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Let's cross-link: diverse functions of the promiscuous cellular transglutaminase, factor XIII-A

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Abstract

Factor (F)XIII is a tranglutaminase enzyme that catalyses the formation of ε-(γ-glutamyl)lysyl isopeptide bonds into protein substrates. The plasma form, $FXIIIA_2B_2$ has an established function in hemostasis, where its primary substrate is fibrin. A deficiency in FXIII manifests as a severe bleeding diathesis underscoring its importance in this pathway. The cellular form of the enzyme, a homodimer of the A subunits, denoted FXIII-A, has not been studied in as extensive detail. FXIII-A was generally perceived to remain intracellular, due to the lack of a classical signal peptide for its release. In the last decade emerging evidence has revealed that this diverse transglutaminase can be externalised from cells, by an as yet unknown mechanism, and can cross-link extracellular substrates and participate in a number of diverse pathways. The FXIII-A gene (F13A1) is expressed in cells of bone marrow and mesenchymal lineage, notably megakaryocytes, monocytes/macrophages, dendritic cells, chrondrocytes, osteoblasts and preadipocytes. The biological processes that FXIII-A is coupled with reflect its expression in these cell types, such as wound healing, phagocytosis and bone and matrix remodelling. This review describes the mounting evidence that this cellular transglutaminase can be externalised, usually in response to stimuli, and participate in extracellular cross-linking reactions. A corollary of being involved in these biological pathways is the participation of FXIII-A in pathological processes. In conclusion, the functions of this transglutaminase extend far beyond its role in hemostasis and our understanding of this enzyme in terms of its secretion, regulation and substrates is in its infancy.

Key words: Factor XIII, Cross-link, Transglutaminase, F13A1, Cellular

Introduction

In 1948 Laki and Lorand characterised a labile component of blood that when combined with Ca^{2+} rendered a clot insoluble [1]. The protein was later isolated and named fibrin stabilising protein [2]. Duckert *et al* [3] then reported that deficiency of fibrin stabilizing factor manifests as a severe bleeding diathesis. Subsequently in 1963, the International Committee on Blood Clotting Factors acknowledged this protein as a clotting factor and termed it factor XIII (FXIII). Our knowledge of FXIII has progressed significantly since these early informative observations. It is now clear that in addition to the essential role of FXIII in hemostasis it functions in a variety of other systems ranging from wound healing and angiogenesis [4] to stabilisation of the bone matrix [5]. Once activated FXIII (FXIIIa) elicits transamidase activity that introduces ε-(γ-glutamyl)lysyl isopeptide cross-links into protein substrates. It can incorporate cross-links into single protein substrates, such as fibrin, or can cross-link different proteins to each other, which can impact on their biological function [6].

In plasma, FXIII exists as a zymogen heterotetramer (FXIII-A_2B_2) [7] with two catalytic A subunits and two inhibitory carrier B subunits. [8]. FXIII-B is synthesised and secreted by hepatocytes [9, 10], however the source of plasma FXIII-A subunit has been debated for some years. The gene, F13A1, is largely expressed in cell of bone marrow origin, but it lacks an identifiable endoplasmic reticulum (ER) signal sequence and is excluded from the classical ER-Golgi pathway in nucleated cells [11]. Platelets were projected to be the source of the FXIII-A subunit in plasma [12, 13], but this was ruled out, as levels were unchanged in thrombocytopenic mice [14]. Recent observations, in tissue specific mouse knockouts of FXIII-A, now pinpoint resident tissue macrophages as the cellular source of plasma FXIII-A [15]. Plasma FXIII-A_2B_2 requires the concerted action of thrombin and calcium to be activated [16, 17]. The activation peptides which flank each of the FXIII-A subunits are initially cleaved by thrombin, which destabilizes the interaction between the FXIII-A and FXIII-B subunits [18]. The subsequent binding of calcium ions to defined sites on the FXIII-A subunits instigates dissociation of the FXIII-B subunits and activation peptides [19].

The cellular form of FXIII is a homodimer of the A-subunits, termed FXIII-A throughout this review [20]. Cellular FXIII-A is non-proteolytically activated by modest increases in intracellular Ca^{2+} concentrations [21, 22]. FXIII-A has been localized in a wide variety of different cells including platelets [23-25], megakaryocytes [26] monocytes [27, 28], circulating [27, 29], and tissue macrophages [29], dendritic cells [30], chondrocytes [31-33] osteoblasts [5] and preadipocytes [34]. The mechanism of FXIII-A release from these cells remains an enigma, due to the lack of signal sequence as stated, and it is also absent from

the ER-Golgi secretory pathway in nucleated cells [11]. However, FXIII-A in monocyte-macrophages is reportedly directed to the plasma membrane in association with Golgi vesicles [14] indicating that it is secreted via an alternative pathway. This review will focus on the multifarious actions of cellular FXIII-A and discuss whether it is capable of mediating extracellular cross-linking as well as intracellular functions despite its clear lack of a signal peptide for secretion.

FXIII-A in Platelets

Platelets harbour remarkably high concentrations of FXIII-A within their cytoplasm [35, 36], with a single platelet accruing 60 ± 10 fg, corresponding to 3% of total platelet protein [37]. α-granules reportedly contain a minor pool of FXIII in the A_2B_2 form which is endocytosed from plasma alongside fibrinogen [35, 38], however the concentrations are so low it is often not detectable in the platelet secretome [39, 40]. Early studies on platelet FXIII-A concluded that it was not involved in haemostasis, as it did not form part of the platelet secretome [39], but our recent observations indicate that FXIII-A is translocated from the cytoplasm to the surface of activated platelets where is actively retained [40].

Platelet FXIII-A in hemostasis

The role of plasma $FXIIIA_2B_2$ in haemostasis is well-established; it confers mechanical stability to thrombi by cross-linking the α- and γ-chains of fibrin, and provides protection against fibrinolytic breakdown by cross-linking inhibitors of fibrinolysis to fibrin [41-43]. Our laboratory has shown that flow is required to visualize the impact of $FXIIIA_2B_2$ on fibrinolysis [44] and that the antifibrinolytic action of this transglutaminase is mediated exclusively by cross-linking α_2-antiplasmin (α_2AP) to fibrin [45]. Rijken and colleagues subsequently reported that compaction or retraction of fibrin clots reveals the strong antifibrinolytic effect of $FXIIIA_2B_2$ [46]. The authors also confirm our observations that cross-linking of α2AP is required for the antifibrinolytic effect of FXIII to be visualised rather than by fibrin-fibrin cross-links [46]. Plasma $FXIIIA_2B_2$, but not platelet FXIII-A, also aids in the retention of red blood cells in clots through fibrin α-chain cross-linking which has a direct impact on the overall size of clots [47-49].

Platelet FXIII-A was previously shown to stabilize clots, by inducing the formation of high molecular weight γ-dimer and α-polymer [50-54] and cross-linking α_2AP to fibrin [50, 53]. The conundrum is that FXIII-A was not found within the secretome of platelets. Our laboratory has now shown that strong agonist stimulation of platelets induces translocation of FXIII-A from the cytoplasm to the platelet membrane where it is actively retained and can participate in extracellular cross-linking reactions [40]. The intensity of FXIII-A staining on the surface of activated platelets increases as a function of time, particularly in those

platelets directly associated with collagen fibres (Figure 1A). Our work clearly highlights a role for FXIII-A, externalised during platelet activation, in stabilizing thrombi via cross-linking of α_2AP to fibrin [40]. The relative contribution of plasma $FXIIIA_2B_2$ versus platelet-derived FXIII-A to thrombus stability requires clarification, but it is unlikely to be uniform throughout the thrombus, with the balance tipping toward FXIII-A in platelet-rich areas of the hemostatic plug, where solute transport of plasma $FXIIIA_2B_2$ is low.

