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Review Article

Substrates of Factor XIII-A: roles in thrombosis and wound healing

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Abstract

FXIII (Factor XIII) is a Ca^{2+}-dependent enzyme which forms covalent ε-(γ-glutamyl)lysine cross-links between the γ-carboxy-amine group of a glutamine residue and the ε-amino group of a lysine residue. FXIII was originally identified as a protein involved in fibrin clot stabilization; however, additional extracellular and intracellular roles for FXIII have been identified which influence thrombus resolution and tissue repair. The present review discusses the substrates of FXIIIa (activated FXIII) involved in thrombosis and wound healing with a particular focus on: (i) the influence of plasma FXIIIa on the formation of stable fibrin clots able to withstand mechanical and enzymatic breakdown through fibrin–fibrin cross-linking and cross-linking of fibrinolysis inhibitors, in particular α_2-antiplasmin; (ii) the role of intracellular FXIIIa in clot retraction through cross-linking of platelet cytoskeleton proteins, including actin, myosin, filamin and vinculin; (iii) the role of intracellular FXIIIa in cross-linking the cytoplasmic tails of monocyte AT_1Rs (angiotensin type 1 receptors) and potential effects on the development of atherosclerosis; and (iv) the role of FXIIIa on matrix deposition and tissue repair, including cross-linking of extracellular matrix proteins, such as fibronectin, collagen and von Willebrand factor, and the effects on matrix deposition and cell–matrix interactions. The review highlights the central role of FXIIIa in the regulation of thrombus stability, thrombus regulation, cell–matrix interactions and wound healing, which is supported by observations in FXIII-deficient humans and animals.

Key words: coagulation, extracellular matrix, Factor XIII, fibrinolysis, thrombosis, wound healing.

INTRODUCTION

FXIII (Factor XIII) structure and mechanism of action

FXIII is a transglutaminase which catalyses the formation of covalent ε-(γ-glutamyl)lysine cross-links between the γ-carboxy-amine group of a glutamine (amine acceptor) and the ε-amino group of a lysine (amine donor) residue. Plasma FXIII has a heterotetrameric structure composed of pairs of A subunits (83 kDa) and B subunits (80 kDa) held together by non-covalent interactions (FXIII-A2B2) [1,2], whereas cFXIII-A (cellular FXIII) exists as an A subunit homodimer [1,3]. The FXIII-A subunit contains the active site of the enzyme and is predominantly synthesised by cells of the monocyte/macrophage, megakaryocyte/platelet, chondrocyte and osteoblast/osteocyte lineages. [2,4]. However hepatocytes have also been reported to express low levels of the enzyme [5,6]. The FXIII-B subunit acts as a carrier protein in plasma for the hydrophobic A subunit and is synthesized and secreted by hepatocytes [7]. The cellular origin of plasma FXIII-A is unclear. Inbal et al. [8] proposed that platelets were the source of plasma FXIII-A; however, in two independent transgenic mouse models of thrombocytopenia, plasma levels of FXIII-A were normal, suggesting that, instead of platelets, cells of the monocyte lineage may be the source of plasma FXIII-A [9].

Activation of plasma FXIII-A2B2 is initiated upon the cleavage of the peptide bond between Arg^{37} and Gly^{38} of FXIII-A by thrombin and the release of the 37-amino-acid activation peptide [10]. Binding of FXIII-A2B2 via FXIII-B to non-cross-linked fibrin polymers allows the optimal orientation for FXIII-A cleavage of the activation peptide [11]. In the presence of Ca^{2+} and fibrin [12], thrombin-cleaved FXIII-A undergoes several conformational changes; initially FXIII-B dissociates from FXIII-A and then re-orientation of FXIII-A leads to exposure of the active site, forming FXIIIa (activated FXIII) [13] (Figure 1). Once cross-linking of the fibrin γ-chains occurs, the effect of fibrin

Abbreviations: ACE, angiotensin-converting enzyme; AngII, angiotensin II; AT_1R, angiotensin type 1 receptor; COAT, collagen- and thrombin-activated; FV, Factor V; FVa, activated FV; FXa, activated Factor X; FXIII, Factor XIII; FXIIIa, activated FXIII; cFXIII-A, cellular FXIII; GPIIb etc., glycoprotein IIb etc.; PA, plasminogen activator; PAI, PA inhibitor; TAFI, thrombin activatable fibrinolysis inhibitor; TAFIa, activated TAFI; TG2, tissue transglutaminase 2; t-PA, tissue-type PA; u-PA, urokinase-type PA; vWF, von Willebrand factor.

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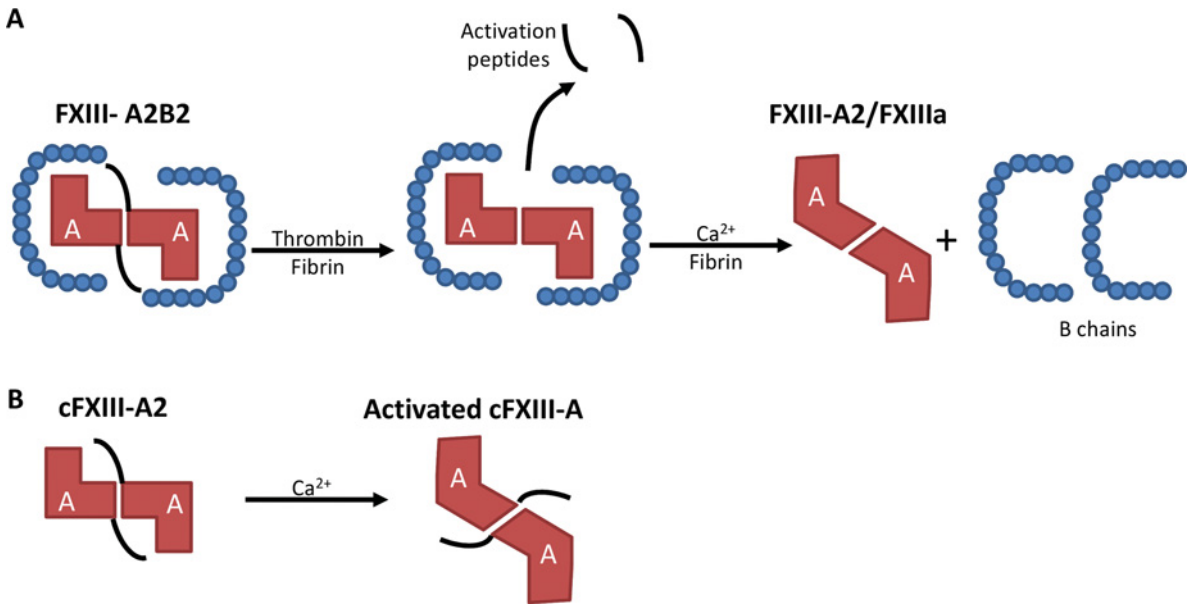

Figure 1 **Mechanism of FXIII activation**
(**A**) Activation of plasma FXIII-A2B2. In the presence of thrombin and Ca^{2+}, the activation peptide is cleaved off, followed by dissociation of the B chains yielding FXIII-A2/FXIIIa. (**B**) Activation of cellular FXIII-A. In the presence of Ca^{2+}, cFXIII-A2 undergoes a conformational change yielding activated cFXIII-A.

on FXIII-A activation is lost, suggesting that fibrin cross-linking down-regulates FXIII-A activation [14]. Activation of cFXIII-A occurs in the presence of Ca^{2+} alone, with intracellular Ca^{2+} concentrations above 2 mM required for activation [15].

The active site of FXIII-A contains a catalytic triad made up of Cys^{314}, which is required for the catalytic activity, and His^{373} and Asp^{396}, which are hydrogen-bonded to Cys^{314} [16,17]. FXIIIa uses a double displacement mechanism for cross-linking proteins, which has been described in detail previously [18]. In the initial stage of cross-link formation, Cys^{314} attacks the glutamine γ-carboxyamide group of a glutamine acceptor protein, displacing a molecule of ammonia to form a thioester intermediate. In the second stage, the reactive thioester intermediate is attacked by the lysine ε-amino group of the amine donor protein, displacing Cys^{314} and resulting in the formation of an isopeptide bond between the two substrate proteins and the release of FXIIIa (Figure 2). In the absence of lysine residues, water reacts with the thioester intermediate converting glutamine into glutamic acid [18].

A variety of methods have been employed to identify and characterize the substrates for FXIIIa. Potential glutamine sites in proteins have been identified by FXIIIa-catalysed incorporation of primary amines, such as putrescine [19–25], dansylcadaverine [21,22,26–28] and 5-(biotinamido)pentylamine [29,30]. Alternatively, labelled synthetic peptides have been designed to incorporate into the lysine residues of FXIIIa substrates [26]. In several cases, reactive glutamine and lysine residues have been characterized by MS and Edman sequencing analysis [19,29–31], as summarized in Figure 2. Recombinant proteins containing mutations in the glutamine [29,32] and lysine sites have also been used to confirm the importance of these residues in the cross-linking reaction.

The following sections provide an overview of the identification and characterization of key substrates for FXIIIa (summarized in Table 1), focusing on those substrates with known or potential roles in thrombosis and wound healing to provide insights into the potentially diverse roles of FXIIIa in the pathophysiology of cardiovascular disease.

SUBSTRATES OF FXIII INFLUENCING THE COMPONENTS OF THE COAGULATION CASCADE

The coagulation cascade is activated upon vascular damage, resulting in the sequential cleavage and activation of coagulation factors and culminating in thrombin generation and fibrin formation. FXIIIa-dependent cross-linking of fibrin results in the formation of a stable fibrin meshwork that is able to withstand mechanical pressures and enzymatic breakdown (Figure 3) [33].

Inter-molecular fibrin chain cross-linking

Fibrin is formed by thrombin-mediated cleavage of fibrinogen ($2A\alpha$, $2B\beta$ and 2γ) in the final stages of activation of the coagulation cascade. Thrombin sequentially cleaves the short N-terminal fibrinopeptides A and B from the fibrinogen $A\alpha$ and $B\beta$ chains respectively, to initiate fibrin protofibril formation, lateral aggregation and formation of trimolecular and tetramolecular branch points, which ultimately lead to the formation of a complex fibrin network [34,35]. The fibrin clot is stabilized by FXIIIa-induced cross-linking between fibrin γ chains and fibrin α chains [33,36], which increase the stiffness and reduce the stretch of the fibrin

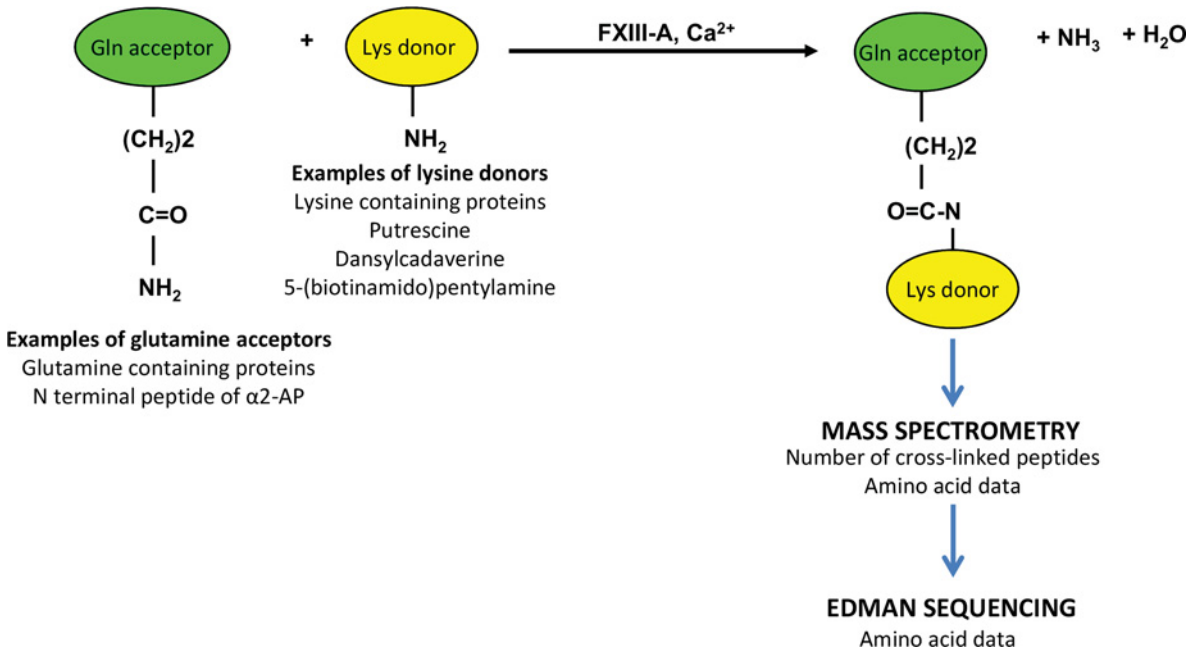

Figure 2 **Methodology for the identification of FXIII-A substrates**
FXIIIa forms an isopeptide bond between a glutamine-containing protein and a lysine-containing protein. Alternatively, the lysine- and glutamine-containing proteins can be replaced by synthetic peptides in the cross-linking reaction. FXIII-A substrates have been identified by MS and sequence information obtained by Edman sequencing.

network [37], thus providing the important structural framework of a thrombus.

