

## Coagulation Factor XII –a Key Pro-inflammatory and Pro-coagulant Protein

Shan-Shan WANG<sup>1</sup>, Jin-Wen GE<sup>2, \*</sup>, Shao-Wu CHENG<sup>3</sup>

<sup>1</sup> College of Integrated Chinese and Western Medicine, Hunan University of Chinese Medicine; Changsha, Hunan 410208, China

<sup>2</sup> Department of Integrated Chinese and Western Medicine, Hunan University of Chinese Medicine; Changsha, Hunan 410208, China

<sup>3</sup> Department of Cytobiology and Molecular Biotechnology, Hunan University of Chinese Medicine, Changsha, Hunan 410208, China

\*Corresponding author

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**Abstract.** Coagulation factor XII (FXII) is a multidomain serine protease that is the starter of intrinsic coagulation pathway. FXII deficiency and clinical hemostasis are not associated with bleeding, so investigators have not considered FXII important in physiology for a long time. In seeking explanation for FXII-independent physiologic hemostasis, investigators found FXII is an essential constitute of contact system that mediates procoagulation and proinflammatory via the intrinsic coagulation pathway or the Kallikrein-kinin system, respectively. Data obtained in infectious inflammation have revealed FXII mediated clotting that limited bacterial or toxin spread in early phases, and regulated fibrinolysis in later. Moreover, FXII mediated two non-infectious inflammation Hereditary angioedema (HAE) and non-specific allergy via bradykinin (BK) formation. In the coagulation, thrombosis not only is involved with coagulation, but is redefined as thrombo-inflammatory disorder. This paper reviews in detail the progresses in roles of FXII intersection between inflammation and coagulation.

### Introduction

FXII was first identified by Oscar Ratnoff in 1955, who also diagnosed the first FXII-/- patient John Hageman the same year. Since the patient showed no bleeding phenotype, FXII was considered to be not involved in blood clot formation[1]. The idea was proved wrong by the genetically engineered FXII-/- mouse, although the role of FXII in vivo is still to be clarified, it was recognized that FXII did participate in pathological clot formation, while its role in physiological clot formation during hemostasis was not significant[2]. Further researches expanded the function of FXII, it was found that besides clot formation, FXII also activated the kallikrein kinin system (KKS) after auto-activation, which generates bradykinin and promote inflammatory response[4,5]. Studies on the multiple roles of FXII/FXIIa in plasma, especially, the differential roles under physiological and pathological conditions, have revealed the comprehensive role of FXII/FXIIa in inflammation and thrombosis.

### FXII in Inflammation.

Bradykinin (BK) is an important inflammatory cytokine in all types of inflammatory response. The classical pathway of BK generation involves three proteins: FXII,

prekallikerein (PK) and high-molecular kininogen (HK) [5]. FXII is a  $\beta$ -globin widely spreading in the circulation, which is auto-activated upon contact with the negatively charged surface through breakage of internal disulfide bonds to form FXIIa with a heavy chain – light chain conformation. FXIIa induces lysis and activation of PK and initiates KKS to generate  $\alpha$ -KK, which then cleaves HK to form bradykinin. Bradykinin acts on the G-protein coupled B1/2 receptor of endothelial cells and smooth muscle cells, elevating cytoplasmic  $Ca^{2+}$  and leading to vasodilation, increased vascular permeability, and leukocyte chemotaxis [3]. So, as an important participant in bradykinin generation, FXII regulates various inflammation response through the above-mentioned cascade.

