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Newly-recognized roles of factor XIII in thrombosis

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Abstract

Arterial and venous thrombosis are major contributors to coagulation-associated morbidity and mortality. Greater understanding of mechanisms leading to thrombus formation and stability is expected to lead to improved treatment strategies. Factor XIII (FXIII) is a transglutaminase found in plasma and platelets. During thrombosis, activated FXIII crosslinks fibrin and promotes thrombus stability. Recent studies have provided new information about FXIII activity during coagulation and its effects on clot composition and function. These findings reveal newly-recognized roles for FXIII in thrombosis. Herein, we review published literature on FXIII biology and effects on fibrin structure and stability, epidemiologic data associating FXIII with thrombosis, and evidence from animal models indicating FXIII has an essential role in determining thrombus stability, composition, and size.

Keywords

Factor XIII; myocardial infarction; venous thromboembolism; stroke; fibrinogen

INTRODUCTION

Intravascular coagulation, or thrombosis, is a primary contributor to coagulation-associated morbidity and mortality. Thrombosis can occur in the arterial or venous circulation, leading to myocardial infarction, stroke, deep vein thrombosis, and pulmonary embolism. Arterial thrombosis is usually associated with atherosclerotic plaque rupture that exposes subendothelial cells and procoagulant material (e.g., tissue factor, collagen) to blood, leading to platelet activation and aggregation in high shear rates and stress. Ultimately, platelet accumulation and fibrin deposition produce an occlusive platelet-rich “white thrombus” (reviewed in[1]). In contrast, venous thrombosis/thromboembolism is thought to be triggered by inappropriate expression of cell adhesion molecules on intact, but dysfunctional endothelium in the presence of plasma hypercoagulability in reduced blood flow (stasis). This group of abnormalities is known as Virchow’s triad.[2] Because venous thrombi have high red blood cell (RBC) content, they are known as “red thrombi.” Fibrin formation is a

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CONFLICT OF INTEREST

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central, etiologic component of both arterial and venous thrombosis, and is the ultimate target of thrombolytic enzymes used to treat both of these clinical presentations.

Factor XIII (FXIII) is a protransglutaminase found in cells and plasma. As the central driver of fibrin stability, the contribution of activated FXIII (FXIII[a]) to arterial and venous thrombosis has been investigated. However, although epidemiologic studies have associated abnormal FXIII levels and/or function with thrombosis risk, these associations are complex and the mechanisms by which FXIII contributes to thrombosis *in vivo* remain unclear.

Herein, we briefly review aspects of FXIII biology and activity. We then review recent findings on the contributions of these functions to thrombus formation, stability, and composition *in vitro*, and how these observations have exposed newly-recognized pathophysiologic roles for FXIII(a) during thrombosis *in vivo*. These discoveries may help reconcile apparently discordant findings from previous studies, and reveal new insight into the role of FXIII in arterial and venous thrombosis.

FXIII BIOLOGY

FXIII is one of 9 members of the transglutaminase superfamily found in both cellular (transglutaminases 1–7 and erythrocyte band 4.2) and plasma (pFXIII) compartments. The cellular forms of FXIII are present in multiple cell types, including monocytes, megakaryocytes, osteoblasts, and platelets (reviewed in[3]). Plasma and platelet FXIII are thought to contribute to blood coagulation.

FXIII in plasma

Plasma FXIII is unique because it circulates as two catalytic subunits (FXIII-A_2) and two non-catalytic subunits (FXIII-B_2) arranged in a non-covalent, heterotetramer (FXIII-A_2B_2, M_r 325-kDa, ~70 nM, 14–28 μg/mL).[4] Mechanisms mediating the interactions between the A and B subunits are reviewed by Schroeder and Kohler in this issue of *Seminars in Thrombosis and Hemostasis*[5]. Essentially all FXIII-A_2B_2 zymogen circulates in complex with fibrinogen.[6] Although older studies implicated the alternatively-spliced fibrinogen γ-chain (termed γ′) in FXIII-A_2B_2 binding[7,8], a more recent study using recombinant fibrinogen suggested FXIII-A_2B_2 binds both γA- and γ′-containing molecules with similar affinity (K_D ~40 nM)[9]. Both older[10] and more recent[11] studies identified regions in the fibrinogen α-chain that bind FXIII. Smith *et al.*[11] found that FXIII-A_2B_2 binds a peptide containing amino acid residues 371–425 of the fibrinogen αC domain with high affinity (K_D 5–30 nM). However, they did not demonstrate binding of FXIII-A_2B_2 to this region on full-length fibrinogen and suggested that the interaction between FXIII-A_2B_2 and the αC region may arise during FXIII activation, and that other fibrinogen residues bind zymogen FXIII-A_2B_2 in circulation.[11] Our group showed that murine fibrin(ogen) containing alanine mutations within γ-chain residues γ390–396 (Fibγ$^{390–396A}$) exhibits decreased binding of FXIII-A_2B_2, suggesting these residues mediate the carrier function.[12] Since residues γ390–396 are immediately N-terminal to the fibrin γ-chain residues that are crosslinked by FXIII (glutamine residues 398/399 and lysine 406), localization of FXIII-A_2B_2 at this site would position FXIIIa for rapid translocation to its nearby substrate recognition residues during thrombus formation.

