline in response to inflammation.[17] Fibrinogen circulates as a large (340 kDa) hexameric glycoprotein consisting of 2 each of 3 polypeptides: 2 Aα-, 2 Bβ-, and 2 γ-chains $(A\alpha B\beta\gamma)_2$.[18] Alternative splicing within the fibrinogen γ-chain leads to a subset of molecules containing one γ’-chain (~8–15% of total circulating fibrinogen). The fibrinogen chains are synthesized and assembled in hepatocytes and the fully-formed fibrinogen hexamer is secreted into the blood (Figure 1A).[19] Following vascular injury, activation of the coagulation cascade leads to production of thrombin. Thrombin proteolytically cleaves fibrinogen, which releases N-terminal fibrinopeptides from the Aα- and Bβ-chains in the central E region to generate fibrin monomers. The newly exposed “knobs” on the α- and β-chains can then insert into “pockets” in the C-terminal globular γC and βC regions of the D domain of another fibrin molecule, enabling formation of fibrin oligomers and protofibrils. Through subsequent lateral aggregation and branching events, the half-staggered, double-stranded protofibrils assemble into fiber polymers, and ultimately produce an insoluble 3-dimensional fibrin network.[20] Several molecules and environmental conditions influence clot structure. In particular, thrombin has a profound effect on fibrin structure; low thrombin concentrations generate thick fibrin fibers in coarse and permeable networks that are susceptible to fibrinolysis, whereas high thrombin concentration produce thin fibrin fibers in densely-packed networks that are less permeable and resistant to fibrinolysis.[21]

Fibrin(ogen) interaction with plasma proteins and cells.—Fibrin(ogen) provides binding sites for plasma proteins involved in clot formation (thrombin), stabilization

(FXIII), and lysis (tissue-type plasminogen activator [tPA], plasmin[ogen], α_2-antiplasmin, plasminogen activator inhibitor-2), and other proteins. Fibrin(ogen) also interacts with receptors on multiple cell types: αMβ2 and αXβ2 on monocytes;[22–24] αIIbβ3 and glycoprotein VI (GPVI) on platelets;[25,26] αvβ3, α5β1, intercellular adhesion molecule-1, and VE-cadherin on endothelial cells;[27–30] and αvβ3 on fibroblasts[31]. These interactions mediate the diverse roles of fibrin(ogen) in hemostasis, immunity, inflammation, and infection.[32] For example, fibrin(ogen) binding to monocytes enhances monocyte activation.[23] Interaction of extravascular fibrin(ogen) with the toll-like receptor-4 on macrophages stimulates chemokine secretion and promotes an immune response.[33] Fibrin(ogen) binding to the activated platelet integrin receptor αIIbβ3 serves as a bridge that mediates platelet aggregation, clot contraction, and thrombus consolidation. Fibrin is also a ligand for platelet collagen receptor GPVI and this interaction is associated with phosphatidylserine exposure.[26] Fibrinogen can also increase the permeability of cultured endothelial cells and may contribute to microvascular leakage in cardiovascular disease.[34] Assembly of fibrin(ogen) on leukocytes and endothelial cells stabilize leukocyte attachment and migration to endothelium.[35] Binding and incorporation of plasma proteins and blood cells into the nascent fibrin network can alter protofibril formation and polymerization, network density, pore size/clot permeability, elasticity, and the rate of fibrinolysis, with direct consequences for the clot's mechanical and fibrinolytic properties.[20,36]

Quantitative and qualitative fibrin(ogen) defects.—Fibrinogen disorders can be congenital or acquired and are classified as quantitative deficiencies marked by no or low plasma fibrinogen (afibrinogenemia or hypofibrinogenemia, respectively), or qualitative abnormalities associated with abnormal function of fibrinogen molecules present at either normal or low levels (dysfibrinogenemia or hypodysfibrinogenemia, respectively). Congenital fibrinogen disorders are typically caused by mutations within the fibrinogen structural genes *FGA*, *FGB,* and *FGG*. Afibrinogenemia is relatively rare and occurs in ~1 in 1 million individuals, whereas hypofibrinogenemia and dysfibrinogenemia are more common. Acquired fibrinogen disorders are usually caused by clinical situations that alter synthesis (e.g., liver disease), increase consumption (e.g., cancer, sepsis with disseminated intravascular coagulation), alter plasma concentration (e.g., hemodilution during transfusion), or promote autoantibody formation (e.g., myeloma, autoimmune disease, or drug-induced). Combinations of these mechanisms (e.g., blood loss, consumption, hemodilution, and hyperfibrinolysis) frequently occur in trauma and contribute to poor outcomes.[37–39] The phenotype of individuals with fibrinogen deficiency is highly variable and can be asymptomatic or can be associated with increased bleeding and/or thrombosis. A more detailed description of fibrinogen deficiencies can be found elsewhere.[40–42]

FXIII

Structure and activation of plasma FXIII.

