Review Article

New Developments in the Area of Factor XIII

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Summary

Coagulation factor XIII (FXIII) is best known for its role in fibrin stabilization and crosslinking of antifibrinolytic proteins to the fibrin clot. From patients with congenital FXIII deficiency it was known that FXIII also has important functions in wound healing and maintaning pregnancy. Over the last decade more and more research groups with different backgrounds have studied FXIII and have unveiled putative novel functions for FXIII. FXIII, with its unique role as a transglutaminase among the other serine protease coagulation factors, is now recognized as a multifunctional protein involved in regulatory mechanisms and construction and repair processes beyond hemostasis with possible implications in many areas of medicine. The aim of this review is to give an overview of exciting novel findings and to highlight the remarkable diversity of functions attributed to FXIII. Of course, more research into the underlying mechanisms and (patho-)physiological relevance of the many described functions of FXIII is needed. It will be exciting to observe future developments in this area and to see if and how these interesting findings may be translated into clinical practice in the future.

Keywords

Bone metabolism, coagulation, factor XIII, inflammation, transglutaminase, wound healing.

Introduction

For a long time, coagulation factor XIII (FXIII) has been known as a neglected coagulation factor at the „less interesting“ end of the clotting cascade beyond the diagnostically more relevant thrombin generation and fibrin polymerization. FXIII research underwent a revival when a role in cardio- and cerebrovascular diseases was first suggested [1-4]. Since then more and more research groups with different backgrounds have studied FXIII and thanks to their great work over the last decade, FXIII is now recognised as a multifunctional protein which is involved in many regulatory and construction and repair processes with possible implications in many areas of medicine. In this article we aim to review many exciting new functions of a protein belonging to the family of transglutaminases, which exhibit fundamental biological reactions in most organisms and are therefore thought to have appeared early in the evolutionary history [5].

Plasma FXIII (pFXIII) is a heterotetramer of two A and two B subunits (FXIII-A_2B_2). The A subunit (FXIII-A) contains the catalytic domain, the B subunit (FXIII-B) serves as carrier and regulatory protein. Figure 1 [6] shows the structure of the FXIII-A_2 homodimer. In pFXIII, the B subunits are thought to be wrapped around FXIII-A_2. pFXIII circulates in plasma at an average concentration of 21.6 μg/ml [8] and is noncovalently bound to fibrinogen. In plasma, all FXIII-A exists in complexed form, whereas there are free FXIII-B_2 homodimers present. In platelets and monocytes/macrophages, cellular FXIII (cFXIII) is present as FXIII-A_2. In plasma, thrombin initiates the physiological conversion of the zymogen into the active enzyme by cleavage of the activation peptide (AP-FXIII), consisting of amino acids 1-37, of FXIII-A resulting in FXIII-A_2'B_2. This reaction is greatly enhanced by polymerized fibrin. The dissociation of the A' and B subunits is induced by conformational changes due to binding of Ca^{2+} and is again enhanced by fibrin. The free thiol group of the active site Cys314 is now exposed for the transglutaminase reaction to form a covalent bond between a peptide-bound glutamine residue and a peptide-bound lysine residue. Figure 2 schematically shows the activation and action of pFXIII [9]. The best known function of pFXIII is clot stabilization

during the hemostatic process: FXIII provides a mechanically stronger clot by crosslinking fibrin chains [10], and by incorporating antifibrinolytic proteins FXIII prevents the clot from premature degradation by the fibrinolytic system [11]. Many other established and emerging functions of FXIII and underlying mechanisms are discussed below and summarized in Figures 3 and 4. An excellent review article by Muszbek et al. [12] gives a very detailed description of FXIII biochemistry and functions.

FXIII deficiency

Since the description of the first case of congenital FXIII deficiency in 1960 until the 1990s, congenital FXIII deficiency was the main area of research on FXIII. With more than 100 mutations so far described, occuring in all exons of the FXIII-A gene [13], we have learned that almost every affected family have their individual mutation. Contrary to hemophilia A, mutations in the FXIII-A gene usually lead to complete absence of FXIII-A protein, preventing clear genotype-phenotype correlations. However, the very first functional mutation was recently discovered at position 37, the thrombin cleavage site, leading to expression of a FXIII-A protein that cannot be cleaved by thrombin [14]. Despite of raised awareness and availability of specific and sensitive assays, diagnosis of FXIII deficiency is still often insufficient and the use of inappropriate assays may lead to missed diagnoses with fatal consequences even in developed countries [15]. Diagnosis should therefore follow the guidelines of the FXIII and Fibrinogen SSC Subcommittee of the ISTH [16]. Treatment of FXIII-A deficiency is undergoing important changes. The first recombinant product has proven to be safe and effective and will be available soon [17,18]. Although the old-fashioned dogma that 5% of FXIII plasma levels is enough for efficient hemostasis is unfortunately still believed by some clinicians, there is increasing evidence that patients with „mild“ FXIII deficiency due to congenital heterozygous deficiency, acquired deficiency due to consumption, or acquired deficiency due to autoantibodies experience bleeding complications in situations such as trauma or surgery [13,19-21]. Data are collected from

such cases in ongoing studies with the aim to improve diagnosis, treatment and ultimately the outcome in those patients.

FXIII and pregnancy

Women with congenital FXIII deficiency usually suffer pregnancy loss within the first trimester, if not treated with prophylactic FXIII substitution therapy [22,23]. In these patients, poor development of the cytotrophoblastic shell and fibrinoid layers, due to impaired protein crosslinking, at the interface of maternal and fetal tissue leads to premature detachment of the placenta [24]. Therefore, FXIII has an important function in maintaining pregnancy. However, it remained unknown whether FXIII plasma levels are altered in FXIII-competent women with unexplained recurrent pregnancy loss. In a first study in women with recurrent pregnancy loss, low FXIII levels did not predict subsequent miscarriages [25]. This study, however, did not compare absolute FXIII levels between patients with and without subsequent miscarriage, nor were FXIII levels compared to a control group of women without history of recurrent miscarriage. We therefore measured FXIII-A and FXIII-B levels in women with two or more unexplained consecutive miscarriages and women without history of miscarriage and at least one successful pregnancy [26]. Since FXIII levels did not differ between these groups, we conclude that recurrent pregnancy loss in the general population is not associated with reduced FXIII plasma levels. Whether locally reduced FXIII-A levels or impaired FXIII function in the placenta may contribute to an increased risk of abortion, remains to be investigated.

