Mechanisms Regulating Platelet Factor XIII Release

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BACKGROUND AND AIM

- Blood vessel injury activates the **coagulation cascade**, a process that forms a blood clot.
- Vessel injury helps generate **thrombin (IIa)**, which cleaves fibrinogen into fibrin, and **coagulation factor XIII (FXIII)** into **FXIIIa**.
- FXIIIa crosslinks fibrin to increase fibrin clot stability.
- FXIII is found in plasma and platelets. Plasma FXIII is an effective crosslinker, whereas mechanisms behind **platelet FXIII** release, fate and functions are unclear.
- **Aim:** To understand the role of platelet FXIII, we investigated mechanisms regulating platelet FXIII-A release

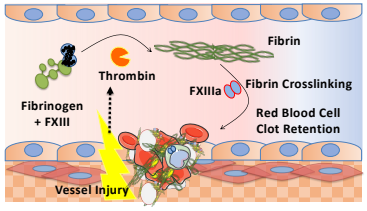
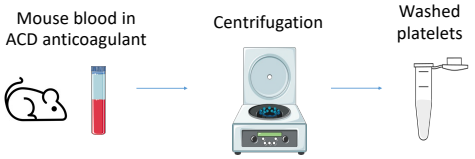


Figure 1. Coagulation process. After vessel injury, tissue factor exposure triggers the coagulation cascade. Subsequent generation of thrombin activates fibrinogen to fibrin, and FXIII to FXIIIa. FXIIIa crosslinks fibrin and antifibrinolytic proteins within clots to help stabilize the clot and retain red blood cells.

METHODS

1. Preparation of mouse platelets



2. Platelet FXIII-A release and retention measured by immunoblotting

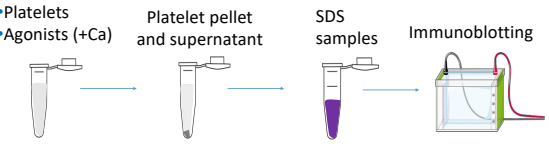


Figure 2. Experimental design. Washed mouse platelets were either unstimulated, activated by 100 ng/mL convulxin (CVX)+ 5 U/mL IIa, or 10 µM calcium ionophore A23187. The mixture was centrifuged (1500×g, 15 minutes) to separate platelet pellet and releasate. The pellet and releasate were boiled in reducing SDS sample buffer (5 min) before FXIII-A was visualized by immunoblotting and quantified by densidometry.

RESULTS

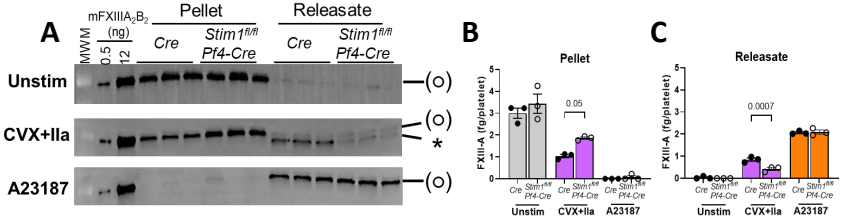


Figure 3. Following dual agonist stimulation, STIM1-deficient mouse platelets release less FXIII-A. (A) Representative immunoblots of FXIII-A in the mouse platelet pellet and releasate**.** The molecular weight marker (MWM) bands indicate 100 kDa. Uncleaved (O) and cleaved FXIII-A (*) are labeled to the right. **(B-C)** Quantification of total FXIII-A. Each dot represents an individual mouse.

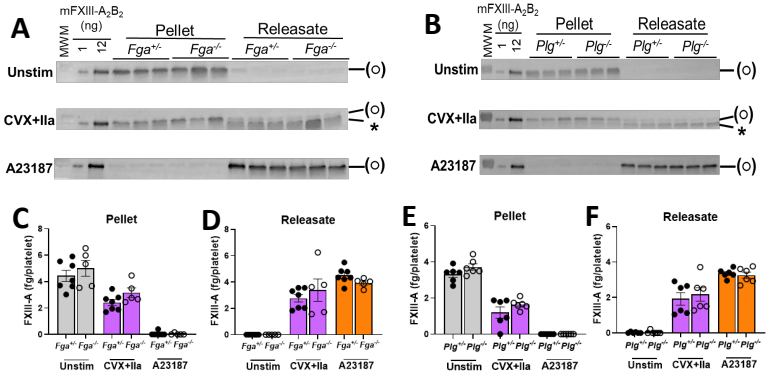


Figure 4. Platelet FXIII release is unaffected in fibrinogen-deficient and plasminogen-deficient mice. Washed mouse platelets from fibrinogen-deficient (*Fga*$^{-/-}$), plasminogen-deficient (*Plg*$^{-/-}$), or littermate controls (*Fga*$^{+/-}$ and *Plg*$^{+/-}$) were studied. **(A-B)** Representative immunoblots of FXIII-A. **(C-F)** Quantification of total FXIII-A. Each dot represents an individual mouse.

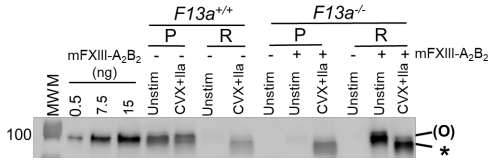


Figure 5. Platelets do not protect exogenous FXIII from cleavage by thrombin. Representative immunoblot of washed platelets from *F13a1*$^{+/+}$ or *F13a1*$^{-/-}$ mice unstimulated or activated by CVX+ IIa in the absence of presence of exogenous mouse FXIII (mFXIIIA$_2$B$_2$) for 30 minutes. P, pellet; R, releasate.

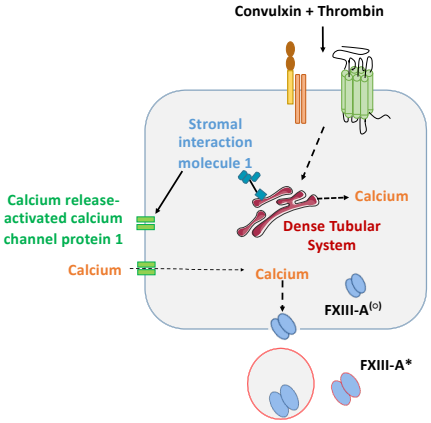


Figure 6. Stromal interaction molecule 1 (STIM1) signaling mediated FXIII-A release. Convulxin and thrombin activate cell receptors, triggering calcium (Ca^{2+}) efflux from the dense tubular system (DTS) to the cytosol. DTS membrane-bound STIM1 is activated and binds to calcium release- activated calcium channel protein 1 (ORAI1) in the cell membrane which opens channels for extracellular Ca^{2+} to enter. FXIII-A is released from the cell.

CONCLUSIONS

- Platelet FXIII-A release is partially regulated by receptor-mediated calcium signaling and is independent of fibrinogen and plasminogen.
- Only platelet-derived and associated FXIII-A is protected from proteolysis.
- Further studies on platelet FXIII physiology can help understand its function and develop therapeutic treatments for thrombosis.

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