FXIII, known as "fibrin stabilizing factor," is a member of a family of nine transglutaminase proteins. FXIII is the only member of this family that is present in plasma, as well as cells. Plasma FXIII consists of a dimer of catalytic A-subunits (FXIII-A_2) and a dimer

of carrier/inhibitory B-subunits (FXIII-B_2) that circulate as a heterotetrameric complex (FXIII-A_2B_2, 320 kDa, 14–28 μg/mL). Each A-subunit is an ~83 kDa molecule with 731 amino acids arranged in four structural domains: an activation peptide (AP-FXIII [residues 1–37]), a β-sandwich domain (residues 38–184), a catalytic core domain (residues 185–515), and two β-barrel domains (residues 516–628 and 629–731). Each B-subunit is an ~80 kDa molecule consisting of 641 amino acids assembled in ten sushi domains, each held together by two internal disulfide bonds.[43] The FXIII A- and B-subunits are synthesized in different tissues; the A-subunits are produced in hematopoietic cells thought to be resident tissue macrophages in the aorta,[44] whereas the B-subunits are synthesized in hepatocytes (Figure 1A).[45] Assembly of the FXIII-A_2B_2 heterotetramer occurs in the plasma (Figure 1A). The equilibrium dissociation constant (K_D) for the interaction between FXIII-A_2 and FXIII-B_2 subunits is ~10^{-10} M, such that 99% of FXIII-A_2 circulates in the FXIII-A_2B_2 complex.[46] The B-subunits stabilize the A-subunits by preventing spontaneous activation, and are essential for maintaining plasma FXIII levels.[47,48] Although early studies suggested FXIII-A_2B_2 circulates bound to the alternatively-spliced γ' sequence in fibrinogen, more recent studies showed the FXIII-B subunits mediate binding of FXIII-A_2B_2 to fibrinogen residues γ390–396 in both humans and mice.[49] In addition to residues γ390–396, fibrinogen residues α371–425, and particularly αGlu396 within the αC domain, have also been implicated in FXIII binding and activation.[50,51] FXIII-B_2 circulates in a ~2-fold molar excess relative to FXIII-A_2. Essentially all FXIII-A_2B_2 and FXIII-B_2 circulate bound to fibrinogen residues γ390–396.[49] A small pool of FXIII-A_2B_2 is found in platelet α-granules, likely endocytosed with circulating fibrinogen.[52]

In concert with fibrin formation, thrombin proteolytically activates FXIII by thrombin-mediated cleavage of the Arg37-Gly38 peptide bond and dissociation of the N-terminal activation peptides. This process is accelerated by the presence of polymerized fibrin.[53] Activation peptide release is followed by calcium-mediated dissociation of B-subunits from the A-subunits and exposure of the active site cysteine.

Structure, activation, and activity of cellular FXIII.

Cellular FXIII consists of only FXIII A-subunits (cFXIIIA, FXIII-A_2). FXIII-A_2 is present in cells of bone marrow origin and mesenchymal lineage, including osteoblasts and chondrocytes,[54,55] monocytes/macrophages,[56–58] and megakaryocytes and platelets[59–61]. Cellular FXIII-A is activated nonproteolytically by increased intracellular Ca^{2+}, can be exposed on the membrane surface[62,63], and is involved in multiple cellular functions.[55] Almost half of circulating FXIII-A is present in platelets[64], and FXIII-A is one of the most prevalent platelet proteins (~83,000 copies per platelet)[65]. Unlike most coagulation proteins in platelets that are located in the α-granules, platelet FXIII-A is localized in the cytoplasm (Figure 2).[61,66] FXIII-A exposure on platelets requires stimulation by strong dual agonists (e.g., convulxin plus thrombin or thrombin receptor activation peptide), but mechanisms regulating the exposure and release of FXIII-A from platelets, as well as other cells, are unclear. Two populations of FXIII-exposing platelets are formed after strong stimulation: ballooned procoagulant (phosphatidylserine-exposing) platelets with FXIII-A on a protruding "cap," and spread platelets with FXIII-A in a diffuse distribution (Figure 2).[67,68] FXIII-A is also exposed on extracellular vesicles released from GPVI and