FXIII in wound healing, angiogenesis and atherosclerosis

Role of FXIII in wound healing after myocardial infarction and atherosclerosis

An interesting observation was made about the role of FXIII in myocardial wound healing after myocardial infarction (MI). In a murine model of MI, transglutaminase activity during acute infarction predicted healing outcome and left ventricular remodelling [27]. FXIII treatment induced a faster resolution of the neutrophil response, enhanced macrophage recruitment, increased collagen content and augmented angiogenesis in the healing infarct. The study also showed decreased FXIII tissue levels in patients with insufficient healing after MI. A small clinical study by the same authors confirmed their experimental study: in three consecutive patients presenting with acute myocardial rupture following MI, FXIII levels were consistently reduced to 50% [28]. To determine whether a truly causative relationship existed between FXIII activity and myocardial healing, myocardial repair after left coronary artery ligation was studied in FXIII-deficient mice. The results showed that mice lacking FXIII suffered from impaired wound healing and fatal rupture of the left ventricle after MI [29].

Transglutaminases in general play an important role in cardiovascular disease by numerous ways [30]. FXIII may act as signal transduction protein and maintains endothelial barrier function by modifying paracellular transport in endothelial cell monolayers [31]. Therefore FXIII administration may prevent capillary leakage syndrome in certain clinical scenarios [32].

Direct evidence for a role for FXIII in atherosclerosis comes from findings that activated FXIII (FXIIIa) crosslinks angiotensin-1 (AT1) receptor dimers of monocytes at the onset of atherosclerosis. Expression of a FXIIIa-inhibiting peptide reduced AT1-stimulated monocyte activation and monocyte entry into the artery wall and inhibited the development of atherosclerosis in hypercholesterolemic Apo E−/− mice [33]. In general, atherosclerosis is a disease in which inflammation plays a significant role and the modulation of inflammatory processes by transglutaminases may be a new approach to further investigate the role of FXIII and other transglutaminases in cardiovascular disease.

Proangiogenic properties of FXIII

Proangiogenic properties of FXIII have been discovered recently showing another until then unknown specific role of this unique coagulation factor. FXIII exerts a direct proangiogenic effect on endothelial cells *in vitro* and promotes angiogenesis in several *in vivo* animal models. Dardik et al. showed for the first time that FXIIIa increased endothelial cell migration and proliferation and inhibited apoptosis [34]. The observed proangiogenic effects of FXIII were dependent on its transglutaminase activity since the proangiogenic capacity of FXIIIa was completely abolished by blockade of its active site. The proangiogenic effect of FXIIIa on endothelial cells was accompanied by downregulation of the anti-angiogenic factor thrombospondin-1 (TSP-1) [34,35]. TSP-1, an extracellular matrix protein, acts as a modulator of various cell processes such as migration, adhesion, proliferation, but also apoptosis [36]. In a rabbit cornea model, FXIIIa enhanced neovascularization which was associated with an almost complete loss of TSP-1 [34].

Substantial *in vivo* evidence for the proangiogenic activity of FXIII was given by two murine models [37]: In a neonatal cardiac allograft transplant model, the number of new vessels was higher in FXIII-injected animals than in controls. In a Matrigel plug model, FXIII-deficient mice showed lower number of new vessels compared with control mice. Furthermore the number of vessels almost reached normal levels following administration of FXIII. Using a different animal model Kilian et al. [38] confirmed that FXIII stimulated neovascularization in bone defects filled with hydroxyapatite paste.

The molecular mechanisms underlying the proangiogenic effects of FXIII are complex. Binding of FXIII-A to endothelial cells requires integrins [39] and FXIIIa induces up-regulation of several transcription factors affecting cell proliferation and differentiation, vasculogenesis and angiogenesis [40]. Dardik et al. [41] showed that the proangiogenic effect of FXIIIa is mediated by 1) enhancement of crosslinked and noncovalent αvβ3/VEGFR-2 complex formation (αvβ3: integrins involved in angiogenesis and vasculogenesis; VEGFR-2: vascular endothelial growth factor receptor 2); 2) tyrosine phosphorylation and activation of VEGFR-2; 3) upregulation of transcription factors c-Jun and Egr-1; and 4) downregulation of TSP-1

induced indirectly by c-Jun through WT-1 (Wilm's tumor-1). These findings shed light on the mechanisms by which FXIII is involved in angiogenesis and tissue repair.

The role of FXIII in bone metabolism and bone disease

Transglutaminases, bone metabolism and extracellular matrix stabilization

Bone represents a dynamic tissue that is under constant remodelling throughout life [42]. The two major cells involved in these processes are osteoclasts that resorb bone and osteoblasts that form new bone tissue. Extracellular matrix (ECM) represents the biological substratum that supports these cells by fascilitating cell attachment, cell differentation, but also regulates bone mineralization. Secretion and assembly of bone ECM is conducted by osteoblasts and is regulated by cytokines, hormones and by their ionic microenvironment and the ECM itself. Stimulation of osteoblasts leads to ECM production and finally matrix mineralization [43]. Fully differentiated osteoblasts deposit bone matrix of which approximately 90% is collagen type-1. The remaining part is composed of proteoglycans and various proteins. Disturbed osteoblast activity contributes to defective bone deposition.

Transglutaminases are well known to be involved in ECM stabilization in different tissues. From the transglutaminase family only transglutaminase 2 (TG2) and FXIII are involved in cartilage and endochondral ossification [44-46]. It is interesting to note that TG2-knockout mice have no overt skeletal phenotype, suggesting that besides TG2 another transglutaminase with at least partially overlapping functions must be involved [47]. Nakano et al. confirmed by immunohistochemistry, *in situ* hybridization, and biochemical methods that FXIII-A was expressed *in vivo* by osteoblasts and osteocytes in bones formed by both intramembranous and endochondral ossification [48]. FXIII-A was present in bone tissue and in osteoblast cultures mostly as a small 37-kDa form, presumably resulting from posttranslational proteolytic processing of the parent enzyme. This 37-kDa form of FXIII-A

was found to be associated with the osteoblast plasma membrane as part of the osteoblast differentiation process.