The interface of FXIII-A_2B_2 that interacts with fibrin(ogen) has not been determined. However, Souri *et al.*[13] suggested the FXIII-B subunits augment the catalytic effect of fibrin(ogen) binding on FXIII activation. Further experiments using truncated FXIII-B subunits suggested sushi domains 1 and 10 mediate this interaction.[13] More studies are needed to characterize the specific FXIII residues involved in the FXIII-fibrinogen interaction, and how these residues contribute to FXIII localization during thrombus formation.

FXIII in platelets

Platelet FXIII is a homodimer of catalytic subunits (FXIII-A_2) present in high concentrations (~46–82 fg/platelet).[14] Most platelet FXIII is derived from megakaryocytes during platelet production, but platelets may also uptake a minor fraction from plasma as fibrinogen-bound FXIII-A_2B_2. Platelets may also translate FXIII mRNA *de novo*.[15] In unactivated platelets, FXIII-A_2 is primarily associated with the cytoplasmic fraction.[16]

FXIII activation

During activation of plasma FXIII-A_2B_2, thrombin first catalyzes the cleavage of activation peptide(s) from the N-termini of the FXIII-A subunits (FXIII-$A_2'B_2$).[17,18] Calcium then promotes dissociation of the FXIII-B subunits from FXIII-A_2', yielding activated FXIII-A_2* (FXIIIa).[19] Plasma FXIII activation occurs early during coagulation and is accelerated when it is bound to fibrinogen, which facilitates dissociation of the FXIII-B subunits.[10,20,21] This function ascribes fibrin(ogen) with an important regulatory role during FXIII(a) activation and activity. Plasma from mice that have reduced binding of FXIII-A_2B_2 to fibrin(ogen) (Fib$\gamma^{390–396A}$ mice) exhibits delayed activation of FXIII-A_2B_2 and slower formation of fibrin γ- and α-chain crosslinks.[12] In addition to demonstrating the catalytic role of FXIII-A_2B_2 binding to fibrinogen in plasma, these findings led to new observations on the impact of FXIII activation kinetics on thrombus formation *in vivo* (discussed below).

Activation of platelet FXIII-A_2 can occur after thrombin- or calpain-mediated cleavage of the activation peptide(s).[22] FXIII-A_2 can also be activated without activation peptide cleavage, in the presence of high calcium concentrations.[23] Following platelet activation, FXIII-A_2* is exposed in protruding caps on the surface of phosphatidylserine-positive platelets, but is not released into the supernatant.[16] The mechanisms mediating FXIII exposure on activated platelets are still poorly-understood.

Although early studies suggested the activation peptide(s) remain bound to FXIII-A_2*, more recent studies indicate the activation peptide is released during FXIII activation[18,24] and can be used as a biomarker of acute ischemic stroke.[25]

FXIIIa inactivation

FXIIIa activity is generated early during clot formation and can still be detected within experimental thrombi 6 or more hours after the onset of clotting.[26] Consequently, mechanisms mediating FXIIIa inactivation are of substantial interest. FXIIIa inactivation has been attributed to reversible oxidation, proteolytic digestion by thrombin, and proteolytic enzymes released by granulocytes.[27,28] Studies have examined the ability of plasmin to

activate and inactivate FXIII(a)[29,30], with conflicting conclusions. Recently, Hur *et al.* showed that both plasma- and platelet-derived FXIII-A_2*, but not zymogen FXIII-A_2B_2, are cleaved by plasmin during fibrinolysis.[31] The specificity of this mechanism for activated FXIII-A_2* may stem from steric protection provided by the FXIII-B subunits when bound to FXIII-A_2. This finding is interesting because it temporally-associates clot dissolution with a mechanism to inactivate any FXIIIa that may be released from the dissolving clot. Thus, this mechanism suggests the coagulation system maintains tight control over transglutaminase activity during hemostasis. It is therefore intriguing to speculate that reduced fibrinolytic activity could promote thrombosis by permitting prolonged transglutaminase activity and excessive crosslinking of proteins within the thrombus and the blood.

