

P010 Differential proteome analysis of stimulated platelets identifies Dok2 as a potential platelet adaptor, regulated downstream of integrin $\alpha_{IIb}\beta_3$
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We have utilised a proteomics approach to identify platelet proteins that are differentially regulated following stimulation with TRAP. By comparing proteomes derived from basal- versus TRAP-stimulated platelets, we were able to detect 62 differentially regulated protein features. Of most interest, was the identification of Dok2, which has not been previously detected in platelets. We also demonstrate that Dok2 is phosphorylated as a general feature of platelet activation, with evidence of phosphorylation following platelet stimulation by thrombin, TRAP, collagen and CRP. Pretreating platelets with the integrin $\alpha_{IIb}\beta_3$ inhibitor lotrafiban, significantly reduces the extent of phosphorylation, establishing the existence of integrin $\alpha_{IIb}\beta_3$ -dependent and -independent pathways, leading to Dok2 phosphorylation. Analysis of GPIIb-/- (α_{IIb}) mice also revealed a significant requirement for integrin $\alpha_{IIb}\beta_3$ in Dok2 phosphorylation. Taken together, our studies have identified for the first time, the existence of Dok2 in human platelets, and establish a key role for G protein-coupled and integrin $\alpha_{IIb}\beta_3$ in Dok2 phosphorylation. Furthermore, these data suggest Dok2 may be important in integrin $\alpha_{IIb}\beta_3$ outside-in signalling.