Al-Jallad et al. presented new functions of FXIII in osteoblast matrix secretion and deposition [49]. They showed that FXIII-A and its crosslinking activity were colocalized with plasma membrane-associated tubulin. Thus FXIII-A crosslinking activity appeared to be directed towards stabilizing the interaction of microtubules with the plasma membrane. These results provide strong evidence how transglutaminase activity could affect protein secretion and matrix deposition in osteoblasts and suggest a novel function for plasma membrane FXIII-A in microtubule dynamics. How FXIII-A activation occurs remains elusive; possible candidates include membrane-bound proteases matrix metalloproteinase-2 (MMP-2) and PHEX (phosphate-regulating gene with homology to endopeptidases on the X-chromosome) [50]. Newer results from the same group [51] showed that osteoblasts secreted a latent, inactive dimeric ECM form of FXIII-A (ecmFXIII-A) which was activated upon binding to the matrix by a so far unknown mechanism. Cross-linking activity was detected at sites where fibronectin colocalized with collagen type-1, indicating that ecmFXIII-A secretion could function to stabilize newly deposited matrix. Thus, FXIII-A may be an integral part of the collagen type-1 deposition machinery and of the ECM-feedback loop, both of which regulate matrix deposition and osteoblast differentiation.

In summary, these data show another important function of FXIII besides its role in coagulation. In bone metabolism, FXIII seems to play a synergistic role to TG2 in ECM deposition and osteoblast differentiation.

The role of FXIII in bone disease

Osteoarthritis is a common cause of disability in the elderly that is characterized by cartilage degradation, synovium and tendon inflammation, osteophyte formation accompanied by subchondral bone remodeling by osteoid substance accumulation, and decreased

mineralization. Sanchez et al. [52] investigated gene expression in human osteoblasts isolated from sclerotic or nonsclerotic areas of subchondral bone. FXIII-A expression was significantly up-regulated in sclerotic osteoblasts compared with nonsclerotic osteoblasts confirming a role of FXIII in bone remodelling [52].

Significance of FXIII antigen levels in severe trauma and surgery

Hemorrhagic shock represents a dangerous complication of severe trauma and is associated with high mortality and morbidity due to inadequate capillary perfusion in vital organs and tissues. The condition is called trauma-hemorrhagic shock (THS) which leads to a reduction of circulatory blood volume. This results in insufficient organ microcirculation, tissue hypoxia and finally organ damage. Most THS patients develop severe coagulopathy due to loss of coagulation and fibrinolytic proteins by bleeding and/or consumption [53,54]. Loss of these proteins cause prolongation of routine coagulation tests such as activated partial thromboplastin time (APTT) and prothrombin time (PT). Thrombelastography (TEG) and rotation thrombelastometry (ROTEM) are ideal bedside tests to provide fast and reliably information on the existance or development of coagulopathies [55-57]. Immediate identification of patients at risk is of critical importance in order to goal-direct transfusion therapy with specific coagulation proteins, platelets and/or antifibrinolytic agents.

FXIII has a significant impact on thrombelastographic parameters suggesting a major role of FXIII in patients with these conditions [58]. However, its benefit in trauma as well as surgical patients is still under debate, especially the question what level of FXIII antigen is required to maintain hemostasis. Clinical studies in surgical patients suggested an increased bleeding tendency at FXIII activity levels below 60% [59-62].

Still, there is insufficient evidence to judge the extent of blood loss or acute coagulopathy that lead to a critical decrease in FXIII antigen levels which could be potentially dangerous during and after a surgical procedure. However, it is well known that trauma-induced shock and

coagulopathy lead to disseminated intravascular coagulation including significant consumption of FXIII [63]. A recent animal study investigated the role of FXIII in shock-induced organ dysfunction: rats were subjected to THS or trauma sham shock and were treated with either recombinant cellular FXIII-A_2 (rcFXIII) or placebo. Administration of rcFXIII diminished THS-induced multiple organ dysfunction, presumably by preservation of the gut barrier function, limitation of polymorphonuclear leukocyte activation, and modulation of the cytokine response [64].

FXIII as part of the insulin resistance syndrome

Insulin resistance represents a common metabolic abnormality increasing the risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease, the major cause of morbidity and mortality in most parts of the world. Insulin resistance is not simply a problem of deficient glucose uptake in response to insulin but represents a multifaceted syndrome called insulin resistance syndrome which is associated with atheromatous risk factor such as dyslipidemia, hyperinsulinemia, obesity, and hypertension, affecting around 25% of a Western population [65,66]. It became more and more evident that not only the clustering of atheromatous risk factors belongs to the syndrome but also atherothrombotic risk factors such as increased plasma levels and/or certain genetic variants of fibrinogen, FVII and most notably plasminogen activator inhibitor-1 (PAI-1) [67]. In addition there is evidence that also FXIII levels cluster with these risk factors contributing to the prothrombotic state which may in turn enhance cardiovascular risk. FXIII-A and FXIII-B antigen levels are elevated in patients with T2DM and FXIII-A antigen levels are increased in relatives of subjects with T2DM [68]. The specific role and the underlying mechanisms of FXIII in this complex syndrome needs further investigation. Since inflammation is another feature of the insulin resistance syndrome and FXIII is also involved in inflammatory processes as outlined below, these may represent a link between FXIII and the insulin resistance syndrome.

FXIII and immune defence and inflammation

The simultaneous activation of coagulation and inflammatory processes after injury is a phylogenetically ancient adaptive response that can be traced back to early eukaryotic evolution [69]. The aim of co-activation and interactions between coagulation and inflammatory processes is to protect the host from blood loss and infection. FXIII plays a role in infection control and interacts with complement factors and inflammatory cells [recently reviewed by 70].

