Phosphorylation of Immunoglobulin-Containing and Proline-Rich Receptor-1 (IGPR-1): Identification and Functional Significance

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Abstract

Background

IGPR-1(Immunoglobulin containing and proline rich receptor (IGPR-1) is a novel adhesion molecule expressed in endothelial cells and epithelial cells. IGPR-1 activity regulates cell-cell interaction and capillary tube formation/angiogenesis. The proline rich cytoplasmic domain of IGPR-1 strongly interacts with several SH3-containing signaling proteins including, SPIN90/WISH (SH3 protein interacting with Nck, 90 kDa/Wiskott–Aldrich syndrome protein (WASP) interacting SH3 protein). SPIN90 plays a central role in IGPR-1 mediated capillary tube formation of endothelial cells. To determine the molecular basis of interaction of IGPR-1 with SPIN90 and establish biological importance of phosphorylation to IGPR-1 function, we analyzed post-translational modifications of IGPR-1, particularly phosphorylation sites that might regulate its interaction with signaling proteins and its function.

Methods

Cells were prepared and lysed, and IGPR-1 was isolated by immunoprecipitation using anti-IGPR-1 antibody. On-bead reduction, alkylation and tryspin digestion were performed while immunoprecipitated proteins were still bound to the affinity beads. Enrichment of phosphopeptides was carried out using TiO_2. Separation on a nano C18 column was followed by MS/MS analysis (both CID and HCD) on an LTQ Orbitrap. LC-MS/MS data were analyzed using Proteome Discoverer. All the phosphopeptides assigned by Mascot and Sequest searches were verified manually.

Results and Conclusions

IGPR-1 is phosphorylated at three serine sites, including S220, S262 and S266. Site-directed mutagenesis analysis demonstrated that phosphorylation of S262 and S266 are not required for recognition of SPIN90 by IGPR-1, suggesting that perhaps phosphorylation of S220 but not S262, and S266, is important for interaction of IGPR-1 with SPIN90. Further studies are underway to elucidate the importance of S220 in the interaction of IGPR-1 with SPIN90 and the putative biological importance of S262 and S266 phosphorylations in IGPR-1 mediated cellular functions in milieu of angiogenesis.

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