FXIIIA TRANSGLUTAMINASE ACTIVITY AND EFFECTS ON FIBRIN

Each FXIII-A subunit contains an active site (cysteine 314, histidine 373, and aspartate 396) that catalyzes the formation of intermolecular ε-N-(γ-glutamyl)-lysyl crosslinks. FXIII-A_2B_2 does not compete with FXIII-A_2* for binding to fibrin[21], indicating these interactions are mediated by unique binding sites. However, apart from the specific residues that are crosslinked, the nature of the intermolecular interaction between FXIII-A_2* and fibrinogen has not been resolved. Smith *et al.* showed that the structural cleft on FXIII-A_2* that is exposed by thrombin-mediated cleavage and subsequent release of the FXIII activation peptide contains a recognition site for the fibrin(ogen) α-chain.[24] A consensus recognition motif for FXIIIa substrates has not been identified, suggesting the interaction between FXIIIa and its substrates is mediated by substrate secondary and/or tertiary structure rather than primary amino acid sequence, and may involve a distinct exosite on the FXIIIa molecule.[32,33]

Cellular FXIII(a) (cFXIII[a]) crosslinks cellular and plasma proteins and regulates a variety of physiologic functions including phagocytosis and cell locomotion, osteoblast differentiation and matrix formation, and preadipocyte maturation and proliferation. The contributions of FXIIIa activity to these and other non-classical functions are reviewed by Schroeder and Kohler[5].

Although FXIIIa has over 140 potential substrates in plasma[34], it actually displays high substrate specificity during clot formation and is best characterized for its role in fibrin crosslinking. Briefly, during coagulation, thrombin cleaves N-terminal fibrinopeptides from the Aα- and Bβ-chains to produce fibrin monomers that polymerize into fibrin fibers (Figure 1). FXIIIa catalyzes the formation of isopeptide bonds between glutamine residues 398/399 and lysine 406 in the fibrin γ-chain and subsequently between glutamine and lysine residues in the α-chain (Figure 1). It is likely that the preference of FXIIIa for fibrin is at least partly mediated by its pre-localization on fibrinogen in circulation, in that fibrin(ogen) “delivers” FXIII(a) to the nascent clot.

Fibrin crosslinking has minor effects on global network structure, but substantial effects on the structure and function of individual fibers, and consequently, clot biochemical and mechanical stability, and clot composition (Figure 2).

Effects of FXIIIa on fibrin structure

Crosslinking has little effect on gross fibrin network morphology, producing, at most, only a minor (~12%) increase in fibrin network density.[35–39] In contrast, fibrin crosslinking substantially alters the structure of individual fibrin fibers by promoting protofibril coupling within the fiber, itself.[40] This effect, which is specifically associated with the formation of α-chain-rich, high molecular weight (HMW) crosslinked species, causes fiber compaction. Compaction decreases the size of pores within individual fibers, with potentially important effects on the diffusion of molecules like tissue-type plasminogen activator (tPA) through the fibers and consequently, on fibrinolysis. Fiber compaction also promotes fiber stiffening, making fibers more resistant to deformation under low strain.[40] Thus, the effect of FXIIIa on thrombus formation likely stems from its effects on the biochemical and biomechanical properties of individual fibrin fibers.

Effects of FXIIIa on fibrin resistance to fibrinolysis

FXIII(a) has critical anti-fibrinolytic functions during coagulation, mediated by its ability to crosslink antifibrinolytic proteins, including α_2-antiplasmin[41,42], thrombin activatable fibrinolysis inhibitor[43], and type-2 plasminogen activator inhibitor[44] to fibrin. This activity is mediated primarily by plasma FXIII-A_2*. However, in situations in which the concentration of plasma FXIII is low (≤10%), platelet FXIII-A_2* exposed on the platelet surface also promotes fibrin stability by crosslinking α_2-antiplasmin to fibrin.[16,45]

Effects of FXIIIa on fibrin mechanical stability

FXIIIa-mediated crosslinking significantly increases the elastic modulus (stiffness) of both individual fibrin fibers[46–48] and whole clot networks[49], and is required to protect the clot against premature disruption or dissolution. Using a synthetic FXIII inhibitor and a patient-derived antibody to selectively inhibit the formation of HMW crosslinked species yet permit γ-γ dimer formation, Ryan *et al.*[50] showed that γ-chain crosslinking, alone, is insufficient to stiffen fibrin networks, and that the increased stiffness is correlated with the formation of α-chain-rich, HMW crosslinked species.[50] More recent studies using a recombinant fibrinogen mutated to eliminate γ-chain crosslinking sites confirmed that α-chain crosslinking is the primary contributor to clot stiffness and elasticity.[37,51,52]

Effects of FXIIIa on clot contraction

Several studies have examined the role of FXIII in platelet-mediated clot contraction, with discordant results. Whereas some investigators reported impaired retraction of FXIII-deficient plasma clots[53–55], others observed normal or even increased contraction of plasma and whole blood clots from FXIII-deficient humans and mice[12,39,56–58]. Some of the discord may stem from the use of a transglutaminase inhibitor, cystamine, that also reduces thrombin activity[59] and therefore, may have indirectly reduced thrombin-mediated platelet activation. Other experimental differences that may account for these discordant findings have not been identified.