FXIIIa rapidly forms cross-links between γ chains of fibrin molecules to form γ-dimers [38,39]. Cross-link formation occurs between Lys^{406} on one γ chain and either Gln^{399} or Gln^{398} on the neighbouring γ chain; however, FXIIIa shows increased specificity for Gln^{398} over Gln^{399} [40]. There has been much debate regarding the orientation of γ chain cross-links [41,42], with several studies suggesting that cross-linked γ chains are longitudinal with isopeptide bonds forming end-to-end between fibrin molecules [43,44], whereas others suggest that isopeptide bonds are aligned between fibrin protofibrils in the transverse orientation [45]. Definitive experiments to clarify the orientation are yet to be reported.

The fibrin α chain is cross-linked by FXIIIa to form α-polymers, albeit at a much slower rate compared with γ–γ cross-links [39]. Reactive glutamine residues of the α chain have been located at positions Gln^{221}, Gln^{237}, Gln^{328} and Gln^{366} using primary amines [46,47]. A number of lysine donor sites in the α chain have been identified by their ability to incorporate an N-terminal peptide of α_2-antiplasmin, including Lys^{556}, Lys^{539}, Lys^{508}, Lys^{580}, Lys^{418}, Lys^{448}, Lys^{601}, Lys^{606}, Lys^{427}, Lys^{429}, Lys^{208}, Lys^{224} and Lys^{219} [47,48]. Cross-link formation occurs between Gln^{223} and either Lys^{508} or Lys^{539}, between Gln^{237} and Lys^{418}, Lys^{508}, Lys^{539} or Lys^{556}, between Gln^{366} and Lys^{539}, and between Gln^{563} and either Lys^{539} or Lys^{601} [49]. The multiple lysine donor sites identified in the α chain provide the opportunity for the formation of a complex fibrin polymer while retaining the possibility of cross-linking numerous other plasma proteins to fibrin [47,48].

Studies measuring the elastic modulus of fibrin clots suggest that α chain cross-linking increases clot stability and rigidity [50,51] by promoting protofibril aggregation and the formation of thicker fibres [52]. Clot rigidity is also dependent on the formation of γ chain cross-links. Site-directed mutagenesis of Lys^{406}, Gln^{399} and Gln^{398} within the γ chain has demonstrated that γ-dimers produce maximal clot stiffness, whereas γ–α heteropolymers produce minimal increases in the strength of the fibrin fibres [38]. Fibrin is degraded by plasmin within the coiled-coil located between regions D and E. Resistance of the fibrin clot to plasmin-mediated fibrinolysis was found to be independent of γ-dimer formation [38,53], whereas the formation of γ-multimers or highly complex α polymers conferred increased resistance to fibrinolysis [53].

Cross-linking of FV (Factor V)

FV is a single-chain glycoprotein found in plasma and platelet α-granules. During activation of the coagulation cascade, FV is proteolytically activated by thrombin, resulting in the release of two activation peptides ($M_r = 150\,000$ and $71\,000$) and the formation of FVa (activated FV). FVa binds to FXa (activated Factor X) and is a co-factor for the FXa-mediated cleavage of pro-thrombin, leading to thrombin generation and fibrin formation. FVa is inactivated by cleavage by the natural anticoagulant activated protein C, leading to down-regulation of thrombin generation [54].

Activated and zymogen FV form high-molecular-mass polymers in the presence of FXIIIa, with cross-linking occurring between the large activation peptide and FVa [22], suggesting that FV contributes both glutamine and lysine residues in

Table 1 Summary of the reactive glutamine and lysine cross-linking sites of FXIIIa substrates and the systems in which the cross-linking reactions were performed
HEK, human embryonic kidney; HUVEC, human umbilical vein endothelial cell.

Protein	Cross-linking sites	Cross-linking substrate	Type of transglutaminase	Purified/plasma/cell system
Fibrin α chain [39,46–49]	Gln^{221}, Gln^{237}, Gln^{328} Gln^{366}, Lys^{556}, Lys^{539}, Lys^{508}, Lys^{580}, Lys^{583}, Lys^{418}, Lys^{448}, Lys^{601}, Lys^{606}, Lys^{427}, Lys^{446}, Lys^{429}, Lys^{208}, Lys^{224} and Lys^{219}	Fibrin α chain and fibrin γ chain	FXIII-A and TG2	Purified fibrin, plasma and thrombi
Fibrin γ chain [38–40]	Gln^{399}, Gln^{398} and Lys^{406}	Fibrin γ chain and fibrin α chain	FXIII-A and TG2	Purified fibrin, plasma and thrombi
FV [22,55]	–	FV	FXIII-A	Purified FV
Thrombospondin-1 [24,25,57]	–	Thrombospondin-1 and fibrin α chain	FXIII-A	Purified thrombospondin-1 and plasma
α_2-Antiplasmin [27,29–31,60,141]	Gln^{2}, Gln^{21}, Gln^{419} and Gln^{447}	Fibrin α chain Lys^{303}	FXIII-A and TG2	Purified α_2-antiplasmin and plasma
TAFI [26,65,66]	Gln^{2}, Gln^{5} and Gln^{292}	TAFI and fibrin α chain Lys^{77}, Lys^{79} and Lys^{212}	FXIII-A and TG2	Purified TAFI and plasma
Vitronectin [19,20]	Gln^{93}, Gln^{73}, Gln^{84} and Gln^{86}	Vitronectin	FXIII-A and TG2	Purified vitronectin
α_2-Macroglobulin [21,28]	Gln^{670} and Gln^{669}	Unknown	FXIII-A and TG2	Purified α_2-macroglobulin
Actin [72]	Gln^{41}	Fibrin α chain	cFXIII-A	Purified actin
Myosin [71]	–	Myosin	cFXIII-A	Purified myosin
Vinculin [73]	–	Unknown	cFXIII-A	Immunoblotting of platelet lysate
Filamin [73]	–	Unknown	cFXIII-A	Immunoblotting of platelet lysate
AT_1R [108]	Gln^{315}	AT_1R	cFXIII-A	Immunoblotting in HEK cells and patient monocytes
Fibronectin [23,32,83,84,111]	Gln^{3}, Gln^{4} and Gln^{16}	Fibrin α chain and collagen	FXIII-A and TG2	Purified fibronectin, immunohistochemistry in pre-osteoblasts, platelet aggregation under flow and fibroblast and megakaryocyte spreading
Type I, II, III and V collagen [90–92,111]	–	Fibronectin and vWF	FXIII-A	Purified collagen, immunohistochemistry in pre-osteoblasts and megakaryocyte spreading
vWF [95–97]	Gln^{313} and Gln^{560}	Fibrin α chain, collagen and laminin	FXIII-A	Purified vWF, plasma and *in vitro* cross-linking to purified collagen and laminin
Plasminogen [99,100]	Lys^{298} and Gln^{322}	Plasminogen, fibronectin and endothelial cells	FXIII-A and TG2	Purified plasminogen and HUVECs
PAI-2 [102,104-106]	Gln^{83}, Gln^{84} and Gln^{86}	Fibrin α chain Lys^{148}, Lys^{176}, Lys^{230} and Lys^{413}	FXIII-A and TG2	Purified PAI-2 and extracts of arterial/venous thrombi
Osteopontin [115–117]	Gln^{34} and Gln^{36}	Osteopontin	FXIII-A and TG2	Purified osteopontin
Glu-tubulin [119]	–	Unknown	cFXIII-A	Immunofluoresence staining in pre-osteoblasts

the cross-linking reaction [55]. Putrescine and dansylcadaverine can be incorporated into the large activation peptide of FV by FXIIIa, as determined by SDS/PAGE and fluorography [22], suggesting the activation peptide contains glutamine residues for the transamidation reaction; however, the specific reactive glutamine and lysine residues of FV have yet to be identified. Cross-linking between FV and other plasma proteins has not been identified and the physiological role of FV cross-linking is unknown; however, cross-linking of either putrescine or dansylcadaverine did not prevent thrombin cleavage of FV,

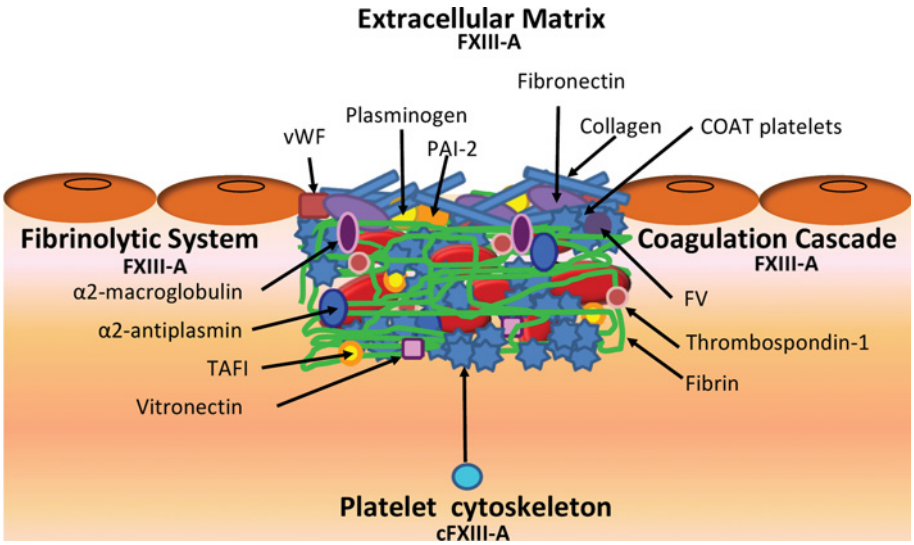

Figure 3 Role of cross-lining in thrombus consolidation and extracellular matrix attachment

Following tissue damage, the coagulation cascade is activated to generate thrombin. Thrombin cleaves fibrinogen to form fibrin and activates platelets and plasma FXIII-A. Activated platelets undergo conformational changes due to the cross-linking of cytoskeleton proteins by cFXIII-A and some form COAT platelets, thus aiding pro-thrombinase activity. Plasma FXIII-A cross-links fibrin to produce the structural framework of a thrombus, whereas cross-linking of the fibrinolysis inhibitors during fibrin formation is essential in protecting the forming clot from plasmin-mediated lysis. Cross-linking of matrix proteins fibronectin, collagen and vWF may be important in cell–matrix interactions and cross-linking of PAI-2 and plasminogen to matrix components suggests a role in localized regulation of plasmin generation and matrix metalloproteinase activation.

suggesting the site for thrombin cleavage is accessible following cross-linking [22].

