Protein S Facilitates Clot Retraction through Tyro3 Signaling on Platelets

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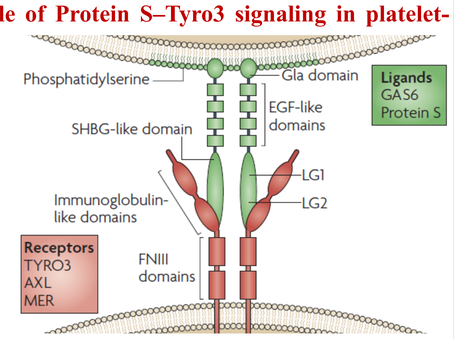
Introduction

Clot retraction is the process by which a blood clot reduces its volume by extruding excess liquid. This retraction is crucial for removing the clot from circulation. Platelets play a central role in this process through the extension of filopodia. Within the clot, platelets are activated and undergo cycles of αIIbβ3-dependent protein phosphorylation and dephosphorylation. TAM receptors contribute significantly to this mechanism by mediating platelet–platelet interactions, enhancing granule secretion, activating PI3K, and promoting β3 integrin phosphorylation. The TAM receptors have two known ligands: Protein S (PS) and GAS6. Previous studies have shown that Protein S specifically binds to Tyro3 and Mer receptors. Protein S is primarily synthesized by hepatocytes, but platelets also produce a small amount. In this study, we aim to elucidate the role of Protein S in clot retraction, and to determine whether platelet-derived or plasma-derived Protein S is responsible for this effect.

Aim: To elucidate the role of Protein S–Tyro3 signaling in platelet-mediated clot retraction.

Protein S binds and activates TAM receptors, initiating inside-out signaling that crosstalks with αIIbβ3 integrin pathways, which in turn interact with myosin — a potential mechanism underlying its role in clot retraction.



Methods

Citrated blood from healthy volunteers was used to isolate platelets by centrifugation, which were then resuspended in PS-deficient plasma. Reaction mixtures contained platelets in PS-deficient plasma (200 µL), HEPES-Tyrode buffer (745 µL), and red blood cells (5 µL). Experimental groups included: control, 500 nM Tyro3 inhibitor, 100 nM Axl/Mer inhibitor, 300 nM exogenous PS, and 300 nM PS with 500 nM Tyro3 inhibitor. Clotting was initiated by adding 50 µL thrombin (20 U/mL). Clot retraction was monitored over time by imaging and weighing the clots.

Collection of fresh blood from volunteers

↓

Centrifuge to separate Platelet Rich Plasma (PRP) and blood cells.

↓

Centrifuge the PRP to pellet down platelets and remove Platelet Poor Plasma (PPP)

↓

Re-suspend platelets in equal volume of PS-Deficient plasma

↓ ← **Tyro3 and Axl/Mer inhibitor was added**

Initiate the reaction in glass tubes and take image and weight at different time interval

Schematic representation of the procedure for clot retraction.

Results

Confocal Microscopy Imaging of Alexa 647 labeled Fibrin Clots

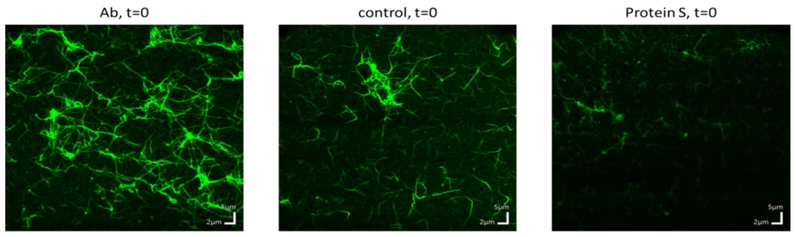


Figure 1: The fibrin mesh (green) remains expanded in the presence of anti-Protein S (anti-PS) antibody, indicating impaired clot retraction. In contrast, addition of Protein S induces physical compaction of the fibrin mesh as the clot retracts. Thus, Protein S promotes clot retraction, whereas anti-PS antibody inhibits it.

Protein S Interacts with TAM Receptors

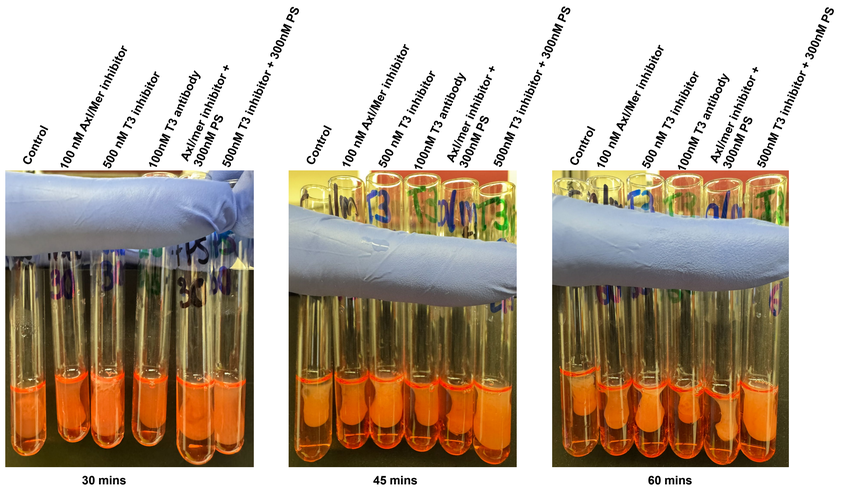


Figure 2: Clot retraction of platelets suspended in PS-deficient plasma in the presence of 500nM Tyro3 inhibitor, 100nM Tyro3 antibody, 100nM Axl/MER inhibitor, and 300nM PS. The clot retraction was delayed the most in the presence of Tyro3 inhibitor.

