Published in final edited form as:
Nat Rev Cancer. 2010 December ; 10(12): 842–857. doi:10.1038/nrc2960.

Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy?

Dominico Vigil[*], **Jacqueline Cherfils**[‡], **Kent L. Rossman**[*], and **Channing J. Der**[*]

[*] University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center, Department of Pharmacology, Chapel Hill, NC 27599

[‡] Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France

Abstract

There is now considerable and increasing evidence for a causal role of aberrant activity of the Ras superfamily of small GTPases in human cancers. These GTPases act as GDP-GTP-regulated binary switches that control many fundamental cellular processes. A common mechanism of GTPase deregulation in cancer is the deregulated expression and/or activity of their regulatory proteins, guanine nucleotide exchange factors (GEFs) that promote formation of the active GTP-bound state and GTPase activating proteins (GAPs) that return the GTPase to its GDP-bound inactive state. We assess the association of GEFs and GAPs with cancer and their druggability for cancer therapeutics.

Ras proteins (H-, N- and K-Ras) are the founding members of a large superfamily of monomeric small GTPases (20–25 kDa) that regulate diverse cellular processes that include cell cycle progression, cell survival, actin cytoskeletal organization, cell polarity and movement, and vesicular and nuclear transport[1, 2]. The Ras superfamily (>150 members in humans) is divided into five main families based on sequence identity and function: Ras, Rho, Rab, Arf, and Ran (BOX 1).

Box 1

Ras superfamily of small GTPases

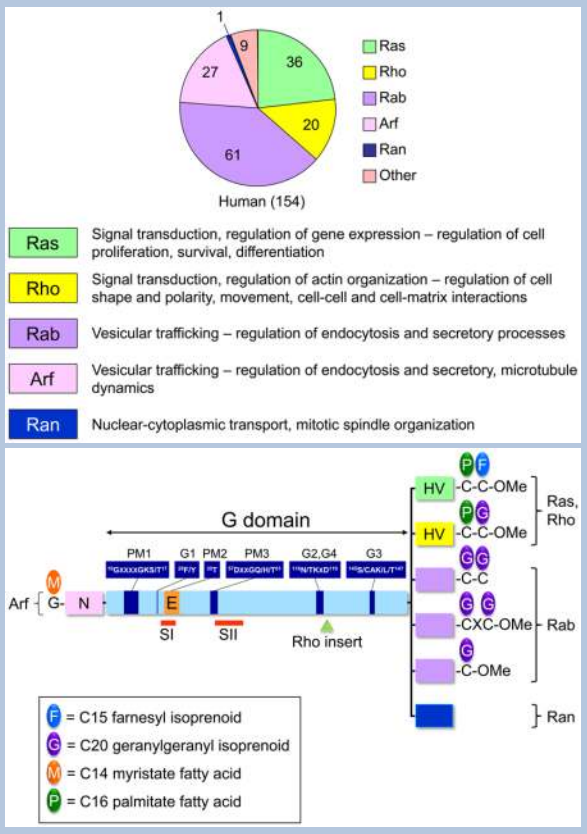
The human Ras superfamily comprised of over 150 members which is divided into five major branches on the basis of sequence and functional similarities[1, 2]. In addition to the three Ras isoforms, other members of the Ras family with important roles in cancer include Rheb and Ral proteins. The ~20 kDa core G domain (corresponding to Ras residues 4–166) is conserved among all Ras superfamily proteins and is involved in GTP binding and hydrolysis[148]. This domain is comprised of five conserved guanine nucleotide consensus sequence elements (Ras residue numbering) involved in binding phosphate/Mg^{2+} (PM) or the guanine base (G). The switch I (Ras residues 30–38) and II (59–76) regions change in conformation during GDP-GTP cycling and contribute to

Correspondence to C.J.D. cjder@med.unc.edu.

Links to websites:
The Cancer Genome Project: http://www.sanger.ac.uk/genetics/CGP/
Smart: http://smart.embl-heidelberg.de/
ClinicalTrials.gov: http://clinicaltrials.gov/
Channing Der's homepage: http://cancer.med.unc.edu/derlab/

Competing interests statement
The authors declare no competing financial interests.

preferential effector binding to the GTP-bound state and the core effector domain (E; Ras residues 32–40). Ras and Rho family proteins have additional C-terminal hypervariable (HV) sequences that commonly terminate with a CAAX motif that signals for farnesyl or geranylgeranyl isoprenoid addition to the cysteine residue, proteolytic removal of the AAX residues and carboxylmethylation of the prenylated cysteine. Some are modified additionally by a palmitate fatty acid to cysteine residues in the HV sequence that contributes to membrane association. Rab proteins also contain a C-terminal HV region that terminates with cysteine-containing motifs that are modified by addition of geranylgeranyl lipids, with some undergoing carboxylmethylation. Arf family proteins are characterized by an N-terminal extension involved in membrane interaction, with some cotranslationally modified by addition of a myristate fatty acid. Ran is not lipid modified but contains a C-terminal extension that is essential for function. Rho proteins are characterized by an up to 13 amino acid “Rho insert” sequence positioned between Ras residues 122 and 123 involved in effector regulation.



