Characterization of a Novel Integrin Binding Protein that is Essential for aIIbß3 Outside-in Signaling and Hemostasis

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Integrins are heterodimeric (α/β) membrane proteins that play fundamental roles in many biological processes, e.g. cell adhesion and spreading, which are important for platelet function and hemostasis. Integrin function is modulated by bi-directional transmembrane signaling: inside-out and outside-in, which is mediated through the interactions between integrin cytoplasmic tails and intracellular, regulatory proteins. Here, we show that VPS33B, a member of the Sec1/Munc18 (SM) family and component of the Class C core of the CORVET/HOPS sorting complexes, binds directly to the β subunit. Overexpression of VPS33B in CHO cells potentiated aIIbß3 outside-in signaling but not inside-out signaling. Platelets, from megakaryocyte- and platelet-specific VPS33B conditional knockout mice we generated, had normal morphology yet their spreading on fibrinogen was impaired and they failed to support clot retraction. Platelet aggregation and ATP secretion in response to low-dose thrombin were reduced in the VPS33B knockout mice. aIIbß3-mediated endocytosis of fibrinogen was also defective. Tail bleeding times were prolonged in the VPS33B knockout mice. Furthermore, VPS33B acted upstream of the RhoA-ROCK-MLC and Rac1 dependent pathways that leads to clot retraction and cell spreading, respectively. Our work demonstrates that vesicular trafficking complexes are a novel class of modifiers of integrin function and in hemostasis. Our data also provide insights into the molecular mechanism and treatment of ARC syndrome.