

Chapter 15

Blood Coagulation Factor XIII: A Multifunctional Transglutaminase

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Abstract Factor XIII is a pro-enzyme of plasma transglutaminase consisting of two enzymatic A subunits and two non-catalytic B subunits, and platelet transglutaminase consisting of two enzymatic A subunits. FXIII plays a critical role in the generation of a stable hemostatic plug, wound healing, maintaining pregnancy, angiogenesis, apoptosis and bacterial immobilization. FXIII catalyzes intermolecular cross-linking reactions between fibrin monomers and α_2 -antiplasmin. These reactions increase the mechanical strength of the fibrin clot and its resistance to proteolytic degradation. Congenital FXIII deficiency is a rare autosomal recessive disorder, most cases of which are caused by defects in the FXIII-A gene, leading to a bleeding tendency. An autoimmune hemophilia-like disease is caused by anti-FXIII antibodies. Platelet surface FXIII-A₂ is involved in fibrin translocation to lipid rafts and outside-in signaling, leading to clot retraction. FXIII-A₂-mediated protein cross-linking is associated with assembly of the extracellular matrix on a variety of cell surfaces in physiological events such as differentiation.

Keywords Factor XIII • Platelets • Surface • Clot retraction • Lipid rafts • Transamidation

15.1 Introduction

The blood coagulation cascade has evolved as a defense mechanism for maintaining hemostasis during blood vessel injury. This process is controlled by a signaling cascade consisting of 13 coagulation factors. There are two separate pathways, the intrinsic and extrinsic. The intrinsic pathway is activated by trauma inside the vascular system, and is activated by platelets, exposed endothelium, or collagen. This pathway involves factors XII, XI, IX, and VIII. The extrinsic pathway is activated by external trauma that causes blood to escape from the vascular system. This pathway involves factor VII. These eventually join together to form the common pathway. The common pathway involves factors I, II, V, and

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X. Activation of the cascade produces thrombin (factor IIa) that activates a variety of components in the cascade. Thrombin cleaves fibrinogen to fibrin resulting in clot formation. At the end of this process, covalent bonds are introduced into the fibrin clot by the activated fibrin stabilizing factor (factor XIIIa) (Lorand 2001).

15.2 FXIII Biochemistry

Factor XIII (FXIII) is a Ca^{2+} -dependent pro-transglutaminase which cross-links proteins by catalyzing the formation of isopeptide bonds between glutamine and lysine residues (Lorand 2001). FXIII has two forms: a plasma form that is a tetramer of two carrier B subunits and two catalytic A subunits, and an intracellular form that consists of two catalytic A subunits.

The primary structures of the A subunit (FXIII-A; ~83 kDa, encoded by the F13A1 gene on human chromosome 6p24-25) and B subunit (FXIII-B; ~80 kDa, encoded by the F13B gene on chromosome 1q31-32.1) have been determined by cDNA cloning and amino acid sequence analysis (Ichinose et al. 1986a, b; Grundmann et al. 1986). FXIII-A consists of four main structural domains, the β -sandwich (amino acids 38–184), the catalytic core (amino acids 185–515), β -barrel 1 (amino acids 516–628), and β -barrel 2 (amino acids 629–731) domains, plus an NH_2 -terminal activation peptide (amino acids 1–37). There are nine cysteine residues, including the active site cysteine (Cys 314), none of which forms disulfide bonds. FXIII-B consists of ten short tandem repeats, called sushi domains, held together by a pair of internal disulfide bonds.

FXIII plays a critical role in the generation of a viable hemostatic plug. Following exposure to thrombin and calcium, a zymogen is activated to FXIIIa, which cross-links proteins by catalyzing the formation of isopeptide bonds between glutamine and lysine residues of fibrin within a clot network. FXIII also catalyzes the posttranslational modification of proteins by transamidation of available glutamine residues. This reaction results in the incorporation of low-molecular-weight amines (e.g., serotonin, dopamine, and polyamine) into suitable protein substrates. Serotonin has been demonstrated to modify small GTPase by transglutaminase-mediated transamidation, during platelet activation. As a result, the small GTPase is constitutively activated, leading to irreversible aggregation and platelet α -granule release (Walther et al. 2003).

In the initial step of FXIII activation, thrombin cleaves off an activation peptide from the NH_2 terminus of FXIII-A by hydrolyzing the Arg37–Gly38 peptide bond. Then, in the presence of Ca^{2+} , the inhibitory B subunits dissociate, which is a prerequisite for the truncated FXIII-A dimer (FXIII-A₂') to assume an enzymatically active conformation (FXIII-A₂*). The conformational change of FXIII-A₂' resulting in an active transglutaminase also requires Ca^{2+} .

FXIII is responsible for the cross-linking of fibrin γ -chains in the early stages of clot formation, whereas α -chain cross-linking occurs at a slow rate. Cross-linking of the γ -chains plays a role in fibrin fiber appearance time and fiber density. Cross-

linking of the α -chains plays a role in the thickening of fibrin fibers (Duval et al. 2014).