Distribution of FXIII-A on the activated platelet surface is dependent on the subpopulation of platelets (Figure 1B), with PS-negative or spread platelets exhibiting diffuse staining across the membrane with a high concentration over the granulomere [40]. In PS-positive procoagulant platelets, FXIII-A was present only in the protruding 'cap' [40]. These 'caps' bind a number of other hemostatic proteins including fibrinogen [55], plasminogen and PAI-1 [56]. The 'caps' of platelets have recently been described as the 'platelet body' which the authors speculate is the remnant of organelles following ballooning of the platelet following strong agonist stimulation [57]. Ballooning transpires due to expansion of the platelet membrane, as a result of rapid influx of water and Na^+ and Cl^- ions. These platelets bind both procoagulant factor Xa and factor Va to the ballooned area and the platelet body thereby augmenting thrombin generation [58]. The collection of both procoagulant and pro- and anti-fibrinolytic proteins in these PS-positive platelets suggests they participate not only thrombus formation, but also the stability and breakdown of the thrombus. FXIII-A also functions in the formation of PS-positive platelets [59], by acting in concert with calpain to reduce the adhesive function of αIIbβ3, in a process that appears to be a prerequisite for their formation.

The method of externalization of platelet FXIII-A remains to be elucidated, but clearly occurs in response to external stimuli [40]. Reduced levels of FXIII-A were detected on stimulation of platelets with TRAP-6, compared to thrombin, and when platelets were treated with the Gly-Pro-Arg-Pro peptide, which inhibits fibrin polymerisation. The connection between platelet-associated fibrin and FXIII-A exposure suggests that αIIbβ3 may serve as a 'bridge' for FXIII-A to traverse to access the fibrin network [40]. Fibrin associated directly with the platelet surface is exceptionally resilient to fibrinolysis [56, 60-63], suggesting that platelet-derived FXIII-A and other anti-fibrinolytic proteins contribute to this heightened resistance at least in the early stages of thrombus formation.

Platelet FXIII-A in clot retraction

Clot retraction is the process by which fully-formed clots are compacted to limit vessel blockage and prevent leakage from the wound site. Platelets operate contractile machinery to reel in the surrounding fibrin network, expedited by bi-directional αIIbβ3 signalling [64].

The integrin αIIbβ3 acts as a molecular bridge between extracellular fibrinogen and the intracellular actin cytoskeleton via sphingomyelin-rich lipid rafts [65]. The cytoskeleton interacts with the α3 subunit tails via the adapter proteins talin and vinculin [66]. During clot retraction, fibrin bound to αIIbβ3 triggers outside-in signalling [67], resulting in the contraction of the actin cytoskeleton. $FXIIIA_2B_2$ contributes to the strength and rigidity of the condensed clot by cross-linking fibrin, and by enhancing platelet spreading [68]. Conflicting evidence exists on the effects of platelet FXIII-A on clot retraction. Early reports found that clot retraction was normal in FXIII-deficient patients [69-71]. More recently, Kasahara *et* al., [65, 72], have demonstrated that clot retraction was significantly impaired in the absence of platelet FXIII-A transglutaminase activity in PRP from FXIII-A knockout mice [65, 72]. In contrast, Kattula *et al*., [49] found that platelet FXIII-A did not contribute to the weight of clots formed from reconstituted FXIII-depleted plasma reconstituted with red blood cells compared with those containing FXIII-deficient platelets. Methodological differences in the two studies may account for these reported discrepancies, however, it is evident that further studies are required to confirm the role of platelet FXIII-A functions in clot retraction *in vivo*.

Other roles of platelet FXIII-A

The adhesive ability of αIIbβ3 is thought to be negatively regulated by FXIII-A and calpain, which limits platelet aggregate formation and thrombus growth [59]. These observations were made primarily in collagen-adherent platelets with prolonged elevations in cytosolic calcium, leading to a specific reduction in αIIbβ3 adhesive function. During thrombus formation, a number of phenotypically different platelet populations arise [59, 73-75]. These populations play different roles in the regulation of the thrombus microenvironment. Adherent platelets, with activated αIIbβ3, bind other platelets and fibrin and ensure thrombus stability [76], while procoagulant platelets lack functional αIIbβ3. Platelet FXIII-A also drives formation of a specific subtype of procoagulant platelets that retain an alpha-granule protein 'coat' on their surface [77-79]. Transglutaminase activity cross-links α-granule proteins to serotonin where they can then be retained on the platelet surface by binding to low affinity serotonin binding sites on fibrinogen or thrombospondin [77, 79]. It has been proposed that platelet FXIII-A is not a requirement for generation of coated platelets, due to their formation in FXIII deficient mice [80]. However, this could be due to a compensatory upregulation of other transglutaminases in FXIII-A deficient platelets [80]. In line with this FXIII-A deficient platelets have also been found to accelerate cross-linking [53].

Under low shear conditions fibrin formation on platelets forms a 'star-like' pattern on the platelet surface [81]. FXIII-A and αIIbβ3 play synergistic roles in aiding formation of these

fibrin protrusions. In the absence of FXIII-A and αIIbβ3, fibrin formation still occurs on the platelet surface, however fibres are orientated in the direction of flow [78], suggesting that early cross-links facilitate fibrin polymerisation against the direction of flow.

It has been suggested that FXIII-A functions in formation of protrusions such as filopodia and lammelipodia which aid in platelet adhesion, spreading and clot retraction [82]. A number of proteins involved in cytoskeletal remodelling are cross-linked by FXIII-A, including; actin [83, 84], GPIIb, GPIII, myosin, tropomyosin [84], talin, vinculin, filamin [82], and Thymosin beta4 [85]. FXIII-A associates with the cytoskeleton upon platelet activation, however, this is dependent on actin polymerisation, as the phallotoxin cytochalasin D inhibited this translocation [82]. Interestingly, cross-linking of vinculin was dependent on aggregation. Vinculin cannot be cross-linked to itself, but is cross-linked to a number of other cytoskeletal proteins [82], suggesting FXIII-A may localise key cytoskeletal proteins during remodelling. FXIII-A directly associates with HSP27 in activated platelets [86]. HSP27 functions as a molecular chaperone and rapidly interacts with the actin cytoskeleton upon platelet stimulation. It is plausible that HSP27 acts as a chaperone for translocation of FXIII-A from the platelet cytoplasm to the outer membrane via the actin cytoskeleton.

FXIII-A in leukocytes

FXIII-A is located in the cytoplasm of macrophages and monocytes [87], Leukocyte FXIII-A has been implicated in a number of intra-and extracellular processes, but as yet there is no defined route of externalisation

FXIII-A is expressed on the cell surface of monocytes and macrophages [88] in response to stimulation with certain immune modulators, which is akin to the situation in platelets [40]. The expression of FXIII-A in macrophages is dynamic in nature and can be altered in response to the external stimulus and the phenotype of the activated macrophage. Macrophages can be ‘alternatively’ or ‘classically’ activated depending on the activating stimulus. ‘Classically activated’ or M1 macrophages are generated in response to stimulation with the immune mediators, IFN-γ, LPS or TNF [89]. These pro-inflammatory ‘type 1’ macrophages [90] tend to exhibit down-regulation of FXIII-A [91, 92]. ‘Alternatively activated’, or M2 macrophages are stimulated in response to anti-inflammatory mediators, such as IL-4 and IL-13 [90]. M2 macrophages are reported to function in matrix remodelling, wound healing, allergy and parasite killing [89] and it is this subtype of macrophages that reveal upregulation of FXIII-A [92-94]. The selective expression of FXIII-A in M2 macrophages is in line with the capacity of this transglutaminase to act as an anti-inflammatory and pro- wound healing molecule.

Phagocytosis is the active ingestion and breakdown of microbes or other foreign particles by cells such as monocytes and macrophages. Phagocytic processes are driven by a finely controlled rearrangement of the actin cytoskeleton [95]. Considering the key role of FXIII-A in regulating cytoskeletal proteins it is perhaps not surprising that it is directly linked to this process [82-84]. Studies have indicated that FXIII-A activity may play a role in increasing the amount of phagocytosis in monocytes and macrophages [96].

