

Prenyl-electrostatic Switch

Principal Investigators:

Mark R. Philips, M.D., Department of Medicine, Cell Biology and Pharmacology

Trevor G. Bivona, Ph. D., Department of Medicine, Cell Biology and Pharmacology/NYU School of Medicine

Background: Ras is the oncogene most frequently mutated in human cancers and has therefore been long considered a prime target for anti-cancer drug discovery. Ras proteins are prototypical GTPases that regulate a wide variety of cellular processes, including growth and differentiation, by cycling between a GDP-bound inactive state and a GTP-bound active state. Ras mutations associated with human cancer impair the ability of the GTPase to hydrolyze GTP and therefore result in constitutively active Ras that drives cellular proliferation in a deregulated fashion. Mammalian genomes encode three Ras isoforms designated N-Ras, H-Ras and K-Ras. Mutations of the K-Ras gene are most frequently associated with human cancers including those of the pancreas, colon and lung such that therapeutic strategies aimed specifically at oncogenic K-Ras are particularly attractive. The biological functions and oncogenic potential of Ras proteins depend on their ability to associate with cellular membranes. Membrane association of Ras proteins depends on the post-translational modification of a C-terminal CAAX motif with a farnesyl lipid. Farnesyltransferase inhibitors have been developed to inhibit this process but have proved to have disappointing efficacy against K-Ras dependent tumors. In addition to farnesylation, N-Ras and H-Ras also require palmitoylation of nearby cysteines for targeting to the plasma membrane (PM). K-Ras lacks palmitoylation sites and is unique among Ras isoforms in requiring a polylysine sequence adjacent to the CAAX motif for association with the PM. In this way, K-Ras is similar to the myristoylated alanine-rich C kinase substrate (MARCKS) protein that is associated with the PM by virtue of a lipid modification that works in conjunction with a polybasic region that affords an electrostatic interaction with the anionic head groups of phospholipids in the cytosolic leaflet of the PM. The membrane association of the MARCKS protein is regulated by a myristoyl-electrostatic switch whereby serine residues interspersed among the basic residues of the polybasic region are sites for phosphorylation by protein kinase C (PKC). Phosphorylation of these sites neutralizes the positive charge to a sufficient degree that the MARCKS protein is released from the PM.

Description of Project: Philips and Bivona have discovered that PM association of K-Ras is regulated by a mechanism analogous to that of the MARCKS protein that they have termed the prenyl-electrostatic switch. The switch consists of serine residues at positions 171 and 181 of K-Ras that are sites for phosphorylation. PKC agonists stimulate phosphorylation of these serines and rapidly lead to the discharge of K-Ras from the PM. Philips and Bivona have further discovered that K-Ras released from the PM by engagement of the prenyl-electrostatic switch rapidly accumulates on intracellular membranes that include the endoplasmic reticulum, Golgi apparatus and outer mitochondrial membrane. Moreover, this translocation is associated with the engagement of a potent pro-apoptotic pathway. GTP-bound, active K-Ras more efficiently engages this pro-apoptotic pathway than does GDP-bound K-Ras. Thus, engagement of the prenyl-electrostatic switch on K-Ras has great potential as a strategy to specifically eliminate cells that harbor oncogenic K-Ras.

As a proof of principle, Philips and Bivona have shown that cells transformed with oncogenic K-Ras, but not oncogenic H-Ras, are sensitive in vitro and in vivo to PKC agonists and that this sensitivity is dependent on serine 181, thus implicating the prenyl-electrostatic switch as the mechanism for elimination of K-Ras transformed cells. This exciting result suggests that agents that activate the prenyl-electrostatic switch of K-Ras may prove to be potent and specific anti-cancer drugs.

Applications: Philips and Bivona have developed novel methods for screening for agents that engage the K-Ras prenyl-electrostatic switch. These methods can be applied to small-scale laboratory efforts to test and optimize agents that engage this process (e.g. PKC agonists such as bryostatin-1) and can be adapted to high throughput screens to identify novel compounds that engage the prenyl-electrostatic switch.

Patent Status: Three US Patents (6,184,016, 6,232, 108 and 6,432, 403) have issued and additional US and foreign applications are pending.

For further information please contact:

New York University/Office of Industrial Liaison

650 First Avenue, New York, N.Y. 10016 Tel: (212)263-8178 Fax: (212)263-8189