The main role of FXIII-B is to prolong the lifespan of FXIII-A in plasma. In FXIII-B-deficient patients, the FXIII-A₂ concentration in plasma is significantly decreased. Administration of recombinant FXIII-B into FXIII-B(-/-) mice accelerates fibrin cross-linking in plasma and assists the maintenance of plasma FXIII-A levels (Souri et al. 2008a). The subunits form an A₂B₂ tetramer, which circulates in plasma with a half-life of >1 week.

The A subunit is synthesized by cells of bone marrow origin and the B subunit is synthesized by hepatocytes in the liver. Formation of the FXIII-A₂B₂ complex must occur in plasma. In a previous study, enzyme-linked immunosorbent assay was carried out to investigate the affinity between the A and B subunits during their interaction, which demonstrated that their affinity constant was on the order of magnitude of $>10^{-8}$. However, this affinity constant implies that most FXIII-A₂ should circulate in the free form; however, in reality, most plasma FXIII-A₂ is present in complex with FXIII-B₂. Thus, this value must substantially underestimate the affinity between the A and B subunits.

Katona et al. (2014) demonstrated that the equilibrium dissociation constant (Kd) for the interaction between the A and B subunits was established in the range of 10^{-10} M using a surface plasmon resonance technique. On the basis of the measured Kd, it was calculated that 99 % of plasma FXIII-A₂ is present in complex with FXIII-B₂ and only ~1 % of circulating FXIII-A₂ is present as a free homodimer.

To locate the epitope on FXIII-B responsible for the interaction with FXIII-A, Souri et al. (2008b) produced various truncated recombinant FXIII-Bs. They demonstrated that those truncated FXIII-B subunits that lacked sushi domain 1 could not form a complex with FXIII-A. Katona et al. (2014) demonstrated that a monoclonal antibody recognizing sushi domains 1 and 2 of the FXIII-B subunit prevents this subunit from forming a complex with the FXIII-A subunit. However, from their subsequent experiments, they were able to localize the epitope to amino acid residues 90 to 103 on sushi domain 2. They noted that these findings are not contradictory and may reflect complex interactions between sushi domains 1 and 2 of the FXIII-B subunit.

Intracellular FXIII is present in the cytoplasm of platelets, monocytes, monocyte-derived macrophages, dendritic cells, chondrocytes, osteoblasts, and osteocytes. FXIII is activated via the nonproteolytic pathway in human platelets during activation induced by thrombin. The FXIII-A in platelets can also be activated by calpain, an endogenous intracellular protease (Ando et al. 1987).

Agonist-induced platelet aggregation in patients with FXIII deficiency and FXIII-A-knockout mice is normal. Platelet aggregation is a relatively fast process (it is completed in 5 min), whereas the cross-linking of platelet proteins is much slower. This suggests that cross-linked polymers are required only for the later phases of platelet activation, e.g., for spreading of platelets following adhesion or for clot retraction. The altered phenotype of FXIII-A-deficient platelets is characterized by a delay in their spreading (Jayo et al. 2009). Furthermore, a reduced

contractile force was found in platelet-rich plasma clots from an FXIII-deficient patient (Carr et al. 2003). Clot retraction is impaired in FXIII-A knockout mice, and the addition of plasma FXIII or recombinant FXIII-A₂ to the platelet-rich plasma from FXIII-A knockout mice only partially restored clot retraction, suggesting that cytosolic FXIII also contributes to this process (Kasahara et al. 2010). To verify this idea, a plasma FXIII-free clot retraction assay is performed using washed platelets, purified fibrinogen, thrombin, and Ca²⁺ (Kasahara et al. 2013). Cross-linking of vinculin and filamin by cytosolic FXIII may be involved in platelet cytoskeleton remodeling during clot retraction (Serrano and Devine 2002).

FXIII targets a wide range of additional substrates that have important roles in health and development (Dickneite et al. 2015; Richardson et al. 2013). These include antifibrinolytic proteins, which function in cross-linking of an α_2 -antiplasmin to fibrin. Clots formed in the absence of FXIII are unstable and easy to lyse by the fibrinolytic system. Proteomic approach in combination with transglutaminase-specific labeling by 5-(biotinamido) pentylamine (5BAPA) identified a total of 147 plasma FXIIIa substrates, and 48 of these were incorporated into the insoluble fibrin clot during the coagulation of plasma (Table 15.1) (Nikolajsen et al. 2014). FXIIIa cross-links a number of different proteins to clots, including factor V, thrombin-activable fibrinolysis inhibitor, von Willebrand factor, complement C3, inter- α -trypsin inhibitor, and plasminogen activator inhibitor type 2. These proteins regulate clot characteristics other than stability. The cross-linking of fibronectin to fibrin by FXIIIa has been shown to affect cell adhesion and migration in a fibrin matrix.

Cross-linking occurs between Gln398 or 399 on the γ -chain of the fibrin molecule and Lys406 on the γ -chain of another fibrin, resulting in the formation of two antiparallel isopeptide bonds that connect the D-regions of two molecules. Glutamine residues involved in the cross-linking of the α -chain are Gln221, 237, 328, and 366. Many lysine residues of the α -chain are involved as acceptor sites. Cross-linking occurs between Gln2 in the amino terminus of an α_2 -antiplasmin and Lys303 on the fibrin α -chain (Richardson et al. 2013).