Cross-linking of thrombospondin-1

Thrombospondin-1 is a glycoprotein released by α-granules of activated platelets and plays a role in platelet aggregation [56]. Thrombospondin-1 covalently incorporates putrescine in the presence of FXIIIa and forms intermolecular cross-links to itself by assembling as homopolymers, suggesting that thrombospondin-1 can provide both glutamine and lysine residues in the cross-linking reaction [24,25], although the specific residues are currently unknown. Thrombospondin-1 partially digested by thrombin incorporated a larger quantity of putrescine compared with the native protein, suggesting that thrombin digestion exposes additional glutamine sites for cross-linking [24]. Radioactively labelled thrombospondin-1 is incorporated into purified and plasma clots, with greater incorporation occurring in the presence of FXIIIa [57], due to the formation of cross-links between thrombospondin-1 and the fibrin α chain [24,25]. Cross-linking of thrombospondin-1 into fibrin clots was found to increase the density of the fibrin clot in a concentration-dependent manner [57], although the effects on fibrin function have not yet been investigated.

In summary, FXIIIa plays a major role in coagulation through the cross-linking of fibrin to produce the structural framework of a thrombus, which is able to withstand mechanical breakdown. The role of FV and thrombospondin-1 cross-linking in thrombus formation remains unclear; however, evidence suggests the function of FV is unaffected by cross-linking, whereas thrombospondin-1 cross-linking influences clot structure and therefore potentially fibrin function.

SUBSTRATES OF FXIII INFLUENCING THE FIBRINOLYTIC SYSTEM

Fibrinolysis is mediated by the serine protease plasmin. Plasmin is produced from the inactive zymogen plasminogen by the PAs (plasminogen activators), u-PA (urokinase-type PA) and t-PA (tissue-type PA). Upon activation, plasmin breaks down fibrin into fibrin degradation products. Regulation of fibrinolysis occurs via several mechanisms, including modulation of plasminogen binding, plasminogen activation and plasmin inhibition. Many of these effects are influenced by FXIIIa-dependent cross-linking reactions, as outlined below and in Figure 3.

Cross-linking of α_2-antiplasmin

The serine protease inhibitor α_2-antiplasmin is a glycoprotein that forms a covalent bond with the active-site serine residue of plasmin, inhibiting plasmin activity and thus plasmin-induced breakdown of fibrin. In plasma there are two N-terminal forms of circulating α_2-antiplasmin: Met-α_2-antiplasmin (464 residues) and Asn-α_2-antiplasmin (452 residues). Met-α_2-antiplasmin is proteolytically cleaved by APCE (antiplasmin-cleaving enzyme) between Pro^{12} and Asn^{13}, resulting in Asn-α_2-antiplasmin. α_2-Antiplasmin is cross-linked to the fibrin α chain and this reaction occurs 13 times faster for Asn-α_2-antiplasmin than Met-α_2-antiplasmin and, as a direct consequence, inhibition of fibrinolysis is more effective upon cross-linking of Asn-α_2-antiplasmin [58]. Gln^2 within Asn-α_2-antiplasmin is specifically cross-linked to Lys^{303} of the fibrin α chain [31]. Gln^2 was found to be the primary residue susceptible to FXIIIa cross-linking as it readily incorporated [^{14}C]histamine [59], and a synthetic peptide

comprising residues 1–12 of Asn-α_2-antiplasmin was found to compete with wild-type α_2-antiplasmin for FXIIIa cross-linking both in a purified [60] and plasma-based [61] system. Mutant Asn-α_2-antiplasmin, formed by replacing Gln^2 with alanine, incorporated 5-(biotinamido)pentylamine into three alternative sites, including Gln^{21}, Gln^{419} and Gln^{447}, in the presence of FXIIIa, suggesting that FXIIIa has the potential to cross-link a number of sites; however, incorporation at these sites was markedly less efficient compared with Gln^2 [29,30].

Clots produced from plasma deficient in α_2-antiplasmin lyse readily, even after suspension into plasma containing physiological concentrations of α_2-antiplasmin, whereas clots formed using α_2-antiplasmin-deficient plasma supplemented with α_2-antiplasmin prior to clotting prevented fibrinolysis in a concentration-dependent manner [62]. Thrombi deficient in either FXIII-A or α_2-antiplasmin lyse at significantly faster rates compared with thrombi containing both FXIII-A and α_2-antiplasmin [63], and incorporation of the synthetic Asn-α_2-antiplasmin residues 1–12 peptide competed with native α_2-antiplasmin for cross-linking and accelerated fibrinolysis [61]. These findings highlight the importance of cross-linking of α_2-antiplasmin to fibrin during clot formation to provide protection from plasmin-mediated lysis.

Cross-linking of thrombin activatable fibrinolysis inhibitor

During fibrinolysis, plasmin cleaves fibrin exposing C-terminal lysine and arginine residues which act as cofactors for plasminogen binding and tPA-mediated plasmin generation. The pro-carboxypeptidase TAFI (thrombin activatable fibrinolysis inhibitor) undergoes proteolysis by the thrombin–thrombomodulin complex to form the carboxypeptidase TAFIa (activated TAFI), which inhibits plasminogen activation and therefore fibrinolysis [64]. TAFIa removes the C-terminal lysine and arginine residues preventing plasminogen binding and plasmin generation. Physiological concentrations (70–250 nM) of TAFIa also inhibit plasmin directly to further inhibit fibrinolysis [64].

TAFI incorporates both dansylcadaverine and dansyl-PGGQQIV in the presence of FXIIIa and TG2 (tissue transglutaminase 2) forming homopolymers, indicating it contains amine acceptor and donor sites. The reactive glutamine sites have been identified as Gln^2, Gln^5 and Gln^{292} [26]; however, currently the reactive lysine sites are unknown. TAFI is also cross-linked to fibrin by FXIIIa and TG2 as determined by SDS/PAGE [26], and Gln^2 and Gln^5 within the activation peptide of TAFI are capable of cross-linking to Lys^{212}, Lys^{77} and Lys^{79} of the fibrin α chain [65]. In plasma and whole-blood thrombi, neutralization of TAFIa by a carboxypeptidase inhibitor conferred an increase in lysis rates [66], suggesting TAFIa remains functional after cross-linking. FXIIIa-mediated cross-linking of TAFI may therefore serve to localize TAFI to sites of thrombus formation.

Cross-linking of vitronectin

Vitronectin is a plasma glycoprotein present in megakaryocytes and platelet α-granules. Vitronectin is involved in the regulation of coagulation, fibrinolysis, complement and cell adhesion, and is a carrier protein for PAI (PA inhibitor)-1 [67]. Putrescine is readily incorporated into vitronectin in the presence of FXIIIa, as determined by MS analysis, and Edman sequencing of the tryptic peptides indicated the predominant incorporation of putrescine at Gln^{93}, with minor incorporation at Gln^{73}, Gln^{84} and Gln^{86} [19]. In the presence of FXIIIa, vitronectin forms homodimers, suggesting that vitronectin contains reactive glutamine and lysine residues [20]; however, the lysine residues have not been identified. Although vitronectin is incorporated into fibrin clots, this was found to be independent of FXIIIa, suggesting that vitronectin binds rather than is cross-linked to fibrin [67]. Fibrin-bound vitronectin is important for PAI-1 binding to fibrin and PAI-1-mediated inhibition of t-PA, suggesting that vitronectin plays an essential role in preventing fibrinolysis [68]. Whether vitronectin is incorporated into other proteins is currently unclear.

Cross-linking of α_2-macroglobulin

α_2-Macroglobulin is a plasma glycoprotein which inhibits several proteolytic enzymes, such as thrombin and plasmin. α_2-Macroglobulin binds and forms a complex with plasmin and, although the rate of complex formation is slower than that of α_2-antiplasmin and plasmin, complex formation begins before α_2-antiplasmin saturation, supporting a role of α_2-macroglobulin as a secondary inhibitor of fibrinolysis with physiological relevance [69]. α_2-Macroglobulin is a substrate for FXIIIa as it readily incorporates dansylcadaverine [21,28] and putrescine [21] into a major site at Gln^{670} and a minor site at Gln^{669} [21]. Although α_2-macroglobulin contains reactive glutamine sites, the substrate(s) to which α_2-macroglobulin is cross-linked has not been identified to date. Unlike α_2-antiplasmin, α_2-macroglobulin is not cross-linked to fibrin [28], suggesting that α_2-macroglobulin may be important for the protection of other clot or matrix proteins from proteolytic cleavage.

In summary, FXIIIa-dependent cross-linking of the fibrinolysis inhibitors during fibrin formation is essential in protecting the forming clot from plasmin-mediated lysis. In particular, cross-linking of α_2-antiplasmin to fibrin appears to be the primary mechanism for FXIIIa-dependent prolongation of fibrinolysis [63]. Cross-linking of TAFI, vitronectin and α_2-macroglobulin may play a secondary more localized role in the modulation of fibrinolysis and/or proteolysis of extracellular matrix proteins.

SUBSTRATES OF FXIII INFLUENCING CELLULAR FUNCTION AND EXTRACELLULAR MATRIX FORMATION AND STABILITY

A number of cell types contain cFXIII-A, which appears to be located primarily in the cytosol; however, in macrophages the association of cFXIII-A with other compartments, including the nucleus and plasma membrane, has also been described [70]. A previous study found that cFXIII-A is enriched in plasma-membrane-associated structures resembling cell adhesions that also contain certain Golgi proteins [9], suggesting a possible pathway for externalization of cFXIII-A, which may contribute to the plasma pool. As outlined below, cFXIII-A and plasma FXIII-A

contribute to both intracellular and extracellular processes to modulate cell/protein interactions (Figure 3).

Cross-linking of the platelet cytoskeleton

The platelet cytoskeleton is made up of actin filaments which provide support to the plasma membrane allowing it to change from a discoidal shape in a resting platelet to a sphere with filopodia in an activated platelet ready for clot retraction. Some of the first cytoskeletal proteins to be identified as FXIIIa substrates were myosin and actin [71,72]. Fluorescence analysis of dansylcadaverine incorporation into myosin by SDS/PAGE suggested that most of the incorporation occurred in the myosin heavy chain; however, a small amount of incorporation was also observed within the myosin light chain. The myosin heavy chain has been shown to form highly cross-linked polymers in the presence of FXIIIa, suggesting the presence of both FXIIIa-reactive glutamine and lysine residues [71]. Dansylcadaverine cross-linking to platelet actin occurs at a slow rate, with incubation times of 8 h and high FXIIIa/actin ratios required [72]. Actin subunits also form intermolecular cross-links in the presence of FXIIIa, suggesting this protein also contains FXIIIa-reactive glutamine and lysine residues [72]. A more recent study failed to demonstrate cross-linking of platelet actin in activated platelets; however, platelet activation was essential for binding of cFXIII-A to actin, allowing it to be translocated to the platelet periphery upon actin polymerization [73]. The translocation of cFXIII-A to the platelet periphery may be essential for the cross-linking of cytoskeleton proteins such as vinculin, which is also redistributed to the platelet periphery during platelet activation [74]. Both vinculin and filamin are cross-linked in activated platelets, as demonstrated by the formation of high-molecular-mass complexes; however, platelet aggregation was required for vinculin cross-linking [73]. In platelets deficient in cFXIII-A, binding of fibrinogen is reduced upon thrombin receptor agonist peptide stimulation and platelet spreading is delayed [75]. These findings suggest a role for cFXIII-A in stabilizing and strengthening the platelet cytoskeleton prior to clot retraction. A role for cFXIII-A in clot retraction is supported by studies in FXIII-A-knockout mice in which clot retraction does not occur [76]. Clot retraction following supplementation with plasma FXIII-A was only partially restored, suggesting both extracellular and intracellular FXIII play a role in clot retraction in mice.

