The role of filamin A in platelet thromboxane A2 secretion

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Platelets and Hemostasis

Platelets are the second most abundant cell type in the blood after erythrocytes; there are 150,000-450,000 platelets per µl of blood. Platelets are critical for hemostasis. Following damage to the blood vessels, von Willebrand factor (vWF) and collagen are exposed at damaged sites. Platelets adhere to these molecules via their surface receptors (e.g. GPIb complex), become activated and aggregate to form a transient hemostatic plug. The subsequent activation of coagulation factors leads to the formation of a fibrin clot.

In addition to their role in hemostasis, platelets store and secrete a multitude of cytokines, chemokines and growth factors, which orchestrate inflammation and wound healing.

Thromboxane A_2 (TXA_2)

TXA_2 is an eicosanoid lipid (eicosa-: has 20 carbons) derived from arachidonic acid. After platelet activation at vascular injury sites, TXA_2 is synthesized and secreted, which secondarily potentiates platelet activation via autocrine signaling. TXA_2 is essential for platelet functions in humans; for example, aspirin, a commonly-used antithrombotic drug, works by inhibiting TXA_2 synthesis in platelets. However, the intracellular signaling pathway which leads to TXA_2 generation in platelets, is incompletely characterized. The current understanding of this pathway involves mitogen activated protein kinase (MAP kinase) signaling, which is depicted in **Fig. 2**. An improved understanding of TXA_2 synthesis could lead to the identification of new anti-platelet targets and the development of improved anticoagulant drugs.

Filamin A (FLNA) as an intracellular signaling scaffold protein

Filamin A (FLNA) is a ubiquitously-expressed protein that crosslinks actin filaments near the cell membranes, and is therefore important for membrane stability. FLNA also binds multiple cell surface receptors and intracellular signalling proteins, including the GPIb complex and the tyrosine kinase SYK (**Fig. 1**). Recent publications show that FLNA-KO platelets exhibit multiple signaling defects. However, the role of FLNA in modulating MAPK signaling and TXA_2 generation is unknown.

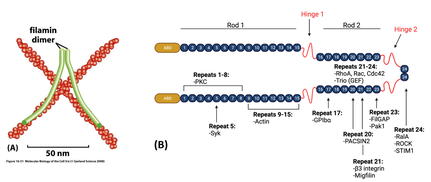

Figure 1. Filamin A. A filamin A (FLNA) dimer crosslinks two actin filaments (shown in red) in platelets (A). FLNA contains 24 immunoglobulin-like domains. Many of these domains bind cell surface receptors and intracellular signalling proteins (B). Figure adapted from De Silva et al., Front. Mol. Biosci., 2022

Thromboxane A_2 (TXA_2) generation pathway

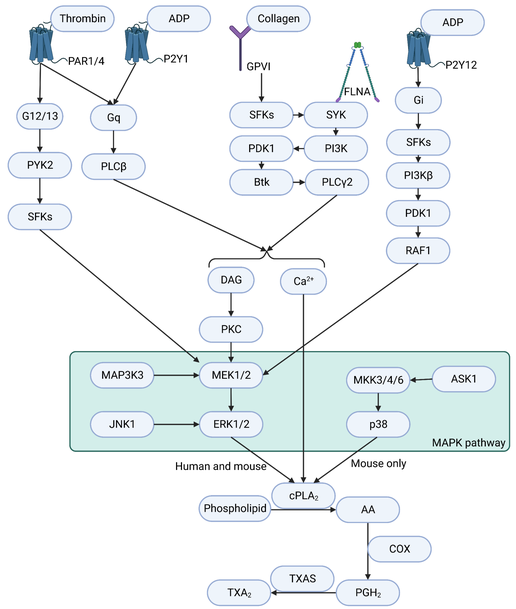

Figure 2. The intracellular signalling pathway of TXA_2 generation in activated platelets. Agonist-activated surface receptors trigger the subsequent activation of downstream intracellular signalling proteins in platelets. These proteins include proteins in the mitogen-activated protein kinase (MAPK) pathway. The MEK/ERK and the p38 modules of MAPK signaling activate cytosolic phospholipase A_2 ($cPLA_2$), which cleaves arachidonic acid (AA) from membrane phospholipids. AA is eventually converted into TXA_2. Notably, FLNA has been shown to interact with SYK and facilitate collagen-induced platelet activation. (Figure created with BioRender.com).

