INTERACTION OF CELL MEMBRANE PERMEABLE PHOSPHOPEPTIDES OF GAB1 ADAPTOR PROTEIN WITH SHP2 PHOSPHATASE INHIBITS B CELL SIGNALING

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Background: The multisite docking protein Gab1 plays essential is role in growth factor, cytokine- and antigen receptor signaling. Gab1 has several tyrosine motifs that get phosphorylated upon cell activation and serves as a platform for SH2 domain containing signaling molecules. One of the main binding partners of Gab1 is SHP-2 tyrosine phosphatase. The intimate interaction between phosphorylated Gab1 motifs and SHP-2 activates the phosphatase. SHP-2 by activating the Erk pathway may play a positive role in signaling, additionally, its role is crucial in many hematological malignancies and cancer.
Methods: For binding affinity measurements fluorescence polarization based method was used, Erk inhibition was investigated by western blotting. Phosphatase activity was measured by the RediPlate 96 EnzChek Tyrosine Phosphatase Assay Kit (Molecular Probes); protein-peptide structure was solved by crystallization and X-ray structure solution.
Results: First we identified the proteins that bind to Gab1 N terminal phosphopeptide, GDKQVEY(p)LDLDLD from B cell lysate. This phosphopeptide of Gab1 binds PLCγ and SHP2. We have analyzed the interaction between the phosphopeptides and the SH2 domains of SHP-2 and found that the N terminal SH2 domain binds GDKQVEY(p)LDLDLD with one order of magnitude higher affinity as compared to the C terminal domain. In the contrary, the C terminal peptide of Gab1, DERVDY(p)VVVDQQK peptide binds only to the C terminal but not to N terminal SH2 domain.
We elucidated the molecular interaction between SHP2 SH2 domains and corresponding tyrosyl phosphorylated motifs of the Gab1 adapter. Comparison of the phosphopeptide unbound and bound crystal structures of N terminal SH2 domain suggests that Tyr66 have a critical role in the allosteric activation of the phosphatase domain. Binding of GDKQVEY(p)LDLDLD to the N terminal SH2 domain enhances SHP2 phosphatase activity in vitro, moreover, when administered as cell membrane permeable phosphopeptide into B cells, the phosphopeptide reduces Erk activity and cell proliferation.

Conclusions: We show that an externally delivered GAB1 phosphopeptide is capable to inhibit ERK signaling in activated B-cells suggesting that specific targeting of SHP2-Gab1 association could be an efficient strategy to combat disorders of Ras/MAPK signaling.

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