### **FXII in Infectious Inflammation**

In the host defense inflammatory response against infections, FXII may be activated to initiate the intrinsic coagulation cascade to limit tissue damage, and to facilitate hemostasis at the site of infection or injury. FXII causes aggregation of fibrin that effectively blocks or delays spreading of bacteria and their toxic products. Meanwhile, FXIIa activates KKS to generate bradykinin, which increases vascular permeability and outflow of proteins from blood into tissues, aggravating the inflammatory process[6]. Studies have revealed that during systemic inflammation, interaction between neutrophils and platelets may help release extracellular nucleosomes, which acts on FXII to form fibrins and thrombosis, promoting separation of bacteria in liver blood vessels and limiting bacterial invasion [7,8]. Blocking this contact system in Leishmania infected mice using Lundep exacerbated infection, possibly because Lundep inhibited endogenous coagulation mediated by FXII, Leishmania can escape from NETs and the infection can spread[9]. This indicates an important role of FXII-mediated coagulation in early phases of disease progression. Recent studies discovered polyphosphate (PloyP), a highly linear anionic polymer, in the subcellular organelles termed acidocalcisomes, which has been proved to be a strong activator of FXII and affect fibrin structure[10,11]. This indicates that FXII, as a key protein of host defense, may also be activated by the invading pathogens themselves to induce fibrin formation and coagulation to stop pathogen spreading. Although the pro-inflammatory and pro-coagulation cascade of FXII has been intensively studied in vitro, how it functions in vivo is not so clear. Studies have shown that collagen-activated platelets release large amount of  $Zn^{2+}$ , and under high concentrations of  $Zn^{2+}$ , FXII may bind to urokinase-type plasminogen activator receptor (u-PAR), complement protein C<sub>1</sub>q receptor, (gC<sub>1</sub>qR) and corner binding protein-1 on the surface of human umbilical vein endothelial cells (HUVECs) [3,12]. During which, the 40-44 and 78-82 residues of the fibronectin Type II domain on the N-terminal of FXII bind to two  $Zn^{2+}$ , and leads to a conformational change of FXII[13,14]. This suggests that FXII can be activated following local activation of endothelial cells. This is consistent with a previous study in which FXII was activated following Hantavirus-induced hypersensitisation of endothelial cells to VEGF[15]. In another in vitro study, the inflammatory cytokine BK stimulated cultured endothelial cells to secrete plasminogen, prostacyclin, thromboxane A<sub>2</sub> and NO, thereby regulating platelet function and promote fibrinolysis[16]. This suggests that FXII also mediates fibrin degradation in the later phase of inflammation while promoting fibrin aggregation in the early phases of inflammation. How such bidirectional regulation functions and how it is regulated, however, is still to be clarified. At least, it can be inferred that absence or excessiveness of such regulation may play a role in

disseminated intravascular coagulation (DIC) after severe inflammation. In addition, such bidirectional regulation also suggests that coagulation in the early phase of severe inflammation may help prevent spreading of the pathogens and anticoagulant administration may accelerate deterioration of the disease; on the other hand, FXII also regulates fibrinolysis during recovery of inflammation, suggesting a physiological role of FXII.

### **FXII in Non-infectious Inflammation**

Hereditary angioedema (HAE) and non-specific allergy are the most typical two non-infectious inflammatory diseases mediated by FXII.

Hereditary angioedema is characterized by skin swelling, abdominal pain and severe laryngeal edema (upper airway obstruction) [17]. Previous studies have revealed that Type I and II HAE are caused by complement-inhibition genes, and the recently identified HAE III only occurs in females, often repeatedly during hormone treatment or birth-control drug administration. Unlike HAE I and II, HAE III patients have normal levels of C<sub>1</sub> esterase inhibitor (C1INH) and C<sub>4</sub> despite BK over-expression [18]. Compared to that of normal control, plasma of HAE III patients showed higher FXII activity, but the FXII pro-coagulant activity remains normal, as indicated by APTT assay, and such activity is not subject to regulation by C<sub>1</sub>INH and agents that inhibit conformational activation FXII [19], suggesting a closer link between HAE III and FXII mutation. Actually, a third of HAE III patients harbored mutations in Thr309Lys, Thr328Lys or Thr309Arg[3,20]. Currently, the reason why HAE III only occurs in females is not clear, but it has been proved that FXII is clearly regulated by estrogen, which makes an explanation.

In specific allergy, IgE antibodies specifically adhere to mast cells and basophils, and cause distortion and rupture of these cells, leading to release of heparin and substances that cause local vasodilation and increased capillary permeability[21]. It has been proved that heparin can initiate the FXII/PK activation cascade that leads to HK lysis and BK release. Studies have shown that mast cells regulate heparin-mediated and FXII-dependent plasma BK generation [22]. Consistent with in vitro data, local administration of heparin increased vascular permeability in a FXII-dependent way. BK actively mediates microvascular leakage, which was proved by in vivo confocal laser scanning microscopy and classical tracer recognition assay. Compared with normal mice, FXII<sup>-/-</sup> and B<sub>2</sub>R<sup>-/-</sup> mice were partially protected from allergen-activated, mast cell-mediated edema, with decrease in incidence[23,24], and allergen/IgE-mediated mast cell hypersensitivity was also significantly decreased in such mice, which can be reversed by infusion of human FXII[25]. These results further emphasize the key role of mast cell-released heparin in activation of the contact system. However, FXI, the substance of FXII, is not activated upon activation of the contact system PK, which is similar to activation of the contact system by misfolded protein complex in Alzheimer's patients[26,27].