Clot retraction experiment with Platelet Poor Plasma (PPP)

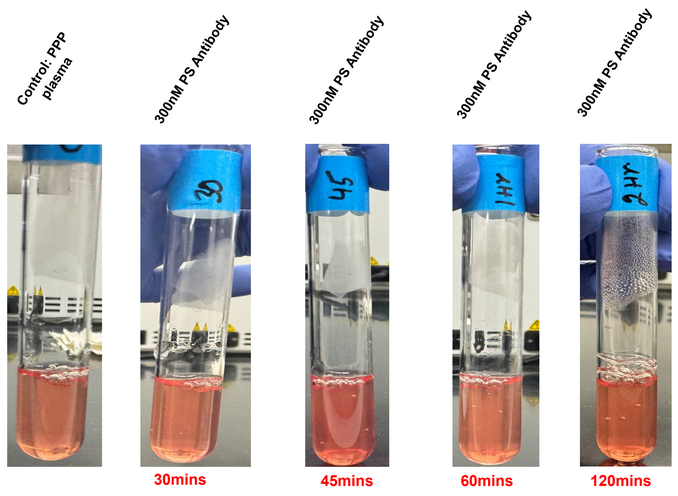


Figure 3: Clot retraction of platelet poor plasma (PPP) in the presence of 300 nM anti-PS antibody. The clots were unable to retract due to a lack of platelet PS.

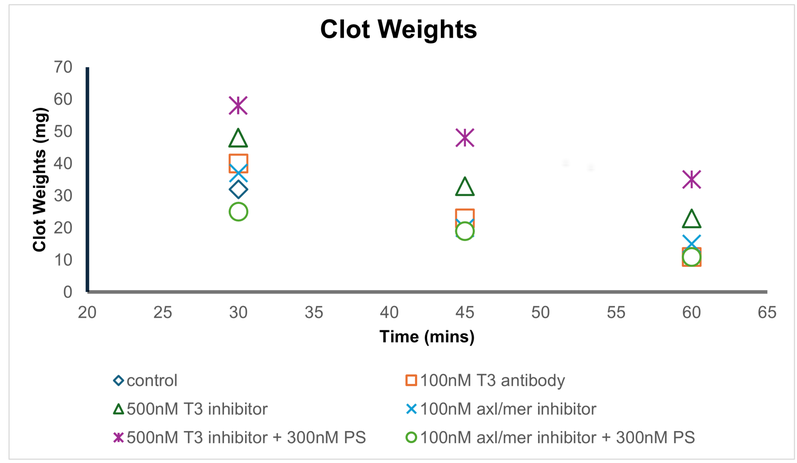


Figure 4 : Clot weight were measured at 30 min, 45 min and 60 min. Clots supplemented with Tyro3 inhibitor retracted the least, thus weighing the most.

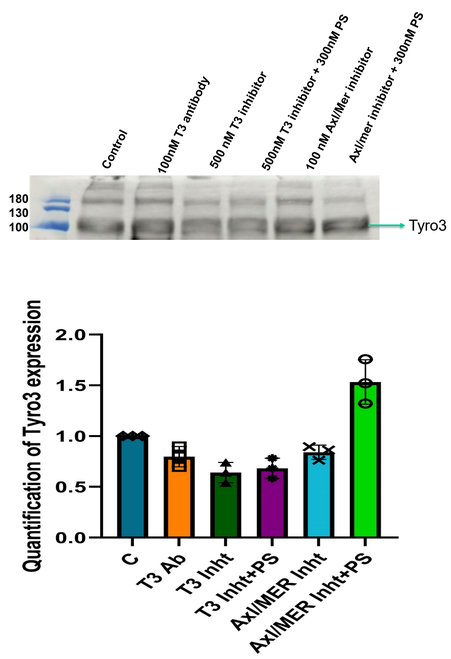


Figure 5: Immunoblot analysis of Tyro3 expression in clots. In control and Axl/Mer inhibitor samples, Tyro3 expression remains unchanged. However, in samples treated with Tyro3 antibody or Tyro3 inhibitor, Tyro3 expression decreases by approximately 40%. Addition of Protein S to the Tyro3 inhibitor–treated samples does not alter Tyro3 expression further.

Conclusion

Platelet-derived Protein S plays a critical role in clot retraction.

Clot retraction was delayed upon addition of Axl/Mer inhibitors, but the delay was more pronounced with the addition of the Tyro3 inhibitor.

The greatest delay in clot retraction was observed with the combination of Tyro3 inhibitor and Tyro3 antibody, indicating that Tyro3 signaling is essential for efficient clot retraction.

Moreover, Tyro3 receptor expression was markedly reduced in the presence of the Tyro3 inhibitor, consistent with its functional role in this process.

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