Cross-linking in COAT (collagen- and thrombin-activated) platelet formation

COAT platelets are a subset of thrombin and type I and V collagen-activated platelets characterized by the presence of high surface concentrations of α-granule proteins, including FV, fibrinogen, vWF (von Willebrand factor), fibronectin, α_2-antiplasmin and thrombospondin. The presence of prothrombotic proteins on the surface of COAT platelets make them highly likely to contribute to thrombosis compared with non-COAT platelets. As highlighted above, these proteins are also known FXIIIa substrates, suggesting that FXIIIa-dependent cross-linking may be important in formation of COAT platelets. To confirm this, Dale et al. [77] monitored COAT platelet formation following incubations with dansycadaverine, S4732 (an FXIIIa active-site inhibitor) and antibodies inhibiting FXIII-A activation and activity. By preventing FXIIIa activity the appearance of protein complexes on COAT platelets was significantly reduced, even though protein was still released from α-granules. That study showed further that cross-link formation occurred between released α-granule proteins and serotonin [77]. Szasz and Dale [78] found that proteins cross-linked to serotonin bound to a currently unknown serotonin-binding site on fibrinogen and thrombospondin and proposed a model for COAT platelet formation whereby fibrinogen and FV initially bind to their respective receptors on platelets [GP (glycoprotein) IIb/IIIa and phosphatidylserine], followed by binding of thrombospondin and other proteins cross-linked to serotonin to fibrinogen and thrombospondin via the serotonin-binding sites to form a complex network of proteins [78]. As COAT platelet formation does not occur on all platelets, it suggests that α-granule secretion, FXIII-A activity, phosphatidyl exposure and GPIIb/IIIa activation all need to coincide. The importance COAT platelets and, in particular, serotonin cross-linking in thrombus formation is highlighted in patients undergoing treatment with fluoxetine (which decreases platelet serotonin levels) where a tendency for mild bleeding is observed [79].

Cross-linking of fibronectin

Fibronectin is a glycoprotein which plays important roles in cell adhesion, migration and tissue repair due to its ability to bind molecules such as cell-surface integrins, fibrin, collagen and heparin [80,81]. Fibronectin exists in two forms. Plasma fibronectin is a disulfide-bonded dimer produced by hepatocytes, which plays a major role in extracellular matrix formation. Cellular fibronectin is synthesized locally in tissues by a variety of cells, including fibroblasts, myoblasts, macrophages, epithelial cells, smooth muscle cells, hepatocytes, chondrocytes, osteoblasts, Schwann cells and astroglial cells, as dimers and multimers and is more important in mediating cell–matrix interactions rather than matrix formation [82].

Fibronectin is cross-linked to the fibrin α chain by FXIIIa [83], initially forming heterodimers, which upon further cross-linking form high-molecular-mass polymers as observed by SDS/PAGE [84]. Early studies of bovine fibronectin demonstrated cross-linking of putrescine to Gln^3 [23]. Corbett et al. [32] subsequently demonstrated that Gln^3, Gln^4 and Gln^{16} were potential cross-linking sites in human fibronectin, as recombinant fibronectin containing mutations at Gln^3 and Gln^4 resulted in a 65% reduction in cross-linking compared with wild-type fibronectin, and additional mutation at Gln^{16} resulted in a complete loss of cross-linking [32]. To date, the lysine residues which participate in fibrin to fibronectin cross-linking have not been determined.

Several studies have investigated the role of fibronectin cross-linking on clot formation. Cross-linking of fibronectin to fibrin produces significantly more dense clots with smaller pores; however, clot rigidity was unaffected by fibronectin at physiological concentrations [84–87]. Perfusion of platelets over a matrix containing either fibrin or fibronectin alone or cross-linked fibronectin–fibrin in a parallel plate flow chamber indicated increased platelet adhesion and aggregation to cross-linked fibronectin–fibrin compared with fibrin or fibronectin alone [88].

The extent of platelet thrombus formation was also shown to be dependent on the concentration of fibronectin cross-linked to fibrin [88]. Cross-linking of fibronectin to fibrin is also required for fibroblast adhesion and spreading on fibronectin, suggesting that covalent interactions may induce structural changes within the protein which provide access to the adhesive domain [32]. Cross-linking by FXIIIa is also required for megakaryocyte spreading and fibronectin matrix assembly on spread megakaryocytes incubated on type I collagen [89]. These findings suggest diverse roles for FXIIIa-dependent cross-linking of fibronectin in thrombosis and wound healing, including extracellular matrix assembly, platelet thrombus formation and cell–matrix interactions.

Cross-linking of collagen

Collagen forms a major component of the extracellular matrix and exposure of collagen to flowing blood initiates thrombus formation. Type I, II, III and V collagens are cross-linked by FXIIIa to fibronectin [90,91]. During the cross-link reaction collagen provides the lysine residues, whereas fibronectin provides the glutamine residues [92]. Although the reactive lysine residue(s) of collagen is currently unknown, type I collagen competitively inhibits the cross-linking of fibronectin to fibrin, suggesting that Gln^{3}, Gln^{4} or Gln^{16} of fibronectin are probable sites for cross-linking to collagen [90]. Cross-linking of fibronectin to collagen may play an important physiological role in extracellular matrix formation and wound healing.

Cross-linking of vWF

vWF is a plasma glycoprotein composed of 250 kDa subunits held together by disulfide links to form large multimers, which are important for vWF–platelet interactions. vWF is present in platelet α-granules and endothelial cell Weibel–Palade bodies, and plays an essential role in the initial adhesion of platelets to subendothelial collagen at sites of vascular injury and as a carrier of FVIII (Factor VIII), providing protection from proteolytic degradation in plasma [93]. Multimeric vWF molecules are not cross-linked to each other by FXIIIa, but can incorporate putrescine, suggesting that only glutamine residues are available for cross-linking [94]. Studies of bovine vWF have shown that Gln^{313}, Gln^{509}, Gln^{560} and Gln^{634} are susceptible to FXIIIa cross-linking as dansylcadaverine is readily incorporated at these residues [95]; however, only Gln^{313} and Gln^{560} are conserved in human vWF, suggesting these may be the residues involved in cross-linking vWF.

In a purified system, multimeric vWF was found to covalently cross-link to the fibrin α chain as both vWF–α chain heterodimers and high-molecular-mass polymers were observed. vWF was also found to incorporate into fibrin in a plasma-based system; however, clotting times of 3 h were required [94]. In the presence of FXIIIa, vWF is also covalently cross-linked to components of the basement membrane, such as type I and III collagen [96] and laminin [97]. Subendothelial vWF plays an essential role in primary haemostasis by binding to platelet GPIb–IX–V receptors to promote platelet adhesion and thrombus formation at sites of vascular damage; however, the specific role of vWF cross-linking to extracellular matrix components remains unclear.

Cross-linking of plasminogen

Plasminogen is produced by the liver as an inactive precursor, which is converted into the serine protease plasmin upon cleavage by u-PA or t-PA. Plasmin is responsible for fibrinolysis and also the activation of matrix metalloproteinases, which play important roles in tissue repair [98]. Plasminogen incorporates dansylcadaverine and dansyl-PGGQQIV in the presence of FXIIIa, indicating the presence of both reactive glutamine and lysine residues [99]. The incorporation sites were identified as Lys^{298} and Gln^{322} by Edman sequencing analysis [100]. Plasminogen molecules become cross-linked to each other within 5 min of incubation with FXIIIa, and plasminogen is also cross-linked to fibronectin by FXIIIa and TG2, forming heteropolymers [99]. Plasminogen cross-linking occurs extensively on endothelial cell surfaces and remains activatable by t-PA to form plasmin [99]. This suggests a localized role of plasminogen at the cell surface and extracellular matrix in generating plasmin to limit thrombus formation and in the activation of matrix metalloproteinases involved in matrix remodelling and angiogenesis [101].

Cross-linking of PAI-2

PAI-1 and PAI-2 form complexes with free PA, preventing plasmin generation and therefore fibrinolysis and proteolysis [102]. PAI-2 is predominantly an intracellular protein expressed in monocytes, endothelial cells, fibroblasts and smooth muscle cells and, as a result, is localized to the extracellular matrix. Although secreted as a 60 kDa glycoprotein, the plasma levels are extremely low [103], suggesting PAI-2 influences tissue repair by modulating proteolysis at sites of vascular injury. PAI-2 has been shown to be cross-linked to fibrin and contains three glutamine residues susceptible to cross-linking: Gln^{83}, Gln^{84} and Gln^{86}, which are located away from the PAI-2 active site in a 33-amino-acid extended loop region between helices C and D [104]. Recombinant peptides of PAI-2 residues 80–90, mutated at either Gln^{83} or Gln^{86} demonstrated reduced competition with wild-type PAI-2 for FXIIIa cross-linking [102]. MS analysis of a tryptic peptide containing the three glutamine residues showed that only one putrescine unit is attached to PAI-2 at any one time, suggesting that these sites are not cross-linked simultaneously [104]; however, the preferred site of incorporation has yet to be defined. PAI-2 is cross-linked to the fibrin α chain [105,106] at positions Lys^{148} and Lys^{176} in the coiled-coil region and Lys^{230} and Lys^{413} located in the flexible C-terminal domain [106]. Fibrin α chain cross-linking was unaffected by the presence of PAI-2 [105], and PAI-2 cross-linked to fibrin remained functional as both u-PA [102] and t-PA [106] were inhibited in a dose-dependent manner thus preventing fibrinolysis. Although PAI-2 is predominantly an intracellular protein, secreted PAI-2 cross-linked to fibrin may provide localized inhibition of fibrinolysis at the site of vascular damage [102]. Whether PAI-2 is cross-linked to other extracellular matrix proteins is currently unknown.

Cross-linking of AT_1R (angiotensin type 1 receptor)

AT_1R is a G-protein-coupled receptor activated by binding of its ligand AngII (angiotensin II), which is formed during activation of the RAS (renin–angiotensin system) [107]. Activation of monocyte AT_1R results in increased cell activation and

