

Progress in Making ‘Ras’ as a Target

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Human RAS gene family consists of 3-functional genes named HRAS, NRAS, and KRAS [1-3]. RAS genes provide instruction through a signaling pathway (e.g. Ras/MAPK pathway) for making a set of proteins called Ras proteins, which relay the signals from outside cells to the nucleus of the cell. Ras proteins are GTPase, which means they exhibit high binding affinity for GDP and GTP. Ras acts as a molecular switch and timer that cycle between inactive GDP-bound “off” and active GTP-bound “on” states (Figure 1). When Ras protein binds to the GDP, it doesn’t relay the signals to the nucleus. To transmit signals, Ras protein must be turned “on” or be active by binding to the GTP. Normally, Ras is in “off” position, but when it receives the message, it briefly switches to turned “on” position (active state) and tells the cell to grow. After which it returns to the “off” position by intrinsic GTP-hydrolysis activated by GAP (Figure 1). In this way, Ras proteins control normal cell growth and development by transmitting regulated signals to the cell nucleus.

Mutations build up in cells over time. When enough mutations happen cells no longer act normally, they grow out of control and cancer is thought to happen. RAS genes are frequently mutated in human cancers (33%) and encode four highly homologous (Figure 2) Ras proteins (H-Ras, N-Ras, and K-RasA, and K-RasB) [4]. Mutated Ras proteins are resistant to GTP-hydrolysis, which renders them chronically active (GTP-bound state) and persistently transmitting deregulated signals to the cell nucleus caused uncontrolled cellular proliferation in cancers. KRAS mutations are the highest (86%) of all RAS mutations documented in human cells and are primarily associated with pancreatic, lung, and colon cancers [2,4]. Because of the mutated RAS genes (Figure 2) have a high prevalence in the progression of human cancers, development of inhibitors of the Ras signaling pathway as anticancer agents is a very promising pharmacologic strategy.

It has been shown in animal models that inhibitors of Ras-signaling cause regression of Ras dependent tumors [1-3]. Farnesyltransferase (FTase) inhibitors are the most promising class of Ras inhibiting potential cancer therapeutics. Ras proteins are membrane associated signal transducers. It has been proved that farnesylation (by FTase) is critical for membrane association and Ras-transforming activity. Mutation of the cysteine residue of Ras-protein C-terminate (tetrapeptide CAAX) motif prevented farnesylation, rendering Ras non-transforming. FTase enzyme was isolated, and it was shown that Ras-CAAX tetra-peptide sequence alone was effective in blocking FTase activity [5]. These findings prompted both pharmaceutical companies and academic researchers to design CAAX peptidomimetics as possible FTase inhibitors (FTIs). Because of the high throughput chemical library screens, numerous chemically diverse FTase inhibitors (FTIs) were developed. Of these, two nonpeptidic, tipifarnib and lonafarnib underwent the most significant clinical evaluation. Tipifarnib is selective for FTase with an IC₅₀ of 7.9 nM. Lonafarnib is a CAAX-competitive inhibitor that is selective for FTase (IC₅₀ 1.9 nM). Some studies have been focused on the development of inhibitors of Ras-membrane association. Couple of small molecules (Salirasib, and TLN-4601) containing farnesyl group have been evaluated. These molecules are proposed to antagonize Ras function by competing for membrane-bound farnesyl-binding dock proteins [2].

FTIs showed promising results by inhibiting cell growth in a large variety of cancer cell lines in vitro and in vivo as tumor xenografts by preventing farnesylation. However, K-Ras and N-Ras in human cancers are subject to alternative prenylation by another enzyme (GGTase I), in FTI treated cells, resulted in persistent membrane localization of K-Ras and N-Ras and thereby upregulation of downstream signaling. This cross prenylation of K-Ras and N-Ras had been proposed to be the main reason why FTI monotherapy showed poor results in clinical trials. The alternative prenylation of K-Ras and N-Ras by GGTase I led to the development of dual prenylation inhibitors, but these inhibitors showed very toxic effect in mice and did not correlate with the inhibition of K-Ras prenylation, which suggests that these inhibitors have a variety of targets in cells [1].