Effects of FXIIIa on clot composition

Our group recently showed that FXIIIa-mediated crosslinking of fibrin is also a major determinant of thrombus RBC content.[12,39] Briefly, reduced plasma FXIII or presence of a FXIII(a) inhibitor decreases RBC retention in contracted human whole blood clots.[12] Whole blood from mice with full or partial deficiency in the FXIII catalytic A-subunit also show decreased retention of RBCs in contracted clots.[12] These data are consistent with the "excessive red cell fallout" previously reported in a family with congenital FXIII deficiency[56], and demonstrate a newly-recognized function for FXIII(a) during clot formation. Further work using recombinant fibrinogen variants that lack γ- or α-chain crosslinking residues, and FXIIIa inhibitor concentrations that preferentially block formation of α-chain-rich HMW crosslinked species versus γ-γ dimers associated this effect specifically with fibrin α-chain crosslinking.[39] Given the effect of fibrin α-chain crosslinks on fibrin biophysical properties[37,50–52], these data suggest FXIIIa promotes RBC retention in clots via its ability to increase fibrin elastic modulus and/or elasticity during clot contraction. Since clot contraction packs and deforms RBCs trapped within the clot and decreases clot permeability[60], these data implicate FXIII activity as a major determinant of clot composition and function. The contribution of these effects to thrombosis *in vivo* is discussed below.

FXIII-ASSOCIATED ISOMERASE ACTIVITY

Some studies have suggested that members of the transglutaminase family are also associated with a protein disulphide isomerase (PDI) activity.[61,62] The thiol isomerase family is part of the intracellular biosynthetic pathway during protein synthesis, but members of this family also have extracellular functions. Curiously, none of the transglutaminases carry the canonical isomerase catalytic motif (cysteine-X-X-cysteine, where X can be any one of a number of amino acids), suggesting the PDI-like activity may reside in a protein that associates with these transglutaminases. Since canonical PDIs contribute to thrombosis in mice and possibly humans (reviewed in[63,64]), the possibility that a FXIII-related thiol isomerase activity promotes thrombus formation warrants further investigation.

EPIDEMIOLOGIC DATA ON THE ROLE OF FXIII IN THROMBOSIS

Excellent reviews have summarized epidemiologic studies investigating the contribution of FXIII to arterial and venous thrombosis.[65–67] In aggregate, findings suggest FXIII antigen, activity, and/or genotype influence thrombosis risk, at least in certain populations and clinical situations. Notably, however, these studies have shown considerable discordance. This discord is thought to reflect several as-yet incompletely-understood aspects of FXIII function that may have influenced conclusions. These potential aspects are discussed below.

First, early studies were confounded by the use of FXIII activity assays that were unknowingly influenced by the FXIII-A Val34Leu polymorphism present in ~25% of European Caucasians.[68] This polymorphism causes accelerated release of the FXIII activation peptide, 2.5-fold earlier FXIII activation, and consequently, faster fibrin crosslinking *in vitro*.[69–71] In certain assays, this accelerated activation erroneously appears

as increased FXIIIa activity, leading to poor correlation between FXIII antigen and activity and overestimation of the normal range for FXIIIa activity.[71] Notably, however, even after this effect was recognized, findings from studies evaluating the association of the Val34Leu polymorphism with venous and arterial thrombosis have been inconsistent.[70,72–78] Meta-analyses of these studies suggest presence of the 34Leu allele offers significant, but modest protection against coronary artery disease (CAD, odds ratio (OR) 0.81, 95% confidence interval (95% CI) 0.70–0.92)[79] and venous thromboembolism (OR 0.85, 95% CI 0.77–0.95).[80]