Phagocytosis is positively correlated with FXIII-A expression in myelomonocytic cells [97]. In accordance with this Fcγ and complement receptor mediated phagocytosis is impaired in monocytes and macrophages following inhibition of FXIII-A and in FXIII-A-deficient mice [96]. FXIII-A is known to be upregulated during the maturation of monocyte-derived dendritic cells and actively assists migration of these cells [98]. Together these data implicate FXIII-A in the phagocytic and/or migration capacity of these cells, however there may be a degree of redundancy in the system, as phagocytosis is only slightly impaired in the absence of FXIII-A [96].

The role of cellular FXIII-A in lymphocytes in haemostasis has not been widely explored, however macrophages and monocytes are able to cross-link fibrin α- and γ-chains [88, 99], suggesting a potential role in thrombus stabilisation. Interestingly, thrombin treatment of monocytes does not augment exposure of FXIII-A [88], suggesting these cells may contribute to haemostasis in a situation where there is also an increase in the type 2 immune response, for example in a wound healing capacity.

FXIII-A in bone

Bone is a dynamic mineralized tissue which undergoes continuous remodelling in the form of bone resorption by osteoclasts and formation by osteoblasts. The processes of bone formation and resorption are influenced by many chemical and mechanical factors and an imbalance can severely impact on bone quality [100]. FXIII-A is present in a number of cell types in mineralized tissues including chondrocytes [31-33], osteocytes [101] and osteoblasts [5, 101] where it is both expressed on the cell surface and secreted into the extracellular matrix. FXIII-A contributes to the formation and stabilisation of connective tissue in bone by cross-linking a number of different substrates. Secreted osteoblast FXIII-A aids in the incorporation of fibronectin into the bone matrix [102, 103] thereby promoting formation of an insoluble matrix. This matrix forms a scaffold for other proteins to adhere to, including type 1 collagen [104]. Surface-associated osteoblast FXIII-A is involved in stabilizing the interaction between microtubules and the plasma membrane, which in turn enhances the secretion of collagen [102, 105-107]. Collagen is the principal component of bone matrix and it appears that both FXIII-A activity and fibronectin are essential for normal

collagen deposition [102, 103]. Extracellular collagen is a prerequisite for secretion of FXIII-A from osteoblasts and in line with this observation, increased levels of collagen enhance expression of FXIII-A mRNA [106]. Osteoblast FXIII-A also contributes to bone quality where its absence has a negative effect on alkaline phosphatase activity, lysyl oxidase levels and bone mineralisation. On an intracellular level, a regulatory role for FXIII-A and other transglutaminase enzymes has been identified in the different stages of osteoclastogenesis, including differentiation, migration and osteoclast fusion. FXIII-A contributed to these processes through its ability to influence actin dynamics [108], suggesting it may also be involved in cytoskeletal-mediated processes in other cell types. Evidence of FXIII-A involvement at the intracellular signalling level in osteoblast calveolae has also been identified in mineralised tissues. Calveolae are lipid raft plasma membrane invaginations involved in the regulation of endocytosis and intracellular signal transduction, via the clustering of receptors and signalling molecules. In differentiating osteoblasts FXIII-A colocalises intracellularly with caveolin-1 on the inner leaflet of calveolae, where it is involved in intracellular signalling by regulating interactions between Cav-1 and c-SRC kinase [109]. This regulatory signalling role in osteoblasts suggests that FXIII-A may also be involved in signalling pathways in other cells.

There are divergent reports on the size of FXIII-A in various cell types including those of mineralized tissue and adipocytes. In preadipocytes two bands of FXIII-A have been identified, one at the expected size of 80 kDa and another of 50-75 kDa [34]. FXIII-A has also been detected as a 37 kDa fragment in chondrocytes [110], cultured MC3T3-E1 osteoblast cells [101, 102] primary mouse osteoblast cells, mouse macrophage and chondrocyte extracts and in rat bone [101]. The 37 kDa fragment, postulated to arise from proteolytic cleavage of the full-length form, has a different subcellular localisation to full length FXIII-A in osteoblasts [101]. However, Cordell and colleagues [111] suggest that the mAb-AC-1A1 antibody used in these studies cross-reacts with transaldolase-1 (37 kDA) and other off-target antigens in cultured cells. Furthermore, this 37 kDa band is present in a number of cell types that lack FXIII-A protein and mRNA [111] and in bone and heart tissue from FXIII-A deficient mice [111]. FXIII-A and TG2 deficient mice also appear to exhibit normal bone deposition [111], suggesting that transglutaminase activity is not required for these processes *in vivo*. It is evident from the tangled literature that further work is necessary to define the forms of FXIII-A in bone and confirm its role in the formation, maintenance and repair of mineralized tissues *in vivo.*

Cellular FXIII-A in disease

The vast number of functions carried out by cellular FXIII-A has inevitably resulted in its contribution to a number of different disease states, where it has been found to play beneficial and detrimental roles.

FXIII-A in lung disease

Cellular FXIII-A and plasma $FXIIIA_2B_2$ have been implicated in the pathogenesis of lung diseases. In many forms of acute and chronic lung inflammation, fibrin deposition occurs as a result of increased vascular permeability eventually leading to fibrosis [112]. Cellular FXIII-A from injured alveolar macrophages and plasma $FXIIIA_2B_2$ from leaky capillaries have been detected in the bronchoalveolar lavage fluid of children with chronic bronchoalveolar inflammatory conditions, along with D-dimer [113]. These data suggest that FXIII is involved in stabilising fibrin deposits in the extravascular compartment of the lung tissues in these diseases. Increased FXIII-A release from dendritic cells is also detectable in bronchiolar lavage fluid following allergen challenge in asthmatic patients. This suggests a potential role for FXIII-A in airway obstruction in this disease [114], perhaps through the reinforcement of fibronectin deposits, which are involved in pathogenic airway remodelling in asthma [115].

FXIII-A in vascular disease

Long-term alterations in blood flow ultimately give rise to vascular remodelling [116], a process in which FXIII-A has been implicated [117]. Blood vessel widening occurs in response to increased blood flow, decreased blood flow results in vessel narrowing [118, 119] and vessel walls thicken in response to high blood pressure [120]. Macrophage FXIII-A may participate in flow-induced remodelling of vessels [121]. Zhou *et* al., [121] demonstrated that expression of the CXCR3 receptor is necessary for inward perivascular remodelling induced by alterations in blood flow. This CXCR3-dependant accumulation of macrophages during perivascular remodelling enhanced expression of FXIII-A mRNA [121], suggesting that the transglutaminase may function by stabilizing the remodelled vascular wall.

Hypertension is characterized as long term elevation in blood pressure and is a significant risk factor for the development of atherosclerosis. Infiltration of the arterial wall by monocytes, macrophages and T-cells leads to formation of new connective tissue, which together with the infiltrating leukocytes, forms an atherosclerotic lesion [122]. The angiotensin II (ATII) signalling system is involved in multiple regulatory processes, including the control of blood pressure through vasoconstriction. ATII signalling is also implicated in a number of pathological diseases, such as hypertension and atherosclerosis [123]. Monocyte FXIII-A plays a pathogenic role in hypertensive disease due to its ability to

increase the signalling capacity of the angiotensin receptor (ATI) [124]. ATI receptor dimerization occurs in the presence of ATII, and FXIII-A subsequently facilitates covalent cross-linking of the ATI monomers, resulting in increased receptor capacity for signalling and desensitization [124]. Hypertensive patients display an increase in both monocyte FXIII-A and angiotensin-converting-enzyme (ACE)-dependant ATII production and storage [124]. Increased cross-linked AT1 dimers have been found in an ApoE-/- model of atherosclerotic mice and inhibition of ACE and cellular FXIII-A reduced atherosclerotic lesion area and attenuated the recruitment of leukocytes into the aorta [124]. Platelet-derived FXIII-A has also been identified in atherosclerotic plaques [125], suggesting that the function of platelet FXIII-A is not confined to hemostasis and may function in pathogenic situations, such as stabilization of atherosclerotic lesions.

