Screening for Novel Growth Factor Induced cyclic-AMP dependent Proetin Kinase A Interactions

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Cyclic-Amp-dependent protein kinase A (PKA) is a serine/threonine kinase that can be activated in response to platelet derived growth factor (PDGF) and epidermal growth factor (EGF) mediated signaling events. While PKA has been shown to play a role in the regulation of cellular proliferation and migration in response to these growth factors, the mechanisms linking PDGF and EGF receptor tyrosine kinase activation to PKA remain largely unknown. We have previously shown that tyrosine residue 330 of the catalytic subunit of PKA (PKA-C) can be phosphorylated by PDGFR and EGFR activation. This phosphorylation event can allow for the formation of novel protein complexes via the recruitment of proteins containing the SH2 (Src Homology 2) and PTB (protein tyrosine binding) domains, both of which bind selectively to phospho-tyrosine residues. As tyrosine phosphorylation often generates a docking site for signaling proteins, we hypothesized that phosphorylation on PKA at Y330 may induce a protein-protein interaction. To begin to investigate our hypothesis, we performed a screen using a commercially available SH2 domain array that contains SH2 domains from 36 of the most commonly expressed SH2-containing proteins in mammalian cells. It was found that 9 of these bound specifically to the phospho-tyrosine residue of PKA. We are currently in the process of determining whether these binding interactions observed in vitro occur in growth-factor-stimulated cells by comparing the ability of wild type PKA-C and mutant PKA-C that cannot be phosphorylated on Y330 to coimmunoprecipitate with these proteins.