Hypothesis and Objective

HYPOTHESIS: FLNA regulates TXA_2 generation in platelets

The objective of this project is to determine the role of FLNA in the generation of TXA_2 in thrombin-activated platelets. The focus is on mitogen-activated protein kinase (MAPK) signalling in thrombin-activated platelets.

Platelet-specific conditional FLNA deletion

Since FLNA deletion is embryonically lethal in mice, we used the Cre-loxP system to generate platelet-specific FLNA-KO mice. Cre recombinase, under the control of the platelet factor 4 (Pf4) promoter, was used to selectively excise the DNA encoding FLNA from the genome of megakaryocytes and platelets (**Fig. 2**).

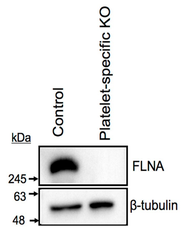

Figure 2. Platelet-specific Filamin A knockout. Filamin A (FLNA) was not detected in FLNA-knockout platelets using Western blot. β-tubulin was blotted as the loading control. Figure adapted from Golla et al., RPTH., 2022

Methods and Results

To study whether FLNA contributes to TXA_2 generation, platelets were isolated from the blood of FLNA conditional KO mice and controls. Platelets were stimulated with 0.1 U/mL thrombin for 5 minutes. The TXA_2 secreted from these platelets spontaneously degraded into TXB_2, the levels of which were determined using ELISA. Notably, thrombin stimulation caused a robust secretion of TXA_2 in control platelets but not in FLNA-KO platelets **(Fig. 4A)**.

To study whether FLNA contributes to MAPK activation, platelets were isolated and stimulated using thrombin as above. The platelets were subsequently lysed and the phosphorylation levels of MAPK components ERK1/2, p38, and $cPLA_2$ - upstream mediators of TXA_2 generation - were measured by Western blotting. The relative densities of the phospho-protein bands reflect defective MAPK signaling in the absence of FLNA **(Figs. 4B-E)**.

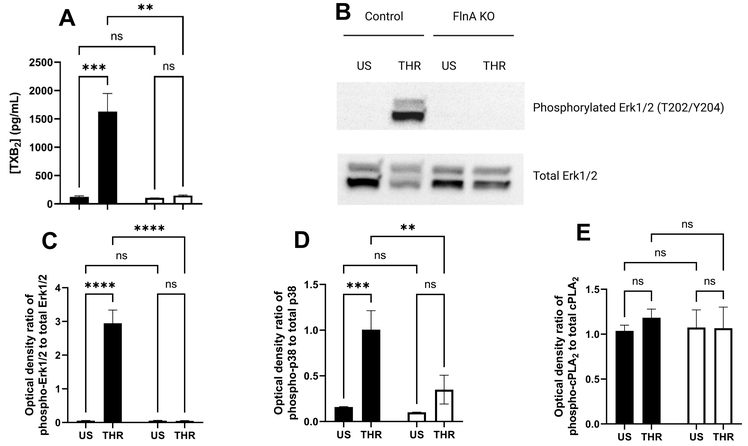

Figure 4. TXA_2 secretion and protein phosphorylation in thrombin-stimulated platelets. FLNA-KO platelets (black bars) and control platelets (white bars) were either stimulated with 0.1U/mL thrombin (THR) or left unstimulated (US). TXB_2 levels were measured in the platelet supernatant using ELISA (A). The phosphorylation levels of ERK1/2 (B and C), ERK1/2 (D), and $cPLA_2$ (E) were measured using Western blot. Two-way ANOVA statistical test and graphs were processed using GraphPad Prism.

Conclusions and Future Directions

FLNA is essential for thrombin-induced TXA_2 generation in platelets by regulating MAPK activation. The intracellular signaling proteins interacting with FLNA will be identified in the future.

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