FXIII-A in cardiac disease

Plasma $FXIIIA_2B_2$ and platelet FXIII-A have been found to contribute to the integrity of the cardiac vessel wall. A number of cardiac pathologies are observed in FXIII deficient mice, most of which are exacerbated by the combined absence of both cellular and plasma FXIII [126]. In these cases, hemorrhage and fibrosis resulting from lack of plasma $FXIIIA_2B_2$ induce initial damage to cardiac tissue, this is followed by delayed wound healing, due to the absence of cellular FXIII-A in leukocytes in these tissues [126]. FXIII-A is present in resident monocytes and macrophages in normal cardiac tissue, and following coronary ligation [127]. FXIII deficient mice exhibit an increased incidence of cardiac rupture, which can be circumvented by infusion of $FXIIIA_2B_2$, although ventricular remodelling in these mice remained diminished [127]. High levels of FXIII transglutaminase activity was observed in healing infarct tissue, suggesting its active participation in the wound healing response. A reduction in leukocytes has been documented in cardiac tissue in FXIII-A deficient mice [128], which could be attributed to the role of FXIII-A in cell migration, thus exacerbate the impaired wound healing observed.

FXIII-A in inflammatory disease

The expression of FXIII-A in a number of inflammatory cells also implicates it in the pathogenesis of certain inflammatory disorders. In ulcerative colitis, reduced expression of both cellular FXIII-A and plasma $FXIIIA_2B_2$ is evident due to upregulation of the M1 immune response. The reduction of FXIII levels in this case will affect its capacity to aid in phagocytosis and cell migration and may contribute to the prolongation and severity of the disease [129]. The contribution of FXIII-A to inflammatory arthritis is also evident in collagen-induced arthritis models FXIII-A deficient mice. There was a significant reduction in osteoclast differentiation in FXIII-A deficient mice which limited disease progression.

FXIII-A deficient mice also displayed reduced deposition of fibrin in the extracellular spaces within the knee joints leading to a reduction in the retention of inflammatory macrophages [130]. These results clearly show that eliminating FXIII-A limits inflammatory arthritis and protects from cartilage and bone destruction, thus suggesting that inhibition of this transglutaminase is a potential therapeutic strategy in arthropathies and other degenerative bone diseases.

FXIII-A in diabetes and obesity

Pancreatic islet β cells harbours FXIII-A which exerts transglutaminase activity in response to prolonged spikes in cytosolic Ca^{2+} [131]. Glucose-stimulated insulin secretion in β-cells is inhibited upon treatment with a transglutaminase inhibitor, suggesting that FXIII-A activity is involved in insulin regulation [131]. Interestingly, a recent study has identified a possible connection between FXIII-A and type 2 diabetes in a mouse model of obesity-induced chronic low-grade inflammation, mimicking that found in type 2 diabetes [132]. Mice treated with low dose pro-inflammatory cytokines exhibited reduced glucose-stimulated insulin secretion and increased basal Ca^{2+} levels, resulting in reduced expression of the FXIII-A gene (F13A1) [132]. Identification of F13A1 as a novel stress-inhibited gene in islets provides a promising lead to pursue in the dysfunction that occurs in these cells during the development of type 2 diabetes.

Single nucleotide polymorphisms SNPs in F13A1 correlate with increased body mass index and an increased incidence of type 2 diabetes [133]. A recent study performed by Myneni *et al*., [34] suggests that FXIII-A may contribute to obesity and weight gain. FXIII-A is expressed in adipose tissue where it is enhances proliferation of preadipocytes and stabilises the fibronectin matrix. [34]. In line with these observations, FXIII-A deficient mice are protected against insulin resistance, they show signs of metabolically healthy obesity [134]. Further work is urgently required to clarify the direct role of FXIII-A in attenuating type 2 diabetes and obesity.

FXIII-A in cancer

FXIII-A has been identified in a number of leukemic cell types including megakaryoblasts, promyeloblasts, monoblasts and lymphoblasts[135, 136] and FXIII-A expression in leukemic cells is associated with reduced patient survival in acute promyelocytic leukemia [137]. In contrast, a recent study has shown that FXIII-A expression in children with B-cell precursor acute lymphoblastic leukemia was associated with patient survival [138]. Further studies in this area are essential to delineate the role of FXIII-A in leukemic cells in contributing towards disease progression.

Summary and future perspectives

Plasma $FXIIIA_2B_2$ was classified as a coagulation factor in the 1960's and is largely found in complex with the precursor of its principal target protein, fibrinogen. The clear bleeding phenotype of individuals deficient in FXIII is testimony to its essential function in hemostasis. However, there has been ambiguity surrounding the true function of the cellular form of FXIII-A. This can in part be ascribed to the fact that while FXIII-A is expressed by numerous cell types, mainly those of hematopoietic origin, it does not contain a classical endoplasmic reticulum signal peptide for secretion in nucleated cells [11]. This has hampered research into FXIII-A, but accumulating evidence now indicates that it is a diverse cellular enzyme that cross-links numerous substrates within the intracellular and extracellular environment. Recent observations accrediting the cellular source of the plasma FXIII-A subunit to resident tissue macrophages [15] has significantly advanced our knowledge, but as yet the mechanism involved in its secretion remain an enigma. Given the nature of this enzyme, and the fact that isopeptide bonds can be formed between glutamine donors and lysine acceptor residues in a wide range of proteins, it is perhaps not surprising that FXIII-A functions in such an array of biological processes. Nonetheless, the absence of an inhibitor of FXIII-A suggests that the environment and kinetics of this transamidase enzyme must regulate its function, but direct evidence on this is scant. In conclusion, it is evident that FXIII-A is a broad spectrum enzyme that is largely indiscriminate in its ability to cross-link protein substrates, but there is still much to be uncovered in relation the mechanism of secretion from cells of bone marrow lineage and direction of its function in different biological and pathophysiological processes.

Disclosure of Conflict of Interest

The authors state that they have no conflict of interest.

References

1 Laki K, Lorand L. On the Solubility of Fibrin Clots. *Science*. 1948; **108**: 280.

2 Lorand L. Fibrin clots. *Nature*. 1950; **166**: 694-5.

3 Duckert F, Jung E, Shmerling DH. A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. *Thromb Diath Haemorrh*. 1960; **5**: 179-86.

4 Dardik R, Solomon A, Loscalzo J, Eskaraev R, Bialik A, Goldberg I, Schiby G, Inbal A. Novel proangiogenic effect of factor XIII associated with suppression of thrombospondin 1 expression. *Arterioscler Thromb Vasc Biol*. 2003; **23**: 1472-7.

5 Nurminskaya M, Kaartinen MT. Transglutaminases in mineralized tissues. *Front Biosci*. 2006; **11**: 1591-606.

6 Folk JE, Finlayson JS. The epsilon-(gamma-glutamyl)lysine crosslink and the catalytic role of transglutaminases. *Adv Protein Chem*. 1977; **31**: 1-133.

7 Schwartz ML, Pizzo SV, Hill RL, McKee PA. The subunit structures of human plasma and platelet factor XIII (fibrin-stabilizing factor). *J Biol Chem*. 1971; **246**: 5851-4.

8 Katona E, Penzes K, Csapo A, Fazakas F, Udvardy ML, Bagoly Z, Orosz ZZ, Muszbek L. Interaction of factor XIII subunits. *Blood*. 2014; **123**: 1757-63.

9 Ikematsu S. An approach to the metabolism of factor XIII. *Nihon Ketsueki Gakkai Zasshi*. 1981; **44**: 1499-505.

10 Nagy JA, Henriksson P, McDonagh J. Biosynthesis of factor XIII B subunit by human hepatoma cell lines. *Blood*. 1986; **68**: 1272-9.

11 Kaetsu H, Hashiguchi T, Foster D, Ichinose A. Expression and release of the a and b subunits for human coagulation factor XIII in baby hamster kidney (BHK) cells. *J Biochem*. 1996; **119**: 961-9.

12 Poon MC, Russell JA, Low S, Sinclair GD, Jones AR, Blahey W, Ruether BA, Hoar DI. Hemopoietic origin of factor XIII A subunits in platelets, monocytes, and plasma. Evidence from bone marrow transplantation studies. *J Clin Invest*. 1989; **84**: 787-92.

