

**P019** Functional Association between the Platelet Integrin  $\alpha$ IIb $\beta$ 3 and a Chloride Channel Identified using Protein Arrays

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The precise molecular mechanism of activation of the platelet integrin  $\alpha$ IIb $\beta$ 3 is not understood although a critical role for the conserved  $\alpha$ -integrin motif, KxGFFKR, has been identified. We scanned a high-density protein expression array (37,000 recombinant human proteins) for high-affinity interactions with a tagged synthetic peptide corresponding to this motif, as a novel mechanism for identifying integrin binding proteins. KVGFFKR but not a control peptide bound with high avidity to one such protein, the volume regulating chloride channel, ICl<sub>n</sub>. We verify the presence of ICl<sub>n</sub> in human platelets by PCR, western blots and immunofluorescence, and its co-association with  $\alpha$ IIb $\beta$ 3 by immunoprecipitation and peptide-pulldown assays. ICl<sub>n</sub> is regulated by nucleotides and inhibited by the anti-viral agent, Acyclovir. We show that ATP(2nM) increases agonist-induced activation of platelets but has no effect on basal platelet activation or secretion levels supporting the role for ICl<sub>n</sub> in  $\alpha$ IIb $\beta$ 3 activation. Furthermore, acyclovir(1mM) and a cell permeable peptide(100 $\mu$ M) identified as the potential integrin-recognition domain on ICl<sub>n</sub>, specifically inhibit agonist-induced integrin activation (PAC-1) and platelet aggregation without affecting P-selectin expression confirming a specific functional role for ICl<sub>n</sub> in platelet integrin activation.