A role for GRK2 in the aberrant de-differentiation of vascular smooth muscle cells.

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The G protein-coupled receptor kinases (GRKs) are best known for their role in phosphorylating and desensitising G protein-coupled receptors (GPCRs). The GRKs also regulate signalling downstream of other families of receptors and have a number of non-receptor substrates and binding partners. Using co-immunoprecipitation and direct binding studies we have identified $RhoA_{GTP}$ and Raf1 as novel binding partners of GRK2, and report a previously unsuspected function for this kinase. GRK2 is a RhoA effector serving as a RhoA-activated scaffold protein for the ERK MAP kinase cascade. The ability of GRK2 to bind to Raf1, MEK1 and ERK2 is dependent on $RhoA_{GTP}$ binding to the catalytic domain of the kinase. Consistent with previously published results (Gao, J., Li, J., and Ma, L. (2005) Acta Biochim Biophys Sin (Shanghai) 37, 525-531, Wan, K. F., Sambi, B. S., Tate, R., Waters, C., and Pyne, N. J. (2003) J Biol Chem 278, 18658-18663), we find that overexpression of GRK2 in HEK-293 cells results in a ~2-fold increase in EGF-induced ERK activation, as detected by western blotting for phospho-ERK. This increased ERK activity is associated with EGF-dependent, $RhoA_{GTP}$ binding and ERK scaffolding by GRK2, as assessed by the EGF-dependent co-immunoprecipitation of these proteins. Depletion of GRK2 expression by RNAi in vascular smooth muscle cells (VSMCs) reveals that GRK2 is required for EGF-induced, Rho- and ERK-dependent thymidine incorporation; thymidine incorporation in VSMCs depleted of GRK2 was reduced by 59 $\pm$ 12% relative to cells nucleofected with a scrambled control. Thus GRK2 is required for EGF-induced proliferation of VSMCs. Increased levels of GRK2 and RhoA have previously been associated with hypertension, in which a shift in the balance of differentiated versus proliferative VSMCs causes aberrant growth of vascular epithelia and restriction of blood flow. It is thought that EGF-induced phenotypic switching of VSMCs to the de-differentiated state may be mediated by GRK2's ERK scaffolding function. Currently, we are looking to characterize the interactions between GRK2 and its ERK-scaffolding binding partners as direct or indirect, as well as investigating the role of GRK2 ERK-scaffolding in VSMCs downstream of EGF signalling.