13 Inbal A, Muszbek L, Lubetsky A, Katona E, Levi I, Karpati L, Nagler A. 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*. 2004; **15**: 249-53.

14 Cordell PA, Kile BT, Standeven KF, Josefsson EC, Pease RJ, Grant PJ. Association of coagulation factor XIII-A with Golgi proteins within monocyte-macrophages: implications for subcellular trafficking and secretion. *Blood*. 2010; **115**: 2674-81.

15 Beckers CML, Simpson KR, Griffin KJ, Brown JM, Cheah LT, Smith KA, Vacher J, Cordell PA, Kearney MT, Grant PJ, Pease RJ. Cre/lox Studies Identify Resident Macrophages as the Major Source of Circulating Coagulation Factor XIII-A. *Arterioscler Thromb Vasc Biol*. 2017; **37**: 1494-502.

16 Lorand L, Konishi K. Activation of the Fibrin Stabilizing Factor of Plasma by Thrombin. *Arch Biochem Biophys*. 1964; **105**: 58-67.

17 Schwartz ML, Pizzo SV, Hill RL, McKee PA. Human Factor XIII from plasma and platelets. Molecular weights, subunit structures, proteolytic activation, and cross-linking of fibrinogen and fibrin. *J Biol Chem*. 1973; **248**: 1395-407.

18 Lorand L, Jeong JM, Radek JT, Wilson J. Human plasma factor XIII: subunit interactions and activation of zymogen. *Methods Enzymol*. 1993; **222**: 22-35.

19 Hornyak TJ, Shafer JA. Interactions of factor XIII with fibrin as substrate and cofactor. *Biochemistry*. 1992; **31**: 423-9.

20 Adany R, Bardos H. Factor XIII subunit A as an intracellular transglutaminase. *Cell Mol Life Sci*. 2003; **60**: 1049-60.

21 Polgar J, Hidasi V, Muszbek L. Non-proteolytic activation of cellular protransglutaminase (placenta macrophage factor XIII). *Biochem J*. 1990; **267**: 557-60.
22 Muszbek L, Polgar J, Boda Z. Platelet factor XIII becomes active without the release of activation peptide during platelet activation. *Thromb Haemost*. 1993; **69**: 282-5.
23 Buluk K. [An unknown action of blood platelets; preliminary communication]. *Pol Tyg Lek (Wars)*. 1955; **10**: 191.
24 Kiesselbach TH, Wagner RH. Fibrin-stabilizing factor: a thrombin-labile platelet protein. *Am J Physiol*. 1966; **211**: 1472-6.
25 Luscher EF. [Fibrin-stabilizing factor from thrombocytes]. *Schweiz Med Wochenschr*. 1957; **87**: 1220-1.
26 Kiesselbach TH, Wagner RH. Demonstration of factor XIII in human megakaryocytes by a fluorescent antibody technique. *Ann N Y Acad Sci*. 1972; **202**: 318-28.
27 Henriksson P, Becker S, Lynch G, McDonagh J. Identification of intracellular factor XIII in human monocytes and macrophages. *J Clin Invest*. 1985; **76**: 528-34.
28 Muszbek L, Adany R, Szegedi G, Polgar J, Kavai M. Factor XIII of blood coagulation in human monocytes. *Thromb Res*. 1985; **37**: 401-10.
29 Adany R, Belkin A, Vasilevskaya T, Muszbek L. Identification of blood coagulation factor XIII in human peritoneal macrophages. *Eur J Cell Biol*. 1985; **38**: 171-3.
30 Nestle FO, Zheng XG, Thompson CB, Turka LA, Nickoloff BJ. Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets. *J Immunol*. 1993; **151**: 6535-45.
31 Nurminskaya M, Linsenmayer TF. Identification and characterization of up-regulated genes during chondrocyte hypertrophy. *Dev Dyn*. 1996; **206**: 260-71.
32 Nurminskaya M, Magee C, Nurminsky D, Linsenmayer TF. Plasma transglutaminase in hypertrophic chondrocytes: expression and cell-specific intracellular activation produce cell death and externalization. *J Cell Biol*. 1998; **142**: 1135-44.
33 Rosenthal AK, Masuda I, Gohr CM, Derfus BA, Le M. The transglutaminase, Factor XIIIA, is present in articular chondrocytes. *Osteoarthritis Cartilage*. 2001; **9**: 578-81.
34 Myneni VD, Hitomi K, Kaartinen MT. Factor XIII-A transglutaminase acts as a switch between preadipocyte proliferation and differentiation. *Blood*. 2014; **124**: 1344-53.
35 Lopaciuk S, Lovette KM, McDonagh J, Chuang HY, McDonagh. Subcellular distribution of fibrinogen and factor XIII in human blood platelets. *Thromb Res*. 1976; **8**: 453-65.
36 Sixma JJ, van den Berg A, Schiphorst M, Geuze HJ, McDonagh J. Immunocytochemical localization of albumin and factor XIII in thin cryo sections of human blood platelets. *Thromb Haemost*. 1984; **51**: 388-91.
37 Katona EE, Ajzner E, Toth K, Karpati L, Muszbek L. Enzyme-linked immunosorbent assay for the determination of blood coagulation factor XIII A-subunit in plasma and in cell lysates. *J Immunol Methods*. 2001; **258**: 127-35.
38 Marx G, Korner G, Mou X, Gorodetsky R. Packaging zinc, fibrinogen, and factor XIII in platelet alpha-granules. *J Cell Physiol*. 1993; **156**: 437-42.
39 Joist JH, Niewiarowski S. Retention of platelet fibrin stabilizing factor during the platelet release reaction and clot retraction. *Thromb Diath Haemorrh*. 1973; **29**: 679-83.
40 Mitchell JL, Lionikiene AS, Fraser SR, Whyte CS, Booth NA, Mutch NJ. Functional factor XIII-A is exposed on the stimulated platelet surface. *Blood*. 2014; **124**: 3982-90.
41 Sakata Y, Aoki N. Cross-linking of alpha 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. *J Clin Invest*. 1980; **65**: 290-7.
42 Valnickova Z, Enghild JJ. Human procarboxypeptidase U, or thrombin-activable fibrinolysis inhibitor, is a substrate for transglutaminases. Evidence for transglutaminase-catalyzed cross-linking to fibrin. *J Biol Chem*. 1998; **273**: 27220-4.