protease-activated receptor (PAR)-activated platelets.[69] Platelet FXIII-A can be activated by calpain (Ca^{2+}-dependent cysteine proteinase) in purified systems.[70] However, during platelet activation, elevated intracellular Ca^{2+} induces a conformational change in FXIII-A_2 that produces enzymatically active FXIII without proteolytic removal of the activation peptide (FXIII-A°).[63,71] After exposure and/or release, FXIII-A° can be proteolytically cleaved by thrombin to produce activated FXIII-A* (FXIIIa). Proteolytically activated FXIII-A* has higher conformational flexibility and increased affinity toward glutamine substrates, suggesting activation peptide removal might make FXIII-A* more accessible to substrates.[72,73] In activated platelets, nonproteolytically activated FXIII-A° can crosslink cytoskeletal proteins including myosin, actin, filamin, and vinculin. These events occur in later stages of platelet activation and may contribute to cytoskeletal remodeling and certain phases of platelet spreading (Figure 2).[74–76] Although some studies suggested platelet FXIII-A is required for platelet contraction,[77,78] others have seen little or no difference in the ability of clots to contract in the absence of FXIII activity.[79–82]

FXIII(a) function in hemostasis.

Plasma FXIIIa catalyzes the formation of covalent ε-(γ-glutamyl)-lysine isopeptide bonds between glutamine and lysine residues. FXIIIa is a relatively promiscuous enzyme, and proteomic analysis has identified almost 150 FXIIIa substrates in plasma, including 48 that may be incorporated into the insoluble fibrin clot during coagulation.[83] Plasma and cellular substrates for FXIIIa and their related crosslinking sites are reviewed elsewhere.[84,85] Nonetheless, the primary physiological function of plasma FXIII(a) is well-established: 1) crosslinking fibrin γ- and α-chains into γ-chain dimers, α-chain polymers, and γ-α species to increase clot mechanical stability; and 2) crosslinking antifibrinolytic proteins (e.g., $α_2$-antiplasmin) to fibrin(ogen) to protect clots against biochemical degradation by the fibrinolytic system (Figure 1B). Fibrin γ-chain dimer formation is a fast process that results from reciprocal intermolecular bond formation between the γ406 lysine of one γ-chain and a γ398/399 glutamine residue of another γ-chain. Crosslinking of α-chains is slower than γ-chain crosslinking and involves multiple glutamine and lysine residues.[86] Crosslinking of $α_2$-antiplasmin to fibrin (primarily at α-chain residue Lys303) lags slightly behind fibrin γ-dimer formation but precedes fibrin α-chain polymer formation.[55,87,88] Platelet FXIII-A may also have antifibrinolytic activity by crosslinking $α_2$-antiplasmin to fibrin(ogen), but this effect is only significant when plasma FXIII is below 20%.[68] Similarly, FXIII(a) exposed on interleukin (IL)-4- and IL-10-stimulated monocytes may also stabilize thrombi against fibrinolytic degradation in settings where plasma FXIII concentrations are low.[89]

FXIII deficiency.

FXIII deficiency is a rare disorder, affecting ~1 in 2–3 million people. Congenital deficiency and FXIII activity below 3% is often identified by delayed umbilical cord bleeding and is associated with severe, life-long bleeding tendency, including intracranial bleeding in ~30% of patients.[90] FXIII deficiency is also associated with abnormal wound healing and spontaneous miscarriage.[90–93] Congenital FXIII deficiency can arise from mutations in genes encoding either the FXIII-A subunit (*F13a1*, type 2) or the FXIII-B subunit (*F13b*, type 1), although defects in FXIII-A account for 95% of FXIII-related bleeding disorders.[91,93] Bleeding in FXIII-B deficiency is generally milder than that seen

in FXIII-A-deficient patients; this has been attributed to low residual levels of plasma FXIII-A_2 in FXIII-B-deficient individuals, or to antifibrinolytic activity of platelet FXIII-A which may compensate for the loss of plasma FXIII-A_2B_2.[90,94] Acquired FXIII is more common than congenital deficiency, and can be caused by autoimmune disease, consumption (e.g., surgery, infection, inflammatory bowel disease, thrombosis), reduced synthesis (e.g., liver disease, leukemia, medication-related) and/or hemodilution. Since conventional coagulation tests available in most clinical settings are not sensitive to FXIII, FXIII deficiency is difficult to identify and may be underdiagnosed.[95–97] Congenital FXIII deficiency is treated with plasma-derived FXIII-A_2B_2 (in patients with either A- or B-subunit deficiency) or recombinant FXIII-A_2 (in patients with genetically-confirmed A-subunit deficiency). Patients with acquired FXIII deficiency associated with autoantibody development are treated with immunosuppressive agents combined with cryoprecipitate and/or FXIII concentrate.[98–100]

FIBRIN(OGEN) AND FXIII IN VENOUS THROMBOSIS

Pathophysiologic mechanisms in VTE.