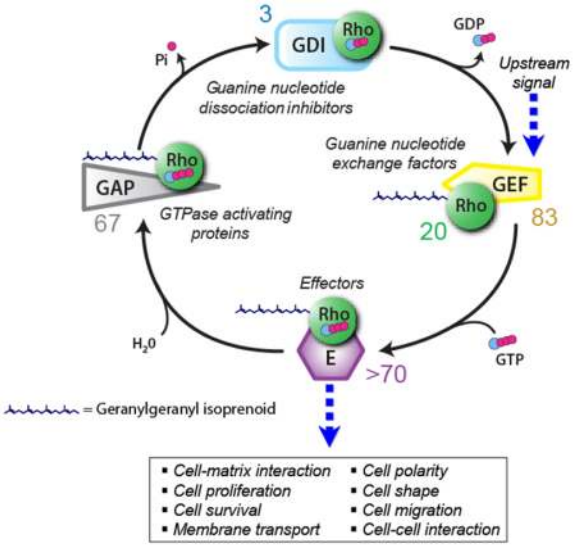
Ras superfamily small GTPases, together with their two key classes of regulatory proteins constitute a three-protein machinery that functions as cellular GDP-GTP-regulated binary switches (BOX 2). Alternation between the active GTP-bound and inactive GDP-bound states of the small GTPase is controlled by guanine nucleotide exchange factors (GEFs), which stimulate the exchange of GDP for GTP, and by GTPase activating proteins (GAPs), which terminate the active state by stimulating GTP hydrolysis[3, 4]. In their GTP-bound state, small GTPases bind effectors to activate biochemical processes. Typically, each small GTPase mediates its functions through association with multiple and functionally distinct effectors, whose selection may depend on the identity of the activating GEF. This may be achieved by each GEF causing a spatially-distinct distribution of GTPase activation and by the function of the GEF as a scaffold that facilitates effector activation. Thus, small GTPases

act as signaling nodes, with multiple input signals converging on GEFs and GAPs and upon GTPase activation, which initiates multiple output signals (FIG. 1). The Rho and Rab families possess a third class of regulatory proteins, guanine nucleotide dissociation inhibitors, which will not be discussed in this review.

Box 2

The GDP-GTP cycle

Ras superfamily proteins possess intrinsic guanine nucleotide exchange and GTP hydrolysis activities. However, these activities are too low to allow efficient and rapid cycling between their active GTP-bound and inactive GDP-bound states. GEFs and GAPs accelerate and regulate these intrinsic activities. Members of the different branches of the superfamily are regulated by GEFs and GAPs with structurally distinct catalytic domains[3, 4, 149–152]. Here we have utilized the Rho family as an example to illustrate the complexity of this process, where multiple GEFs and GAPs may regulate one specific GTPase. For the 20 human Rho GTPases there are 83 GEFs and 67 GAPs and a subset of Rho GTPases are not likely regulated by GEFs and GAPs (e.g., Rnd3/RhoE). Rho GTPases are activated by distinct RhoGEF families. Dbl family RhoGEFs (68) possesses a tandem Dbl homology (DH) catalytic and pleckstrin homology (PH) regulatory domain topology. DOCK family RhoGEFs (11) are characterized by two regions of high sequence conservation that are designated Dock-homology region regulatory DHR-1 and catalytic DHR-2 domains. Two other RhoGEFs have been described (SWAP70 and SLAT) contain a PH but no DH domain (2) and smgGDS (1) is an unusual GEF in that it functions as a GEF for some Rho as well as non-Rho family GTPases. At least 24 Dbl RhoGEFs have been reported to activate RhoA[151]. Rho (and Rab) GTPases are also controlled by a third class of regulatory proteins, Rho dissociation inhibitors (RhoGDI) (of which there are 3) whose main function involves regulation of Rho GTPase membrane association by masking the isoprenoid group.



The best validated connection between small GTPases and cancer comprise the three Ras proteins[5]. Mutational activation of *Ras* is found in 33% of human cancers (collated from COSMIC database)[6]. Consequently, intensive efforts have been made to identify pharmacologic approaches to block Ras function for cancer treatment. To date, no successful “anti-Ras” strategies have reached the clinic. The low micromolar binding affinity of protein kinases for ATP, where potent nanomolar affinity ATP-competitive

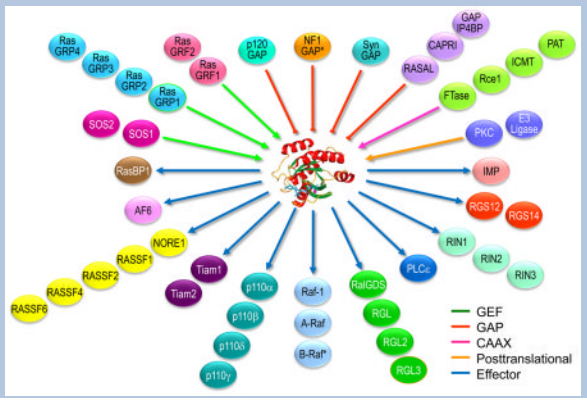
inhibitors have been developed (imatinib for example), has been a very successful avenue for anti-cancer drug development[7]. In contrast, the low picomolar binding affinity of small GTPases for GTP and milimolar cellular concentrations of GTP renders a similar strategy for Ras implausible[8]. Thus, past and current efforts have focused on indirect approaches for disruption of Ras function: inhibition of components that regulate Ras membrane association[9] and inhibition of downstream effector signaling[10] (BOX 3).