Induction of coagulation leads to immobilization and killing of bacteria inside the clot. This entrapment is mediated via crosslinking of bacteria to fibrin fibers by FXIIIa. FXIII knock-out mice developed severe signs of inflammation at the site of infection, whereas FXIII treatment of wild-type animals reduced bacterial dissemination during early infection [71]. In sepsis, FXIII protected mucosal capillary perfusion against endotoxin-induced impairment in a rat model [72]. Administration of FXIII also reduced hemorrhagic shock-induced organ dysfunction in rats by preserving lung and gut endothelial barrier function and limiting leukocyte activation [64]. While activation of FXIII is beneficial in fighting infection and improving endothelial barrier function (first shown by Noll et al. [31]), it also has negative effects in sepsis by increasing the risk of intravascular thrombosis, as depletion of FXIII was shown to prevent disseminated intravascular coagulation-induced organ damage in rabbits [73].

The complement system is an important part of innate immunity and FXIII interacts with proteins of the complement system. Its central component complement C3 is incorporated into fibrin clots and prolongs fibrinolysis [74,75]. Incorporation occurs by noncovalent binding to fibrinogen/fibrin and covalent crosslinking by FXIIIa. Thus, complement C3 is a novel substrate for FXIIIa [76,77]. Mannan-binding lectin-associated serine protease-1 (MASP-1) of the complement lectin pathway has a similar substrate specificity to thrombin, and we and

others have shown that MASP-1 also activates FXIII [78]. These interactions may contribute to the prothrombotic state accompanying many inflammatory diseases.

FXIII also interacts with cells of the immune system (recently reviewed by Bagoly et al. [79]. Interactions include activation of FXIII by human neutrophil elastase, downregulation of FXIIIa within the clot by granulocyte proteases, and enhancing effects of FXIII on monocyte proliferation and migration and inhibition of monocyte apoptosis [79]. Monocytes/macrophages have been discussed as a source of FXIII-A in plasma, and despite of some evidence for a non-classical secretion pathway of cFXIII from these cells [79,80] the origin of plasma FXIII-A is not yet proven. An association between FXIIIVal34Leu polymorphism and monocyte and neutrophil cell counts following LPS infusion in humans has been suggested [81], however, due to the small sample size in this study larger studies are needed to confirm this finding and investigate possible underlying mechanisms.

Inflammatory bowel diseases (IBD) have long been associated with decreased FXIII levels. A recent study in Crohn's disease has shown, however, that FXIII levels can not be recommended as a marker for disease activity [82]. The mechanisms leading to decreased FXIII levels in IBD are controversially discussed. While one study suggested FXIII consumption due to coagulation activation based on findings of elevated D-dimer and prothrombin fragment 1+2 in patients with active ulcerative colitis or Crohn's disease [83], another study did not find increased thrombin-antithrombin complex levels in patients with active Crohn's disease and suggested that FXIII was not consumed due to coagulation activation but due to repair of injured tissue [84]. This was supported by a histological study which detected tissue transglutaminase and FXIII-A in damaged areas of the colon underpinning the important role of transglutaminases in mucosal healing [85]. However, it is not yet clear whether patients with IBD benefit from administration of pFXIII as clinical studies have yielded contradictory results [86,87]. Severe graft-versus-host disease (GvHD) of the gut is a relatively frequent complication of haematopoietic stem cell transplantation and manifests with similar symptoms as IBD. There is also a significant decrease in FXIII plasma

levels in patients with GvHD [88] and these patients may benefit from FXIII replacement therapy [89].

FXIII in neoplasm

In regard to neoplasm FXIII has come to attention 1) as a marker for certain types of leukemia and carcinoma and 2) with decreased plasma levels due to coagulation activation and consumption.

The cellular form of FXIII (FXIII-A_2) is present in platelets, megakaryocytes, monocytes and macrophages and thus has been detected in mono- and megakaryocytic leukemias [90]. In patients with acute myeloblastic leukemia (AML) M4 and M5, FXIII-A was a sensitive marker for blast cells [90,91] in which expression levels were markedly increased compared to normal cells. Recently, FXIII-A has also been detected as a marker in acute promyelocytic leukemia (APL) M3 [92]. Surprisingly, FXIII-A expression was found in 19 out of 47 cases of newly diagnosed B cell acute lymphoblastic leukemia (ALL) [93]. Expression of FXIII-A can be considered as leukemia-associated immunophenotype which may be of value for diagnosis and disease monitoring [90,92].

Leukemia is also associated with consumption of pFXIII. In a child presented in a case report, FXIII plasma levels of only 56% and increased D-dimer levels preceded diagnosis of ALL by 6 weeks and FXIII levels normalized when the child was in remission [94]. In a young woman, retro-bulbar hematoma associated with FXIII-A antigen levels as low as 7.6% preceded diagnosis of APL by 2.5 weeks [95].

FXIII has also been studied in regard to solid tumors. FXIII is related to certain types of neoplasms of the skin. In normal skin, FXIII-A is expressed in specific dermal dendrocytes (DD) derived from the monocyte/macrophage lineage or from mesenchymal origin [96]. In tumor pathology, expression of FXIII-A is used e.g. to distinguish between dermatofibroma and dermatofibrosarcoma protuberans [97,98]. In addition, FXIII-A+ DD are found in

fibrovascular lesions including fibrous papules of the nose, acquired digital fibrokeratomas, angiofibromas, oral fibromas [99] or desmoplastic neoplasms where FXIII-A is possibly acting as a growth factor. FXIII-A+ DD may also be involved in the progression and regression of some malignancies including cutaneous melanoma and basal cell carcinoma [96]. A study in 130 patients with oral squamous cell carcinoma and 135 healthy controls suggested that the Leu allele of the FXIIIVal34Leu polymorphism was associated with increased risk for this type of cancer. As a possible mechanism it was poposed that a less porous fibrin network composed of thinner fibers may facilitate tumor stroma formation and tumor cell proliferation [100]. In 110 patients with breast cancer, significantly lower expression levels of FXIII were found in tumor tissues compared with normal mammary tissues (n=27) [101].

FXIII may also play a role in metastasis. Wild-type and FXIII-deficient mice were injected with Lewis lung carcinoma and B16–BL6 melanoma cells. Metastatic potential was significantly diminished in FXIII-A deficient mice relative to control animals. FXIII was shown to support metastasis primarily by limiting natural killer cell-mediated clearance of micrometastatic tumor cells [102]. Human data, however, are lacking so far.

