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Title

Defining the Clinical Contribution of Platelet-Derived FXIII-A to Regulation of Fibrinolysis

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Introduction

Platelets contain an abundance of cellular FXIII-A in their cytoplasm which is released upon stimulation and participates in extracellular cross-linking reactions. As FXIII-A lacks a signal release peptide, the mechanisms involved in its externalisation remain unclear.
Defining the clinical contribution of platelet-derived FXIII-A to regulation of fibrinolysis and identify the signalling mechanisms of their exposure on the platelet surface following stimulation.

Methodolgy

Washed human platelets were activated with (thrombin 100 nM + convulxin 100 ng/ml) ± $\alpha_{IIb}\beta_3$ inhibitor (tirofiban 1 µg/ml), pan-caspase inhibitor (ZVAD 100 µM), caspase-3 inhibitor (Z-DEVD 100 µM), Src family kinases (SFKs) inhibitor (dasatinib 4 µM) or PTP1B inhibitor (MSI-1436 2 µM). FXIII-A and phosphatidylserine (PS) exposure were detected by flow cytometry and confocal microscopy. Model thrombi formed from FXIII deficient plasma + FITC-labelled fibrinogen ± platelets, transglutaminase inhibitor (1mM) or the inhibitors described above. Thrombi were bathed in tissue plasminogen activator (1 µM) and lysis rate determined as fluorescence release.

Results

The number of platelets expressing FXIII-A and PS was significantly increased following strong dual activation with thrombin + convulxin (PS 79.6 ± 6.6 vs. 3.4 ± 1.1 %; FXIII-A 74.3 ± 6.3 vs. 5.4 ± 1.6 %) compared to resting platelets. Flow cytometry and confocal microscopy revealed a significant reduction in FXIII-A and PS exposure after inhibition of $\alpha_{IIb}\beta_3$, caspases, caspase-3 and SFKs. Inhibition of PTP1B had no effect on FXIII-A or PS exposure. Platelet FXIII-A stabilized FXIII-depleted thrombi however, this stabilising effect was reduced upon inhibition of $\alpha_{IIb}\beta_3$, caspases, caspase-3 and SFKs.

Conclusion

Platelet-derived FXIII-A promote the thrombus stability against the degradation and the intracellular signalling of the integrin $\alpha_{IIb}\beta_3$, caspases and SFKs are required for their externalisation and activity to enhance the extracellular cross-lined functions.