43 Ritchie H, Robbie LA, Kinghorn S, Exley R, Booth NA. Monocyte plasminogen activator inhibitor 2 (PAI-2) inhibits u-PA-mediated fibrin clot lysis and is cross-linked to fibrin. *Thromb Haemost*. 1999; **81**: 96-103.
44 Mutch NJ, Koikkalainen JS, Fraser SR, Duthie KM, Griffin M, Mitchell J, Watson HG, Booth NA. Model thrombi formed under flow reveal the role of factor XIII-mediated cross-linking in resistance to fibrinolysis. *J Thromb Haemost*. 2010; **8**: 2017-24.
45 Fraser SR, Booth NA, Mutch NJ. The antifibrinolytic function of factor XIII is exclusively expressed through alpha(2)-antiplasmin cross-linking. *Blood*. 2011; **117**: 6371-4.
46 Rijken DC, Abdul S, Malfliet JJ, Leebeek FW, Uitte de Willige S. Compaction of fibrin clots reveals the antifibrinolytic effect of factor XIII. *J Thromb Haemost*. 2016; **14**: 1453-61.
47 Aleman MM, Byrnes JR, Wang JG, Tran R, Lam WA, Di Paola J, Mackman N, Degen JL, Flick MJ, Wolberg AS. Factor XIII activity mediates red blood cell retention in venous thrombi. *J Clin Invest*. 2014; **124**: 3590-600.
48 Byrnes JR, Duval C, Wang Y, Hansen CE, Ahn B, Mooberry MJ, Clark MA, Johnsen JM, Lord ST, Lam WA, Meijers JC, Ni H, Ariens RA, Wolberg AS. Factor XIIIa-dependent retention of red blood cells in clots is mediated by fibrin alpha-chain crosslinking. *Blood*. 2015; **126**: 1940-8.
49 Kattula S, Byrnes JR, Martin SM, Holle LA, Cooley BC, Flick MJ, Wolberg AS. Factor XIII in plasma, but not in platelets, mediates red blood cell retention in clots and venous thrombus size in mice. *Blood Adv*. 2018; **2**: 25-35.
50 Reed GL, Matsueda GR, Haber E. Fibrin-fibrin and alpha 2-antiplasmin-fibrin cross-linking by platelet factor XIII increases the resistance of platelet clots to fibrinolysis. *Trans Assoc Am Physicians*. 1991; **104**: 21-8.
51 Reed GL, Matsueda GR, Haber E. Platelet factor XIII increases the fibrinolytic resistance of platelet-rich clots by accelerating the crosslinking of alpha 2-antiplasmin to fibrin. *Thromb Haemost*. 1992; **68**: 315-20.
52 Francis CW, Marder VJ. Rapid formation of large molecular weight alpha-polymers in cross-linked fibrin induced by high factor XIII concentrations. Role of platelet factor XIII. *J Clin Invest*. 1987; **80**: 1459-65.
53 Hevessy Z, Haramura G, Boda Z, Udvardy M, Muszbek L. Promotion of the crosslinking of fibrin and alpha 2-antiplasmin by platelets. *Thromb Haemost*. 1996; **75**: 161-7.
54 Rubens FD, Perry DW, Hatton MW, Bishop PD, Packham MA, Kinlough-Rathbone RL. Platelet accumulation on fibrin-coated polyethylene: role of platelet activation and factor XIII. *Thromb Haemost*. 1995; **73**: 850-6.
55 Abaeva AA, Canault M, Kotova YN, Obydennyy SI, Yakimenko AO, Podoplelova NA, Kolyadko VN, Chambost H, Mazurov AV, Ataullakhanov FI, Nurden AT, Alessi MC, Panteleev MA. Procoagulant platelets form an alpha-granule protein-covered "cap" on their surface that promotes their attachment to aggregates. *J Biol Chem*. 2013; **288**: 29621-32.
56 Whyte CS, Swieringa F, Mastenbroek TG, Lionikiene AS, Lance MD, van der Meijden PE, Heemskerk JW, Mutch NJ. Plasminogen associates with phosphatidylserine-exposing platelets and contributes to thrombus lysis under flow. *Blood*. 2015; **125**: 2568-78.
57 Agbani EO, van den Bosch MT, Brown E, Williams CM, Mattheij NJ, Cosemans JM, Collins PW, Heemskerk JW, Hers I, Poole AW. Coordinated Membrane Ballooning and Procoagulant Spreading in Human Platelets. *Circulation*. 2015; **132**: 1414-24.
58 Agbani EO, Hers I, Poole AW. Temporal contribution of the platelet body and balloon to thrombin generation. *Haematologica*. 2017; **102**: e379-e81.
59 Kulkarni S, Jackson SP. Platelet factor XIII and calpain negatively regulate integrin alphaIIbbeta3 adhesive function and thrombus growth. *J Biol Chem*. 2004; **279**: 30697-706.

60 Collet JP, Montalescot G, Lesty C, Mishal Z, Soria J, Choussat R, Drobinski G, Soria C, Pinton P, Barragan P, Thomas D. Effects of Abciximab on the architecture of platelet-rich clots in patients with acute myocardial infarction undergoing primary coronary intervention. *Circulation*. 2001; **103**: 2328-31.
61 Collet JP, Montalescot G, Lesty C, Soria J, Mishal Z, Thomas D, Soria C. Disaggregation of in vitro preformed platelet-rich clots by abciximab increases fibrin exposure and promotes fibrinolysis. *Arterioscler Thromb Vasc Biol*. 2001; **21**: 142-8.
62 Collet JP, Montalescot G, Lesty C, Weisel JW. A structural and dynamic investigation of the facilitating effect of glycoprotein IIb/IIIa inhibitors in dissolving platelet-rich clots. *Circ Res*. 2002; **90**: 428-34.
63 Shenkman B, Einav Y, Livnat T, Budnik I, Martinowitz U. In vitro evaluation of clot quality and stability in a model of severe thrombocytopenia: effect of fibrinogen, factor XIII and thrombin-activatable fibrinolysis inhibitor. *Blood Transfus*. 2014; **12**: 78-84.
64 Ginsberg MH, Du X, Plow EF. Inside-out integrin signalling. *Curr Opin Cell Biol*. 1992; **4**: 766-71.
65 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. Clot retraction is mediated by factor XIII-dependent fibrin-alphaIIbbeta3-myosin axis in platelet sphingomyelin-rich membrane rafts. *Blood*. 2013; **122**: 3340-8.
66 Knezevic I, Leisner TM, Lam SC. Direct binding of the platelet integrin alphaIIbbeta3 (GPIIb-IIIa) to talin. Evidence that interaction is mediated through the cytoplasmic domains of both alphaIIb and beta3. *J Biol Chem*. 1996; **271**: 16416-21.
67 Shattil SJ. Signaling through platelet integrin alpha IIb beta 3: inside-out, outside-in, and sideways. *Thromb Haemost*. 1999; **82**: 318-25.
68 Cohen I, Gerrard JM, White JG. Ultrastructure of clots during isometric contraction. *J Cell Biol*. 1982; **93**: 775-87.
69 Jelenska M, Kopec M, Breddin K. On the retraction of collagen and fibrin induced by normal, defective and modified platelets. *Haemostasis*. 1985; **15**: 169-75.
70 Niewiarowski S, Markiewicz M, Nath N. Inhibition of the platelet-dependent fibrin retraction by the fibrin stabilizing factor (FSF, factor 13). *J Lab Clin Med*. 1973; **81**: 641-50.
71 Rao KM, Newcomb TF. Clot retraction in a factor XIII free system. *Scand J Haematol*. 1980; **24**: 142-8.
72 Kasahara K, Souri M, Kaneda M, Miki T, Yamamoto N, Ichinose A. Impaired clot retraction in factor XIII A subunit-deficient mice. *Blood*. 2010; **115**: 1277-9.
73 Alberio L, Safa O, Clemetson KJ, Esmon CT, Dale GL. Surface expression and functional characterization of alpha-granule factor V in human platelets: effects of ionophore A23187, thrombin, collagen, and convulxin. *Blood*. 2000; **95**: 1694-702.
74 Heemskerk JW, Vuist WM, Feijge MA, Reutelingsperger CP, Lindhout T. Collagen but not fibrinogen surfaces induce bleb formation, exposure of phosphatidylserine, and procoagulant activity of adherent platelets: evidence for regulation by protein tyrosine kinase-dependent Ca2+ responses. *Blood*. 1997; **90**: 2615-25.
75 Kempton CL, Hoffman M, Roberts HR, Monroe DM. Platelet heterogeneity: variation in coagulation complexes on platelet subpopulations. *Arterioscler Thromb Vasc Biol*. 2005; **25**: 861-6.
76 Munnix IC, Kuijpers MJ, Auger J, Thomassen CM, Panizzi P, van Zandvoort MA, Rosing J, Bock PE, Watson SP, Heemskerk JW. Segregation of platelet aggregatory and procoagulant microdomains in thrombus formation: regulation by transient integrin activation. *Arterioscler Thromb Vasc Biol*. 2007; **27**: 2484-90.