The amino acid sequences around the substrate glutamine site show no consensus sequence could be derived from the primary structure. However, screening for the preferred substrate sequence of transglutaminase using a phage-displayed peptide library demonstrated that Qxx ϕ xWP (where x and ϕ represent a nonconserved amino acid and a hydrophobic amino acid, respectively) is preferred as a glutamine donor substrate (Sugimura et al. 2006).

15.3 FXIII Deficiency

Congenital FXIII deficiency is caused by defects in the F13A or F13B genes, leading to a bleeding tendency and abnormal wound healing in affected patients and spontaneous miscarriage in female patients. Several hundred autosomal,

Table 15.1 FXIII substrates cross-linked to the plasma clot

Accession no.	Protein	Reactive Gln	Cross-linking sites
P02671	Fibrinogen α -chain	8	Gln ²²¹ , Gln ²³⁷ , Gln ³²⁸ , Gln ³⁶⁶
P01023	α_2 -Macroglobulin	15	Gln ⁶⁶⁹ , Gln ⁶⁷⁰
P00488	Coagulation factor XIII A chain	8	
P00747	Plasminogen	20	Gln ³²² , Lys ²⁹⁸
P00734	Prothrombin	11	
P19823	Inter- α -trypsin inhibitor heavy chain H2	16	
P06727	Apolipoprotein A-IV	18	
P01024	Complement C3	27	
P02787	Serotransferrin	10	
P19827	Inter- α -trypsin inhibitor heavy chain H1	11	
P10909	Clusterin	5	
P02679	Fibrinogen γ -chain	5	Gln ³⁹⁸ , Gln ³⁹⁹ , Lys ⁴⁰⁶
P08697	α_2 -Antiplasmin	11	Gln ² , Gln ²¹ , Gln ⁴¹⁹ , Gln ⁴⁴⁷
P02751	Fibronectin	22	Gln ³ , Gln ⁴ , Gln ¹⁶
P02649	Apolipoprotein E	13	
P06396	Gelsolin	7	
P04004	Vitronectin	6	
Q16610	Extracellular matrix protein 1	8	
P01042	Kininogen-1	11	
P04003	C4b-binding protein α -chain	9	
P01857	Ig γ -1 chain C region	3	
P23142	Fibulin-1	8	
P02760	Protein AMBP	3	
P02647	Apolipoprotein A-I	4	
Q14624	Inter- α -trypsin inhibitor heavy chain H4	5	
P04275	von Willebrand factor	12	Gln ³¹³ , Gln ⁵⁶⁰
P62736	Actin, aortic smooth muscle	3	Gln ⁴¹
P02765	α_2 -HS-glycoprotein	2	
P01876	Ig α -1 chain c region	5	
P01871	Ig mu chain C region	5	
P69905	Hemoglobin subunit α	1	
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	3	
P01834	Ig κ -chain C region	2	
P02675	Fibrinogen β -chain	6	
Q9Y490	Talin-1	4	
P0C0L4	Complement C4-A	21	
P01031	Complement C5	8	
P02768	Serum albumin	8	

(continued)

Table 15.1 (continued)

Accession no.	Protein	Reactive Gln	Cross-linking sites
Q12805	EGF-containing fibulin-like extracellular matrix protein 1	3	
P00739	Haptoglobin-related protein	1	
O43866	CD5 antigen-like	1	
P00450	Ceruloplasmin	4	
P09871	Complement C1s subcomponent	4	
P04406	Glyceraldehyde-3-phosphate dehydrogenase	1	
Q9UGM5	Fetuin-B	3	
P68871	Hemoglobin subunit β	1	
P0CG04	Ig λ -1 chain C regions	1	
P01766	Ig heavy chain V-III region BRO/TIL	1	

Nikolajsen et al. (2014)

recessively inherited cases of human FXIII deficiency are known worldwide. Most are due to loss-of-function missense mutations in FXIII-A (Iismaa et al. 2009).

Autoimmune hemophilia-like disease caused by anti-FXIII antibodies (termed AH13) or “autoimmune FXIII deficiency” is a life-threatening bleeding disorder. AH13 is rare worldwide (Souri et al. 2015). The AH13 cases are immunologically classified into three types: Aa, Ab, and B. Type Aa autoantibodies are directed against native FXIII-A and block FXIII activation. The autoantibodies not only prevent the assembly of new FXIII-A₂B₂ heterotetramers, but also remove FXIII-A from native FXIII-A₂B₂ heterotetramers by forming an FXIII-A-IgG complex. Type Ab autoantibodies preferentially bind to activated FXIII-A and inhibit its activity. Type B antibodies are non-neutralizing anti-FXIII-B subunit autoantibodies that possibly accelerate FXIII clearance. An excellent review article by Muszbek et al. (2011) gives a detailed description of FXIII biochemistry and FXIII deficiency.