Second, effects of the Val34Leu polymorphism are modulated by complex gene-environment interactions that were not appreciated in early studies. For example, an analysis of 474 patients with deep vein thrombosis enrolled in the Leiden Thrombophilia Study (LETS) showed only a possible weak protective effect of Leu34 homozygosity in men (OR 0.7, 95% CI 0.4–1.3).[81] However, a subsequent analysis of the same samples that considered plasma fibrinogen level suggested slightly stronger protection in both men and women, especially in those over 45 years old (OR 0.4, 95% CI 0.2–1.0).[82] Similarly, in a large prospective study (Norfolk cohort of the European Prospective Investigation in Cancer and Nutrition study [EPIC-Norfolk]), the Val34Leu variant was not broadly associated with risk of CAD. However, when fibrinogen levels were considered in the analysis, Leu34 homozygotes in the lowest tertile of fibrinogen concentration had increased risk of developing CAD (OR 2.88, 95 CI 1.24–6.74, P=0.003), whereas those in the highest tertile of fibrinogen concentrations trended towards reduced risk of developing CAD (OR 0.47, 95% CI 0.18–1.17, P=0.1).[83] Similarly, in a high-risk Hungarian population, although presence of the Leu34 allele did not appear to alter the risk of coronary sclerosis or myocardial infarction (MI) in the general population, when patients with fibrinogen levels in the upper quartile (>4.6 g/L) were analyzed, the Leu34 allele was associated with protection against MI (OR 0.41, 95% CI 0.18–0.93).[84] The proposed mechanism for this effect is interesting. In plasmas with normal fibrinogen levels, the Leu34 allele produces clots with thinner fibers and decreased permeability, whereas in plasmas with high fibrinogen, it produces clots with thicker fibers, and increased permeability and susceptibility to fibrinolysis.[85] Since abnormal fiber thickness and altered clot permeability and stability are associated with increased thrombosis risk (reviewed in[86]), these observations indicate that both FXIII genotype and plasma fibrinogen concentration should be considered when calculating thrombosis risk in population studies.

Third, other FXIII polymorphisms that are not frequently incorporated into epidemiological analyses may also modulate the association between FXIII and thrombosis risk in certain populations. For example, the FXIII-A Tyr204Phe polymorphism has been associated with high (9-to-11-fold) increased risk for MI and ischemic stroke in a Dutch cohort[87,88], although there was no association seen in a Brazilian population[89]. The FXIII-B*2 polymorphism (His95Arg) primarily found in African populations is associated with accelerated dissociation of the B-subunit.[90] This polymorphism was associated with mildly increased risk of venous thrombosis in Dutch Caucasians (OR 1.5, 95% CI 1.1–2.0)[90], but not in Iranians[91], and is not associated with CAD or MI in a Hungarian population[92]. The FXIII-B*3 polymorphism that predominantly occurs in Asians results in a novel splice acceptor site which replaces the final 10 residues of the C-terminus with 25 new residues

containing one additional acidic and two extra basic residues.[93] In a Hungarian population, this FXIII-B*3 polymorphism results in lower FXIII activity and antigen levels and is protective against CAD and MI, but only in the presence of both high fibrinogen levels and the FXIII-A Leu34 allele (OR 0.23, 95% CI 0.06–0.85 and OR 0.10, 95% CI 0.02–0.53, respectively, adjusted for synergy).[92] Together, these studies suggest that incorporating information about these alleles may refine interpretations on the contribution of FXIII to thrombosis in certain populations.

Fourth, the effect of FXIII on thrombosis risk also appears to reflect sex-specific factors that have not been considered in all studies, many of which were not powered to detect a modulating effect of sex. For example, in a Hungarian population, FXIII antigen correlates with moderately increased risk of MI (OR 1.999, 95% CI 1.051–3.765, P=0.03)[94] and peripheral artery disease (PAD, OR 2.000, 95% CI 0.943–4.240, P=0.07)[95] in women, but not men. Moreover, although FXIII activity is slightly increased in both male and female PAD patients compared to controls, FXIII activity in the upper tertile (>120%) is correlated with increased risk of PAD in women (OR 2.316, 95% CI 1.157–4.635, P=0.02), but not men.[95] The effect of the Val34Leu polymorphism on risk may also be sex-specific; female, but not male, homozygotes for the Leu34 allele have increased risk of fatal atherothrombotic stroke.[96] The sex-specific factors that mediate these differences have not been identified.

Finally, but importantly, the possibility that publication bias has inflated the apparent impact of FXIII antigen, activity, and/or genotype on thrombosis risk must be considered when evaluating these data in aggregate. A meta-analysis of 16 studies on the Val34Leu polymorphism suggested that smaller studies with negative findings are less frequently published than studies with positive findings.[79] Thus, the relationship between FXIII and thrombosis risk may be weaker than it appears.

ANIMAL MODELS INVESTIGATING THE ROLE OF FXIII IN THROMBOSIS

Animal models are a useful tool for studying thrombosis in physiologically-relevant settings that lack the heterogeneity of human populations. Studies using animal models have revealed early and rapid contributions of FXIII(a) activity to clot formation, stabilization, and composition during thrombosis *in vivo*.