VTE pathophysiology is usually described as the intersection of three major abnormalities (venous stasis, vascular dysfunction/injury, and blood hypercoagulability) known as Virchow's Triad. In this conceptual model, reduced (stasis) or turbulent (nonlaminar) flow of blood around the venous valve pocket creates a hypoxic environment that activates endothelial cells and leads to abnormal expression of adhesion molecules that bind and retain leukocytes and platelets at the endothelial surface.[101] Activated leukocytes, and potentially also the dysfunctional endothelial cells themselves, express tissue factor, triggering the coagulation cascade. Leukocytes, red blood cells (RBCs) and platelets that accumulate in the valve pockets promote thrombin generation and ultimately, the formation of a thrombus rich in RBCs and fibrin (Figure 1C). Venous thrombi can occlude venous flow and/or dislodge and migrate through the heart to the pulmonary vasculature.

Epidemiologic data associating changes in fibrin(ogen) and FXIII with VTE.

Previous reviews have summarized epidemiologic studies investigating relationships between fibrin(ogen) and FXIII in venous thrombosis[102–104] and are only summarized here. Briefly, elevated fibrinogen is associated with increased risk of venous thrombosis. Risk persists even after adjusting for potential confounded effects of ongoing inflammation,[102] but is complicated by the relative presence of the alternatively-spliced fibrinogen γ'-chain, which offers protection against VTE.[104] Similar discord in the literature investigating the role of FXIII in thrombosis has arisen through complexities in interpreting FXIII activity assays, complex relationships between FXIII and fibrinogen levels, polymorphisms that alter FXIII function, and potential sex-specific effects.[103] However, together the findings suggest FXIII antigen, activity, and/or genotype influence thrombosis risk in certain populations and clinical situations. For example, meta-analyses show the common FXIII Val34Leu polymorphism (rs5985) protects against VTE.[105] Presence of this polymorphism leads to faster activation by thrombin, and therefore, faster fibrin crosslinking.[106] Interestingly, the functional impact of the FXIII Val34Leu polymorphism is manifested through a "gene-environment interaction" in which homozygous presence of the Leu34 allele promotes

the formation of clots with thicker fibers and more permeable clots when fibrinogen concentrations are high, but formation of thinner fibers and denser networks when fibrinogen concentrations are low.[107] Collectively, these studies provide strong support for the premise that both fibrin(ogen) and FXIII are major contributors to VTE.

Fibrin and FXIIIa as determinants of venous thrombus formation and composition.

Conventionally, venous clots are referred as “red” RBC- and fibrin-rich thrombi, and their fibrin content has been used as an imaging target.[108] Platelets also contribute to both thrombus initiation[109,110] and composition. Scanning and transmission electron microscopy of thrombi retrieved from patients as well as contracted whole blood clots formed *in vitro*, reveal the presence of closely packed, distorted RBCs (termed “polyhedrocytes”) in clot core.[111] Platelet-mediated clot contraction generates the force required to compress these resident RBCs into polyhedrocytes. RBCs in circulation or in the thrombus may contribute mechanistically to VTE by interacting with other cells, supporting thrombin generation, altering fibrin structure, and/or slowing the diffusion of lytic enzyme into the clot and enhancing clot resistance to fibrinolysis (reviewed in [112]).

In experimental models of venous thrombosis, elevated fibrinogen shortens the time to vessel occlusion, increases fibrin deposition within thrombi, and increases fibrin stability.[113] Clots with increased fibrin network density, including those formed in the presence of high tissue factor concentrations, retain higher numbers of RBCs.[114] Notably however, FXIII(a) crosslinking of fibrin promotes RBC retention in clots independent of fibrin network density.[81,114,115] Following inferior vena cava ligation, FXIII-deficient mice (*F13a1*$^{-/-}$) produce thrombi with decreased RBC content and consequently, reduced mass than wild-type mice.[81,115] Similarly, human whole blood clots retain fewer RBCs in the absence of FXIII.[115] Experiments with FXIIIa inhibitors and recombinant fibrinogen variants associated this effect with the production of α-chain-rich high molecular weight crosslinks.[114] Subsequent studies suggested these crosslinks are produced primarily by plasma, but not platelet, FXIII.[81] The ability of clots to retain RBCs depends not only on the presence of FXIII, but also on the timing of its activation. Studies using a FXIIIa-sensitive near-infrared fluorescence imaging agent (A15 peptide) showed significant crosslinking in acute thrombi, but less incorporation in aged thrombi[116], consistent with the early activation of FXIII that is synchronized with fibrin formation. Delayed FXIII activation associated with reduced FXIII (*F13a1*$^{+/-}$) or reduced FXIII-A_2B_2 binding to fibrinogen (as in mice bearing mutated fibrinogen, Fib$\gamma^{390-396A}$) decreases RBC retention in venous thrombi in mice.[49,81,115] Interestingly, accelerated FXIII activation also decreases RBC retention in clots, but in a gene/environment mechanism; compared to the FXIII 34Val allele, presence of the FXIII 34Leu allele reduces the impact of elevated fibrin(ogen) on whole blood clot mass *in vitro*, and thus may protect from venous thrombosis *in vivo*.[117]