15.4 FXIII Function

The pivotal role of plasma FXIII in hemostasis is well established (Richardson et al. 2013; Schroeder and Kohler 2013). In human blood coagulation, the activated form of plasma FXIII stabilizes fibrin matrix assembly during clot formation by catalyzing the covalent linkage of fibrin monomers. In addition, plasma FXIIIa enhances the incorporation of an α_2 -antiplasmin into the clot network, being the principal mechanism whereby plasmin-mediated clot degradation is minimized.

FXIII plays a key role in a range of physiological functions. In addition to its role in maintaining hemostasis, these functions include (1) wound healing, (2) maintenance of pregnancy, (3) angiogenesis, (4) apoptosis, and (5) bacterial immobilization.

15.4.1 Wound Healing

Impaired wound healing was noted in a patient with congenital FXIII deficiency (Duckert et al. 1960). A key role of FXIII in wound healing has been demonstrated in FXIII-deficient transgenic mice, where healing of an excisional wound was markedly delayed compared with healing in normal mice (Inbal et al. 2005). Plasma FXIII plays a role in wound healing through a number of mechanisms: (A) FXIII enhances aggregation of platelets to the endothelium at the site of injury; (B) FXIIIa promotes the cross-linking of fibrin, thus increasing the integrity and tensile strength of a clot; (C) FXIIIa-induced cross-linking of the provisional matrix enables the infiltration of leukocytes; (D) Cross-linked macromolecules facilitate the invasion of fibroblasts and endothelial cells into the wound, enabling collagen deposition and angiogenesis (Richardson et al. 2013).

15.4.2 Maintenance of Pregnancy

Clinical findings prove that plasma FXIII is essential for carrying out normal pregnancy. Although homozygous female FXIII-A knockout mice are capable of becoming pregnant, most of them die owing to excessive vaginal bleeding during gestation (Koseki-Kuno et al. 2003). A series of histologic examinations of the pregnant animals suggest that massive placental hemorrhage and the subsequent necrosis occurred in the uteri of the FXIII knockout mice on day 10 of gestation. These findings indicate that maternal FXIII plays a critical role in uterine hemostasis and maintenance of the placenta during gestation. The cross-linking of fibrinoid components in the placenta (fibrin and fibronectin) is likely important for the sealing/anchoring effect and barrier function of Nitabuch's layer (Muszbek et al. 2011).

15.4.3 Angiogenesis

In FXIII knockout mice, the formation of new vessels into a subcutaneously injected Matrigel™ plug was significantly inhibited compared with control mice (Dardik et al. 2006a). Dardik et al. (2003) demonstrated that FXIIIa dose-dependently enhanced endothelial tube formation and endothelial cell migration.

FXIII binds to endothelial cell $\alpha v \beta 3$ integrin, resulting in interaction between $\alpha v \beta 3$ and vascular endothelial growth factor receptor-2 (VEGFR-2), and autophosphorylation and activation of VEGFR-2. Activation of VEGFR-2 results in phosphorylation and activation of ERK, which promotes endothelial cell survival. Activated VEGFR-2 also induces endothelial cell proliferation, through phosphorylation of ERK and upregulation of Egr-1. Finally, activation of

VEGFR-2 leads to upregulation of c-Jun, and downregulation of thrombospondin-1 (TSP-1) via the transcription factor Wilm's tumor-1, resulting in enhanced cell proliferation, migration, and survival, ultimately promoting angiogenesis (Dardik et al. 2006b). TSP-1 is one of the best characterized antiangiogenic factors. The importance of TSP-1 in angiogenesis is demonstrated by animal studies. In transgenic mice, targeted overexpression of TSP-1 in the skin was associated with potent inhibition of cutaneous tissue repair, granulation tissue formation, and wound angiogenesis (Streit et al. 2000). Therefore, the antiangiogenic properties of TSP-1 suggest the potential significance of this observation in FXIIIa-mediated promotion of angiogenesis (Richardson et al. 2013).

15.4.4 Apoptosis

Plasma FXIII targets an apoptotic molecule to downregulate its signal when cell death is initiated by an agonist. The Fas antigen is a member of the tumor necrosis factor receptor superfamily and can mediate apoptotic cell death in various cell types. FXIII inhibits apoptosis induced by a cytotoxic anti-Fas monoclonal antibody in Jurkat cells. Furthermore, an antibody against FXIII strongly accelerates the Fas-mediated apoptosis, indicating that FXIII is involved in cross-linking of Fas and downregulates Fas-mediated apoptotic cell death (Kikuchi et al. 2014).

15.4.5 Bacterial Immobilization

FXIII plays an important role in host defense against invasive bacteria. In normal plasma, the induction of coagulation by *Streptococcus pyogenes* results in immobilization of bacteria cells within a clot (Loof et al. 2011). This bacterial entrapment within a plasma clot was shown to be FXIII-dependent; cross-linking was not observed when FXIII-deficient plasma was used. Immunostaining with an antibody against N- ϵ - γ -glutamyl-lysine elucidated the nature of the interaction, revealing a covalent interaction between one terminal of a streptococcal M1 surface protein and a globular domain of fibrinogen. FXIII-dependent bacterial killing and cross-linking of a streptococcal M1 protein to fibrin networks were detected in tissue biopsy material from patients with streptococcal necrotizing fasciitis (Richardson et al. 2013).

