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Analysis of Myosin 5A recruitment to endothelial Weibel Palade bodies.

V. Llombart[1], S. Le Trionnaire[1], N. Hellen[2], R. Bierings[3], M. Hannah[4], T. Carter[1];
[1]St George's University of London, London, United Kingdom, [2]Imperial College, London, United Kingdom, [3]Sanquin Research, Amsterdam, Netherlands, [4]Public Health England, London, United Kingdom

Endothelial cells store Von Willebrand-factor (vWF) in secretory granules called Weibel-Palade bodies (WPB). vWF trafficking and secretion depends on the interplay between WPBs and the cell cytoskeleton. Interaction with actin filaments is thought to be mediated through a Rab27A-MyRIP complex involving two elements; a direct MyRIP-actin interaction and an indirect actin interaction mediated through recruitment of Myosin 5A (Myo5A). In other cell types Myo5A can bind Rab3 isoforms, Slp4-a, or other effectors (e.g. rabphilin-3A) to mediate organelle-actin interactions. Because WPBs can also recruit Rab3 isoforms and Slp4-a the possibility exists that multiple mechanisms may operate for Myo5A recruitment to WPBs. In this study we aimed to examine the contribution of Rab27-MyRIP, Rab3 isoforms and Slp4-a to Myo5A recruitment to WPBs.
To investigate the role of secretory Rabs and their effectors in WPB-Myo5A recruitment HUVEC were transfected with specific siRNAs to deplete expression of Rab27A, MyRIP, Rab3B and Slp4-a or treated with the potent RabGGTII inhibitor 3-(3-Pyridyl)-2-hydroxy-2-phosphonopropanoic acid (3-PEHPC) to disrupt Rab-protein membrane localization. Depletion was assessed by quantitative PCR, western blot and immunocytochemistry and the subcellular localisation of Rabs, effectors and Myo5A determined by immunocytochemistry.
Myo5A immuno-localised to WPBs in HUVEC. Pre-absorption of the Myo5A antibody with the peptide antigen against which it was raised abolished WPB-Myo5A immunoreactivity. Analysis of endogenous Rab27a, MyRIP and Myo5A localisation on WPBs revealed 1) that WPBs in some cells showed Myo5A but not Rab27A or MyRIP localisation and 2) Myo5A immunoreactivity was associated with newly formed WPBs at the trans-Golgi network which lack Rab27A, MyRIP or Slp4-a. siRNA depletion of Rab27A removed Rab27A and MyRIP from WPBs but failed to deplete Myo5A immunoreactivity. siRNA depletion of MyRIP also failed to deplete WPB-Myo5A. Dual siRNA mediated depletion of Rab27A and Rab3GEP removed all detectable immunoreactivity to Rab27A, Rab3B or D, but failed to deplete Myo5A from WPBs. siRNA mediated depletion of Slp4-a, or Slp4-a and MyRIP failed to deplete Myo5A from WPBs. Selective inhibition of RabGGTAse II to block all Rab prenylation and thereby remove all Rab proteins (confirmed by complete removal of all detectable Rab27A, MyRIP and Rab3 isoforms) from WPBs failed to deplete Myo5A from WPBs. PCR and RNA seq analysis showed that rabphilin-3A, is not expressed in the HUVEC cultures used here.
Our results point toward the existence of a Rab- and rabphilin-3A independent mechanism for Myo5A recruitment to WPBs. Future studies aim to determine what the mechanism might be.