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Ras and Rho Post-translational Modification by Prenylation: Role in Cancer Drug Discovery

Ras and Rho GTPases are small G-proteins that cycle between an active GTP-bound form and inactive GDP-bound form. Ras proteins, known for their role in cell proliferation, and Rho proteins, known for their involvement in cell morphology, have common post-translational modifications (PTMs) that have been identified as contributors to oncogenesis[1,2]. Understanding Ras and Rho PTMs have been of interest for drug discovery groups for many years. Recent studies of signaling pathways mediated by the Ras and Rho PTMs prenylation and/or palmitoylation have identified potential cancer drug targets[1,2].

Lipid Modification of Ras: A PTM's Role in Cancer

Mutations resulting in abnormal activation of Ras isoforms (H, N, & K) are a common cause of human cancers, including pancreatic, cervical, colon, lung, thyroid, bladder, breast, skin, and leukemias[1,3,4]. Although activated Ras isoforms have been frequently associated with cancers, no effective Ras signaling inhibitor has been developed. While all three isoforms are worthy drug targets[1], recent efforts have focused on K-Ras[5]. (Fig. 1).

K-Ras undergoes the PTM farnesylation (a type of prenylation) that involves adding an isoprenyl group on the C-terminus[6,7]. In short, either a farnesyl or geranylgeranyl lipid is covalently attached to the cysteine residue on the C termini of Ras proteins (CAAX tetrapeptide motif), followed by Rce1-mediated amino acid cleavage of -AAX, and Icmt-mediated methylation of the prenylated cysteine[6,7]. The farnesyl tails of K-Ras tether the protein to cell membranes and help restrict free diffusion of K-Ras through the cytoplasm[6-8]. These farnesyl tails also play an important role in trafficking Ras to proper subcellular compartments for cell signaling events. PDEδ (a.k.a. PDE6δ) is a prenyl-binding protein involved in intracellular localization of Ras-like membrane bound proteins within the cytosol[9,10]. PDEδ's beta sandwich fold composes a hydrophobic pocket which binds to K-Ras farnesyl tails, allowing the complex to be solubilized in the cytosol for trafficking to the acceptor membrane[6-10]. K-Ras is released at the acceptor membrane upon binding of GTP-bound Arl2/3 to PDEδ[6-9] (Fig. 1).

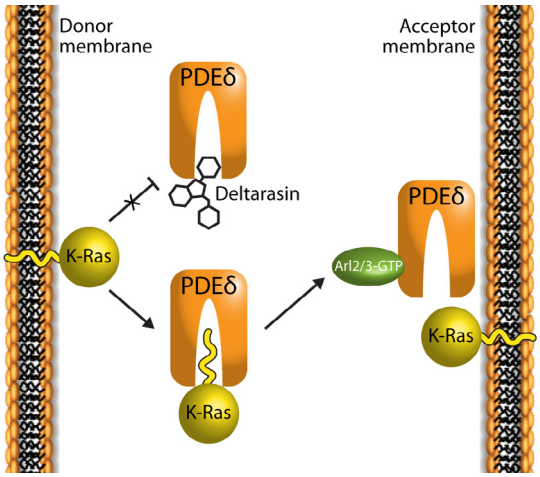

Figure 1: Deltarasin (a benzimidazole-based compound) inhibits the interaction of K-Ras and PDEδ by binding in the PDEδ pocket. K-Ras typically binds to PDEδ via the farnesylated lipid PTM at its C-terminus [5,6,8].

High-throughput screening has identified the benzimidazole-based compound Deltarasin as a disrupter of the interaction of PDEδ and K-Ras[5]. Deltarasin occupies the farnesyl-binding cavity, preventing the lipid tail of the Ras protein from binding to PDEδ; hence altering the subcellular localization of K-Ras. Cancer cells dependent upon K-Ras signaling for survival show increased cell death when treated with deltarasin. Furthermore, deltarasin treatment effectively reduces tumor growth rates in mice with tumor cell xenografts[5]. These findings support the importance of the Ras/PDEδ interaction

Small G-protein PRODUCTS

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on cancer development[5]. Interestingly, Ras protein function can remain intact without PDEδ[11], possibly due to redundant cytosolic chaperones acting in the absence of PDEδ[6,7].