15.5 Cell Surface FXIII

FXIII is present in platelets in large quantities, making the local platelet FXIII-A concentration approximately 150-fold greater than that in plasma. Platelets stabilize FXIII-depleted thrombi in a transglutaminase-dependent manner. Fluorescence confocal microscopy and flow cytometry revealed exposure of FXIII-A on an activated platelet surface (Mitchell et al. 2014). Therefore, the detection of FXIII-A that binds to activated, but not resting, platelets may be useful for the detection of pathological *in vivo* platelet activation (Devine et al. 1993). The Fab fragment of a polyclonal antibody against platelet FXIII inhibited the collagen-induced platelet aggregation, suggesting that FXIII-A functions on the platelet surface (Kasahara et al. 1988). However, it is unclear how platelet FXIII-A functions on the cell surface because it is not released by classical secretion mechanisms.

Lipid rafts are dynamic assemblies of sphingolipids, cholesterol, and signaling molecules such as src-family kinases that can be stabilized into platforms involved in the regulation of a number of vital cellular processes (Simons and Gerl 2010). The important functions of lipid rafts are signal transduction and membrane trafficking. Coalescence of lipid rafts on the cell surface leads to activation of src-family kinases (Kasahara and Sanai 2000). Lipid rafts are compositionally and functionally heterogeneous in the cell membrane. Platelet lipid rafts are critical membrane domains involved in physiological responses such as adhesion, aggregation and clot retraction (Bodin et al. 2003).

Cell surface FXIII-A transglutaminase is required for efficient clot retraction (Kasahara et al. 2013). Clot retraction is mediated by the interaction between the extracellular fibrin fiber and intracellular actomyosin via integrin α IIb β 3, together with the activation of the platelet contractile apparatus. Clot retraction is regulated through multiple signaling pathways. The src-dependent actomyosin contraction mediates clot retraction (Suzuki-Inoue et al. 2007). The src-dependent activation of phospholipase C γ 2 induces calcium mobilization, activation of myosin light chain kinase, and phosphorylation of myosin light chain. Flow cytometric analysis demonstrated that 5BAPA is incorporated into the surface of washed platelets by transamidation with thrombin stimulation. 5BAPA incorporation is completely impaired in FXIII-A-deficient mouse platelets. The 5BAPA-incorporated protein was identified as fibrin, which colocalizes with activated myosin in sphingomyelin-rich lipid rafts. Thrombin causes the rapid translocation of fibrin and myosin to the lipid raft fraction of washed platelets on a sucrose density gradient. The fibrin translocation to the lipid raft fraction is impaired in FXIII-A-deficient mouse platelets. These results suggest that fibrinogen is released from α -granules of platelets, converted to fibrin by the cleavage of fibrinopeptides A and B by thrombin, and translocated to sphingomyelin-rich rafts of thrombin-stimulated platelets in an intracellular FXIII-dependent manner, and activates myosin beneath sphingomyelin-rich rafts. Furthermore, raft disruption with methyl- β -cyclodextrin (which removes membrane cholesterol) inhibits outside-in signaling and clot retraction. Sphingomyelin-rich-raft-depleted platelets from sphingomyelin

synthase knockout mice exhibit delayed clot retraction (Kasahara et al. 2013). These findings suggest that platelet sphingomyelin-rich rafts act as platforms where fibrin and actomyosin efficiently join via integrin $\alpha\text{IIb}\beta\text{3}$ to promote outside-in signal for clot retraction (Fig. 15.1). The mechanism of FXIII-dependent fibrin translocation to lipid rafts remains to be explored.

Platelets activated simultaneously by collagen and thrombin constitute a sub-population of activated platelets. The activated platelets likely contribute significantly to thrombotic processes because they become “coated” with a number of procoagulant proteins, including factor V, fibrinogen, fibronectin, thrombospondin, and von Willebrand factor on the platelet surface. Many of these procoagulant proteins are posttranslationally modified by transamidase-mediated conjugation with serotonin, a process that increases their procoagulant activity. This mechanism may be important in the initial stabilization of the platelet plug at the site of injury (Dale 2005).