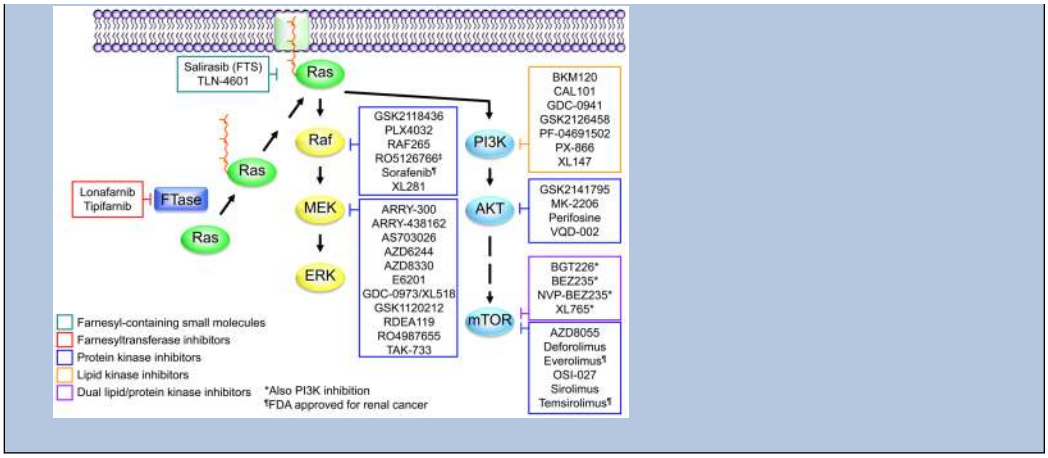
Box 3

Clinical evaluation of candidate anti-Ras inhibitors

Since the identification and development of small molecule inhibitors that directly target Ras have not been successful, a majority of past and ongoing efforts have targeted Ras indirectly, to modulate the functions of proteins that influence or mediate Ras oncogenesis. Shown here are proteins that regulate Ras posttranslational processing, either signaled through the C-terminal CAAX tetrapeptide motif (farnesyltransferase, FTase; Rac converting enzyme1; Rce1; Isoprenylcysteine carboxyl methyltransferase; Icmt) or by protein kinase C alpha (PKCα)-dependent phosphorylation or ubiquitination. Similar to Rho GTPases, Ras proteins are also regulated by multiple GEFs and GAPs. GTP-bound Ras interacts with catalytically-diverse downstream effectors that possess Ras-binding (RBD) or Ras-association (RA) domains. Although shown here as interactions with Ras, some are interactions restricted to specific Ras isoforms. Question mark indicates that more interacting partners are yet to be discovered.

Considerable past efforts centered on the development of FTase inhibitors (FTIs), with many identified, and with two remaining in clinical trial analyses (lonafarnib and tipifarnib). The prenylation of KRAS and NRAS by a related enzyme, geranylgeranyltransferase-I, when farnesyltransferase activity is blocked by treatment with an FTI, proved to be the downfall of FTIs as effective Ras inhibitors. A second class of inhibitor of Ras membrane association is comprised of two small molecules with farnesyl lipid groups (salirasib and TLN-4601) and proposed to compete with Ras for membrane-associated docking proteins for the Ras isoprenoid group. Efforts to target Ras effector signaling first centered on the Raf-MEK-ERK MAPK cascade. Small molecule protein kinase inhibitors of MEK1/2 and later Raf have been developed, with many now in clinical evaluation. More recently, inhibitors of the p110 catalytic subunits of PI3K, AKT and mTOR have entered clinical trials and two mTOR inhibitors have been FDA approved for renal cell cancers. Compiled from information at http://www.clinicaltrials.gov.





Beyond Ras, the aberrant function of an expanding roster of Ras superfamily proteins has been implicated in human cancer growth and development. However, whereas mutational activation of Ras is seen commonly in human cancers, direct mutation of other Ras superfamily GTPases is not seen frequently. Instead, the deregulated gene expression and/or deregulated protein function of GEFs and GAPs, in particular for specific Ras and Rho family proteins but also Arf[11, 12], have been found to play important roles in cancer (supplementary information S1 and S2 (tables) lists the mechanisms and roles of GEF and GAP deregulation in human cancers). Genome-wide sequence analyses of breast, colon, pancreatic and brain cancers have now been completed[13–16] and a search of the COSMIC database reveals isolated mutations in numerous GEFs and GAPs from sequence analyses of 173 regulators of Ras superfamily GTPases. However, whether these mutated genes are passengers or drivers of oncogenesis, whether they encode proteins with altered function, are not known for most of these situations.

In this review, we summarize representative studies in which aberrant GEF or GAP function is observed in cancer cells and where sufficient validation has been done to show causal roles of individual GEFs or GAPs in the aberrant growth properties of human cancer cells or in mouse models of cancer. We will focus primarily on Ras and Rho family GTPases and summarize the current evidence validating a causal role for their regulators in causing aberrant small GTPase function in human cancer or cancer-related processes. We also discuss the issues surrounding pharmacologic manipulation of GEF or GAP function. Our conventional targets and approaches for anti-cancer drug discovery have been hampered by tradition and past success. While it is still early days in target validation, and our current success in therapeutic targeting of these regulators is more proof-of-concept than clinical reality, we believe that GEFs and GAPs hold exciting prospects for cancer therapy.

GEFs in cancer

The potential involvement of GEFs in cancer was first suggested by the isolation of RhoGEFs[17,18–20] and later RasGEFs[21–23] as transforming proteins in expression library functional screens using genomic DNA or mRNA derived from human cancer cells. However, the transforming RhoGEFs identified were activated by genomic deletion of coding sequences during the process of experimental manipulation rather than due to genetic events that occurred in the cancer cells[24]. Nevertheless, these observations supported their potential role as oncogenes in cancer development. Since GEF activation is the most common mechanism for signal-mediated GTPase activation, the theme that has emerged is that aberrant signaling from growth factor receptors, in particular, transmembrane receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs), leading to aberrant GEF

regulation, contributes to small GTPase activation in cancer. Another common mechanism of aberrant GEF activation is upregulated gene expression, and to a lesser degree, missense mutations and the consequent expression of catalytically-altered GEFs (supplementary information S1). While it is possible that there is upregulation of Ras or Rho GTPase activity by multiple GEFs simultaneously or inactivity of multiple GAPs, since there are many family members that could regulate the same GTPases, we present examples where the roles of individual GEFs or GAPs are clear.