77 Dale GL, Friese P, Batar P, Hamilton SF, Reed GL, Jackson KW, Clemetson KJ, Alberio L. Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface. *Nature*. 2002; **415**: 175-9.
78 Mattheij NJ, Swieringa F, Mastenbroek TG, Berny-Lang MA, May F, Baaten CC, van der Meijden PE, Henskens YM, Beckers EA, Suylen DP, Nolte MW, Hackeng TM, McCarty OJ, Heemskerk JW, Cosemans JM. Coated platelets function in platelet-dependent fibrin formation via integrin alphaIIbbeta3 and transglutaminase factor XIII. *Haematologica*. 2015.
79 Szasz R, Dale GL. Thrombospondin and fibrinogen bind serotonin-derivatized proteins on COAT-platelets. *Blood*. 2002; **100**: 2827-31.
80 Jobe SM, Leo L, Eastvold JS, Dickneite G, Ratliff TL, Lentz SR, Di Paola J. Role of FcRgamma and factor XIIIA in coated platelet formation. *Blood*. 2005; **106**: 4146-51.
81 Cosemans JM, Schols SE, Stefanini L, de Witt S, Feijge MA, Hamulyak K, Deckmyn H, Bergmeier W, Heemskerk JW. Key role of glycoprotein Ib/V/IX and von Willebrand factor in platelet activation-dependent fibrin formation at low shear flow. *Blood*. 2011; **117**: 651-60.
82 Serrano K, Devine DV. Intracellular factor XIII crosslinks platelet cytoskeletal elements upon platelet activation. *Thromb Haemost*. 2002; **88**: 315-20.
83 Cohen I, Blankenberg TA, Borden D, Kahn DR, Veis A. Factor XIIIa-catalyzed cross-linking of platelet and muscle actin. Regulation by nucleotides. *Biochim Biophys Acta*. 1980; **628**: 365-75.
84 Cohen I, Glaser T, Veis A, Bruner-Lorand J. Ca2+-dependent cross-linking processes in human platelets. *Biochim Biophys Acta*. 1981; **676**: 137-47.
85 Yu FX, Lin SC, Morrison-Bogorad M, Atkinson MA, Yin HL. Thymosin beta 10 and thymosin beta 4 are both actin monomer sequestering proteins. *J Biol Chem*. 1993; **268**: 502-9.
86 Zhu Y, Tassi L, Lane W, Mendelsohn ME. Specific binding of the transglutaminase, platelet factor XIII, to HSP27. *J Biol Chem*. 1994; **269**: 22379-84.
87 Adany R. Intracellular factor XIII: cellular distribution of factor XIII subunit a in humans. *Semin Thromb Hemost*. 1996; **22**: 399-408.
88 Kradin RL, Lynch GW, Kurnick JT, Erikson M, Colvin RB, McDonagh J. Factor XIII A is synthesized and expressed on the surface of U937 cells and alveolar macrophages. *Blood*. 1987; **69**: 778-85.
89 Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep*. 2014; **6**: 13.
90 Spellberg B, Edwards JE, Jr. Type 1/Type 2 immunity in infectious diseases. *Clin Infect Dis*. 2001; **32**: 76-102.
91 Pabst MJ, Pabst KM, Handsman DB, Beranova-Giorgianni S, Giorgianni F. Proteome of monocyte priming by lipopolysaccharide, including changes in interleukin-1beta and leukocyte elastase inhibitor. *Proteome Sci*. 2008; **6**: 13.
92 Torocsik D, Szeles L, Paragh G, Jr., Rakosy Z, Bardos H, Nagy L, Balazs M, Inbal A, Adany R. Factor XIII-A is involved in the regulation of gene expression in alternatively activated human macrophages. *Thromb Haemost*. 2010; **104**: 709-17.
93 Chaitidis P, O'Donnell V, Kuban RJ, Bermudez-Fajardo A, Ungethuem U, Kuhn H. Gene expression alterations of human peripheral blood monocytes induced by medium-term treatment with the TH2-cytokines interleukin-4 and -13. *Cytokine*. 2005; **30**: 366-77.
94 Gratchev A, Kzhyshkowska J, Utikal J, Goerdt S. Interleukin-4 and dexamethasone counterregulate extracellular matrix remodelling and phagocytosis in type-2 macrophages. *Scand J Immunol*. 2005; **61**: 10-7.
95 May RC, Machesky LM. Phagocytosis and the actin cytoskeleton. *J Cell Sci*. 2001; **114**: 1061-77.

96 Sarvary A, Szucs S, Balogh I, Becsky A, Bardos H, Kavai M, Seligsohn U, Egbring R, Lopaciuk S, Muszbek L, Adany R. Possible role of factor XIII subunit A in Fcgamma and complement receptor-mediated phagocytosis. *Cell Immunol*. 2004; **228**: 81-90.
97 Kavai M, Adany R, Pasti G, Suranyi P, Szucs G, Muszbek L, Bojan F, Szegedi G. Marker profile, enzyme activity, and function of a human myelomonocytic leukemia cell line. *Cell Immunol*. 1992; **139**: 531-40.
98 Jayo A, Conde I, Lastres P, Jimenez-Yuste V, Gonzalez-Manchon C. Possible role for cellular FXIII in monocyte-derived dendritic cell motility. *Eur J Cell Biol*. 2009; **88**: 423-31.
99 Conkling PR, Achyuthan KE, Greenberg CS, Newcomb TF, Weinberg JB. Human mononuclear phagocyte transglutaminase activity cross-links fibrin. *Thromb Res*. 1989; **55**: 57-68.
100 Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simoes MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. *BioMed research international*. 2015; **2015**: 421746.
101 Nakano Y, Al-Jallad HF, Mousa A, Kaartinen MT. Expression and localization of plasma transglutaminase factor XIIIA in bone. *J Histochem Cytochem*. 2007; **55**: 675-85.
102 Al-Jallad HF, Nakano Y, Chen JL, McMillan E, Lefebvre C, Kaartinen MT. Transglutaminase activity regulates osteoblast differentiation and matrix mineralization in MC3T3-E1 osteoblast cultures. *Matrix Biol*. 2006; **25**: 135-48.
103 Cui C, Wang S, Myneni VD, Hitomi K, Kaartinen MT. Transglutaminase activity arising from Factor XIIIA is required for stabilization and conversion of plasma fibronectin into matrix in osteoblast cultures. *Bone*. 2014; **59**: 127-38.
104 McKee MD, Addison WN, Kaartinen MT. Hierarchies of extracellular matrix and mineral organization in bone of the craniofacial complex and skeleton. *Cells Tissues Organs*. 2005; **181**: 176-88.
105 Al-Jallad HF, Myneni VD, Piercy-Kotb SA, Chabot N, Mulani A, Keillor JW, Kaartinen MT. Plasma membrane factor XIIIA transglutaminase activity regulates osteoblast matrix secretion and deposition by affecting microtubule dynamics. *PLoS One*. 2011; **6**: e15893.
106 Piercy-Kotb SA, Mousa A, Al-Jallad HF, Myneni VD, Chicatun F, Nazhat SN, Kaartinen MT. Factor XIIIA transglutaminase expression and secretion by osteoblasts is regulated by extracellular matrix collagen and the MAP kinase signaling pathway. *J Cell Physiol*. 2012; **227**: 2936-46.
107 Wang S, Cui C, Hitomi K, Kaartinen MT. Detyrosinated Glu-tubulin is a substrate for cellular Factor XIIIA transglutaminase in differentiating osteoblasts. *Amino Acids*. 2014; **46**: 1513-26.
108 Sun H, Kaartinen MT. Transglutaminase activity regulates differentiation, migration and fusion of osteoclasts via affecting actin dynamics. *J Cell Physiol*. 2018.
109 Wang S, Kaartinen MT. Cellular Factor XIIIA Transglutaminase Localizes in Caveolae and Regulates Caveolin-1 Phosphorylation, Homo-oligomerization and c-Src Signaling in Osteoblasts. *J Histochem Cytochem*. 2015; **63**: 829-41.
110 Ulici V, James CG, Hoenselaar KD, Beier F. Regulation of gene expression by PI3K in mouse growth plate chondrocytes. *PLoS One*. 2010; **5**: e8866.
111 Cordell PA, Newell LM, Standeven KF, Adamson PJ, Simpson KR, Smith KA, Jackson CL, Grant PJ, Pease RJ. Normal Bone Deposition Occurs in Mice Deficient in Factor XIII-A and Transglutaminase 2. *Matrix Biol*. 2015; **43**: 85-96.
112 Idell S, Mazar AP, Bitterman P, Mohla S, Harabin AL. Fibrin turnover in lung inflammation and neoplasia. *Am J Respir Crit Care Med*. 2001; **163**: 578-84.