Fibrin and FXIIIa as determinants of PE and venous thrombus resolution.

PE is the most serious complication of DVT and happens when part or all the thrombus detaches from the venous vessel wall, travels to a pulmonary artery, and prevents oxygen exchange. Failure of timely dissolution of PE can result in chronic thromboembolic pulmonary hypertension, right heart failure, and cardiogenic shock.[118]

Clinical observations[119–123] and experimental studies with mice[124–126] support roles for both fibrin(ogen) and FXIII(a) in maintaining venous thrombus stability and preventing embolization.

Plasmas from patients with a history of VTE produce clots with abnormal characteristics, including reduced permeability and delayed clot lysis.[119] Even in patients with DVT who have discontinued anticoagulation, the formation of plasma clots with reduced permeability and prolonged clot lysis in vitro are associated with increased risk of recurrent DVT.[127] These same characteristics are also associated with increased risk of developing chronic thromboembolic pulmonary hypertension, post-thrombotic syndrome, and persistent venous obstruction.[120,121,128–131] The fibrinogen β chain (*FGB* [rs1800790]) and FXIII Val34Leu polymorphisms have been implicated as determinants of altered clot properties in acute PE.[123]

Using intravital video microscopy and lung histology of a $FeCl_3$-induced model of acute thrombosis, Gross and co-workers found reduced thrombus stability and increased emboli in mice treated with the direct thrombin inhibitor dabigatran and in FXIII-deficient mice, leading to the hypothesis that by reducing thrombin production, anticoagulant treatment reduces activation of FXIII and the thrombin-activatable fibrinolysis inhibitor and decreases thrombus stability.[132] They subsequently showed that supplementation with FXIII stabilizes venous thrombi and decreases embolization without altering thrombus size.[124] We confirmed the role of FXIII in venous thrombus stabilization using a new mouse model of VTE. In this model, DVT are first induced by ligating the inferior vena cava (generating blood stasis) to slowly produce large RBC-enriched thrombi similar to human DVT, and then these thrombi are allowed to embolize by releasing the ligature. Using this model, we observed that complete FXIII deficiency increases PE incidence, but partial deficiency (i.e., *F13a1*$^{+/-}$) does not,[125] likely due to preservation of fibrin crosslinking in mice with reduced FXIII.[81] Mice with mutations in the fibrin γ-chain crosslinking sites (FGG3X) also show increased PE following $FeCl_3$-induced femoral vein thrombosis.[126] Collectively, these studies suggest the thrombus-stabilizing effect of FXIII is manifested at least in part through mechanical stability provided by fibrin γ-γ crosslink formation. FXIIIa may also protect against PE through its ability to crosslink α_2-antiplasmin to the clot and increase thrombus biochemical stability.

Interestingly, the ability of FXIII to stabilize thrombi may also potentiate PE sequelae by hindering resolution of thrombi that embolize. Compared to wild-type mice, mice with α_2-antiplasmin deficiency (*SERPINF2*$^{-/-}$) have fewer thrombi in the lungs and decreased mortality after photochemical-induced PE in the jugular vein.[133] In a model in which preformed thrombi are deployed in the jugular vein, α_2-antiplasmin inhibition facilitates thrombus dissolution similar to effects of recombinant tPA, without increasing bleeding.[134]

FIBRIN(OGEN) AND FXIII AS POTENTIAL THERAPEUTIC TARGETS

All existing anticoagulants reduce thrombin generation or activity, and therefore inhibit thrombin-mediated platelet activation and fibrin formation. Consequently, each of these anticoagulants are associated with bleeding.[101] Given the prominent roles of fibrin(ogen)

and FXIII in thrombus formation, composition, size, permeability, and stability, it is interesting to consider the potential of fibrin(ogen) and FXIII as therapeutic targets. These approaches, which target coagulation at a step downstream of current therapeutic targets, may allow for normal thrombin generation and therefore, preservation of hemostasis in high-risk settings. For example, in patients with recurrent VTE in spite of apparently therapeutic levels of anticoagulants, fibrin(ogen) and/or FXIII(a)-targeting strategies may be an effective adjunct therapy for preventing recurrent VTE. Moreover, by reducing thrombus stability and facilitating clot dissolution, strategies that target fibrin(ogen) and/or FXIII may reduce long-term sequelae of VTE, including post-thrombotic syndrome and chronic thromboembolic pulmonary hypertension.