Novel functions of FXIII

FXIII in tears

FXIII-A and FXIII-B subunits and FXIII tetramer have been detected in human tears at low concentration [103,104]. The source of FXIII in tears remains unknown, but possible sources include leakage from plasma or production in conjunctival macrophages or corneal epithelial cells. Since most of FXIII in tears exists as FXIII-A_2, non-proteolytic activation in presence of Ca^{2+} is likely to occur. Alternatively, proteolytic cleavage by thrombin that has leaked from plasma or by granulocyte elastase is possible. In patients undergoing corneal transplantation, FXIII concentrations (normalised for protein concentration) increased up to 25 fold on the first postoperative day, followed by a gradual decrease over the next seven

days. Patients who later developed the complication of neovascularisation of the donor cornea showed the highest FXIII levels. It was suggested that FXIII in tears may be involved in corneal wound healing, whereas high FXIII levels may represent a risk factor for neovascularisation by promoting angiogenesis [104].

Optic nerve regeneration

A novel function for cellular FXIII-A in neuronal regeneration has been proposed in fish [105]. In fish, unlike in mammals, neurons of the central nervous system are capable of self-repair and regeneration, and research goes on to identifiy factors inducing/involved in repair processes. Upon optic nerve injury in goldfish, *in situ* FXIII activity increased accompanied by expression of FXIII-A mRNA. The cells producing FXIII-A were identified as astrocytes/microglial cells in the optic nerve. In retinal cell culture, overexpression of FXIII-A promoted neurite sprouting and elongation [105]. Further studies are needed to elucidate the underlying mechanisms. Whether this may have future implications in human medicine is so far unknown.

Liver remodeling

In a murine model of acute liver injury, FXIII-A deficiency led to increased hepatocyte apoptosis and delay in hepatocyte regeneration. It was concluded that the effects of FXIIIa on ECM protein crosslinking and matrix formation could promote survival of hepatocytes in liver remodeling [106].

FXIII-B subunit is not only a carrier for the FXIII-A subunit

The gene encoding FXIII-B is located on chromosome 1 and belongs to the regulator of complement activation (RCA) gene cluster [107]. FXIII-B with its 10 short consensus repeats, also called Sushi domains, resembles other binding and regulatory proteins such as complement factor H or C4b-binding protein. Furthermore, FXIII-B plasma concentration is twice as high as FXIII-A concentration, i.e. 50% of FXIII-B is free and in excess over FXIII-A. Therefore it would be plausible if the FXIII-B subunit had other functions in addition to its carrier function for FXIII-A. One novel function has been recently described by the group of L. Muszbek [108]. They have shown that FXIII-B binds Staphylococcus aureus protein A (SpA) with high affinity. SpA on the bacterial surface binds human IgG in incorrect orientation preventing recognition and phagocytosis by macrophages. FXIII-B saturates SpA and inhibits incorrect binding of IgG and may thus promote opsonization and subsequent phagocytosis of the bacteria. This may represent a novel role for FXIII-B in immune defence.

AP-FXIII as marker of thrombosis and regulator of coagulation

It has long been known that thrombin initiates the physiological conversion of FXIII-A_2B_2 zymogen into the active enzyme by cleavage of the N-terminal activation peptide (AP-FXIII). Until recently, however, it was unclear whether the AP-FXIII is indeed released into plasma under physiological conditions. Neither had it been explored whether free AP-FXIII, in case it was indeed released into circulation, might have any physiological functions.

We therefore developed an ELISA method with two sensitive and specific monoclonal antibodies against free AP-FXIII and we showed that AP-FXIII is released into plasma upon FXIII activation [6,109]. We then performed a pilot study to provide proof-of-principle, that *in vivo* generated AP-FXIII can be detected in patients with an acute thrombotic event [110]: we investigated FXIII activation in the early phase of acute ischaemic stroke by repeated measurements of free AP-FXIII, FXIII-A and FXIII-B subunit antigen levels in plasma samples from patients within 48 hours of acute ischaemic stroke. Free AP-FXIII could be

detected in 34 out of 66 patients upon hospital admission (range 0.2-26.3 ng/ml), on day 1 in 15 patients (0.2-10.4 ng/ml), and on day 2 in 11 patients (0.1-15.1 ng/ml). AP-FXIII was higher in patients with severe stroke. Lower AP-FXIII levels upon admission were associated with clinical improvement. Larger studies are needed to assess whether AP-FXIII might serve as a diagnostic and/or prognostic marker for acute thrombotic diseases.

We are currently investigating whether free AP-FXIII may affect FXIII function and fibrin formation and structure. Preliminary results show that free AP-FXIII, but not a scrambled peptide of the same amino acid composition but in random order, reduces thrombin-induced FXIII activation and affects fibrin clot structure, suggesting that free AP-FXIII may interact with thrombin and compete with the thrombin substrates FXIII and fibrinogen [111; and unpublished own data]. Whether free AP-FXIII may act as a negative feedback regulator of thrombin-induced clot formation remains to be confirmed.

Concluding remarks

The aim of this review was to highlight the remarkable diversity of functions attributed to FXIII. This diversity may partly originate from its enzymatic characteristics as a transglutaminase, since transglutaminase reactions represent important posttranslational modifications by covalently crosslinking proteins which can change their properties and biological effects. This makes FXIII uniques among the hemostatic proteins which are mainly protein-cleaving serine proteases. Indeed, there is increasing evidence that FXIII actually has more functions beyond than within hemostasis. Therefore and as the only transglutaminase circulating in plasma, the name „plasma transglutaminase“ may be more appropriate than „coagulation FXIII“.

