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Meeting abstract

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The docking protein and proto-oncogene product Gab2 is regulated via a novel negative feedback mechanism mediated by 14-3-3 binding

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In vertebrates, Grb2-associated binder (Gab)1–3 constitute a family of conserved docking proteins. Gab2 is tyrosine-phosphorylated upon activation of a variety of growth factor, hormone, antigen, cytokine and cell matrix receptors, leading to the recruitment of specific src homology (SH)2 domain-containing effectors, which include the p85 subunit of phosphatidylinositol (PI)3-kinase and the protein tyrosine phosphatase Shp2. These Gab2 effectors potentiate the activation of the PI3-K/AKT and Ras/ERK pathways, respectively. Studies using gene knock-out mice indicate that Gab2 is required for normal mast cell-mediated allergic responses and osteoclast differentiation, and in combination with Gab1, for cardiac function. In addition, Gab2 signals downstream of several oncogenic tyrosine kinases, and are overexpressed in breast cancer, and promotes erbB2-induced mammary tumourigenesis. Therefore, it is critical to define how Gab2 signalling is regulated in normal and pathological states. One critical event in Gab2 signalling is its interaction with the adaptor protein Grb2, which promotes its association with specific receptors and thereby sustains its tyrosine phosphorylation dependent recruitment of the aforementioned effectors. However, the molecular mechanisms that attenuate or limit Gab2 signals have remained unclear.

In the presented study, we have addressed Gab2 regulation using an integrated approach that combines a proteomics-based definition of the Gab2 'phosphomap" with bioinformatics, biochemistry and cell biology. Here we report the discovery of 21 novel phosphorylation sites on human Gab2. Furthermore, we demonstrate that growth factor-induced and PI3K-dependent phosphorylation of Gab2 on two of these novel residues, S210 and T391, leads to recruitment of 14-3-3 proteins. These events mediate negative feedback regulation, since a Gab2 mutant that cannot be phosphorylated on these sites exhibits sustained receptor association and signalling, and promotes cell proliferation and transformation. Importantly, site-specific introduction of constitutive 14-3-3 binding sites into Gab2 renders it refractory to receptor activation, demonstrating that site-selective binding of 14-3-3 proteins is sufficient to terminate Gab2 signalling. Furthermore, this is associated with drastically reduced recruitment of Grb2 to the Gab2 signalosome suggesting a competition between 14-3-3 and Grb2 for Gab2 binding. These findings lead to a model where signal attenuation occurs, because 14-3-3 promotes dissociation of Gab2 from Grb2, and thereby uncouples Gab2 from the receptor complex. This represents a novel regulatory

mechanism with implications for diverse tyrosine kinase signalling systems in various cell types.