RasGEFs associated with cancer

RasGEFs activate Ras and additionally may also act as GEFs for the related Rap or R-Ras, but not Ral, subfamily members of the Ras family. The most common mechanism by which RasGEFs are involved in cancer involves their activation by growth factor-activated cell surface RTKs or GPCRs. This is best represented by the "classical" Ras signaling pathway, where activation of the epidermal growth factor receptor (EGFR) causes activation of wild type Ras through GRB2-mediated activation of the two son of sevenless (Sos1 and Sos2) RasGEFs. EGFR overexpression, mutational activation or hyperactivation by autocrine mechanisms are commonly seen in many cancers, leading to persistent Ras activation[25]. RTK and GPCR activation can also cause Ras activation through downstream activation of phospholipase Cγ (PLCγ) and PLCβ, respectively. PLC activation and diacylglycerol production directly activates the Ras guanyl releasing protein (RasGRP) subfamily of RasGEFs[26]. Mutationally activated Ras may also still require RasGEF activity, perhaps to activate wild type Ras isoforms concurrently[27].

Germline gain-of-function mutation of SOS1 RasGEF has been observed in Noonan syndrome (13%), a developmental disorder also associated with increased risk of cancer[28, 29]. This implies that SOS1 could be an oncoprotein. However, an extensive sequence analysis of samples from 810 primary malignancies found only three *SOS1* mutations and concluded that *SOS1* mutational activation is not common in human cancers[30]. Hence, similar to other mutations found in developmental syndromes that activate K-Ras, Raf-1, and MEK1/2, the SOS1 mutations are weakly activating and may not be potent enough to cause cancer[31]. Mutations in other RasGEFs are also rare in cancer (Supplementary information S1 and COSMIC).

Finally, another association between RasGEFs and cancer involves their roles as downstream effectors of Ras (BOX 3). PLCε, a downstream effector of Ras[32–34], contributes to mutant *HRAS*-mediated skin tumor formation; whether the RasGEF function is relevant for this role is not known. However, caution in interpreting these experiments is warranted, as followup studies found that PLCε loss reduced a stromal tissue inflammatory response and that isolated PLCε-deficient keratinocytes displayed no reduction in proliferative capacity[35]. Hence, whether PLCε loss caused reduced tumorigenesis in its role as a critical downstream Ras effector in cancer cells, or serves a tumor cell autonomous function, is unclear. Additionally, while there is evidence that PLCε can activate Ras, most evidence supports its role as a Rap activator[36].

Other CDC25 domain-containing RasGEFs that are not activators of Ras and instead, are activators of the RalA and RalB small GTPases (also members of the Ras GTPase family) include RalGDS, Rgl2(Rlf), Rgl2 and Rgl3[37] (Supplementary FIG. 1) Mice deficient in RalGDS show impaired tumor formation in mutationally-activated *HRAS*-driven skin tumor formation[38]. Rgl2 overexpression was described in pancreatic tumors and cell lines and suppression of Rgl2 expression impaired tumor cell anchorage-independent growth and Matrigel invasion[39]. Moreover, Ral is activated in human tumors and promotes the growth of bladder, pancreatic, prostate and other cancers[40–43]. Ral GTPases function as GDP/GTP-regulated binary switches that are regulated by distinct GEFs and GAPs and activate distinct

downstream effectors that regulate endocytosis, exocytosis and actin organization. Thus, targeting GTPase activation by GEFs or GTPase activation of GEF effectors are two potential applications of GEF inhibitors (Supplementary FIG. 2).

RhoGEFs associated with cancer

It is now clear that Rho GTPases play a major role in many different aspects of tumorigenesis[44, 45]. Most Rho GTPases promote tumorigenesis, and thus hyperactivation of their GEFs would likewise be oncogenic. However, there are examples, such as RhoB, that exert tumor suppressor properties, and thus activation of their GEFs would likewise be considered tumor suppressive. Unlike Ras, which is mutated in a large percentage of human cancers, mutations in Rho GTPases are rare. Instead, Rho GTPase hyperactivation occurs through overexpression, loss of GAP-mediated inactivation, and upstream activation (FIG. 2) or overexpression of the RhoGEFs. Below we highlight some examples, with others summarized in Table S1.

Vav RhoGEFs have been implicated in the growth of several cancers. First, the normally haematopoietic cell-specific *VAV1* was overexpressed in pancreatic carcinoma cells as a consequence of promoter demethylation, leading to Rac activation and signalling[46]. VAV1 was activated by Src-dependent phosphorylation, which in turn was activated by EGFR, and led to activation of a Rac-Pak-NF-κB signalling pathway and cyclin D1 upregulation. RNAi depletion of *VAV1* abrogated anchorage-independent growth *in vitro* and tumour growth in mouse xenografts. Moreover, VAV1 expression in pancreatic carcinomas was associated with decreased survival.

The related RhoGEF VAV2 is hyperactivated in head and neck squamous cell carcinoma (HNSCC) through an autocrine loop dependent on EGFR. Knockdown of *VAV2* inhibited RAC1 activation and EGFR-stimulated invasion through Matrigel[47]. Another member, *VAV3,* was overexpressed at the mRNA and protein level in human glioblastomas compared to unmatched normal brain samples, and knockdown in cell lines decreased migration *in vitro* and in an *ex vivo* organotypic brain slice invasion assay[48]. Finally, $Vav2^{-/-}Vav3^{-/-}$ double knockout mice had reduced xenograft tumor growth when transplanted with lung or melanoma cells, in part due to deficient angiogenesis, largely due to a defect in tumor-induced endothelial cell migration[49]. This suggests a role for RhoGEF signaling in the host microenvironment, highlighting the many ways in which RhoGEFs may affect tumorigenesis.