113 Katona E, Nagy B, Kappelmayer J, Baktai G, Kovacs L, Marialigeti T, Dezso B, Muszbek L. Factor XIII in bronchoalveolar lavage fluid from children with chronic bronchoalveolar inflammation. *J Thromb Haemost*. 2005; **3**: 1407-13.
114 Esnault S, Kelly EA, Sorkness RL, Evans MD, Busse WW, Jarjour NN. Airway factor XIII associates with type 2 inflammation and airway obstruction in asthmatic patients. *J Allergy Clin Immunol*. 2015.
115 Meerschaert J, Kelly EA, Mosher DF, Busse WW, Jarjour NN. Segmental antigen challenge increases fibronectin in bronchoalveolar lavage fluid. *Am J Respir Crit Care Med*. 1999; **159**: 619-25.
116 Langille BL. Arterial remodeling: relation to hemodynamics. *Can J Physiol Pharmacol*. 1996; **74**: 834-41.
117 Bakker EN, Pistea A, Spaan JA, Rolf T, de Vries CJ, van Rooijen N, Candi E, VanBavel E. Flow-dependent remodeling of small arteries in mice deficient for tissue-type transglutaminase: possible compensation by macrophage-derived factor XIII. *Circ Res*. 2006; **99**: 86-92.
118 Kamiya A, Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am J Physiol*. 1980; **239**: H14-21.
119 Langille BL, Bendeck MP, Keeley FW. Adaptations of carotid arteries of young and mature rabbits to reduced carotid blood flow. *Am J Physiol*. 1989; **256**: H931-9.
120 Baumbach GL, Heistad DD. Remodeling of cerebral arterioles in chronic hypertension. *Hypertension*. 1989; **13**: 968-72.
121 Zhou J, Tang PC, Qin L, Gayed PM, Li W, Skokos EA, Kyriakides TR, Pober JS, Tellides G. CXCR3-dependent accumulation and activation of perivascular macrophages is necessary for homeostatic arterial remodeling to hemodynamic stresses. *J Exp Med*. 2010; **207**: 1951-66.
122 Alexander RW. Theodore Cooper Memorial Lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. *Hypertension*. 1995; **25**: 155-61.
123 Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol*. 2007; **292**: C82-97.
124 AbdAlla S, Lother H, Langer A, el Faramawy Y, Quitterer U. Factor XIIIA transglutaminase crosslinks AT1 receptor dimers of monocytes at the onset of atherosclerosis. *Cell*. 2004; **119**: 343-54.
125 Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, McRedmond JP, Cahill DJ, Emili A, Fitzgerald DJ, Maguire PB. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. *Blood*. 2004; **103**: 2096-104.
126 Souri M, Koseki-Kuno S, Takeda N, Yamakawa M, Takeishi Y, Degen JL, Ichinose A. Male-specific cardiac pathologies in mice lacking either the A or B subunit of factor XIII. *Thromb Haemost*. 2008; **99**: 401-8.
127 Nahrendorf M, Hu K, Frantz S, Jaffer FA, Tung CH, Hiller KH, Voll S, Nordbeck P, Sosnovik D, Gattenlohner S, Novikov M, Dickneite G, Reed GL, Jakob P, Rosenzweig A, Bauer WR, Weissleder R, Ertl G. Factor XIII deficiency causes cardiac rupture, impairs wound healing, and aggravates cardiac remodeling in mice with myocardial infarction. *Circulation*. 2006; **113**: 1196-202.
128 Jaffer FA, Sosnovik DE, Nahrendorf M, Weissleder R. Molecular imaging of myocardial infarction. *J Mol Cell Cardiol*. 2006; **41**: 921-33.
129 Soendergaard C, Kvist PH, Seidelin JB, Pelzer H, Nielsen OH. Systemic and intestinal levels of factor XIII-A: the impact of inflammation on expression in macrophage subtypes. *J Gastroenterol*. 2015.

130 Raghu H, Cruz C, Rewerts CL, Frederick MD, Thornton S, Mullins ES, Schoenecker JG, Degen JL, Flick MJ. Transglutaminase factor XIII promotes arthritis through mechanisms linked to inflammation and bone erosion. *Blood*. 2015; **125**: 427-37.
131 Bungay PJ, Owen RA, Coutts IC, Griffin M. A role for transglutaminase in glucose-stimulated insulin release from the pancreatic beta-cell. *Biochem J*. 1986; **235**: 269-78.
132 Sharma PR, Mackey AJ, Dejene EA, Ramadan JW, Langefeld CD, Palmer ND, Taylor KD, Wagenknecht LE, Watanabe RM, Rich SS, Nunemaker CS. An Islet-Targeted Genome-Wide Association Scan Identifies Novel Genes Implicated in Cytokine-Mediated Islet Stress in Type 2 Diabetes. *Endocrinology*. 2015; **156**: 3147-56.
133 Naukkarinen J, Surakka I, Pietilainen KH, Rissanen A, Salomaa V, Ripatti S, Yki-Jarvinen H, van Duijn CM, Wichmann HE, Kaprio J, Taskinen MR, Peltonen L, Consortium E. Use of genome-wide expression data to mine the "Gray Zone" of GWA studies leads to novel candidate obesity genes. *PLoS genetics*. 2010; **6**: e1000976.
134 Myneni VD, Mousa A, Kaartinen MT. Factor XIII-A transglutaminase deficient mice show signs of metabolically healthy obesity on high fat diet. *Sci Rep*. 2016; **6**: 35574.
135 Invernizzi R, De Fazio P, Iannone AM, Zambelli LM, Rastaldi MP, Ippoliti G, Ascari E. Immunocytochemical detection of factor XIII A--subunit in acute leukemia. *Leuk Res*. 1992; **16**: 829-36.
136 Kiss F, Hevessy Z, Veszpremi A, Katona E, Kiss C, Vereb G, Muszbek L, Kappelmayer JN. Leukemic lymphoblasts, a novel expression site of coagulation factor XIII subunit A. *Thromb Haemost*. 2006; **96**: 176-82.
137 Raval JS, Berg AN, Djokic M, Roth CG, Rollins-Raval MA. Factor XIII Subunit A Immunohistochemical Expression is Associated With Inferior Outcomes in Acute Promyelocytic Leukemia. *Appl Immunohistochem Mol Morphol*. 2018; **26**: 202-5.
138 Karai B, Hevessy Z, Szantho E, Csathy L, Ujfalusi A, Gyurina K, Szegedi I, Kappelmayer J, Kiss C. Expression of Coagulation Factor XIII Subunit A Correlates with Outcome in Childhood Acute Lymphoblastic Leukemia. *Pathol Oncol Res*. 2018; **24**: 345-52.

Legends

Figure 1: Platelets externalise FXIII-A during activation. (A) Washed platelets (5 x 10^7/ml) were left unstimulated or activated with 20 μg/ml collagen/ 20 μM TRAP-6 and stained using FITC-labelled anti-FXIII-A antibody (green) and Alexa-fluor®647 Annexin-V to detect phosphatidylserine (red). A time course of activation is shown. Scale bar represents 10 μM. (B) Platelets were stimulated and FXIII-A and annexin detected as described in (A). Images focus on phosphatidylserine (PS)-positive and PS-negative staining. Scale bar represents 5 μM. Representative images of *n=4* separate experiments. (C) Three dimensional reconstructions of platelets stimulated as above showing PS-positive platelets (red) with FXIII-A (green) concentrated in the 'cap'. PS-negative platelets that stain only for FXIII-A can also be visualised. Images were recorded on a Zeiss LSM70 confocal microscope with 63x 1.40 oil immersion objective and analyzed using Zen 2012 software.

Figure 2: Extracellular functions of cellular FXIIII-A. FXIII-A is expressed primarily in cells of bone marrow lineage and is now appreciated to function in many extracellular processes from phagocytosis to stabilization of bone. The range of its extracellular functions intimately aligns with the expression of the FXIII-A gene (F13A1) in hematopoietic stem cells.