Having discussed FXIII as a protein with so many important functions in different processes beyond hemostasis, it may seem at odds that FXIII deficiency does exist and hence is compatible with life. One conclusion may be that FXIII has a rather modulating than exclusive

role and that FXIII and other transglutaminases may have evolved as redundant systems supporting each other in fulfilling certain tasks. The specific contributions and interrelations of the different transglutaminases especially on cellular level are still not well known. In regard to its function in blood coagulation, however, there is no physiological replacement or compensation for FXIII, and the severity of congenital FXIII deficiency is highlighted by two facts: 1) Without medical treatment, most of the affected individuals die at young age. An extensive Swiss family pedigree going back to the 17th century [9,112] illustrated the devastating effect FXIII deficiency had on that family over the years. 2) Normal pregnancy is unlikely in affected women which possibly makes FXIII deficiency more difficult to pass on than other hereditary diseases.

Clearly, more research into the underlying mechanisms and (patho-) physiological relevance of the many described functions of FXIII is needed. It will be exciting to observe future developments in this area and to see if and how these interesting findings may be translated into clinical practice in the future.

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Figure legends

Figure 1. Structure of the FXIII A_2 homodimer. (Reproduced from [6] with permission). One monomer is shown in ribbons with the activation peptide colored in pink and the N-terminus indicated as a ball. The β-sandwich domain is colored in blue, the catalytic core domain in green, and the barrel 1 and barrel 2 domains in orange and red, respectively. The other monomer is represented as surface. The coordinates from the protein data base (PDB) originate from the crystal structure [7].

Figure 2. Activation and action of plasma FXIII. Thrombin initiates FXIII activation by cleavage of the FXIII activation peptide. Then A- and B-subunits dissociate in the presence of Ca^{2+}. Both FXIII activation steps are enhanced by fibrinogen/fibrin. Thrombin also initiates conversion of fibrinogen into soluble fibrin by cleaving off fibrinopeptides A and B. Activated FXIII (FXIIIa) crosslinks lysine (Lys) and glutamine (Gln) residues of fibrin α- and γ-chains in a transglutaminase reaction leading to a three-dimensional, insoluble fibrin network.

Figure 3. Diversity of FXIII functions. As a result of its multiple functions, FXIII is involved in many different physiological and pathophysiological processes and hence FXIII is of interest in different areas of biology and medicine.

Figure 4. Mechanisms and substrates of FXIII. As a transglutaminase, activated FXIII crosslinks many different proteins of the coagulation cascade, extracellular matrix, complement system and intracellular proteins. FXIII also interacts – directly or indirectly – with various cell types. (TAFI: thrombin-activatable fibrinolysis inhibitor; PAI-2: plasminogen-activator inhibitor-2; ECM: extracellular matrix; VEGFR: vascular endothelial growth factor receptor; TSP-1: thrombospondin-1; AT1: angiotensin-1; PMN: polymorphonuclear; MASP-1: mannan-binding lectin-associated serine protease-1.)