A Rac-specific GEF, phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1 (PREX1), has been implicated in prostate cancer cell invasion[50]. The three Rac isoforms RAC1-3 are known to be important in many cancers by through a variety of ways, including stimulating migration and invasion through induction of lamellipodia as well as growth[51]. *PREX1* gene and protein expression was highest in metastatic prostate cancer cell lines and protein expression was highest in metastatic prostate tumor tissue. Suppression of endogenous *PREX1* expression in the PC-3 metastatic prostate cancer cell line inhibited Rac activity and reduced ligand-stimulated cell migration and invasion *in vitro* and ectopic *PREX1* overexpression in PC-3 cell xenografts did not enhance tumourigenic growth but did promote metastasis to lymph nodes. In addition, *PREX1* overexpression was associated with activation of ERK-MAPK signalling in melanomas[52]. Finally, a recent study identified the related PREX2 protein as a binding partner for the PTEN tumor suppressor[53]. PTEN is a lipid phosphatase that converts phosphatidylinositol-3,4,5-trisphosphate to phosphatidylinositol-4,5-disphosphate and thus antagonizes PI3K activity. *PREX2* mRNA was overexpressed in *PTEN* wild type breast cancers and RNAi depletion reduced the levels of activated AKT and impaired the growth of PTEN wild type tumours. Taken together,

these studies with Rac-selective GEFs underscore their importance in migration, invasion and metastasis.

ECT2, an activator primarily of RhoA, but also of Rac and Cdc42[54, 55], mRNA or protein has been found to be overexpressed in a variety of human tumor cell lines and tissues, including lung and esophageal squamous cell carcinomas[48, 56–59], and correlated with poor prognosis[57, 58]. *ECT2* overexpression at the mRNA and protein level was found in patient glioblastoma samples compared to non-matched normal brains and RNAi-mediated suppression of *ECT2* expression in glioblastoma cells reduced migration and growth rates *in vitro* and invasion in an *ex vivo* organotypic rat brain slice model[48]. Finally, a recent study found *ECT2* mRNA and protein overexpression in non-small cell lung carcinomas (NSCLCs)[60]. ECT2 expression was mislocalized to the cytoplasm and was associated with Rac, and surprisingly not RhoA, activation. RNAi-mediated knockdown of ECT2 blocked the anchorage-independent growth and Matrigel invasion in vitro, and tumor xenograft growth in vivo, of NSCLC cell lines.

Three different RhoGEFs are structurally-mutated in human cancers by chromosome rearrangement and formation of chimeric fusion proteins. One involves the ARHGEF12 (also known as LARG) RhoA-specific GEF, which was identified initially in tumor cells from a patient with acute myelogenous leukemia[61]. The rearrangement encodes a MLL-ARHGEF12 fusion protein that retains the DH-PH catalytic domains of ARHGEF12. Whether the fusion protein represents a constitutively activated variant of ARHGEF12 has not been determined. LARG and the related PDZ domain-containing RhoGEFs (p115-RhoGEF and PDX-RhoGEF) may also be activated by GPCRs that are coupled to $G\alpha_{12/13}$ or by $G\alpha_{12/13}$ overexpression (FIG. 2)[62, 63]. The second example is the BCR-ABL1 fusion protein encoded by the translocation associated with the Philadelphia chromosome found in 90% of chronic myelogenous leukemias. BCR possesses a RhoGEF and a RhoGAP domain and ABL1 is a protein tyrosine kinase. In the resulting BCR-ABL1 chimera, the RhoGEF but not RhoGAP domain is retained and fused to a truncated ABL1, resulting in constitutive activation of the kinase activity critical for BCR-ABL1-mediated oncogenesis. BCR-ABL1 transforming activity, as measured by anchorage-independent growth, is also dependent, in part, on the RhoGEF activity, which results in activation of RhoA[64]. Finally, a third RhoGEF, TRIO, is activated in adult T-cell leukaemias by alternative splicing, which results in a truncated protein with the second catalytic DH domain attached to a unique 15-residue peptide (designated TGAT)[65]. The TGAT transcript was detected in peripheral blood mononuclear cells of 14 of 21 T-cell leukaemia patients, but not in four control subjects. Ectopic expression of TGAT caused tumorigenic transformation of NIH 3T3 mouse fibroblasts, although no evidence for T-cell leukemia growth was determined.

TIAM1, a Rac-specific GEF, is associated with a variety of cancer types. First, it can function as a downstream effector of Ras[66]. *Tiam1*$^{-/-}$ mice had impaired carcinogen-induced *HRAS* activation and squamous cell skin carcinoma formation, including fewer tumors and smaller tumor size, although the tumors that did form metastasized more readily[67]. Second, mouse models of APC-induced colon cancer and ERBB2 (also known as Neu)-induced mammary cancer also show impaired tumor formation in the absence of *Tiam1*, although in the case of the mammary cancer model the tumors were more invasive[68, 69]. Third, there are several reports of altered TIAM1 (mutation and overexpression) in various human cancers (supplementary information S1). Importantly, although TIAM1 may be important in tumor initiation, the increased malignancy and invasion in the skin and mammary models upon loss of TIAM1 and the observation that TIAM1 protein expression is lower during breast cancer progression[70] suggests that TIAM1 may act as a metastasis suppressor, and thus inhibiting its activity in some settings may not be beneficial.