Fibrin(ogen) reduction as a therapeutic strategy.

In addition to thrombosis, fibrin(ogen) also contributes to inflammatory and immune diseases and malignancy. Accordingly, a large body of work with genetically engineered mice expressing reduced or functionally altered fibrin(ogen) has shown benefit of fibrinogen reduction in diverse clinical settings. However, means to reduce fibrin(ogen) therapeutically are not established for widespread clinical use. Ancrod, a defibrinogenating agent purified from venom of the Malaysian pit viper, has been tested as a therapy for treating acute ischemic stroke.[135] However, clinical trials showed mixed results, including an increased risk of bleeding[136–138], and this approach invokes concerns about potentially biologically active fibrin(ogen) degradation products inducing pathological effects in the patients. Single-stranded antisense oligonucleotides and small interfering RNA (siRNA) targeting specific fibrinogen chains that reduce circulating fibrinogen without generating fibrin(ogen) degradation products have shown benefit in mouse models of cancer, diet-induced obesity, endotoxemia, peritonitis, and tumor metastasis, without compromising hemostasis.[139,140] Thus, these strategies may also be useful for reducing venous thrombosis in settings of heightened venous thrombosis risk, including constitutively elevated fibrinogen or hyperfibrinogenemia secondary to an inflammatory process.

FXIII inhibition as a therapeutic strategy.

FXIII-directed antagonists may have a relatively wide therapeutic range. FXIII reduction decreases thrombus mass in a dose-dependent manner[125], whereas patients with at least 30 U/dL FXIIIa activity are usually asymptomatic and spontaneous bleeding only occurs when FXIIIa activity is below 15 IU/dL.[141,142] The finding that plasma FXIII, but not platelet FXIIIA, promotes RBC retention in venous thrombi[81] suggests a FXIII(a) inhibitor would only need to reach the plasma compartment to effectively reduce thrombus mass. Indeed, the relative protection of platelet FXIII from plasma inhibitors may provide sufficient transglutaminase activity to crosslink hemostatic clots and reduce bleeding risk with this strategy.

Several potential inhibitors of FXIII(a) or its crosslinked substrates (e.g., α_2-antiplasmin)[143] have been tested in vitro and in animal models. These include antibodies that inhibit FXIII activation, competitive substrates that reduce crosslinking of fibrin and other plasma proteins, and direct inhibitors that bind to the active site of FXIIIa and inhibit its activity.[134,144] Peptide-based inhibitors of FXIII(a), including leech-derived tridegin

and synthetic peptidic transglutaminase-inhibiting Michael acceptors, that decrease FXIIIa activity have gained particular interest.[145–148] The drug-like FXIIIa inhibitor ZED3197, which has selectivity over other transglutaminases, decreases thrombus weight and facilitates flow restoration in a rabbit venous stasis model without increasing bleeding.[146] Given the nature of venous thrombus formation, FXIII-targeting strategy for VTE prevention will require specific inhibitors with longer half-life than current molecules. Leveraging the dependence of circulating plasma FXIII-A on FXIII-B[47,48], Strilchuk et al recently achieved sustained depletion of plasma FXIII-A using siRNA against hepatic FXIII-B.[48] This approach led to enhanced reperfusion in a mouse model of carotid artery thrombosis, suggesting this method may also reduce thrombus mass in models of venous thrombosis.

CONCLUSIONS

The studies summarized here have identified mechanisms by which fibrin(ogen) and FXIII contribute to venous thrombus size, composition, and stability. Mechanisms include function-driving gene polymorphisms, interactions between fibrin(ogen) and FXIII that modify fibrin network structure and mechanical and/or biochemical stability, and the ability of clots to retain RBCs during platelet-mediated contraction. Abnormalities in fibrin structure and/or crosslinking may promote PE and/or facilitate the resolution of venous thrombi or PE. Although still quite speculative, development of molecules that target and modify fibrin(ogen) and FXIII(a) may alter the course of venous thrombogenesis and/or resolution. Future research is necessary to understand the effect of fibrin(ogen) and FXIII interaction with blood cells and the extracellular matrix on initiation, development, and resolution of VTE.

