Implication of RAPGEF2 in a Novel PKC-Mediated Pathway of Platelet RAP1 Activation

Platelets are small anucleate blood cells that are critical for hemostatic clot formation following injury of a blood vessel. Adhesion of platelets to the extracellular matrix and platelet-platelet aggregation are highly dependent activation of the small GTPase RAP1, a “molecular switch” that cycles between a GDP-bound inactive state and a GTP-bound active state. The Bergmeier Lab has previously proposed a two-pathway model of platelet integrin activation in which the calcium-binding guanine nucleotide exchange factor (GEF) CalDAG-GEFI is responsible for a rapid and reversible activation of RAP1, and engagement of the P2Y12 G-protein coupled receptor (GPCR) results in inhibition of the GAP RASA3, permitting sustained activation of RAP1. However, further studies (not shown) have shown that high doses of platelet agonists are capable of inducing aggregation via a PKC-mediated pathway in platelets deficient in both CalDAG-GEFI and P2Y12, suggesting the existence of an alternative signaling cascade. Recent proteomics studies in platelets have shown significant expression of RAPGEF2, another GEF with RAP specificity. This work investigates the involvement of RAPGEF2 in a third pathway of platelet RAP1 activation, using several experimental methods including platelet aggregometry, assays to quantify integrin activation, and fluorescence-activated cell sorting. Platelets deficient in both RAPGEF2 and CalDAG-GEFI were found to have impaired aggregation and integrin activation in comparison to platelets lacking only CalDAG-GEFI. Furthermore, P2Y12-inhibited platelets deficient in CalDAG-GEFI were capable of significant integrin activation and aggregation. These findings implicate RAPGEF2 as a GEF in this newly described PKC-mediated pathway of activation of the small GTPase RAP1 (and subsequent integrin-dependent platelet-platelet aggregation). Future studies will be conducted to further characterize this novel RAPGEF2-dependent pathway of platelet RAP1 activation.