Contribution of FXIII to arterial thrombosis

Mouse models have been used to extend clinical observations and examine the association of cellular FXIII with arterial thrombosis. For example, Mansfield et al. detected increased levels of circulating FXIII in patients with type 2 diabetes relative to healthy controls.[97] Using mouse models of diabetes, Elgheznawy et al. subsequently associated calpain- and miR-223 dependent upregulation of platelet FXIII with increased ferric chloride-mediated carotid artery thrombosis.[15] Although preliminary, these findings suggest FXIII is involved in thrombotic complications in diabetes. In addition, patients who experienced post-MI heart failure or death have lower FXIII levels at hospital admission and increased FXIII consumption 5 days after MI.[98] Reduced FXIII levels are also associated with increased risk of ventricular rupture.[99,100] Studies using mice to examine the mechanism of this association indicate the relationship between FXIII level and myocardial repair is causative; FXIII-

deficient mice die from left ventricular rupture within 5 days after MI, whereas wild type mice or FXIII-deficient mice infused with FXIII replacement have normal survival rates.[100] These data suggest FXIII plays a role in healing the infarcted area and that FXIII levels may be an early prognostic indicator during and immediately following acute MI. In addition, since reduced FXIII decreases survival after acute MI, this outcome may bias retrospective studies on the role of FXIII in MI.

Two studies used animal models to test the ability of FXIIIa inhibitors to reduce arterial thrombus stability. In a rabbit model of femoral artery thrombosis and tPA-mediated thrombolysis, administration of a FXIIIa inhibitor reduced thrombus weights more than administration of tPA, alone, and facilitated thrombolysis in 50% of treated rabbits.[101] Similarly, in a canine model of coronary artery thrombosis, FXIIIa inhibition accelerated reperfusion and reduced residual thrombus mass following tPA administration.[102] Notably, in both models this effect was limited to animals *pretreated* with the FXIIIa inhibitor, and was not observed in animals infused with FXIIIa inhibitor after thrombus induction.[101,102] This finding suggests FXIII activation and crosslinking activity occur early during thrombogenesis. Accordingly, using a fluorescent peptide derived from α_2-antiplasmin to probe for FXIIIa activity in mice, Jaffer *et al.*[103] detected signal enhancement within 30 minutes of thrombus induction, indicating early generation of FXIIIa activity during thrombogenesis.

Contribution of FXIII to venous thrombosis

Our group used mice to investigate the effect of FXIII on venous thrombosis *in vivo*. Consistent with *ex vivo* observations of reduced RBC retention in FXIII-deficient clots[12,39,56], thrombi from FXIII-deficient mice are smaller than thrombi from wild type mice and have reduced RBC content.[12] These findings indicate a central role for FXIII activity during venous thrombosis *in vivo*. Intriguingly, mice with normal levels of FXIII, but reduced binding of plasma FXIII-A_2B_2 to fibrinogen (Fib$\gamma^{390–396A}$ mice) phenocopy FXIII-deficient mice, producing smaller venous thrombi with reduced RBC content.[12] Notably, the loss of FXIII binding to fibrinogen in Fib$\gamma^{390–396A}$ mice delays, but does not abolish, FXIII activation and fibrin crosslinking[12]; Fib$\gamma^{390–396A}$ thrombi are fully-crosslinked 24 hours after thrombus induction (M.M. Aleman and A.S. Wolberg, unpublished observation). These observations in mice suggest biological rationales for FXIII binding to circulating fibrinogen *in vivo*: 1) binding and localization to fibrin(ogen) facilitates delivery of FXIII to the nascent thrombus, 2) localization of FXIII on fibrin(ogen) accelerates FXIIIa activation during thrombogenesis, and 3) FXIII activation at the thrombus site spatially and temporally associates FXIIIa activity with an essential function thrombus contraction. These data suggest not only the generation, but also the *timing*, of FXIIIa activity is a critical determinant of venous thrombus composition and consequently, size.

Contribution of FXIII to thrombus stability

FXIIIa activity may also influence risk of embolization of both arterial and venous thrombi. Platelet FXIII-A mRNA is significantly lower in patients with non-valvular atrial fibrillation and thrombus embolization than in similar patients with left atrial appendage thrombus and no history of embolization.[104] Accordingly, in mice, FXIIIa-mediated crosslinking of

plasma fibronectin into thrombi formed in mesenteric arterioles enhances platelet aggregation and increases the stability of platelet-rich thrombi.[105–107] In a ferric chloride model of venous thrombosis, FXIII-deficient mice show increased embolization compared to wild type mice.[108]

Together, these observations demonstrate critical FXIIIa contributions at several steps during thrombosis.

CONCLUSIONS AND REMAINING QUESTIONS

FXIII is unique among coagulation proteins, not only in the nature of its enzymatic activity that crosslinks, rather than proteolyzes proteins, but also in its ability to directly impact *both* biochemical and biophysical properties of thrombi. In the almost 70 years since its first description as a "fibrin stabilizing factor[109]", defining the role of FXIII in coagulation has been a fundamental goal. Growing data from biochemical, epidemiological, and animal model studies suggest FXIII(a) is an important determinant of thrombus composition and stability.