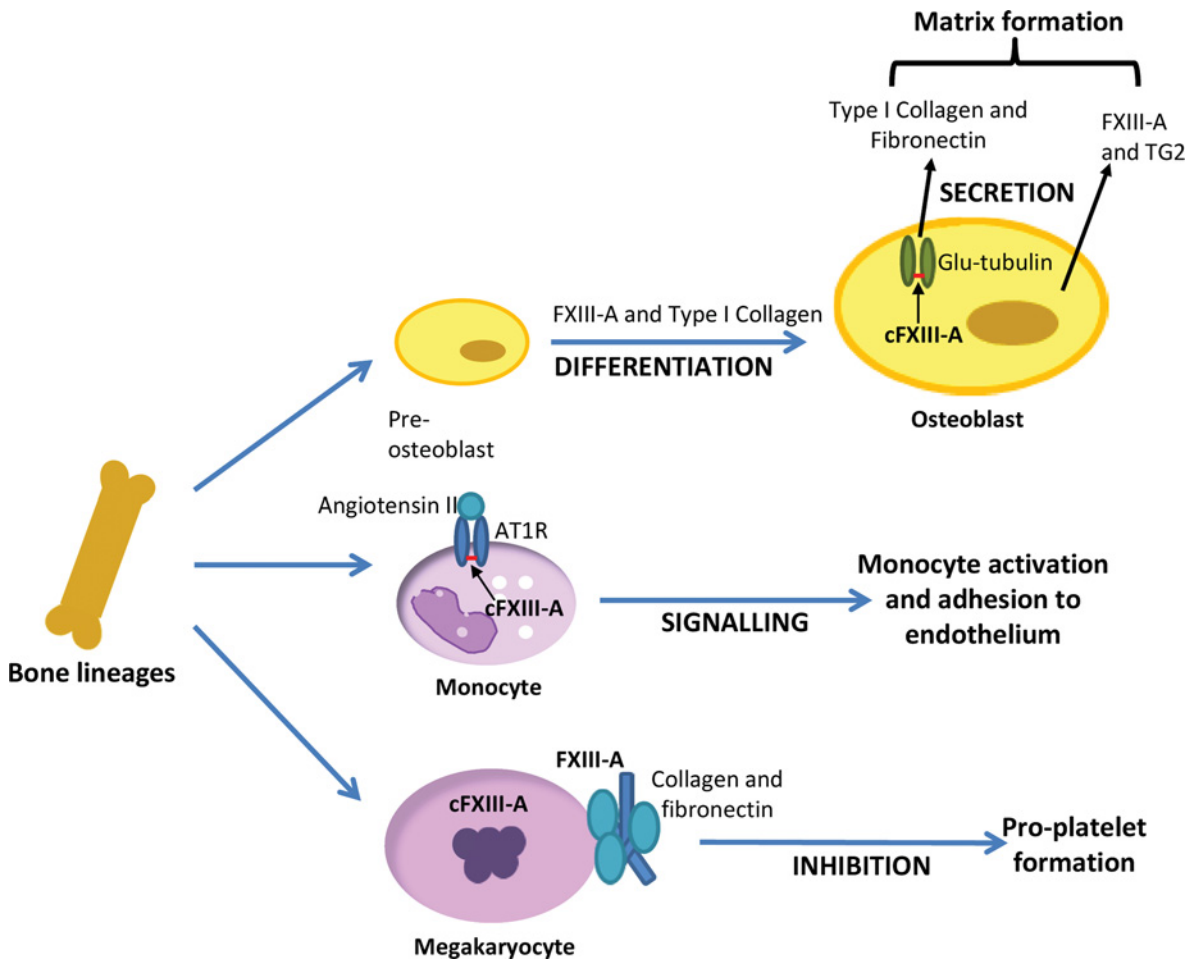

Figure 4 **Role of FXIII in cells of a bone lineage**

In osteoblasts, FXIII-A aids osteoblast differentiation. cFXIII-A promotes secretion of type I collagen and fibronectin due to cross-linking of glu-tubulin, thus aiding matrix formation. In monocytes, cFXIII-A cross-links the cytoplasmin tails of AT_1Rs following AngII binding, resulting in downstream signalling leading to monocyte activation and adhesion to the endothelium. In megakaryocytes, FXIII-A stabilizes the megakaryocyte–type I collagen interaction through fibronectin fibrillogenesis, a process that inhibits pro-platelet formation.

adhesion to the endothelium, a process thought to contribute to the increased risk of atherosclerosis seen in hypertensive individuals [107]. Intriguingly AT_1R signalling is increased in the monocytes of these individuals by the formation of substantial numbers of covalently stabilized AT_1R homodimers [108] (Figure 4). Upon ligand-induced receptor dimerization, monocyte cFXIII-A was shown to cross-link Gln^{315} to a currently unknown lysine residue in the cytoplasmic tail domains of the receptor [108]. This cross-linking required both AngII (to promote receptor activation and dimerization) and increased cytosolic Ca^{2+} (to promote cFXIII-A activation) and was absent from the monocytes of FXIII-A-deficient patients.

Although monocytes from hypertensive individuals have a greater proportion of AT_1R molecules present in cross-linked dimers compared with those from healthy controls, lowering AngII levels by treatment with an ACEI [ACE (angiotensin-converting enzyme) inhibitor] significantly reduced receptor cross-linking. Furthermore, inhibition of ACE or of cFXIII-A reduced the development of atherosclerosis in ApoE (apolipoprotein E)-deficient mice, linking *in vivo* FXIII-A activity to this process [108].

Cross-linking in cartilage and bone

The potential for FXIII-A to modulate extracellular matrix formation and function is supported by studies of bone and cartilage formation (Figure 4). FXIII-A and TG2 are the principle transglutaminases expressed in cartilage and bone, being synthesized and secreted into the extracellular matrix by chondrocytes and osteoblasts in respective tissues [109–111]. Increased expression of both enzymes has been linked to the maturation of both chondrocytes and osteoblasts [112,113]. In chondrocytes, extracellular FXIII-A has been reported as a ligand of $\alpha 1\beta 1$ integrin, promoting externalization of TG2 [114]. FXIII-A-mediated cross-linking activity has been demonstrated within chondrocytes and in the cartilage and bone matrix, with potential extracellular substrates including, fibronectin and osteopontin [110,112,115–117]. Elevation of FXIII-A expression has also been linked to

the differentiation of pre-osteoblasts to osteoblasts [118] promoted by type 1 collagen [111]. cFXIII-A activity has also been shown to enhance the secretion of type I collagen and fibronectin from osteoblasts by stabilization of the microtubule system via glu-tubulin cross-linking, suggesting a role in matrix formation [111,119]. Several studies have also suggested that this cross-linking is required for proper bone matrix deposition and mineralization, as determined by the use of transglutaminase inhibitors [113,119]. No obvious abnormal skeletal phenotypes have been reported for FXIII-A$^{-/-}$ or TG2$^{-/-}$ mice, suggesting a compensatory role of the other transglutaminases in skeletal development [120]. For example, TG2$^{-/-}$ mice have been found to express higher levels of FXIII-A and TGF-β (transforming growth factor-β) [121]. The synergistic effect of FXIII-A and TG2 has also been observed in chondrocyte differentiation [122], suggesting that cross-link formation is important for bone and cartilage growth and maintenance; however, these actions can occur via FXIII-A and/or TG2.

FXIIIa-mediated fibronectin cross-linking has also been demonstrated to play a role in megakarocyte function in the bone marrow [89] (Figure 4). Malara et al. [89] found that FXIII-A is the predominant transglutaminase expressed in megakaryocytes and that, in culture, these cells demonstrate constitutive transglutaminase activity both in the cytoplasm and on the plasma membrane where FXIII-A and fibronectin were found to co-localize. They concluded from these observations, and the effect of small-molecule transglutaminase inhibitors, that FXIIIa is required for stabilization of the megakaryocyte–type I collagen interaction through fibronectin fibrillogenesis, a process that inhibits pro-platelet formation [89] and links bone function to haemostasis.

In summary, cFXIII-A can be found in several cell types, including platelets, monocytes/macrophages, chondrocytes and osteoblast/osteocytes. Although there is increasing evidence to suggest cFXIII-A cross-linking plays a role in platelet cytoskeleton remodelling following platelet activation, the downstream signalling of monocyte AT_1Rs and bone matrix deposition and mineralization, the intracellular functions of FXIII-A remain largely unexplored and require further investigation. FXIIIa-dependent cross-linking of matrix proteins including fibronectin, collagen and vWF may be important in cell–matrix interactions and cross-linking of PAI-2 and plasminogen to matrix components at sites of vascular injury suggests a role in localization and regulation of plasmin generation and matrix metalloproteinase activation to support an important role in wound healing.

THE SPECIFICITY OF FXIII FOR ITS SUBSTRATES

The transglutaminase family contains a group of structurally related enzymes that catalyse the formation of cross-links. To study the role of the transglutaminase active site in defining substrate specificity Hettasch et al. [123] produced a recombinant chimaera of FXIII-A by exchanging exon 7 of FXIII-A, which codes for the active site, with that of TG2 and evaluated the fibrin cross-link pattern. The chimaera produced fibrin α–γ cross-links, a cross-link pattern similar to that of TG2; however, the efficiency was lower than both wild-type enzymes [123]. Although the findings suggests that the primary amino acid residues which make up the active site play a central role in substrate recognition and catalytic activity, this chimaera did not contain the complete catalytic triad of FXIIIa, which could possibly account for the reduced efficiency of the chimaera compared with the wild-type enzyme.

FXIIIa substrates have been studied to determine consensus sequences that confer FXIII specificity. For example, the γ chain of fibrin and β-casein are both known to be good substrates for FXIIIa; however, they do not contain any common sequence which would suggest specificity of FXIIIa for a particular substrate, suggesting substrate recognition residues are also located away from the reactive glutamine residue [40]. Sugimura et al. [124] screened a phage-displayed peptide library for cross-linking of dansylcadaverine and putrescine by FXIIIa to determine common amino acid sequences which aid recognition of reactive glutamine residues. The FXIIIa-preferred substrate sequence was determined to be QxxØxWP, where x is a non-conserved amino acid and Ø is a hydrophobic amino acid [124]. However, this sequence does not correspond to major plasma substrates of FXIIIa such as fibrin, suggesting the importance of substrate protein structure in modulating interactions with FXIIIa.

As no consensus sequence has been identified, studies have focused on the interactions between FXIII-A and its substrates using nuclear magnetic resonance spectroscopy. Marinescu et al. [125] found that residues located C-terminally from the reactive glutamine within α_2-antiplasmin interact directly with the active site first by the attachment of the glutamine and neighbouring amino acids to the catalytic triad and secondly by the clustering of hydrophobic amino acid side chains at a secondary site. The possibility of a secondary site was also confirmed by Pénzes et al. [126] as the serial cleavage of residues 7–12 of the Asn-α_2-antiplasmin peptide (residues 1–12) resulted in the gradual loss of catalytic activity. Within this region hydrophobic residues Leu^{10} and Lys^{12} were found to promote α_2-antiplasmin binding to FXIII-A [126]. These findings suggest that the glutamine and surrounding hydrophobic residues are important in substrate recognition and binding.

Consequences of FXIII deficiency *in vivo*

The importance of FXIII-A in thrombosis and wound healing is emphasized in FXIII deficiency, which is characterized by bleeding, abnormal wound healing and spontaneous miscarriage in females, and occurs as a result of defects in the FXIII-A and FXIII-B subunit genes [127,128]. In FXIII-A-knockout mice, tail-tip bleeding times are doubled and clot stability, assessed by thromboelastography, was impaired compared with wild-type mice [129]. Equally, the rate of clot retraction is significantly reduced [76]. Impaired tissue repair was observed in the left ventricles of FXIII-A-knockout mice after myocardial infarction. Furthermore, molecular imaging of cross-linking within the infarct of wild-type mice confirmed high FXIII-A activity [130]. In male FXIII-A-knockout mice, fibrosis of the myocardium was observed with deposition of the haemorrhage marker haemosiderin at the site of fibrosis [131]. Wound closure after 11 days

was reduced 27% in FXIII-A-knockout mice compared with wild-type mice, with necrotized fissure formation and delayed re-epithelialization [132]. In FXIII-deficient patients, bleeding is delayed to between 12–36 h following injury [127], with subcutaneous bleeding the common clinical manifestation of severe FXIII deficiency, where FXIII activity levels are below 5% of normal [128]. Analysis of whole blood and platelet-free plasma thrombi from an FXIII-A-deficient patient (FXIII-A activity 8% of normal) under flow, revealed that lysis occurred 4.4- and 5.6-fold faster respectively, compared with thrombi from healthy controls, with only the formation of γ-dimers apparent after 30 min in the FXIII-A-deficient patient [133]. This increase in lysis rate in FXIII-A-deficient patients may be accounted for by the lack of α_2-antiplasmin cross-linking, as plasma depleted in either FXIII-A and/or α_2-antiplasmin prolong lysis 9-fold [63]. Impaired wound healing also coincided with reduced FXIII levels in three patients following a myocardial infarction with recurrent myocardial rupture within 7 days [134]. Prophylaxis with FXIII has been shown to be effective at correcting the clinical manifestations of FXIII deficiency in both FXIII-knockout mice [76,129,132] and FXIII-deficient patients [127,128,133], supporting the importance of plasma FXIII in thrombus formation and wound healing.

FUTURE DIRECTIONS

In the future, proteomic technologies should be used to extensively catalogue the plasma and cellular substrates of FXIII-A [124] if we are to fully appreciate the influence of FXIII-A in thrombosis and wound healing. A recent study analysing perfused solubilized *ex vivo* plasma clots from FXIII-A-deficient plasma and pooled plasma from healthy individuals identified proteins involved in inflammation as potentially novel FXIIIa substrates, suggesting FXIIIa-dependent cross-linking influences numerous biological processes [135]. Previous strategies used to identify substrates of TG2 in a protein mixture and an epithelial cell line [136,137] could be adapted to identify novel substrates of FXIII-A in both cells and plasma; however, the dynamic range of proteins in plasma may require targeted depletion of more abundant proteins to reveal substrates of lower concentrations.

