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Identification of a farnesol analog as a Ras function inhibitor using both an in vivo Ras activation sensor and a phenotypic screening approach

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Abstract Mutations in Ras isoforms such as K-Ras, N-Ras, and H-Ras contribute to roughly 85, 15, and 1 % of human cancers, respectively. Proper membrane targeting of these Ras isoforms, a prerequisite for Ras activity, requires farnesylation or geranylgeranylation at the C-terminal CAAX box. We devised an in vivo screening strategy based on monitoring Ras activation and phenotypic physiological outputs for assaying synthetic Ras function inhibitors (RFI). Ras activity was visualized by the translocation of RBD_{Raf1}-GFP to activated Ras at the plasma membrane. By using this strategy, we screened one synthetic farnesyl substrate analog (AGOH) along with nine putative inhibitors and found that only m-CN-AGOH inhibited Ras activation. Phenotypic analysis of starving cells could be used to monitor polarization, motility, and the inability of these treated cells to aggregate properly during fruiting body formation. Incorporation of AGOH and m-CN-AGOH to cellular proteins was detected by western blot. These screening assays can be incorporated into a high throughput screening format using *Dictyostelium discoideum* and automated microscopy to determine effective RFIs. These RFI candidates can then be further tested in mammalian systems.

Keywords Ras function inhibitors · RBD · Polarity · Development

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Introduction

Ras proteins are monomeric small guanosine triphosphatases (GTPases) which regulate normal cellular proliferation [1]. Aberrant signaling through Ras pathways occurs both as the result of mutations in Ras and from the misregulation of genes upstream and downstream of Ras [1–3]. 20 % of human tumors have activating point mutations in Ras, with most found in *KRAS* (about 85 % of total), then *NRAS* (about 15 %), and lastly *HRAS* (<1 %) [2]. These mutations all affect the GTPase activity of RAS, preventing GTPase-activating proteins from promoting hydrolysis of GTP on RAS and therefore causing RAS to accumulate in the GTP-bound active form [2, 4]. Ras GTPases activate four major effector pathways including Raf protein kinases, phosphatidyl inositol 3-kinase (PI3K), Ral guanine nucleotide dissociation stimulator (RAL GDS), and phospholipase C-epsilon. While Raf regulates cell cycle progression

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