Pharmacology & Therapeutics 162 (2016) 35–57

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate Editor: Y. Zhang

Pharmacological modulation of oncogenic Ras by natural products and their derivatives: Renewed hope in the discovery of novel anti-Ras drugs

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ARTICLE INFO

Available online 22 March 2016

Keywords:
Oncogenic Ras
Anti-Ras
Cancer
Natural products
Derivatives
Strategy

ABSTRACT

Oncogenic rat sarcoma (Ras) is linked to the most fatal cancers such as those of the pancreas, colon, and lung. Decades of research to discover an efficacious drug that can block oncogenic Ras signaling have yielded disappointing results; thus, Ras was considered "undruggable" until recently. Inhibitors that directly target Ras by binding to previously undiscovered pockets have been recently identified. Some of these molecules are either isolated from natural products or derived from natural compounds. In this review, we described the potential of these compounds and other inhibitors of Ras signaling in drugging Ras. We highlighted the modes of action of these compounds in suppressing signaling pathways activated by oncogenic Ras, such as mitogen-activated protein kinase (MAPK) signaling and the phosphoinositide-3-kinase (PI3K) pathways. The anti-Ras strategy of these compounds can be categorized into four main types: inhibition of Ras–effector interaction, interference of Ras membrane association, prevention of Ras–guanosine triphosphate (GTP) formation, and downregulation of Ras proteins. Another promising strategy that must be validated experimentally is enhancement of the intrinsic Ras–guanosine triphosphatase (GTPase) activity by small chemical entities. Among the inhibitors of Ras signaling that were reported thus far, salirasib and TLN-4601 have been tested for their clinical efficacy. Although both compounds passed phase I trials, they failed in their respective phase II trials. Therefore, new compounds of natural origin with relevant clinical activity against Ras-driven malignancies are urgently needed. Apart from salirasib and TLN-4601, some other compounds with a proven inhibitory effect on Ras signaling include derivatives of salirasib, sulindac, polyamine, andrographolide, lipstatin, levoglucosenone, rasfonin, and quercetin.

Contents

Abbreviations: AGP, Andrographolide; APT1, Acyl protein thioesterase 1; COX-2, cyclooxygenase-2; DPI, Dual prenylation inhibitor; ERK, Extracellular signal-regulated kinase; FBDD, Fragment-based drug discovery; FTase, Farnesyltransferase; FTIs, Farnesyltransferase inhibitors; G12, Glycine-12; G13, Glycine-13; Q61, Glutamine-61; GAP, GTPase-activating protein; GDP, Guanosine diphosphate; GEF, Guanine nucleotide exchange factor; GGTase I, Geranylgeranyltransferase I; GGTI, Geranylgeranyltransferase inhibitor; GTP, Guanosine triphosphate; GTPase, Guanosine triphosphatase; H-Ras, Harvey-Ras; HTS, High-throughput screening; HVR, Hypervariable region; IC_{50}, Half-maximal inhibitory concentration; ICMT, Isoprenylcysteine carboxymethyltransferase; K-Ras, Kirsten-Ras; MAPK, Mitogen-activated protein kinase; MEK, Mitogen-activated protein/extracellular signal-regulated kinase kinase; Mg^{2+}, Magnesium ion; N-Ras, Neuroblastoma-Ras; NMR, Nuclear magnetic resonance; NSCLC, Non-small cell lung cancer; PDAC, Pancreatic ductal adenocarcinoma; PDB, Protein Data Bank; PFTS, Phosphofarnesylthiosalicylic acid; PI3K, Phosphoinositide-3-kinase; Raf, Rapidly accelerated fibrosarcoma; Ras, Rat sarcoma; SAR, Structure–activity relationship; SBDD, Structure-based drug design; SRJ23, 3,19-(3-chloro-4-fluorobenzylidene) andrographolide; SOS, Son of Sevenless.

