In vivo roles of Disabled-2 (DAB2) in haemostasis and platelet function: studies using a megakaryocyte lineage-restricted DAB2 knockout

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Disabled-2 (DAB2) is a novel adaptor protein associated with megakaryocytic differentiation and platelet function. In this study, DAB2 expression in mouse megakaryocyte and platelet was characterized and the role of DAB2 in platelet biology and haemostasis was elucidated. Both p82 and p59 DAB2 isoforms were expressed in the bone marrow culture-derived primary megakaryocytes, while p59 represented the major DAB2 isoform in mouse platelets. By cross-breeding the $DAB2^{fl/fl}$ and PF4-Cre mice, lineage-specific DAB2 knockout mice ($PF4\text{-}DAB2^{-/-}$) were generated to delineate DAB2 function in megakaryocyte and platelet. The $DAB2^{fl/fl}$ and PF4-Cre alleles were demonstrated to present in the $PF4\text{-}DAB2^{-/-}$ genomic DNA, whereas DAB2 knockout in the megakaryocyte and platelet was confirmed by Western blot and immunofluorescent staining. The $PF4\text{-}DAB2^{-/-}$ mice appeared normal in size. However, the bleeding time and the rebleeding rate were 18.2 ± 1.8 min and 65.5% for $PF4\text{-}DAB2^{-/-}$ (n = 31) when compared with 6.5 ± 1.7 min and 15.8% for $DAB2^{fl/fl}$ (n = 19), respectively ($p < 0.01$). Upon $FeCl_3$-induced mesenteric venules/arterioles injury, the $PF4\text{-}DAB2^{-/-}$ mouse was less likely to develop the thrombus. The platelet count and mean platelet volume were normal and did not account for the observed $PF4\text{-}DAB2^{-/-}$ phenotypes. In contrast, the $PF4\text{-}DAB2^{-/-}$ platelets displayed a heterogeneous morphology with leaky regular platelet shape, and the defects in clot retraction and spreading on fibrinogen. Accordingly, collagen-activated $PF4\text{-}DAB2^{-/-}$ platelets were not efficiently spread on the immobilized fibrinogen. Upon phalloidin staining, the number of platelets with early actin nodules structure was increased and the formation of stress fibers was decreased upon phalloidin staining. This study thereby presents novel evidence demonstrating that DAB2 is a key platelet protein involved in modulating platelet cytoskeleton reorganization and contributes to our understanding for the molecular basis of normal platelet function associated to haemostasis.