Recently, Zimmermann and coworkers [6] developed a new class of small molecules which inhibit KRAS- PDEδ (prenyl-binding protein) interaction by binding the prenyl-binding pocket of PDEδ with nanomolar affinity (Figure 3). Mammalian prenyl-binding protein (PDEδ) facilitates the diffusion of farnesylated K-Ras in the cytoplasm for membrane association. Initial studies with human pancreatic ductal adenocarcinoma cells showed that these molecules interfere the binding of PDEδ to K-Ras by affecting the spatial organization of -Ras and thus provides a novel opportunity to suppress the oncogenic Ras signaling.

Another strategy of Ras-targeted therapy could be the direct attack on the mutant Ras. The search for small molecules that bind to the surface of Ras protein to prevent GTP binding is very challenging. Recently, Ostrem et al. [7] developed small molecules (Figure 4) that irreversibly bind to K-Ras (G12C), a common oncogenic mutant. These compounds selectively bind to the cysteine thiol of mutated K-Ras and therefore, do not affect the wild-type K-Ras. Binding these inhibitors to Ras shifts the relative nucleotide affinities of Ras to prefer GDP over GTP, which leads to the accumulation of Ras to its inactive state. Initial evaluation of these compounds in lung cancer cell lines suggests allele-specific impairment of K-Ras function. Further studies on these interesting compounds are in progress.