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ABBREVIATIONS

VTE	venous thromboembolism
DVT	deep vein thrombosis
PE	pulmonary embolism
F	factor
FXIII	factor XIII
GPVI	glycoprotein VI
FXIIIa	activated factor XIII
RBC	red blood cell

siRNA	small interfering RNA

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HIGHLIGHTS

- Fibrin(ogen) interactions with plasma proteins and blood cells mediates the diverse roles of fibrin(ogen) in hemostasis, immunity, inflammation, and infection.
- Factor XIII catalyzes the formation of covalent bonds between glutamine and lysine residues in fibrin and other proteins, which protects clots against biochemical degradation and mechanical disruption, and promotes retention of red blood cells in contracted clots.
- Both fibrin(ogen) and factor XIII contribute to venous thromboembolism.
- Targeting fibrin(ogen) or factor XIII may decrease the incidence and size of venous thrombi and reduce the pathologic consequences of venous thrombosis.

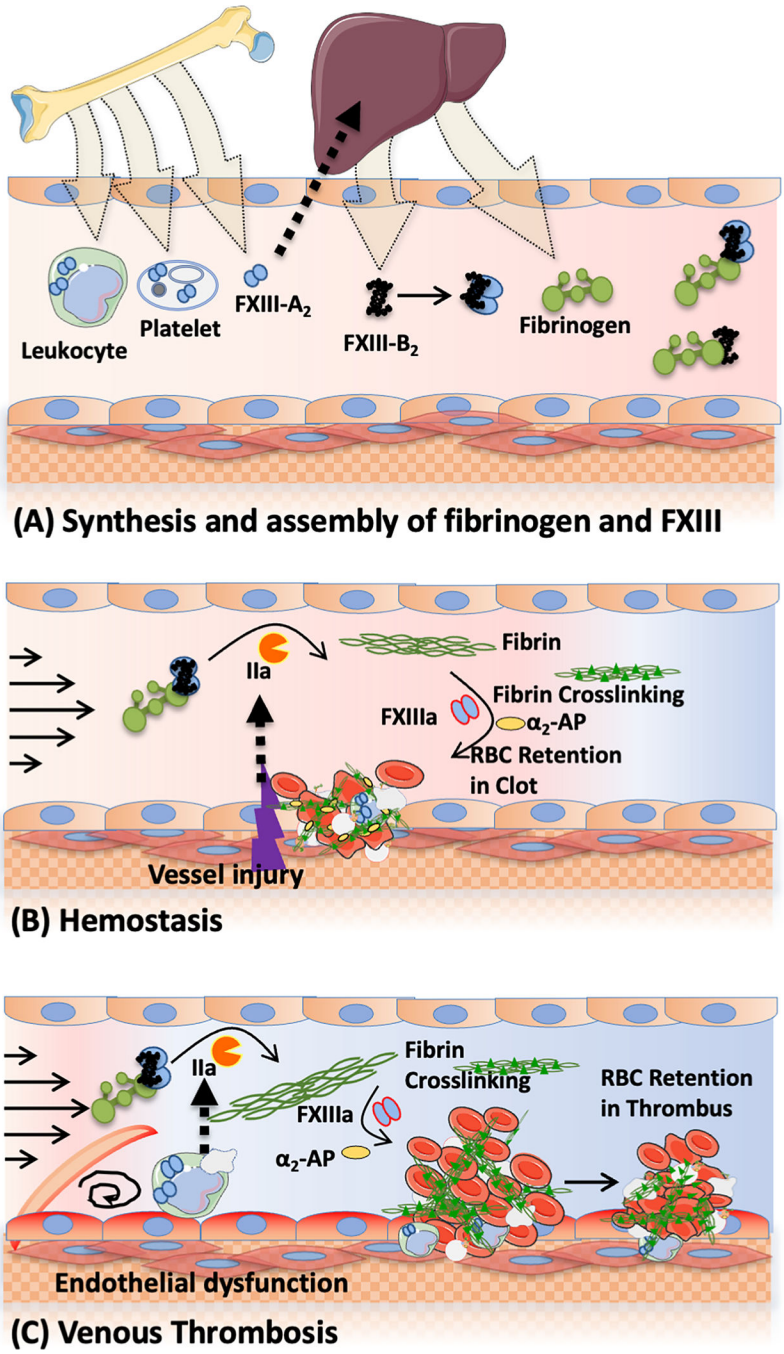

Figure 1. Fibrinogen and FXIII in hemostasis and venous thrombosis.