Recent findings have resolved several long-standing questions, but also exposed new ones. For example, studies examining the contributions of the Val34Leu polymorphism to clotting have revealed complex gene-environment interactions that mediate this activity. However, these studies used purified proteins and cell-free plasma systems. Given the newly-recognized effect of FXIII activation kinetics on whole blood clot composition (RBC retention)[12,39], what is the effect of the Val34Leu polymorphism in more physiologically-relevant whole blood systems? Studies have shown that both plasma and platelet FXIII can contribute to clot stability *in vitro*.[16,41–45] Do both of these pools contribute to clot composition and stability *in vivo*? Data from animal models show FXIII(a) contributes to thrombus size and stability and subsequent tissue repair. Is there a potential role for FXIII(a) inhibitors as a primary or adjunct approach for anticoagulation? Continued investigations of both basic FXIII biology and the effect of FXIII on thrombosis in humans and animal models are expected to uncover central information about these (patho)physiologic mechanisms.

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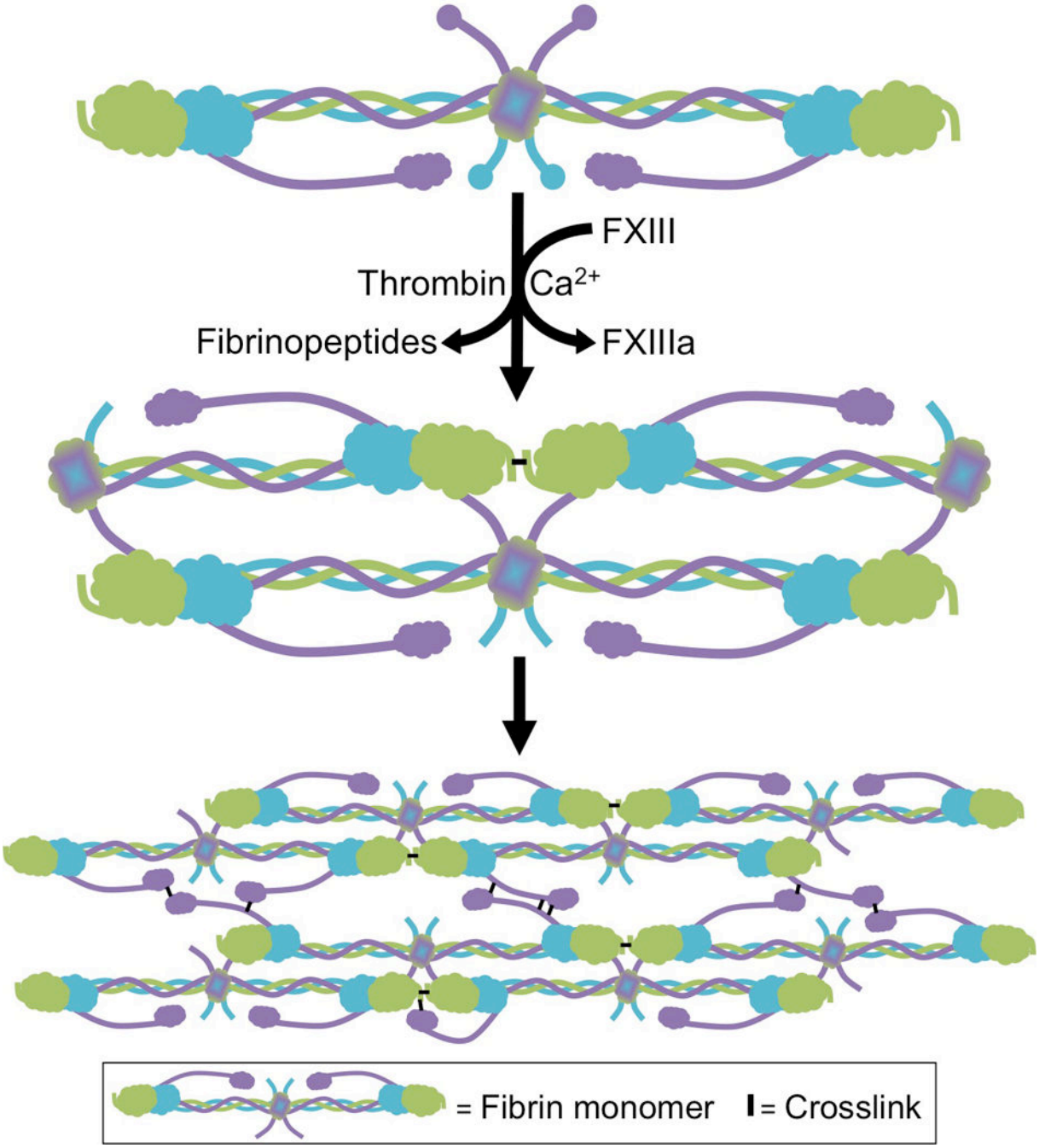