Future diagnostic applications for FXIII-A may include the detection of acute thrombosis using molecular probes, such as a near-IR fluorescent contrast agents [138] and gadolinium-chelating magnetic resonance probes [139], which can be cross-linked to fibrin by FXIIIa and indicate ongoing thrombin generation and thrombus formation. This technology may be important in the future for determining FXIII-deficient individuals and those with acute thrombosis for targeted therapy. Alternatively, FXIII itself may be analysed as a potential marker for acute thrombosis, as a study has been able to monitor the FXIIIa peptide by ELISA in healthy subjects [140]. The role of FXIII-A in thrombosis makes it a highly desirable target for future antithrombotic agents; however, future agents need to be highly specific and targeted to prevent uncontrolled bleeding.

CONCLUSIONS

FXIII is a Ca^{2+}-dependent pro-transglutaminase which forms covalent ε-(γ-glutamyl)lysine cross-links between fibrin fibres and numerous other plasma and intracellular proteins. FXIIIa-mediated cross-linking is vital for normal clot structure and function, promoting stabilization by preventing enzymatic and mechanical breakdown, supporting extracellular matrix attachment and influencing clot retraction. During wound healing, FXIIIa-dependent cross-linking is vital to extracellular matrix assembly and cell–matrix interactions. In some cases, the role of substrate cross-linking on clot properties and wound healing has not been studied. FXIIIa is a potentially attractive therapeutic target for the development of novel antithrombotic agents; however, detailed understanding of the role of FXIII in wound healing may be required to gain a clearer understanding of potential adverse effects of FXIII-A inhibition.

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REFERENCES

1 Schwartz, M. L., Pizzo, S. V., Hill, R. L. and McKee, P. A. (1973) Human Factor XIII from plasma and platelets. Molecular weights, subunit structures, proteolytic activation, and cross-linking of fibrinogen and fibrin. J. Biol. Chem. **248**, 1395–1407

2 Komaromi, I., Bagoly, Z. and Muszbek, L. (2011) Factor XIII: novel structural and functional aspects. J. Thromb. Haemostasis **9**, 9–20

3 Henriksson, P., Becker, S., Lynch, G. and McDonagh, J. (1985) Identification of intracellular factor XIII in human monocytes and macrophages. J. Clin. Invest **76**, 528–534

4 Nurminskaya, M. and Kaartinen, M. T. (2006) Transglutaminases in mineralized tissues. Front. Biosci. **11**, 1591–1606

5 Weisberg, L. J., Shiu, D. T., Conkling, P. R. and Shuman, M. A. (1987) Identification of normal human peripheral blood monocytes and liver as sites of synthesis of coagulation factor XIII a-chain. Blood **70**, 579–582

6 Kappelmayer, J., Bacsko, G., Birinyi, L., Zakany, R., Kelemen, E. and Adany, R. (1995) Consecutive appearance of coagulation factor XIII subunit A in macrophages, megakaryocytes, and liver cells during early human development. Blood **86**, 2191–2197

7 Nagy, J. A., Henriksson, P. and McDonagh, J. (1986) Biosynthesis of factor XIII B subunit by human hepatoma cell lines. Blood **68**, 1272–1279

8 Inbal, A., Muszbek, L., Lubetsky, A., Katona, E., Levi, I., Karpati, L. and Nagler, A. (2004) Platelets but not monocytes contribute to the plasma levels of factor XIII subunit A in patients undergoing autologous peripheral blood stem cell transplantation. Blood Coagul. Fibrinolysis **15**, 249–253

9 Cordell, P. A., Kile, B. T., Standeven, K. F., Josefsson, E. C., Pease, R. J. and Grant, P. J. (2010) Association of coagulation factor XIII-A with Golgi proteins within monocyte-macrophages: implications for subcellular trafficking and secretion. Blood **115**, 2674–2681

10 Takagi, T. and Doolittle, R. F. (1974) Amino acid sequence studies on factor XIII and the peptide released during its activation by thrombin. Biochemistry **13**, 750–756
11 Hornyak, T. J. and Shafer, J. A. (1992) Interactions of Factor-XIII with fibrin as substrate and cofactor. Biochemistry **31**, 423–429
12 Moaddel, M., Falls, L. A. and Farrell, D. H. (2000) The role of γA/γ' fibrinogen in plasma factor XIII activation. J. Biol. Chem. **275**, 32135–32140
13 Lorand, L., Gray, A. J., Brown, K., Credo, R. B., Curtis, C. G., Domanik, R. A. and Stenberg, P. (1974) Dissociation of the subunit structure of fibrin stabilizing factor during activation of the zymogen. Biochem. Biophys. Res. Commun. **56**, 914–922
14 Lewis, S. D., Janus, T. J., Lorand, L. and Shafer, J. A. (1985) Regulation of formation of Factor-XIIIa by its fibrin substrates. Biochemistry **24**, 6772–6777
15 Kristiansen, G. K. and Andersen, M. D. (2011) Reversible activation of cellular factor XIII by calcium. J. Biol. Chem. **286**, 9833–9839
16 Pedersen, L. C., Yee, V. C., Bishop, P. D., Le, T. I., Teller, D. C. and Stenkamp, R. E. (1994) Transglutaminase factor XIII uses proteinase-like catalytic triad to crosslink macromolecules. Protein Sci. **3**, 1131–1135
17 Yee, V. C., Pedersen, L. C., Le, T. I., Bishop, P. D., Stenkamp, R. E. and Teller, D. C. (1994) Three-dimensional structure of a transglutaminase: human blood coagulation factor XIII. Proc. Natl. Acad. Sci. U.S.A. **91**, 7296–7300
18 Ariens, R. A., Lai, T. S., Weisel, J. W., Greenberg, C. S. and Grant, P. J. (2002) Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. Blood **100**, 743–754
19 Skorstengaard, K., Halkier, T., Hojrup, P. and Mosher, D. (1990) Sequence location of a putative transglutaminase cross-linking site in human vitronectin. FEBS Lett. **262**, 269–274
20 Sane, D. C., Moser, T. L., Pippen, A. M., Parker, C. J., Achyuthan, K. E. and Greenberg, C. S. (1988) Vitronectin is a substrate for transglutaminases. Biochem. Biophys. Res. Commun. **157**, 115–120
21 Sottrup-Jensen, L., Stepanik, T. M., Wierzbicki, D. M., Jones, C. M., Lonblad, P. B., Kristensen, T., Mortensen, S. B., Petersen, T. E. and Magnusson, S. (1983) The primary structure of α_2-macroglobulin and localization of a Factor XIIIa cross-linking site. Ann. N.Y. Acad. Sci. **421**, 41–60
22 Francis, R. T., McDonagh, J. and Mann, K. G. (1986) Factor V is a substrate for the transamidase factor XIIIa. J. Biol. Chem. **261**, 9787–9792
23 McDonagh, R. P., McDonagh, J., Petersen, T. E., Thogersen, H. C., Skorstengaard, K., Sottrup-Jensen, L., Magnusson, S., Dell, A. and Morris, H. R. (1981) Amino acid sequence of the factor XIIIa acceptor site in bovine plasma fibronectin. FEBS Lett. **127**, 174–178
24 Lynch, G. W., Slayter, H. S., Miller, B. E. and McDonagh, J. (1987) Characterization of thrombospondin as a substrate for factor XIII transglutaminase. J. Biol. Chem. **262**, 1772–1778
25 Bale, M. D. and Mosher, D. F. (1986) Thrombospondin is a substrate for blood coagulation factor XIIIa. Biochemistry **25**, 5667–5673
26 Valnickova, Z. and Enghild, J. J. (1998) Human procarboxypeptidase U, or thrombin-activable fibrinolysis inhibitor, is a substrate for transglutaminases. Evidence for transglutaminase-catalyzed cross-linking to fibrin. J. Biol. Chem. **273**, 27220–27224
27 Sakata, Y. and Aoki, N. (1980) Cross-linking of α 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. J. Clin. Invest. **65**, 290–297
28 Mosher, D. F. (1976) Action of fibrin-stabilizing factor on cold-insoluble globulin and α_2-macroglobulin in clotting plasma. J. Biol. Chem. **251**, 1639–1645
29 Lee, K. N., Lee, C. S., Tae, W. C., Jackson, K. W., Christiansen, V. J. and McKee, P. A. (2000) Cross-linking of wild-type and mutant α_2-antiplasmins to fibrin by activated factor XIII and by a tissue transglutaminase. J. Biol. Chem. **275**, 37382–37389
30 Lee, K. N., Lee, C. S., Tae, W. C., Jackson, K. W., Christiansen, V. J. and McKee, P. A. (2001) Crosslinking of α 2-antiplasmin to fibrin. Ann. N.Y. Acad.Sci. **936**, 335–339
31 Kimura, S. and Aoki, N. (1986) Cross-linking site in fibrinogen for α_2-plasmin inhibitor. J. Biol. Chem. **261**, 15591–15595
32 Corbett, S. A., Lee, L., Wilson, C. L. and Schwarzbauer, J. E. (1997) Covalent cross-linking of fibronectin to fibrin is required for maximal cell adhesion to a fibronectin-fibrin matrix. J. Biol. Chem. **272**, 24999–25005
33 Weisel, J. W. (2005) Fibrinogen and fibrin. Adv. Protein Chem. **70**, 247–299
34 Weisel, J. W., Veklich, Y. and Gorkun, O. (1993) The sequence of cleavage of fibrinopeptides from fibrinogen is important for protofibril formation and enhancement of lateral aggregation in fibrin clots. J. Mol. Biol. **232**, 285–297
35 Mosesson, M. W., DiOrio, J. P., Siebenlist, K. R., Wall, J. S. and Hainfeld, J. F. (1993) Evidence for a second type of fibril branch point in fibrin polymer networks, the trimolecular junction. Blood **82**, 1517–1521
36 Standeven, K. F., Ariens, R. A. and Grant, P. J. (2005) The molecular physiology and pathology of fibrin structure/function. Blood Rev. **19**, 275–288
37 Liu, W., Carlisle, C. R., Sparks, E. A. and Guthold, M. (2010) The mechanical properties of single fibrin fibers. J. Thromb. Haemostasis **8**, 1030–1036
38 Standeven, K. F., Carter, A. M., Grant, P. J., Weisel, J. W., Chernysh, I., Masova, L., Lord, S. T. and Ariens, R. A. (2007) Functional analysis of fibrin γ-chain cross-linking by activated factor XIII: determination of a cross-linking pattern that maximizes clot stiffness. Blood **110**, 902–907
39 Siebenlist, K. R., Meh, D. A. and Mosesson, M. W. (2001) Protransglutaminase (factor XIII) mediated crosslinking of fibrinogen and fibrin. Thromb. Haemostasis **86**, 1221–1228
40 Gorman, J. J. and Folk, J. E. (1980) Structural features of glutamine substrates for human plasma factor XIIIa (activated blood coagulation factor XIII). J. Biol. Chem. **255**, 419–427
41 Weisel, J. W. (2004) Cross-linked γ-chains in fibrin fibrils bridge transversely between strands: no. J. Thromb. Haemostasis **2**, 394–399
42 Mosesson, M. W. (2004) Cross-linked gamma-chains in fibrin fibrils bridge 'transversely' between strands: yes. J. Thromb. Haemostasis **2**, 388–393
43 Weisel, J. W., Francis, C. W., Nagaswami, C. and Marder, V. J. (1993) Determination of the topology of factor XIIIa-induced fibrin γ-chain cross-links by electron microscopy of ligated fragments. J. Biol. Chem. **268**, 26618–26624
44 Veklich, Y., Ang, E. K., Lorand, L. and Weisel, J. W. (1998) The complementary aggregation sites of fibrin investigated through examination of polymers of fibrinogen with fragment E. Proc. Natl. Acad. Sci. U.S.A. **95**, 1438–1442
45 Mosesson, M. W., Siebenlist, K. R., Meh, D. A., Wall, J. S. and Hainfeld, J. F. (1998) The location of the carboxy-terminal region of γ chains in fibrinogen and fibrin D domains. Proc. Natl. Acad. Sci. U.S.A. **95**, 10511–10516
46 Cottrell, B. A., Strong, D. D., Watt, K. W. and Doolittle, R. F. (1979) Amino acid sequence studies on the α chain of human fibrinogen. Exact location of cross-linking acceptor sites. Biochemistry **18**, 5405–5410
47 Matsuka, Y. V., Medved, L. V., Migliorini, M. M. and Ingham, K. C. (1996) Factor XIIIa-catalyzed cross-linking of recombinant α C fragments of human fibrinogen. Biochemistry **35**, 5810–5816