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http://dx.doi.org/10.1016/j.pharmthera.2016.03.010
0163-7258/

1. Introduction

The rat sarcoma (Ras) protein superfamily comprises small guanosine triphosphate (GTP)-binding proteins with inherent guanosine triphosphatase (GTPase) activity, hydrolyzing GTP to guanosine diphosphate (GDP) (Wennerberg et al., 2005). The members of the Ras family include Harvey-Ras (H-Ras); neuroblastoma-Ras (N-Ras); and the splice variants of Kirsten-Ras (K-Ras), K-Ras-4A, and K-Ras-4B. These isoforms share highly conserved GDP/GTP-binding motif elements (also known as G boxes) that span the N-terminal G domain (Wennerberg et al., 2005). The C-terminal hypervariable region (HVR) of Ras is subjected to posttranslational modifications to facilitate the lipid-mediated membrane localization of Ras (Konstantinopoulos et al., 2007). The G domain possesses two switches: switch I (residues 25–40) and switch II (residues 59–75) (Vetter & Wittinghofer, 2001; Kapoor & Travesset, 2015). These switches are essential for the recognition of the two nucleotide-bound states of Ras by regulatory proteins and effectors, thus determining the protein–protein interactions between Ras and these Ras-binding proteins (Vetter & Wittinghofer, 2001; Herrmann, 2003; Wennerberg et al., 2005).

Ras proteins are molecular switches that can be turned "on" and "off" by effecting conformational changes of switch I and II *via* the binding of GTP and GDP, respectively (Vetter & Wittinghofer, 2001). As Ras exhibits slow intrinsic activities of GDP–GTP exchange and GTP hydrolysis, the interconversion between its active and inactive conformations must be catalyzed by two regulatory proteins: guanine nucleotide exchange factor (GEF) and GTPase-activating protein (GAP). The GEFs include Son of Sevenless (SOS) and Ras guanine nucleotide-releasing factor 1 (RasGRF1). GEF promotes an active GTP-bound state of RAS by facilitating GDP–GTP exchange, whereas GAP maintains Ras in the inactive GDP-bound state by accelerating the intrinsic GTPase activity of Ras (Schmidt & Hall, 2002; Bernards & Settleman, 2004; Fig. 1).

RAS is a proto-oncogene, mutations of which have been implicated in abnormalities of cell proliferation, survival, migration, and differentiation, which ultimately lead to cancer initiation and progression (Wennerberg et al., 2005). When extracellular signals, such as epidermal growth factor (EGF), nerve growth factor (NGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), activate receptor tyrosine kinases (RTKs), Ras activates two canonical cancer-driving mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways (Rodriguez-Viciana et al., 1994; Lodish et al., 2000; Vivanco & Sawyers, 2002; Sever & Brugge, 2015; Fig. 2). Mutations of Ras have been found in 25%–30% of human cancers, including 90% of pancreatic ductal adenocarcinomas (PDACs) (Scheffzek et al., 1997; Prior et al., 2012). Among the Ras isoforms, K-Ras mutations account for 86% of *RAS*-driven cancers, followed by N-Ras (11%) and H-Ras (3%) (Forbes et al., 2011). Ras mutations most commonly occur at codon glycine-12 (G12), glycine-13 (G13), and glutamine-61 (Q61) (Prior et al., 2012). G12 mutations cause steric hindrance that prevents Ras–GAP interaction, whereas Q61 mutations attenuate the GAP-activating GTPase activity of Ras, resulting in prolonged activation of Ras signaling, ultimately leading to cancer initiation and progression (Gideon et al., 1992; Scheffzek et al., 1997). The effect of G13 mutations on the biochemical properties of Ras remains to be elucidated (Gideon et al., 1992). It follows from the structure of the Ras–GAP complex that G13 mutations involving structurally larger amino acids would cause steric hindrance in the Ras–GAP interaction (Prior et al., 2012). Nevertheless, the structural biology of G13 mutations must be investigated further.