Figure 1

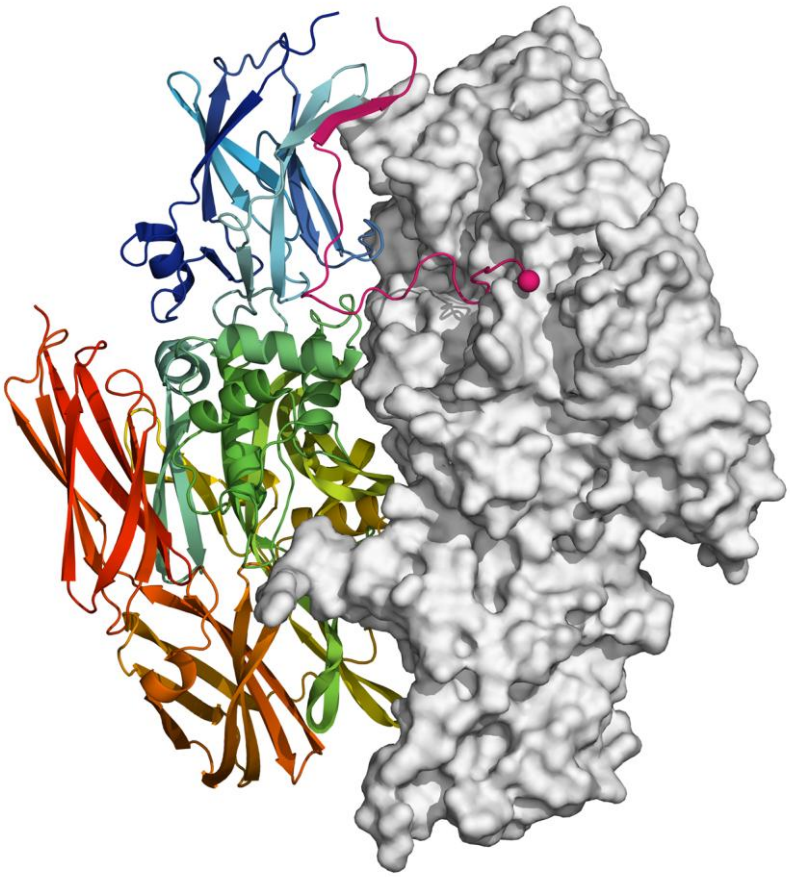


Figure 2

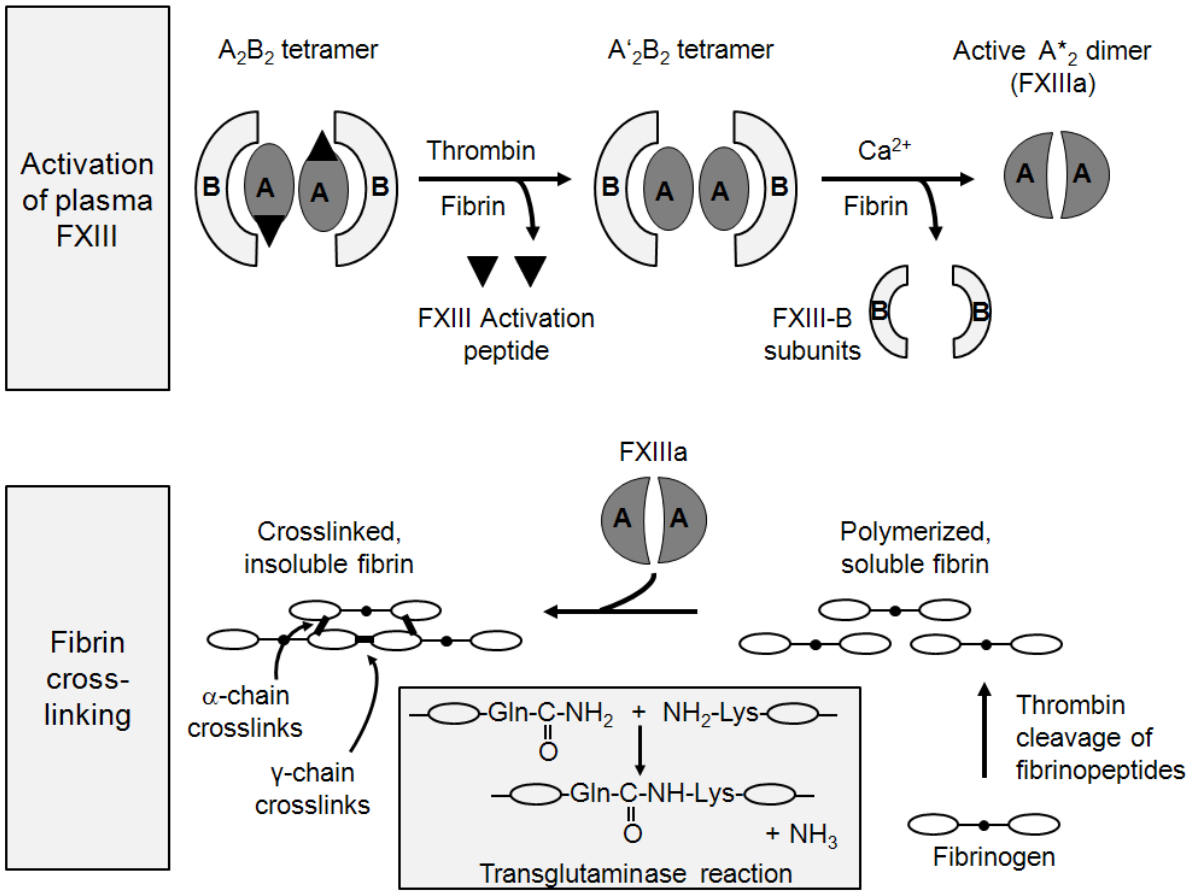

Activation of plasma FXIII
A2B2 tetramer
A'2B2 tetramer
Active A*2 dimer (FXIIIa)
B
A
A
B
Thrombin
Fibrin
FXIII Activation peptide
Ca2+
Fibrin
FXIII-B subunits
Fibrin cross-linking
FXIIIa
Crosslinked, insoluble fibrin
Polymerized, soluble fibrin
α-chain crosslinks
γ-chain crosslinks
Gln-C-NH2 + NH2-Lys
Gln-C-NH-Lys
+ NH3
Transglutaminase reaction
Thrombin cleavage of fibrinopeptides
Fibrinogen