Cell surface FXIII-A is found in various cell types. FXIII-mediated protein cross-linking has been associated with extracellular matrix formation on the cell surface in physiological events such as differentiation. Myneni et al. (2014) demonstrated that cell surface FXIII-A transglutaminase acts as a switch between preadipocyte proliferation and differentiation. FXIII-A has recently been identified as a potential causative obesity gene in human white adipose tissue. It was demonstrated that preadipocyte FXIII-A forms an active transglutaminase that

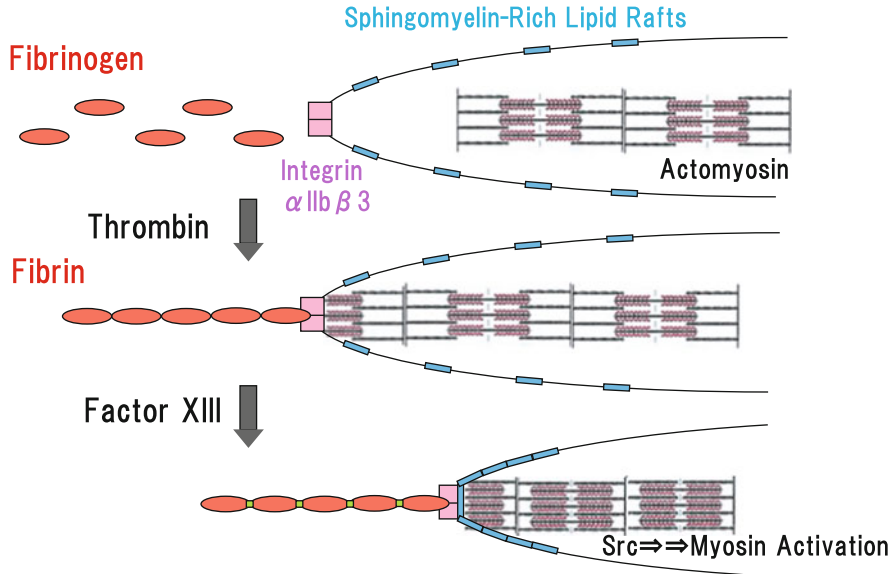


Fig. 15.1 Model of FXIII-dependent fibrin- $\alpha\text{IIb}\beta\text{3}$ -myosin axis in platelet sphingomyelin-rich lipid rafts. Fibrin is translocated to sphingomyelin-rich lipid rafts of thrombin-stimulated platelets in an FXIII-dependent manner. Sphingomyelin-rich lipid rafts act as platforms for fibrin-mediated outside-in signaling, leading to clot retraction

translocates to the cell surface and acts as a negative regulator of adipogenesis by promoting the assembly of fibronectin from plasma into a preadipocyte extracellular matrix.

Plasma membrane FXIII-A transglutaminase also regulates osteoblast differentiation (Al-Jallad et al. 2011). The fibrillary type I collagen matrix plays a major role in regulating osteoblast activity and is required for expression of osteoblast markers. FXIII-A-deficient mice showed decreased type I collagen production during remodeling after induced myocardial infarction. In these mice, type I collagen levels were not corrected by exogenously administered plasma FXIII therapy, indicating that type I collagen matrix synthesis is regulated by cellular FXIII-A. The cross-linking activity of FXIII-A stabilizes the interaction of microtubules with the plasma membrane. Microtubule association with the plasma membrane is required for the promotion of secretory vesicle (exosome) trafficking and protein delivery to the cell surface and secretion to the matrix. Tubulin undergoes palmitoylation for membrane insertion in some cell types and it has been demonstrated to be present in lipid rafts and interacts with GM1 and GM3 gangliosides (Palestini et al. 2000; Janich and Corbeil 2007). Although the precise interaction mechanism between FXIII-A and microtubules at the plasma membrane is not clear, tubulin is a substrate of cellular FXIII-A in differentiating osteoblasts (Wang et al. 2014). Serotonin can be incorporated covalently into proteins via a transglutaminase-mediated serotonylation reaction, which in turn can alter protein function. Serotonin inhibits FXIII-A-mediated plasma fibronectin matrix assembly and cross-linking in osteoblast cultures via direct competition with transamidation (Cui and Kaartinen 2015).

One possible mechanism of FXIII-A transport to the plasma membrane could be via sphingomyelin-rich lipid rafts. Sphingomyelin-rich rafts may act as membrane transport vesicles from the trans-Golgi network to the cell surface. FXIII-A is detected in the lipid raft fraction in stimulated but not resting platelets (Kasahara et al. 2013). Alternatively, FXIII-A secretion could be mediated by Golgi matrix protein-130 (GP130), which colocalizes with FXIII-A in macrophages. GP130 functions in nonclassical protein secretion to the plasma membrane prior to excretion through membrane pores. FXIII-A is not detected in classical secretory vesicles containing trans-Golgi network protein-46 (Cordell et al. 2010). Further work is necessary to define the exact mechanism involved in FXIII-A translocation across the plasma membrane and the signaling events.