### **FXII in Thrombosis-inflammation Response**

Although the mechanism of pathogenic thrombosis formation is not entirely clear, we do know that FXII mainly participates in pathogenic thrombosis and is irrelevant to physiological hemostasis. During inflammatory responses, activation FXII is often accompanied by thrombosis formation, so it was suspected that inflammation plays an indispensable role in FXII-mediated thrombosis formation. Current studies confirmed this hypothesis that symptoms of inflammation, such as up-regulation of adhesion

molecules and cytokines, and infiltration of lymphocytes, polymorphonuclear leukocytes, monocytes, and macrophages, are found in the mouse model of cerebral ischemia[28]. Furthermore, lack of T cells protects mice from acute stroke. In deep vein thrombosis, neutropenia, genetic deficiency of FXII, or NETs disintegration each confers protection against DVT amplification[29]. These results suggest that thrombosis is not only the results of dysregulation of the coagulation cascade, but is also caused by dysregulation of the coagulation-inflammation cascade, in which FXII plays a central role. This provides a new direction for studies on the mechanisms of thrombotic diseases. Over the years, the number of drugs for prevention and treatment of thrombosis is very limited, and all these drugs are correlated with severe, sometimes lethal bleedings, since all these drugs target the key proteins (e.g. FX, thrombin) of the coagulation cascade[30]. On the contrary, FXII is not only specific to pathological thrombosis, but also regulates excess inflammatory response, providing a new target for prevention and treatment of ischemic stroke. 3F7, 9A2, and 15H8 are representatives of highly specific FXII inhibitors, but they show differences in regulation of the pro-coagulant and pro-inflammatory effects of FXII. 3F7 specifically binds to the FXIIa enzyme capsule to block contact-induced FXIIa formation, in vivo blood clotting and thrombosis formation in rabbit and rat. In the preliminary treatment of the in vitro rabbit extracorporeal membrane oxygenation system, single dose of 3F7 prevented blood clot formation like heparin but didn't affect hemostasis. Compared to heparin that activate FXII and mediates inflammation, 3F7 inhibits activation of FXII by dextran sulfate and interferes activation of KKS[31]; 9A2 inhibits auto-activation of FXII by binding to the Type I domain of fibronectin or Type II domain of EGF, and inhibits fibrin deposition but doesn't affect FXIIa-mediated KK generation during re-calcification perfusion of collagen-coated surface. 15H8 binds to the Type II domain and Kringle domain of EGF, exerting a stronger inhibitory effect than 9A2, not only on auto-activation of FXII but also on activation of PK by FXIIa[32]. RNA aptamer highly specific to FXII also shows effects only on the pro-coagulation effect of FXII, but no effect on activation of KKS by FXII[33]. Whether different pathways the FXII inhibitors act on are correlated with their different binding sites requires further verification.

## Conclusions

It is now recognized that FXII mainly participate in pathological thrombosis and inflammatory responses. Despite discovery of many candidate activators of FXII, the exact endogenous activator of FXII remains unknown. Although it is clear that FXII can also be activated by the reticuloendothelial system in vivo, the extent and specific mechanism of such activation remain to be clarified. FXII exerts its pro-coagulant and pro-inflammatory effect through activation of the coagulation cascade and the KK system under normal circumstances, but upon FXII mutation or binding by antibodies, FXII may response differently in its pro-coagulant and pro-inflammatory effects. For example, despite the increase in KKS system in HAE patients with FXII abnormality, coagulation remains normal; while blocking certain domain of FXII with specific antibody reduced coagulation but not BK generation. This indicates that activation of FXII doesn't mean simultaneous upregulation of inflammation and coagulation, possibly because the pro-coagulant and pro-inflammatory effect of FXII relies on different domains of FXII, or alternatively, PK and FXI, the two key substances of FXII are also regulated by other factors. These questions would require further verification. A clear correlation between FXII and thrombosis and inflammation has

not yet been established, and some studies have demonstrated a “U” shaped correlation between plasma FXII concentration and mortality of various reasons[34], suggesting that partial inhibition of FXII may actually increase mortality. Complete or nearly complete inhibition of FXII/FXIIa is difficult to achieve, bringing extra obstacles to development of therapeutic FXII/FXIIa inhibitor. More experiments in multiple species are required to clarify the underlying mechanisms of FXII action, so as to promote development of new drugs and to improve the prognosis of inflammatory and thrombotic diseases.

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