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Poster presentation

cGMP-dependent protein kinase regulates Rap1 signaling in platelets

Albert Smolenski*, Meike Hoffmeister, Pavel Riha, Olga Neumüller, Oliver Danielewski and Jan Schultess

Address: Institute for Biochemistry II, University of Frankfurt Medical School, 60590 Frankfurt, Germany

Email: Albert Smolenski* - smolenski@biochem2.de

* Corresponding author

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cGMP- and cAMP-dependent protein kinases (cGK and cAK) mediate the inhibitory effects of endothelium-derived messenger molecules nitric oxide and prostacyclin on platelets. To understand the mechanisms involved in platelet inhibition we searched for new substrates of cGK and cAK. We identified Rap1GAP2, the only GTPase-activating protein of Rap1 in platelets. Rap1 is a guanine-nucleotide binding protein that controls integrin activity, platelet adhesion and aggregation. Rap1GAP2 is required to turn over Rap1-GTP to Rap1-GDP resulting in the inactivation of integrins and reduced cellular adhesion. Using phospho-specific antibodies we demonstrate phosphorylation of endogenous Rap1GAP2 on serine 7 by cGK and cAK in intact platelets. Yeast-two-hybrid screening revealed an interaction of the phosphoserine/-threonine binding adapter protein 14-3-3 with Rap1GAP2, and we mapped the 14-3-3 binding site to the N-terminus of Rap1GAP2 close to the cGK/cAK phosphorylation site. We could show that 14-3-3 binding to Rap1GAP2 requires phosphorylation of serine 9. Platelet activation by ADP and thrombin treatment induces Rap1GAP2 serine 9 phosphorylation and enhances the attachment of 14-3-3 to Rap1GAP2. In contrast, phosphorylation of serine 7 by cGK/cAK leads to the detachment of 14-3-3. Furthermore, Rap1GAP2 serine 7 phosphorylation correlates with the inhibition of Rap1-GTP formation by cGMP and cAMP in platelets. Cell adhesion experiments provide additional evidence that Rap1GAP2 is activated by the detachment of 14-3-3. Point mutants of Rap1GAP2 deficient in 14-3-3 binding inhibit Rap1-mediated cell adhesion significantly stronger than a Rap1GAP2 mutant that binds 14-3-3 constitutively. Our findings define a novel regulatory mechanism that might contribute to both platelet activation and endothelial inhibition of platelet adhesion and aggregation.