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References

- Al-Jallad HF, Myneni VD, Piercy-Kotb SA, Chabot N, Mulani A, Keillor JW, Kaartinen MT (2011) Plasma membrane factor XIIIa transglutaminase activity regulates osteoblast matrix secretion and deposition by affecting microtubule dynamics. *PLoS One* 6:e15893
- Ando Y, Imamura S, Yamagata Y, Kitahara A, Saji H, Murachi T, Kannagi R (1987) Platelet factor XIII is activated by calpain. *Biochem Biophys Res Commun* 144:484–490
- Bodin S, Tronchère H, Payrastra B (2003) Lipid rafts are critical membrane domains in blood platelet activation processes. *Biochim Biophys Acta* 1610:247–257
- Carr ME, Carr SL, Tildon T, Fisher LM, Martin EJ (2003) Batroxobin-induced clots exhibit delayed and reduced platelet contractile force in some patients with clotting factor deficiencies. *J Thromb Haemost* 1:243–249
- Cordell PA, Kile BT, Standeven KF, Josefsson EC, Pease RJ, Grant PJ (2010) Association of coagulation factor XIII-A with Golgi proteins within monocyte-macrophages: implications for subcellular trafficking and secretion. *Blood* 115:2674–2681
- Cui C, Kaartinen MT (2015) Serotonin (5-HT) inhibits Factor XIII-A-mediated plasma fibronectin matrix assembly and crosslinking in osteoblast cultures via direct competition with transamidation. *Bone* 72:43–52
- Dale GL (2005) Coated-platelets: an emerging component of the procoagulant response. *J Thromb Haemost* 3:2185–2192
- Dardik R, Solomon A, Loscalzo J, Eskaraev R, Bialik A, Goldberg I, Schiby G, Inbal A (2003) Novel proangiogenic effect of factor XIII associated with suppression of thrombospondin 1 expression. *Arterioscler Thromb Vasc Biol* 23:1472–1477
- Dardik R, Leor J, Skutelsky E, Castel D, Holbova R, Schiby G, Shaish A, Dickneite G, Loscalzo J, Inbal A (2006a) Evaluation of the pro-angiogenic effect of factor XIII in heterotopic mouse heart allografts and FXIII-deficient mice. *Thromb Haemost* 95:546–550
- Dardik R, Loscalzo J, Inbal A (2006b) Factor XIII (FXIII) and angiogenesis. *J Thromb Haemost* 4:19–25
- Devine DV, Andestad G, Nugent D, Carter CJ (1993) Platelet-associated factor XIII as a marker of platelet activation in patients with peripheral vascular disease. *Arterioscler Thromb* 13:857–862
- Dickneite G, Herwald H, Korte W, Allanore Y, Denton CP, Matucci Cerinic M (2015) Coagulation factor XIII: a multifunctional transglutaminase with clinical potential in a range of conditions. *Thromb Haemost* 113:686–697
- DUCKERT F, JUNG E, SHMERLING DH (1960) A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. *Thromb Diath Haemorrh* 5:179–186
- Duval C, Allan P, Connell SD, Ridger VC, Philippou H, Ariëns RA (2014) Roles of fibrin α - and γ -chain specific cross-linking by FXIIIa in fibrin structure and function. *Thromb Haemost* 111:842–850
- Grundmann U, Amann E, Zettlmeissl G, Küpper HA (1986) Characterization of cDNA coding for human factor XIIIa. *Proc Natl Acad Sci U S A* 83:8024–8028
- Ichinose A, Hendrickson LE, Fujikawa K, Davie EW (1986a) Amino acid sequence of the a subunit of human factor XIII. *Biochemistry* 25:6900–6906
- Ichinose A, McMullen BA, Fujikawa K, Davie EW (1986b) Amino acid sequence of the b subunit of human factor XIII, a protein composed of ten repetitive segments. *Biochemistry* 25:4633–4638
- Iismaa SE, Mearns BM, Lorand L, Graham RM (2009) Transglutaminases and disease: lessons from genetically engineered mouse models and inherited disorders. *Physiol Rev* 89:991–1023
- Inbal A, Lubetsky A, Krapp T, Castel D, Shaish A, Dickneite G, Modis L, Muszbek L (2005) Impaired wound healing in factor XIII deficient mice. *Thromb Haemost* 94:432–437
- Janich P, Corbeil D (2007) GM1 and GM3 gangliosides highlight distinct lipid microdomains within the apical domain of epithelial cells. *FEBS Lett* 581:1783–1787