(A) FXIII-A- and B-subunits are synthesized in bone marrow and liver, respectively and assembled in plasma. Fibrinogen is also synthesized in the liver. FXIII-A_2B_2 circulates in plasma bound to fibrinogen. FXIII-A_2 also circulates in platelets and leukocytes. (B-C) Vessel injury (hemostasis, B) or endothelial dysfunction associated with blood stasis (venous thrombosis, C) triggers the activation of coagulation and results in the production of thrombin which cleaves fibrinogen into fibrin and activates FXIII to FXIIIa. FXIIIa catalyzes crosslinks between fibrin molecules and between fibrin and antifibrinolytic

proteins (i.e., α_2-antiplasmin [α_2-AP]). Crosslinking provides mechanical and biochemical stability to the clot and promotes retention of red blood cells within contracted clots. Controlled clot formation seals the injury site during hemostasis and facilitates wound healing, whereas formation of large intravascular thrombi occludes the vessel and leads to venous thrombosis.

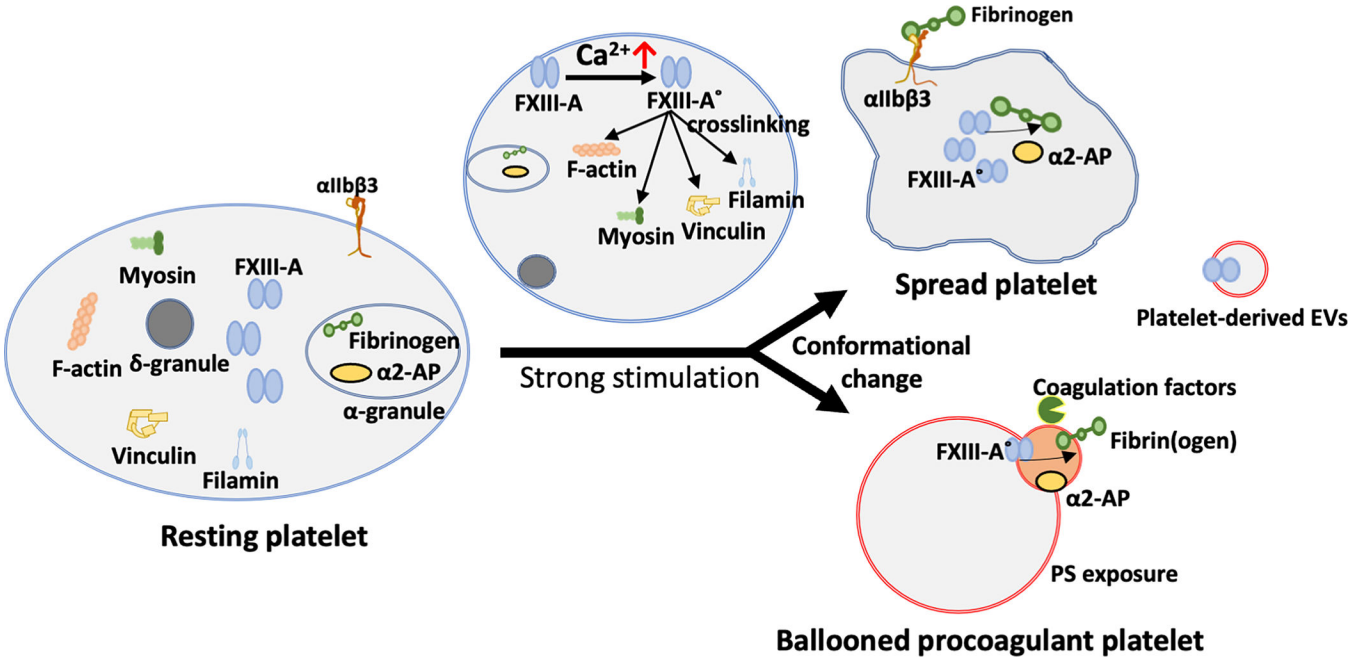

Figure 2. Platelet FXIII-A activation and externalization.

In unstimulated platelets, FXIII-A exists in the cytoplasm in a diffuse distribution. After platelet activation, increased intracellular Ca^{2+} nonproteolytically activates FXIII-A to FXIII-A° which crosslinks cytoskeletal proteins and contributes to cytoskeletal rearrangement and platelet conformational change. After stimulation by strong dual agonists (convulxin + thrombin), two populations of FXIII-A-exposing platelets are formed: spread platelets with FXIII-A in a diffuse distribution, and ballooned procoagulant (phosphatidylserine-exposing) platelets with FXIII-A on a protruding “cap”. FXIII-A is also exposed on extracellular vesicles (EVs).