Lipid Modification of Rho: A PTM's Role in Cancer

Similar to the Ras GTPases, Rho GTPases (isoforms A, B, & C) are intimately involved in cancer cell morphogenesis and migration[2,12,13]. Furthermore, the PTMs prenylation and/or palmitoylation also regulate Rho GTPase trafficking and activity[2]. Like Ras, Rho proteins can be modified by covalent addition of a farnesyl or geranylgeranyl lipid and/or a palmitoyl lipid at the CAAX tetrapeptide motif[14]. Such modifications allow trafficking of Rho proteins to membranes, a necessity for normal biological activity[2]. Given the importance of lipid-based PTMs in the Ras and Rho signaling pathways, their inhibition is considered a promising target for cancer treatments[14]. Roberts et al.[14] demonstrated that the CAAX motif is involved in proper Rho localization and activity through the use of various pharmacological inhibitors of farnesyltransferase, Rce1, and Icmt. These results strongly suggest that the Rce1 and Icmt CAAX-processing enzymes are important targets for therapeutic cancer inhibitors[14].

As researchers reveal additional Ras and Rho regulatory mechanisms, methods to measure activated levels of these proteins take on increasing importance. To aid researchers with identifying and characterizing cancer therapeutics, Cytoskeleton, Inc. offers many small G-protein tools such as pull-down and G-LISA® activation assays which are widely used to measure the activated levels of small G-proteins. Pull-down methods measure activated protein levels utilizing a domain of an effector protein coupled to agarose beads and the activated level of the protein is measured by Western blot. G-LISA activation assays are faster and more sensitive than traditional pull-down techniques. G-LISAs require much less cell material and provide numerical data which allow easy comparison between samples. Small G-protein antibodies, activators, and inhibitors are also available to help elucidate Rho and Ras activation pathways.

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Rho and Ras Research Tools

Protein	Purity	Cat. #	Amount
H-Ras His Protein, wild-type	>80%	RS01-A RS01-B	1 x 100 ug 3 x 100 ug
RhoA His Protein, wild-type	>80%	RH01-A RH01-C	1 x 100 ug 3 x 100 ug
RhoC His Protein, wild-type	>90%	RH03-A RH03-C	1 x 100 ug 3 x 100 ug
Rhotekin-RBD Protein Binds specifically to active (GTP-bound) Rho	>90%	RT01-A RT01-B	1 x 500 ug 3 x 500 ug
Rhotekin-RBD Beads Binds specifically to active (GTP-bound) Rho	>85%	RT02-A RT02-B	2 x 2 mg 6 x 2 mg
Raf-RBD Beads Binds specifically to active (GTP-bound) Ras	>85%	RF02-A RF02-B	1 x 2 mg 4 x 2 mg

G-protein Modulator	Cat. #	Amount
Rho Activator II Cell permeable	CN03-A CN03-B	3 x 20 ug 9 x 20 ug
Rho Inhibitor I Cell permeable	CT04-A CT04-B	1 x 20 ug 5 x 20 ug

Kit	Cat. #	Amount
Ras G-LISA® Activation Assay, colorimetric	BK131	96 Assays
RhoA G-LISA® Activation Assay, colorimetric	BK124	96 Assays
RhoA G-LISA® Activation Assay, luminescence	BK121	96 Assays
Total RhoA ELISA	BK150	96 Assays
RhoGAP Assay Biochem Kit™	BK105	80-160 Assays
RhoGEF Exchange Assay Biochem Kit™	BK100	60-300 Assays

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