- Jayo A, Conde I, Lastres P, Jiménez-Yuste V, González-Manchón C (2009) New insights into the expression and role of platelet factor XIII-A. *J Thromb Haemost* 7:1184–1191
- Kasahara K, Sanai Y (2000) Functional roles of glycosphingolipids in signal transduction via lipid rafts. *Glycoconj J* 17:153–162
- Kasahara K, Takagi J, Sekiya F, Inada Y, Saito Y (1988) “A” subunit of factor XIII is present on bovine platelet membrane and mediates collagen-induced platelet activation. *Thromb Res* 50:253–263
- Kasahara K, Souri M, Kaneda M, Miki T, Yamamoto N, Ichinose A (2010) Impaired clot retraction in factor XIII A subunit-deficient mice. *Blood* 115:1277–1279
- Kasahara K, Kaneda M, Miki T, Iida K, Sekino-Suzuki N, Kawashima I, Suzuki H, Shimonaka M, Arai M, Ohno-Iwashita Y, Kojima S, Abe M, Kobayashi T, Okazaki T, Souri M, Ichinose A, Yamamoto N (2013) Clot retraction is mediated by factor XIII-dependent fibrin- α IIb β 3-myosin axis in platelet sphingomyelin-rich membrane rafts. *Blood* 122:3340–3348
- Katona E, Pénez K, Csapó A, Fazakas F, Udvardy ML, Bagoly Z, Orosz ZZ, Muszbek L (2014) Interaction of factor XIII subunits. *Blood* 123:1757–1763
- Kikuchi H, Kuribayashi F, Imajoh-Ohmi S (2014) Down-regulation of Fas-mediated apoptosis by plasma transglutaminase factor XIII that catalyzes fetal-specific cross-link of the Fas molecule. *Biochem Biophys Res Commun* 443:13–17
- Koseki-Kuno S, Yamakawa M, Dickneite G, Ichinose A (2003) Factor XIII A subunit-deficient mice developed severe uterine bleeding events and subsequent spontaneous miscarriages. *Blood* 102:4410–4412
- Loof TG, Mörgelin M, Johansson L, Oehmcke S, Olin AI, Dickneite G, Norrby-Teglund A, Theopold U, Herwald H (2011) Coagulation, an ancestral serine protease cascade, exerts a novel function in early immune defense. *Blood* 118:2589–2598
- Lorand L (2001) Factor XIII: structure, activation, and interactions with fibrinogen and fibrin. *Ann N Y Acad Sci* 936:291–311
- Mitchell JL, Lionikiene AS, Fraser SR, Whyte CS, Booth NA, Mutch NJ (2014) Functional factor XIII-A is exposed on the stimulated platelet surface. *Blood* 124:3982–3990
- Muszbek L, Bereczky Z, Bagoly Z, Komáromi I, Katona É (2011) Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. *Physiol Rev* 91:931–972
- Myneni VD, Hitomi K, Kaartinen MT (2014) Factor XIII-A transglutaminase acts as a switch between preadipocyte proliferation and differentiation. *Blood* 124:1344–1353
- Nikolajsen CL, Dyrland TF, Poulsen ET, Enghild JJ, Scavenius C (2014) Coagulation factor XIIIa substrates in human plasma: identification and incorporation into the clot. *J Biol Chem* 289:6526–6534
- Palestini P, Pitto M, Tedeschi G, Ferraretto A, Parenti M, Brunner J, Masserini M (2000) Tubulin anchoring to glycolipid-enriched, detergent-resistant domains of the neuronal plasma membrane. *J Biol Chem* 275:9978–9985
- Richardson VR, Cordell P, Standeven KF, Carter AM (2013) Substrates of Factor XIII-A: roles in thrombosis and wound healing. *Clin Sci (Lond)* 124:123–137
- Schroeder V, Kohler HP (2013) New developments in the area of factor XIII. *J Thromb Haemost* 11:234–244
- Serrano K, Devine DV (2002) Intracellular factor XIII crosslinks platelet cytoskeletal elements upon platelet activation. *Thromb Haemost* 88:315–320
- Simons K, Gerl MJ (2010) Revitalizing membrane rafts: new tools and insights. *Nat Rev Mol Cell Biol* 11:688–699
- Souri M, Kaetsu H, Ichinose A (2008a) Sushi domains in the B subunit of factor XIII responsible for oligomer assembly. *Biochemistry* 47:8656–8664
- Souri M, Koseki-Kuno S, Takeda N, Degen JL, Ichinose A (2008b) Administration of factor XIII B subunit increased plasma factor XIII A subunit levels in factor XIII B subunit knock-out mice. *Int J Hematol* 87:60–68

- Souri M, Osaki T, Ichinose A (2015) Anti-factor XIII A subunit (FXIII-A) autoantibodies block FXIII-A2 B2 assembly and steal FXIII-A from native FXIII-A2 B2. *J Thromb Haemost* 13:802–814
- Streit M, Velasco P, Riccardi L, Spencer L, Brown LF, Janes L, Lange-Asschenfeldt B, Yano K, Hawighorst T, Iruela-Arispe L, Detmar M (2000) Thrombospondin-1 suppresses wound healing and granulation tissue formation in the skin of transgenic mice. *EMBO J* 19:3272–3282
- Sugimura Y, Hosono M, Wada F, Yoshimura T, Maki M, Hitomi K (2006) Screening for the preferred substrate sequence of transglutaminase using a phage-displayed peptide library: identification of peptide substrates for TGASE 2 and Factor XIIIa. *J Biol Chem* 281:17699–17706
- Suzuki-Inoue K, Hughes CE, Inoue O, Kaneko M, Cuyun-Lira O, Takafuta T, Watson SP, Ozaki Y (2007) Involvement of Src kinases and PLCgamma2 in clot retraction. *Thromb Res* 120:251–258
- Walther DJ, Peter JU, Winter S, Höltje M, Paulmann N, Grohmann M, Vowinkel J, Alamo-Bethencourt V, Wilhelm CS, Ahnert-Hilger G, Bader M (2003) Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell* 115:851–862
- Wang S, Cui C, Hitomi K, Kaartinen MT (2014) Detyrosinated Glu-tubulin is a substrate for cellular Factor XIIIa transglutaminase in differentiating osteoblasts. *Amino Acids* 46:1513–1526