Adhesion and degranulation promoting adapter protein (ADAP) is a central hub for phosphotyrosine-mediated interactions in T cells

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TCR stimulation leads to an increase in cellular adhesion among other outcomes. The adhesion and degranulation promoting adapter protein (ADAP) is known to be rapidly phosphorylated after T cell stimulation and relays the TCR signal to adhesion molecules of the integrin family. While three tyrosine phosphorylation sites have been characterized biochemically, the binding capabilities and associated functions of several other potential phosphotyrosine motifs remain unclear. Here, we utilize in vitro phosphorylation and mass spectrometry to map novel phosphotyrosine sites in the C-terminal part of human ADAP (486-783). Individual tyrosines were then mutated to phenylalanine and their relevance for cellular adhesion and migration was tested experimentally. Functionally important tyrosine residues include two sites within the folded hSH3 domains of ADAP and two at the C-terminus. Furthermore, using a peptide pulldown approach in combination with stable isotope labeling in cell culture (SILAC) we identified SLP-76, PLCgamma, PIK3R1, Nck, CRK, Gads, and RasGAP as phospho-dependent binding partners of a central YDDV motif of ADAP. The phosphorylation-dependent interaction between ADAP and Nck was confirmed by yeast two-hybrid analysis, immunoprecipitation and binary pulldown experiments, indicating that ADAP directly links integrins to modulators of the cytoskeleton independent of SLP-76.