Figure 1. FXIIIa crosslinking during fibrin formation

Fibrinogen is a hexamer composed of 2 Aα- (purple), 2 Bβ- (blue), and 2 γ-chains (green). During coagulation, thrombin cleaves N-terminal fibrinopeptides from the Aα- and Bβ-chains, producing fibrin monomers which polymerize into protofibrils and subsequently, fibers.[110] FXIIIa increases clot stability by introducing ε-N-(γ-glutamyl)-lysyl crosslinks between residues in the γ- and α-chains of fibrin monomers within individual fibers. FXIIIa first introduces crosslinks between γ-chains (forming γ-γ dimers) and subsequently

between γ- and α-chains (forming high molecular weight species [γ-multimers, α-polymers, and αγ-hybrids]).[37,50–52]

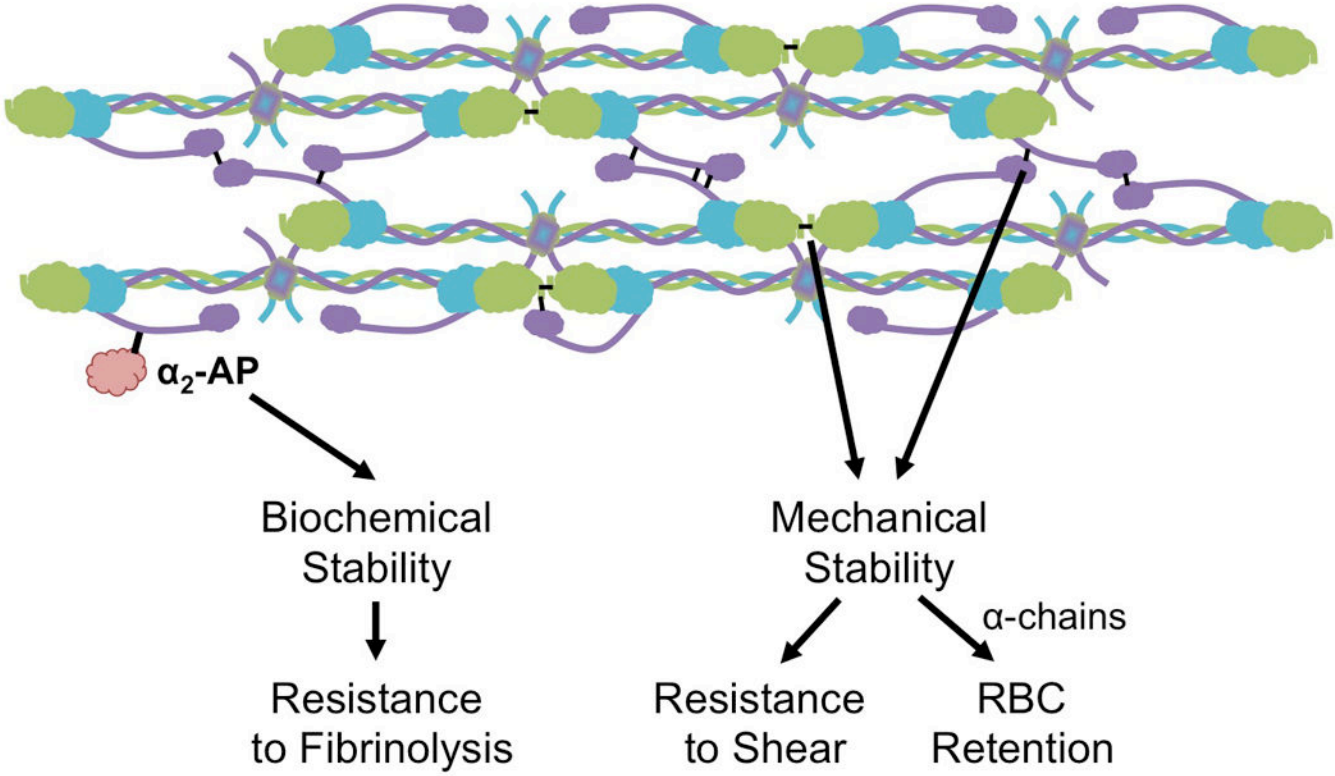

Figure 2. Contributions of FXIIIa to clot biochemical and mechanical stability

FXIIIa crosslinking of plasma proteins [i.e. α_2-antiplasmin, (α_2-AP)] increases the resistance of the clot to fibrinolysis. Crosslinking of the fibrin α- (purple) and γ-chains (green) stiffens fibrin fibers and increases the mechanical stability of the clot. Increased mechanical stability renders the clot more resistant to shear forces. α-chain crosslinking enables RBC retention during clot contraction.[39]