48 Sobel, J. H. and Gawinowicz, M. A. (1996) Identification of the α chain lysine donor sites involved in factor XIIIa fibrin cross-linking. J. Biol. Chem. **271**, 19288–19297
49 Wang, W. (2011) Identification of respective lysine donor and glutamine acceptor sites involved in factor XIIIa-catalyzed fibrin α chain cross-linking. J. Biol. Chem. **286**, 44952–44964
50 Shen, L. L., McDonagh, R. P., McDonagh, J. and Hermans, Jr, J. (1974) Fibrin gel structure: influence of calcium and covalent cross-linking on the elasticity. Biochem. Biophys. Res. Commun. **56**, 793–798
51 Shen, L. and Lorand, L. (1983) Contribution of fibrin stabilization to clot strength. Supplementation of factor XIII-deficient plasma with the purified zymogen. J. Clin. Invest. **71**, 1336–1341
52 Standeven, K. F., Grant, P. J., Carter, A. M., Scheiner, T., Weisel, J. W. and Ariens, R. A. (2003) Functional analysis of the fibrinogen Aα Thr312Ala polymorphism: effects on fibrin structure and function. Circulation **107**, 2326–2330
53 Siebenlist, K. R. and Mosesson, M. W. (1994) Progressive cross-linking of fibrin γ chains increases resistance to fibrinolysis. J. Biol. Chem. **269**, 28414–28419
54 Rosing, J. and Tans, G. (1997) Factor V. Int. J. Biochem. Cell Biol. **29**, 1123–1126
55 Huh, M. M., Schick, B. P., Schick, P. K. and Colman, R. W. (1988) Covalent crosslinking of human coagulation factor V by activated factor XIII from guinea pig megakaryocytes and human plasma. Blood **71**, 1693–1702
56 Roberts, W., Magwenzi, S., Aburima, A. and Naseem, K. M. (2010) Thrombospondin-1 induces platelet activation through CD36-dependent inhibition of the cAMP/protein kinase A signaling cascade. Blood **116**, 4297–4306
57 Bale, M. D., Westrick, L. G. and Mosher, D. F. (1985) Incorporation of thrombospondin into fibrin clots. J. Biol. Chem. **260**, 7502–7508
58 Lee, K. N., Jackson, K. W., Christiansen, V. J., Chung, K. H. and McKee, P. A. (2004) α_2-Antiplasmin: potential therapeutic roles in fibrin survival and removal. Curr. Med. Chem. Cardiovasc. Hematol. Agents **2**, 303–310
59 Tamaki, T. and Aoki, N. (1982) Cross-linking of α 2-plasmin inhibitor to fibrin catalyzed by activated fibrin-stabilizing factor. J. Biol. Chem. **257**, 14767–14772
60 Ichinose, A., Tamaki, T. and Aoki, N. (1983) Factor XIII-mediated cross-linking of NH_2-terminal peptide of α 2-plasmin inhibitor to fibrin. FEBS Lett. **153**, 369–371
61 Kimura, S., Tamaki, T. and Aoki, N. (1985) Acceleration of fibrinolysis by the N-terminal peptide of α_2-plasmin inhibitor. Blood **66**, 157–160
62 Sakata, Y. and Aoki, N. (1982) Significance of cross-linking of α_2-plasmin inhibitor to fibrin in inhibition of fibrinolysis and in hemostasis. J. Clin. Invest. **69**, 536–542
63 Fraser, S. R., Booth, N. A. and Mutch, N. J. (2011) The antifibrinolytic function of factor XIII is exclusively expressed through α-antiplasmin cross-linking. Blood **117**, 6371–6374
64 Wang, W., Boffa, M. B., Bajzar, L., Walker, J. B. and Nesheim, M. E. (1998) A study of the mechanism of inhibition of fibrinolysis by activated thrombin-activable fibrinolysis inhibitor. J. Biol. Chem. **273**, 27176–27181
65 Mutch, N. J., Moore, N. R., Wang, E. and Booth, N. A. (2003) TAFI is cross-linked by its activation peptide to sites on fibrin(ogen) distict from other inhibitors of fibrinolysis. J. Thromb. Haemostasis **1** (Suppl. 1), Abstract OC064.2003
66 Mutch, N. J., Thomas, L., Moore, N. R., Lisiak, K. M. and Booth, N. A. (2007) TAFIa, PAI-1 and α-antiplasmin: complementary roles in regulating lysis of thrombi and plasma clots. J. Thromb. Haemostasis **5**, 812–817
67 Podor, T. J., Campbell, S., Chindemi, P., Foulon, D. M., Farrell, D. H., Walton, P. D., Weitz, J. I. and Peterson, C. B. (2002) Incorporation of vitronectin into fibrin clots. Evidence for a binding interaction between vitronectin and γA/γ' fibrinogen. J. Biol. Chem. **277**, 7520–7528
68 Podor, T. J., Peterson, C. B., Lawrence, D. A., Stefansson, S., Shaughnessy, S. G., Foulon, D. M., Butcher, M. and Weitz, J. I. (2000) Type 1 plasminogen activator inhibitor binds to fibrin via vitronectin. J. Biol. Chem. **275**, 19788–19794
69 Harpel, P. C. (1981) α_2-Plasmin inhibitor and α_2-macroglobulin-plasmin complexes in plasma. Quantitation by an enzyme-linked differential antibody immunosorbent assay. J. Clin. Invest. **68**, 46–55
70 Adany, R., Bardos, H., Antal, M., Modis, L., Sarvary, A., Szucs, S. and Balogh, I. (2001) Factor XIII of blood coagulation as a nuclear crosslinking enzyme. Thromb. Haemostasis **85**, 845–851
71 Cohen, I., Young-Bandala, L., Blankenberg, T. A., Siefring, Jr, G. E. and Bruner-Lorand, J. (1979) Fibrinoligase-catalyzed cross-linking of myosin from platelet and skeletal muscle. Arch. Biochem. Biophys. **192**, 100–111
72 Cohen, I., Blankenberg, T. A., Borden, D., Kahn, D. R. and Veis, A. (1980) Factor XIIIa-catalyzed cross-linking of platelet and muscle actin. Regulation by nucleotides. Biochim. Biophys. Acta **628**, 365–375
73 Serrano, K. and Devine, D. V. (2002) Intracellular factor XIII crosslinks platelet cytoskeletal elements upon platelet activation. Thromb. Haemostasis **88**, 315–320
74 Asijee, G. M., Sturk, A., Bruin, T., Wilkinson, J. M. and Ten Cate, J. W. (1990) Vinculin is a permanent component of the membrane skeleton and is incorporated into the (re)organising cytoskeleton upon platelet activation. Eur. J. Biochem. **189**, 131–136
75 Jayo, A., Conde, I., Lastres, P., Jimenez-Yuste, V. and Gonzalez-Manchon, C. (2009) New insights into the expression and role of platelet factor XIII-A. J. Thromb. Haemostasis **7**, 1184–1191
76 Kasahara, K., Souri, M., Kaneda, M., Miki, T., Yamamoto, N. and Ichinose, A. (2010) Impaired clot retraction in factor XIII A subunit-deficient mice. Blood **115**, 1277–1279
77 Dale, G. L., Friese, P., Batar, P., Hamilton, S. F., Reed, G. L., Jackson, K. W., Clemetson, K. J. and Alberio, L. (2002) Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface. Nature **415**, 175–179
78 Szasz, R. and Dale, G. L. (2002) Tbrombospondin and fibrinogen bind serotonin-derivatized proteins on COAT-platelets. Blood **100**, 2827–2831
79 Dale, G. L. (2005) Coated-platelets: an emerging component of the procoagulant response. J. Thromb. Haemostasis **3**, 2185–2192
80 Pankov, R. and Yamada, K. M. (2002) Fibronectin at a glance. J. Cell Sci. **115**, 3861–3863
81 Brotchie, H. and Wakefield, D. (1990) Fibronectin: structure, function and significance in wound healing. Australas. J. Dermatol. **31**, 47–56
82 Mosher, D. F. and Furcht, L. T. (1981) Fibronectin: review of its structure and possible functions. J. Invest. Dermatol. **77**, 175–180
83 Matsuka, Y. V., Migliorini, M. M. and Ingham, K. C. (1997) Cross-linking of fibronectin to C-terminal fragments of the fibrinogen α-chain by factor XIIIa. J. Protein Chem. **16**, 739–745
84 Procyk, R. and Blomback, B. (1988) Factor XIII-induced crosslinking in solutions of fibrinogen and fibronectin. Biochim. Biophys. Acta **967**, 304–313
85 Chow, T. W., McIntire, L. V. and Peterson, D. M. (1983) Importance of plasma fibronectin in determining PFP and PRP clot mechanical properties. Thromb. Res. **29**, 243–248

86 Niewiarowska, J. and Cierniewski, C. S. (1982) Inhibitory effect of fibronectin on the fibrin formation. Thromb. Res. **27**, 611–618

87 Okada, M., Blomback, B., Chang, M. D. and Horowitz, B. (1985) Fibronectin and fibrin gel structure. J. Biol. Chem. **260**, 1811–1820

88 Cho, J. and Mosher, D. F. (2006) Enhancement of thrombogenesis by plasma fibronectin cross-linked to fibrin and assembled in platelet thrombi. Blood **107**, 3555–3563

89 Malara, A., Gruppi, C., Rebuzzini, P., Visai, L., Perotti, C., Moratti, R., Balduini, C., Tira, M. E. and Balduini, A. (2011) Megakaryocyte-matrix interaction within bone marrow: new roles for fibronectin and factor XIII-A. Blood **117**, 2476–2483

90 Mosher, D. F. and Schad, P. E. (1979) Cross-linking of fibronectin to collagen by blood coagulation Factor XIIIa. J. Clin. Invest. **64**, 781–787

91 Mosher, D. F. (1984) Cross-linking of fibronectin to collagenous proteins. Mol. Cell. Biochem. **58**, 63–68

92 Mosher, D. F., Schad, P. E. and Vann, J. M. (1980) Cross-linking of collagen and fibronectin by factor XIIIa. Localization of participating glutaminyl residues to a tryptic fragment of fibronectin. J. Biol. Chem. **255**, 1181–1188

93 Sadler, J. E. (1998) Biochemistry and genetics of von Willebrand factor. Annu. Rev. Biochem. **67**, 395–424

94 Hada, M., Kaminski, M., Bockenstedt, P. and McDonagh, J. (1986) Covalent crosslinking of von Willebrand factor to fibrin. Blood **68**, 95–101

95 Takagi, J., Aoyama, T., Ueki, S., Ohba, H., Saito, Y. and Lorand, L. (1995) Identification of factor-XIIIa-reactive glutaminyl residues in the propolypeptide of bovine von Willebrand factor. Eur. J. Biochem. **232**, 773–777