Decades of research into drugs that can inhibit oncogenic Ras have been unsuccessful. For this reason, Ras is described as "undruggable." The undruggability of Ras is mainly attributed to the lack of a well-defined binding pocket in the Ras protein structure to accommodate a drug (Ledford, 2015). As the GTP loading of Ras is the hallmark of Ras activation, using a GTP-competitive inhibitor to block oncogenic Ras

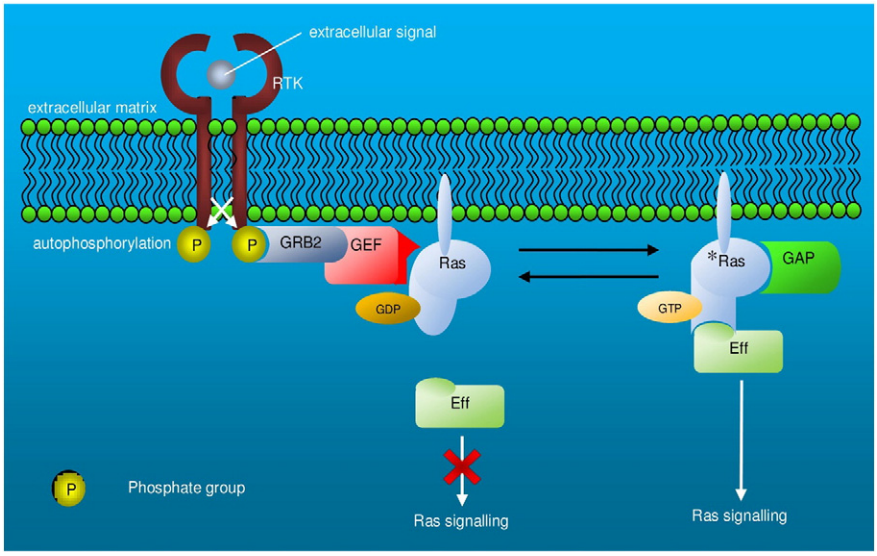

Fig. 1. Regulation of Ras functions. Extracellular signaling molecules (*e.g.*, EGF) activate RTKs, leading to dimerization of the receptor and reciprocal phosphorylation between the two adjacent kinases (also known as autophosphorylation). An adaptor protein, GRB2, binds activated RTK at its phosphotyrosine-binding domain and subsequently recruits GEF to the inner plasma membrane. Ras latches onto the plasma membrane *via* a lipid molecule and interacts with GEF for its activation. The GEF facilitates the GTP loading of Ras by removing GDP, allowing the intracellularly abundant GTP to diffuse into the GDP/GTP-binding pocket and bind to Ras. The active GTP-bound form of Ras interacts with the effector (Eff) to relay a cascade of signaling. To stop this process, GAP interacts physically with GTP-bound active Ras and activates its GTPase activity. The formation of the Ras–GAP complex favors the hydrolysis of GTP into GDP. The inactive GDP-bound form of Ras acquires a protein conformation incompatible for Ras–effector binding, thus turning off Ras signaling. *Oncogenic Ras harbors mutations at codon G12, G13, and Q61. These mutations attenuate or completely disable the GAP-activating GTPase activity of Ras, thus leading us to conclude that oncogenic Ras is constitutively active. Nevertheless, oncogenic Ras retains its low intrinsic GTPase activity, which slowly hydrolyzes Ras-bound GTP to GDP. Therefore, oncogenic Ras is not constitutively active *per se*; rather, its active state is prolonged. *Abbreviations*: EGF, Epidermal growth factor; GAP, GTPase-activating protein; GDP, Guanosine diphosphate; GEF, Guanine nucleotide exchange factor; GRB2, Growth factor receptor-bound protein 2; GTP, Guanosine triphosphate; GTPase, Guanosine triphosphatase; Ras, Ras sarcoma; RTK, Receptor tyrosine kinase.