Figure 3

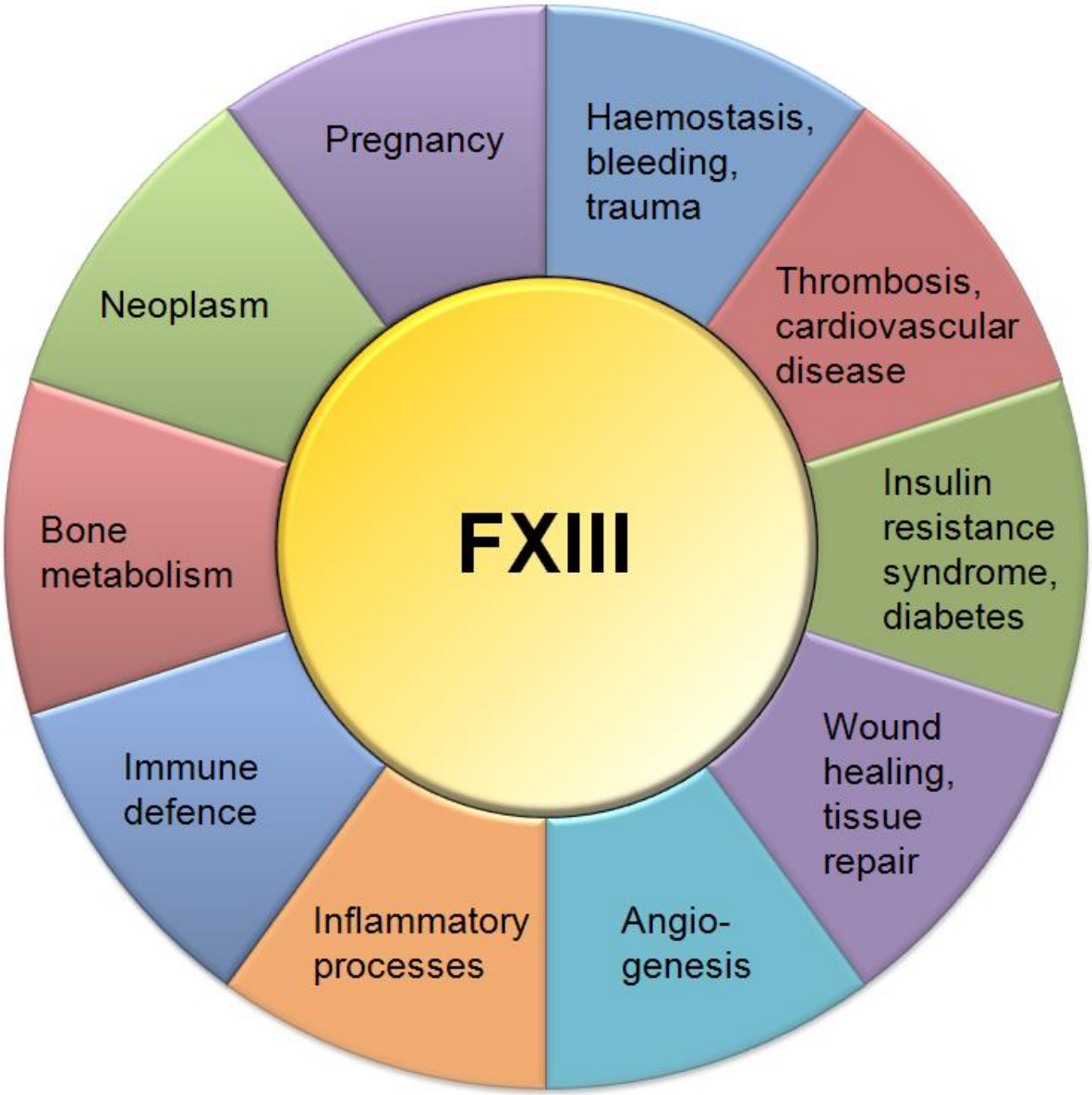
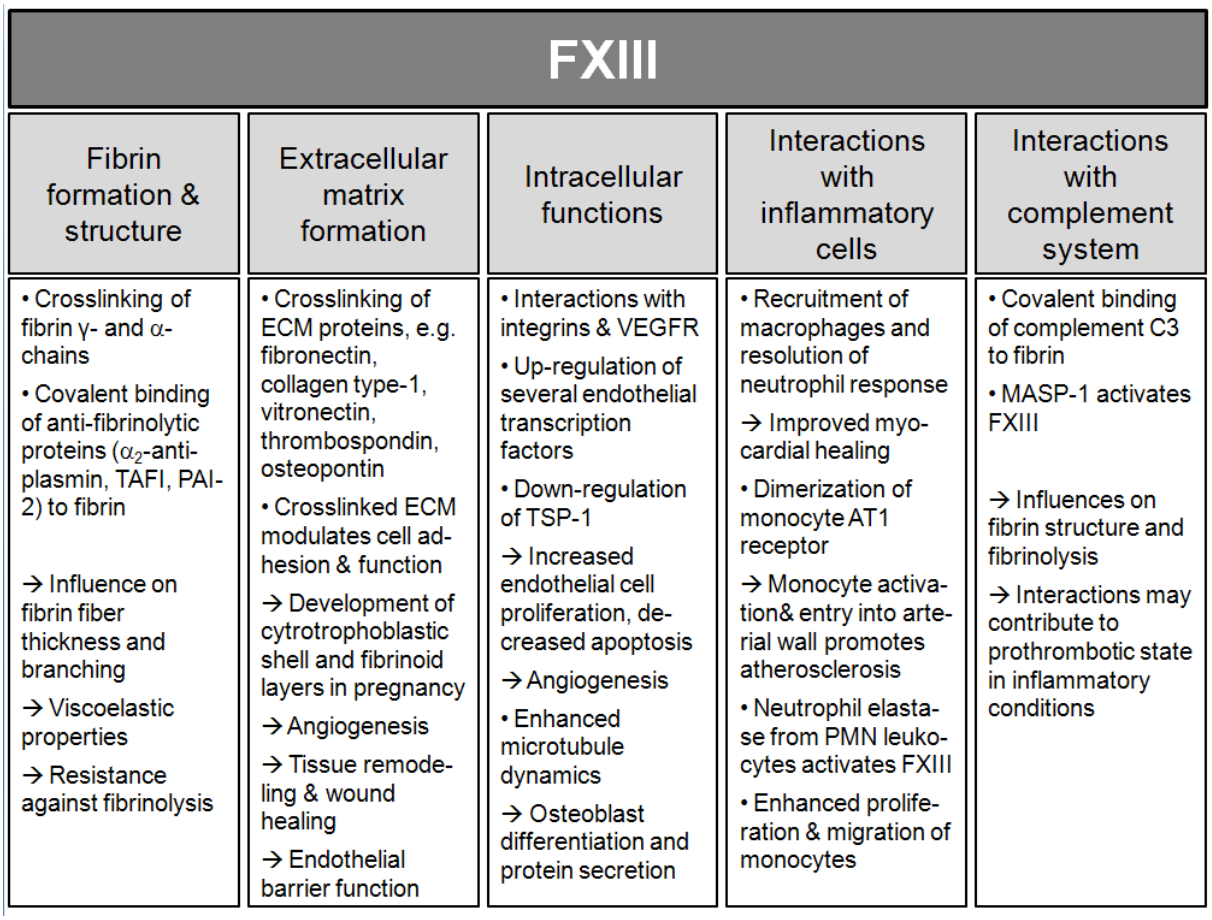

Pregnancy
Haemostasis, bleeding, trauma
Thrombosis, cardiovascular disease
Insulin resistance syndrome, diabetes
Wound healing, tissue repair
Angio-genesis
Inflammatory processes
Immune defence
Bone metabolism
Neoplasm
FXIII

Figure 4


FXIII
Fibrin formation & structure
Extracellular matrix formation
Intracellular functions
Interactions with inflammatory cells
Interactions with complement system
• Crosslinking of fibrin γ- and α-chains
• Covalent binding of anti-fibrinolytic proteins (α2-anti-plasmin, TAFI, PAI-2) to fibrin
→ Influence on fibrin fiber thickness and branching
→ Viscoelastic properties
→ Resistance against fibrinolysis
• Crosslinking of ECM proteins, e.g. fibronectin, collagen type-1, vitronectin, thrombospondin, osteopontin
• Crosslinked ECM modulates cell adhesion & function
→ Development of cytrotrophoblastic shell and fibrinoid layers in pregnancy
→ Angiogenesis
→ Tissue remodeling & wound healing
→ Endothelial barrier function
• Interactions with integrins & VEGFR
• Up-regulation of several endothelial transcription factors
• Down-regulation of TSP-1
→ Increased endothelial cell proliferation, decreased apoptosis
→ Angiogenesis
• Enhanced microtubule dynamics
→ Osteoblast differentiation and protein secretion
• Recruitment of macrophages and resolution of neutrophil response
→ Improved myocardial healing
• Dimerization of monocyte AT1 receptor
→ Monocyte activation& entry into arterial wall promotes atherosclerosis
• Neutrophil elastase from PMN leukocytes activates FXIII
• Enhanced proliferation & migration of monocytes
• Covalent binding of complement C3 to fibrin
• MASP-1 activates FXIII
→ Influences on fibrin structure and fibrinolysis
→ Interactions may contribute to prothrombotic state in inflammatory conditions