In addition to the DH-PH family of RhoGEFs, there is evidence for the aberrant function of DOCK family RhoGEFs in cancer. This family is comprised of 11 members in humans and possess a structurally-distinct RhoGEF catalytic domain[71]. Interestingly, to date, DOCK family proteins activate Rac or Cdc42 but not RhoA[72], although the structure of the RhoGEF catalytic DHR-2 domain bound to Cdc42 suggests that they may activate a broader spectrum of Rho GTPases[73].

DOCK1 (also known as DOCK180) is a RacGEF and its overexpression together with its activator, ELMO, was found to promote glioblastoma cell invasion *in vitro* and *in vivo*[74]. DOCK RhoGEFs are also implicated in distinct facets of melanoma cell migration. DOCK10, a Cdc42GEF, was identified as a key regulator of protease-independent amoeboid melanoma cell migration[75]. In contrast, protease-dependent mesenchymal-type movement was driven by DOCK3, a RacGEF[76]. These results suggest distinct roles for Cdc42 and Rac in promotion of tumor cell migration.

ArfGEFs associated with cancer

ARF1 and ARF6, the most studied isoforms of the Arf GTPase subfamily, are active regulators of proliferative and/or invasive properties of cancer cells, notably in melanoma and breast cancer cell lines[77, 78], and have also been linked to resistance to apoptosis[79]. Their functions in invasion may stem from their role at the crossroad between membrane trafficking and Rho GTPase-controlled actin remodelling, notably in the formation of invadopodia[12, 80]. Several subfamilies of ArfGEFs have recently emerged as candidate regulators that support invasion of cancer cells. GEP100 (also known as BRAG2), a GEF for Arf6, has been implicated in breast cancer invasion[81]. GEP100 was overexpressed in primary ductal breast carcinomas, commonly with EGFR overexpression, with overexpression correlating with higher grade tumours[81]. RNAi knockdown of GEP100, but not other ArfGEFs, reduced breast cancer cell invasion through Matrigel *in vitro* and reduced metastasis to the lung in a mouse model of breast cancer[82]. The expression of EFA6, an Arf6 GEF, is increased glioma tissue samples, and its expression in a human gliobastoma cell line enhanced ERK-dependent invasion[83]. Overexpression of ARF6 has been reported in highly invasive breast cancer cell lines[82]. This may result from loss of expression of FBX8, an unconventional ArfGEF that mediates the ubiquitination of Arf6 and suppresses its activity[84]

GAPs in cancer

GAPs are the flip-side of the coin to GEFs, and although less is known about them in general, many studies have demonstrated their crucial roles in curtailing GTPase activity in cancer. Since activation of GEFs for the Ras superfamily GTPases has many roles in cancer, it is perhaps not surprising that loss of GAP activity allows uncontrolled GTPase activity and can promote cancer. We discuss some pertinent examples that demonstrate their importance and ways in which their activities are regulated (Supplementary information S2). Even though in general GAPs are tumor supressors, there are also examples of oncogenic GAPs.

RasGAPs

The mutants of Ras are missense mutations (primarily at residues 12, 13, or 61) that impair the intrinsic and GAP-stimulated ability to hydrolyse GTP, rendering Ras constitutively GTP-bound and active in the absence of extracellular stimuli. Although the intrinsic activity of the GAPs is not altered in these cancers, the fact that they can no longer deactivate Ras indirectly implicates them in the oncogenic process. Although the RasGAPs in this case would not be considered drivers of this process, one of the earliest unsuccessful efforts made

to develop anti-Ras drugs was to develop small molecules that restored GAP sensitivity to mutant Ras.

Germline mutational loss of the *NF1* tumor suppressor, which encodes the RasGAP neurofibromin, is found in patients with neurofibromatosis type 1 (NF1)[85, 86]. Two recent sequencing studies established frequent somatic mutation of *NF1* in glioblastoma (15–23%), representing the fifth most frequently mutated gene in this cancer[15, 87]. Although some of the mutations are null mutations or truncations resulting in loss of RasGAP catalytic function, consistent with its role as a tumor suppressor, the function of the several point mutations found remains to be determined. Post-translational loss of neurofibromin - induced by protein kinase C-mediated proteasomal degradation – has also been observed in sporadic glioblastomas[88]. Since the only known catalytic function of neurofibromin is its RasGAP activity, the functional consequences of neurofibromin loss is attributed to the observed hyperperactivation of wild type Ras. However, since the RasGAP domain comprise but a small portion of the total protein, non-Ras functions associated have been speculated.

The loss of other RasGAPs, Ras homolog enriched in brain (RHEB1) and RHEB (also known as RHEB2)[89], is associated with tuberous sclerosis complex (TSC), which is a syndrome characterized by the formation of tumor-like lesions, hamartomas, in kidney, lung, brain and skin[90]. This autosomal dominant disease is caused by germline and somatic mutational loss of either *TSC1* (harmartin) or *TSC2* (tuberin). Tuberin contains the RhebGAP catalytic domain whereas harmartin stabilizes tuberin and prevents its degradation; hence, the harmartin:tuberin complex is required for RhebGAP activity[91]. Although the tumor phenotype is distinct, the increased incidence of renal cell and other cancers in Eker rats, which contain germline heterozygous *Tsc2* mutations that inactivate the RhebGAP activity, supports the role of *TSC2* as a tumor suppressor[92–94]. Loss of *TSC1/2* RhebGAP function results in Rheb activation and persistent activation of its downstream effector, mTOR. The functions of the harmartin-tuberin complex as a RhebGAP are also regulated by phosphorylation, in particular AKT phosphorylates and thereby inactivates tuberin. Thus, genetic and biochemical activation of the PI3K signaling pathway (e.g., *PIK3CA* gain-of-function or *PTEN* loss-of-function mutations) in cancer cells can also cause Rheb-mediated activation of the rapamycin-sensitive mTOR complex 1 (mTORC1). mTOR regulates mRNA translation and ribosome biogenesis, regulating cell cycle progression, cellular proliferation and growth, autophagy and angiogenesis.