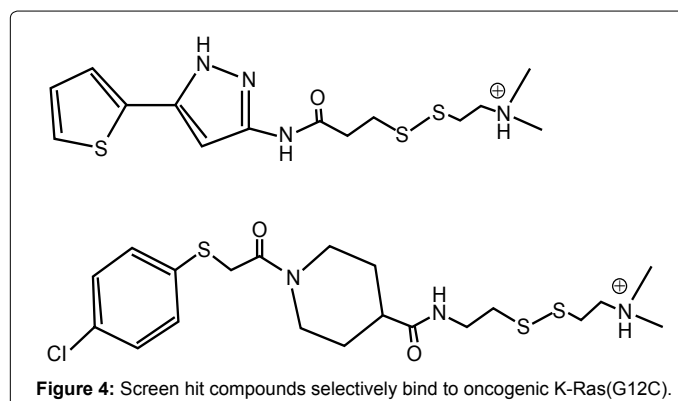
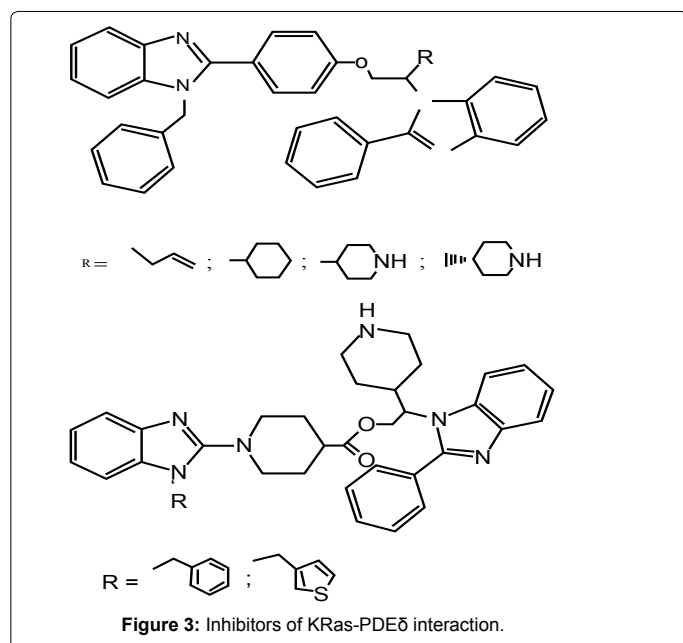
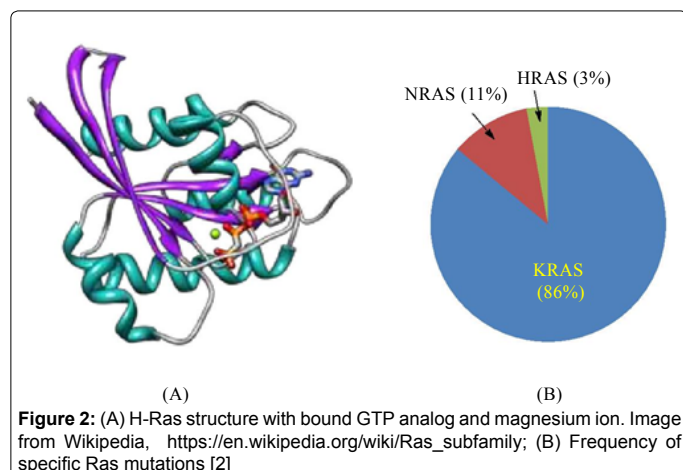
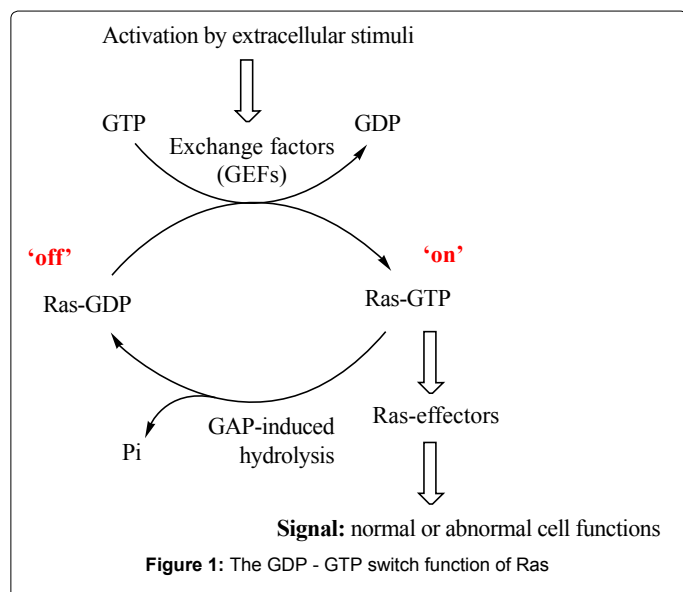
Ras proteins were considered drug targets since 30 years ago. Still now, no drugs that target Ras proteins directly or control the Ras-driven cancers have been developed successfully. Tumors harboring Ras mutations remain the most difficult to treat [1]. For example, conventional EGFR therapy doesn’t work to treat the colon cancer when the tumor has K-Ras mutation. Many medical facilities recommend

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Received March 6, 2014; Accepted March 7, 2014; Published March 19, 2014

Citation: Bhattacharyya S (2014) Progress in Making Ras as a ‘Druggable’ Target. *Biochem Pharmacol* 3: e157. doi:10.4172/2167-0501.1000e157

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that patients with hyperactive K-Ras in their tumors should avoid treatment with cetuximab or panitumumab.

Efforts are ongoing to attack Ras proteins directly based on high throughput molecular screening and on a better understanding of Ras processing and membrane localization. These efforts are still in their early stage of drug discovery. There is a long way to go before these molecules become drugs that could treat the Ras-driven cancer patients. A nationwide program on Ras has been planned by NCI leadership team with the concurrence of the National Cancer Advisory Board (NCAB). Frederick National Laboratory for Cancer Research with its state-of-the-art cancer research facilities will anchor this nationwide program that will unite academic, nonprofit, and pharmaceutical experts to flesh out scientific understanding of the potent gene family and to translate that knowledge into candidate drugs for clinical testing [8].

Finally, our hope is that continued efforts will lead to novel and unexpected new directions for anti-Ras drug discovery which will help to make Ras as a druggable target in the near future.

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U. S. government.

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