96 Bockenstedt, P., McDonagh, J. and Handin, R. I. (1986) Binding and covalent cross-linking of purified von Willebrand factor to native monomeric collagen. J. Clin. Invest. **78**, 551–556

97 Usui, T., Takagi, J. and Saito, Y. (1993) Propolypeptide of von Willebrand factor serves as a substrate for factor XIIIa and is cross-linked to laminin. J. Biol. Chem. **268**, 12311–12316

98 Castellino, F. J. and Ploplis, V. A. (2005) Structure and function of the plasminogen/plasmin system. Thromb. Haemostasis **93**, 647–654

99 Bendixen, E., Borth, W. and Harpel, P. C. (1993) Transglutaminases catalyze cross-linking of plasminogen to fibronectin and human endothelial cells. J. Biol. Chem. **268**, 21962–21967

100 Bendixen, E., Harpel, P. C. and Sottrup-Jensen, L. (1995) Location of the major epsilon-(γ-glutamyl)lysyl cross-linking site in transglutaminase-modified human plasminogen. J. Biol. Chem. **270**, 17929–17933

101 Pepper, M. S. (2001) Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. Arterioscler. Thromb. Vasc. Biol. **21**, 1104–1117

102 Ritchie, H., Robbie, L. A., Kinghorn, S., Exley, R. and Booth, N. A. (1999) Monocyte plasminogen activator inhibitor 2 (PAI-2) inhibits u-PA-mediated fibrin clot lysis and is cross-linked to fibrin. Thromb. Haemostasis **81**, 96–103

103 Kruithof, E. K., Baker, M. S. and Bunn, C. L. (1995) Biological and clinical aspects of plasminogen activator inhibitor type 2. Blood **86**, 4007–4024

104 Jensen, P. H., Schuler, E., Woodrow, G., Richardson, M., Goss, N., Hojrup, P., Petersen, T. E. and Rasmussen, L. K. (1994) A unique interhelical insertion in plasminogen activator inhibitor-2 contains three glutamines, Gln^{83}, Gln^{84}, Gln^{86}, essential for transglutaminase-mediated cross-linking. J. Biol. Chem. **269**, 15394–15398

105 Ritchie, H., Lawrie, L. C., Crombie, P. W., Mosesson, M. W. and Booth, N. A. (2000) Cross-linking of plasminogen activator inhibitor 2 and α_2-antiplasmin to fibrin(ogen). J. Biol. Chem. **275**, 24915–24920

106 Ritchie, H., Lawrie, L. C., Mosesson, M. W. and Booth, N. A. (2001) Characterization of crosslinking sites in fibrinogen for plasminogen activator inhibitor 2 (PAI-2). Ann. N.Y. Acad. Sci. **936**, 215–218

107 Wassmann, S. and Nickenig, G. (2004) The rote of the AT(1) receptor in the cardiovascular continuum. Eur. Heart J. (Suppl.) **6**, H3–H9

108 AbdAlla, S., Lother, H., Langer, A., el, F. Y. and Quitterer, U. (2004) Factor XIIIA transglutaminase crosslinks AT1 receptor dimers of monocytes at the onset of atherosclerosis. Cell **119**, 343–354

109 Aeschlimann, D., Mosher, D. and Paulsson, M. (1996) Tissue transglutaminase and factor XIII in cartilage and bone remodeling. Semin. Thromb. Hemostasis **22**, 437–443

110 Nurminskaya, M. V., Recheis, B., Nimpf, J., Magee, C. and Linsenmayer, T. F. (2002) Transglutaminase factor XIIIA in the cartilage of developing avian long bones. Dev. Dyn. **223**, 24–32

111 Piercy-Kotb, S. A., Mousa, A., Al-Jallad, H. F., Myneni, V. D., Chicatun, F., Nazhat, S. N. and Kaartinen, M. T. (2012) Factor XIIIA transglutaminase expression and secretion by osteoblasts is regulated by extracellular matrix collagen and the MAP kinase signaling pathway. J. Cell. Physiol. **227**, 2936–2946

112 Nurminskaya, M., Magee, C., Nurminsky, D. and Linsenmayer, T. F. (1998) Plasma transglutaminase in hypertrophic chondrocytes: expression and cell-specific intracellular activation produce cell death and externalization. J. Cell Biol. **142**, 1135–1144

113 Al-Jallad, H. F., Nakano, Y., Chen, J. L., McMillan, E., Lefebvre, C. and Kaartinen, M. T. (2006) Transglutaminase activity regulates osteoblast differentiation and matrix mineralization in MC3T3-E1 osteoblast cultures. Matrix Biol. **25**, 135–148

114 Johnson, K. A., Rose, D. M. and Terkeltaub, R. A. (2008) Factor XIIIA mobilizes transglutaminase 2 to induce chondrocyte hypertrophic differentiation. J. Cell Sci. **121**, 2256–2264

115 Sorensen, E. S., Rasmussen, L. K., Moller, L., Jensen, P. H., Hojrup, P. and Petersen, T. E. (1994) Localization of transglutaminase-reactive glutamine residues in bovine osteopontin. Biochem. J. **304**, 13–16

116 Prince, C. W., Dickie, D. and Krumdieck, C. L. (1991) Osteopontin, a substrate for transglutaminase and Factor-Xiii activity. Biochem. Biophys. Res. Commun. **177**, 1205–1210

117 Kaartinen, M. T., El-Maadawy, S., Rasanen, N. H. and Mckee, M. D. (2002) Tissue transglutaminase and its substrates in bone. J. Bone Mineral Res. **17**, 2161–2173

118 Nakano, Y., Al-Jallad, H. F., Mousa, A. and Kaartinen, M. T. (2007) Expression and localization of plasma transglutaminase factor XIIIA in bone. J. Histochem. Cytochem. **55**, 675–685

119 Al-Jallad, H. F., Myneni, V. D., Piercy-Kotb, S. A., Chabot, N., Mulani, A., Keillor, J. W. and Kaartinen, M. T. (2011) Plasma membrane factor XIIIA transglutaminase activity regulates osteoblast matrix secretion and deposition by affecting microtubule dynamics. PLoS ONE **6**, e15893

120 Nurminskaya, M. and Kaartinen, M. T. (2006) Transglutaminases in mineralized tissues. Front. Biosci. **11**, 1591–1606

121 Tarantino, U., Oliva, F., Taurisano, G., Orlandi, A., Pietroni, V., Candi, E., Melino, G. and Maffulli, N. (2009) FXIIIA and TGF-β over-expression produces normal musculo-skeletal phenotype in $TG2^{-/-}$ mice. Amino Acids **36**, 679–684

122 Johnson, K. A., Rose, D. M. and Terkeltaub, R. A. (2008) Factor XIIIA mobilizes transglutaminase 2 to induce chondrocyte hypertrophic differentiation. J. Cell Sci. **121**, 2256–2264

123 Hettasch, J. M., Peoples, K. A. and Greenberg, C. S. (1997) Analysis of factor XIII substrate specificity using recombinant human factor XIII and tissue transglutaminase chimeras. J. Biol. Chem. **272**, 25149–25156

124 Sugimura, Y., Hosono, M., Wada, F., Yoshimura, T., Maki, M. and 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

125 Marinescu, A., Cleary, D. B., Littlefield, T. R. and Maurer, M. C. (2002) Structural features associated with the binding of glutamine-containing peptides to Factor XIII. Arch. Biochem. Biophys. **406**, 9–20
126 Penzes, K., Kover, K. E., Fazakas, F., Haramura, G. and Muszbek, L. (2009) Molecular mechanism of the interaction between activated factor XIII and its glutamine donor peptide substrate. J. Thromb. Haemostasis **7**, 627–633
127 Bhattacharya, M., Biswas, A., Ahmed, R. P., Kannan, M., Gupta, M., Mahapatra, M., Choudhry, V. P. and Saxena, R. (2005) Clinico-hematologic profile of factor XIII-deficient patients. Clin. Appl. Thromb. Hemostasis **11**, 475–480
128 Ivaskevicius, V., Seitz, R., Kohler, H. P., Schroeder, V., Muszbek, L., Ariens, R. A., Seifried, E. and Oldenburg, J. (2007) International registry on factor XIII deficiency: a basis formed mostly on European data. Thromb. Haemostasis **97**, 914–921
129 Lauer, P., Metzner, H. J., Zettlmeissl, G., Li, M., Smith, A. G., Lathe, R. and Dickneite, G. (2002) Targeted inactivation of the mouse locus encoding coagulation factor XIII-A: hemostatic abnormalities in mutant mice and characterization of the coagulation deficit. Thromb. Haemostasis **88**, 967–974
130 Nahrendorf, M., Hu, K., Frantz, S., Jaffer, F. A., Tung, C. H., Hiller, K. H., Voll, S., Nordbeck, P., Sosnovik, D., Gattenlohner, S. et al. (2006) Factor XIII deficiency causes cardiac rupture, impairs wound healing, and aggravates cardiac remodeling in mice with myocardial infarction. Circulation **113**, 1196–1202
131 Souri, M., Koseki-Kuno, S., Takeda, N., Yamakawa, M., Takeishi, Y., Degen, J. L. and Ichinose, A. (2008) Male-specific cardiac pathologies in mice lacking either the A or B subunit of factor XIII. Thromb. Haemostasis **99**, 401–408
132 Inbal, A., Lubetsky, A., Krapp, T., Castel, D., Shaish, A., Dickneitte, G., Modis, L., Muszbek, L. and Inbal, A. (2005) Impaired wound healing in factor XIII deficient mice. Thromb. Haemostasis **94**, 432–437
133 Mutch, N. J., Koikkalainen, J. S., Fraser, S. R., Duthie, K. M., Griffin, M., Mitchell, J., Watson, H. G. and Booth, N. A. (2010) Model thrombi formed under flow reveal the role of factor XIII-mediated cross-linking in resistance to fibrinolysis. J. Thromb. Haemostasis **8**, 2017–2024
134 Nahrendorf, M., Weissleder, R. and Ertl, G. (2006) Does FXIII deficiency impair wound healing after myocardial infarction? PLoS ONE **1**, e48
135 Howes, J. M., Richardson, VR., Smith, KA., Schroeder, V., Somani, R., Shore, A., Hess, K., Ajjan, R., Pease, R. J., Keen, JN. et al. (2012) Complement C3 is a novel plasma clot component with anti-fibrinolytic properties. Diab. Vasc. Dis. Res. **9**, 216–225
136 Orru, S., Caputo, I., D'Amato, A., Ruoppolo, M. and Esposito, C. (2003) Proteomics identification of acyl-acceptor and acyl-donor substrates for transglutaminase in a human intestinal epithelial cell line. Implications for celiac disease. J. Biol. Chem. **278**, 31766–31773
137 Ruoppolo, M., Orru, S., D'Amato, A., Francese, S., Rovero, P., Marino, G. and Esposito, C. (2003) Analysis of transglutaminase protein substrates by functional proteomics. Protein Sci. **12**, 1290–1297
138 Jaffer, F. A., Tung, C. H., Wykrzykowska, J. J., Ho, N. H., Houng, A. K., Reed, G. L. and Weissleder, R. (2004) Molecular imaging of factor XIIIa activity in thrombosis using a novel, near-infrared fluorescent contrast agent that covalently links to thrombi. Circulation **110**, 170–176
139 Tung, C. H., Ho, N. H., Tang, Q., Jaffer, F. A., Reed, G. L. and Weissleder, R. (2003) Novel factor XIII probes for blood coagulation imaging. Chembiochem **4**, 897–899
140 Ortner, E., Schroeder, V., Walser, R., Zerbe, O. and Kohler, H. P. (2010) Sensitive and selective detection of free FXIII activation peptide: a potential marker of acute thrombotic events. Blood **115**, 5089–5096
141 Hevessy, Z., Patthy, A., Karpati, L. and Muszbek, L. (2000) α_2-Plasmin inhibitor is a substrate for tissue transglutaminase: Aa *in vitro* study. Thromb. Res. **99**, 399–406

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