RhoGAPs in cancer

One RhoGAP in particular has stood out recently as having a central role as a tumor suppressor in several different cancer types: deleted in liver cancer 1 (DLC1, also known as ARHGAP7)[95, 96]. *DLC1* was first discovered as a gene which is under-represented in a human hepatocellular carcinoma (HCC) specimen and is deleted in HCC cell lines and tumors[97]. Subsequent studies found *DLC1* was deleted or transcriptionally silenced by promoter methylation in many cancer types (Supplementary Table S2). A comprehensive analysis of the genomic loss of *DLC1* showed that heterozygous loss in tumors happens at a rate that approaches that of *TP53* (which encodes p53) mutation or loss in breast, lung, liver, colon and pancreatic tumors[98]. Additional studies identified two genes that encode DLC1 related proteins, *DLC2* (also known as *STARD13*)[99, 100] and *DLC3* (also known as *STARD8*)[101], and the expression of both genes lost in a variety of human cancers, although it is unknown whether they are lost separately from or concurrently with DLC1. Finally, protein-protein interactions with 14-3-3 isoforms and another GAP, p120RasGAP, may also cause loss of DLC1 function[102, 103].

Together with loss of expression in cancer, genetic and biochemical analyses in cell culture and mice provide functional evidence for DLC1 as a tumor suppressor. Ectopic re-expression of DLC1 in *DLC1*-deficient human tumor cell lines suppressed proliferation, anchorage-independent growth, invasion through Matrigel and tumor formation in xenograft mouse models of a variety of cancer types[104–107] and re-expression in breast cancer cell lines reduced metastasis in a mammary fat pad orthotopic injection model[108]. In an *ex vivo* mouse model of *Myc*-induced tumorigenesis, knockdown of endogenous DLC1 accelerated the onset of tumorigenesis and resulted in more aggressive tumors that resembled aggressive human HCC, providing strong evidence for the role of DLC1 as a tumor suppressor[98]. Similarly, ectopic expression of DLC2 or DLC3 in expression-deficient human tumor cell lines caused impairment in tumor cell growth[99, 101].

Although DLC proteins are multi-domain proteins comprised of sterile alpha motif (SAM), RhoGAP and StAR-related lipid transfer (START) domains, evidence supports the crucial role of the RhoGAP domain in DLC1 tumor suppression. The substrates of DLC1 are RhoA, RhoB, RhoC, and to a lesser degree CDC42, but not Rac[107] and cell-based studies suggest that RhoA activation is a major consequence of DLC1 loss of function. In the *ex vivo* mouse model of *Myc*-induced liver tumorigenesis, activated RHOA phenocopied loss of DLC[98]. Because of the high frequency of reduced DLC expression in many different types of tumors and the functional evidence that DLC1-3 are tumour suppressors, inactivation of which primarily inactivates Rho GTPases, alterations in this RhoGAP protein family represent the most common mechanism of altering Rho GTPase activity in human cancer.

Surprisingly, in contrast to the many RhoGEFs that are altered in cancer, aside from the DLC family, there is limited evidence for the role of other RhoGAPs in cancer. However, putative tumor suppressors, such as GRAF, ARHGAP25, ARHGAP5 and ARHGAP8 may exist (Supplementary Table S2), but more work is required to validate these and to determine whether their RhoGAP activity is crucial. It could be that in many cancers, GAP activity is normal, but that the excessive activation through GEFs or GTPase overexpression overrides normal GAP-mediated inactivation.

ArfGAPs in cancer

Two subfamilies of ArfGAPS, AGAPs and ASAPs, have been implicated in oncogenesis[11, 109], although whether this is through their GAP activity towards Arf GTPases is not yet established. *AGAP2* (also called PIKE, Centaurin γ1 or GGAP2) is amplified and overexpressed in glioblastoma, prostate carcinoma and other cancers[79, 110, 111]. Cancer cells with AGAP2 overexpression resist apoptosis more strongly than those with normal levels, and ectopic expression of AGAP2 activates the AKT pathway and inhibits apoptosis in human glioblastoma cells, suggesting that the oncogenic properties of AGAP2 are achieved through the AKT pathway[79, 110–112], AGAP2 is a multi-domain protein, which includes a domain remotely related to small GTPases[113] in addition to its ArfGAP domain. Whether these domains and/or the GAP domain are involved in the oncogenic effect remain unclear.

ASAP1 (also called AMAP1, DDEF1 or Centaurin β4) overexpression is associated with invasive phenotypes in melanoma, prostate cancer and breast cancer cells[114–116]. ASAP1 has been best studied in breast cancer cells, where it co-localizes with Arf6 to invadopodia, and it is associated with proteins involved in actin remodeling[116]. A peptide derived from the C-terminal SH3 domain [G] of ASAP1 was able to block breast cancer cell invasion and metastasis[117]. A related ArfGAP, ASAP3 (also called UPCL1, DDEFL1 or ACAP4), was identified by its up-regulation in hepatocellular carcinomas[118] and is involved in migration and invasion in a mammary carcinoma cell line, although it is not involved in invadopodia formation its localization is very different than that of ASAP, thus the two likely play nonredundant roles[119].

In summary, as with GEFs, there are a diversity of genetic and biochemical mechanisms by which GAP function, most commonly as tumor suppressors, is deregulated in cancer. However, to date, despite the large numbers of GAPs for Ras and Rho GTPases, those that have been implicated in cancer remain limited. Perhaps this reflects the fact that there has traditionally been a greater focus on GEFs or perhaps there is greater functional redundancy with GAPs, making it unlikely that loss of function of any one GAP will be sufficient to cause significant deregulation of GTPase activity.

Targeting GEFs and GAPs: are they druggable?

As with most proteins propagating information by intracellular protein-protein interactions, with large contact surfaces that lack the grooves and pockets for small molecule interactions, GEFs and GAPs are not classically considered as "druggable" targets[120]. However, it is important to remember that the development of ATP-competitive inhibitors of protein kinases, which were once considered undruggable, now comprise the major class of clinically-useful signal transduction anti-cancer drugs. Hence, druggability is defined primarily on current success and not a static concept. Instead, the strength of target validation, rather than conventional wisdom, should prioritize efforts to establish target druggability.

GEFs: targets for anti-cancer drug discovery?

With increasing evidence for aberrant GEF or GTPase activity in cancer, a logical issue is whether these regulatory proteins are attractive targets for anti-cancer drug discovery, particularly those GEFs that exhibit gain-of-function mutations or are overexpressed. Additionally, GEF activation defines where and when a GTPase is activated and probably what the downstream events are, and are thus likely to convey high signaling specificity. This may limit off-target effects when inhibited. The structures of representative GTPase-GEF complexes have been determined[121–123]: all feature a very large protein-protein interface resulting from the structural remodeling of the small GTPase upon binding. The shape, structural dynamics and chemistry of GEF-GTPase interaction surfaces are thus very different from those of catalytic sites of enzymes, such as the ATP-binding site of signaling kinases, and may therefore appear inappropriate for small molecule binding. However, despite this perception, below we summarize experimental evidence indicates that it may be feasible to develop small molecule inhibitors of GEFs.

Brefeldin A (BFA) is a natural product isolated from the fungus *Eupenicillium brefeldianum* and is the first known inhibitor of a GEF. BFA was discovered in the late 1950's[124] and some 30 years later demonstrated to inhibit trafficking at the Golgi network by blocking the activation of Arf GTPases by Sec7 domain containing ArfGEFs, specifically Arf1 and Arf5[125, 126]. The molecular basis for this activity took another decade to be resolved by a combination of yeast genetics, biochemistry and structural biology[127–129]. BFA targets the complex between Arf-GDP and the catalytic domain of the ArfGEF (the Sec7 domain) at the beginning of the exchange reaction and freezes the complex in an abortive conformation that cannot proceed to nucleotide exchange (FIG. 3)[127, 130, 131]. Despite a modest apparent inhibition constant of 15 μM, and a stabilization of the Arf-GDP-Sec7 complex by only a factor of 10, BFA is remarkably efficient in live cells due to the nature of its inhibition mechanism. The inhibitor contact both Arf-GDP and the ArfGEF in the abortive complex, k which allows it to have a restricted specificity for a subset of both ArfGEFs and Arf proteins. On the ArfGEF side, BFA-sensitivity depends on a small number of residues in the BFA-binding site that differ, either alone or combined, between BFA-sensitive and BFA-insensitive ArfGEFs. A remarkable property of BFA is that is also discriminates between Arf1-GDP and Arf6-GDP, the major cellular Arf isoforms, although the two proteins have the same sequence in the BFA-binding site - yet probably not the same structure and/or

structural dynamics. BFA has also demonstrated a number of anti-cancer effects in cells, which in light of these mechanistic studies, are thus likely to result from its impairment of ArfGEF functions.

The extensive analysis of the mechanism of action of BFA led to the general concept of 'interfacial inhibition', which refers to inhibitors that act by stabilization of protein complexes and target regions in or near interfaces[128] (FIG. 3b). Some inhibitors of natural original origin already used in the clinic have been recognized as interfacial inhibitors, such as the anti-cancer drugs vinblastin or camptothecin, suggesting a novel avenue to therapeutic intervention that has started to be explored[132].

LM11 was discovered by an *in silico* screen based on this concept, and was shown to target an interfacial depression at the surface of the complex between Arf1-GDP and BFA-insensitive GEFs such as ARNO and to block ARNO-dependent cellular migration[131]. A few other promising examples of cell-active small molecule ArfGEF inhibitors have been selected by *in vitro*[133, 134] and phenotypic screens[135]. These studies demonstrate that despite the high homologies that are found within a given GEF family, GEF-specific inhibitors can be developed. Therefore, the specific flexibility and conformational changes that characterize small GTPase-GEF complexes are likely to be advantageous to drug development, notably for interfacial inhibitors. However, the design of high throughput biochemical assays to screen effectively for such inhibitors remains a challenge[132].

There has also been a recent increase in discovery of inhibitors of Rho GTPase activation. Inhibitors that target specific RhoGEFs have been discovered by high throughput screens. The first example was an aptamer screen, in which peptides coupled to thioredoxin were selected in yeast for their binding to the catalytic DH2 domain of TRIO[136]. This identified a potent inhibitor of TRIO, which was subsequently optimized to inhibit its oncogenic splice variant TGAT[137]. The corresponding optimized peptide was active in cells *in vitro* and in reducing TGAT-induced tumour formation in nude mice xenograft models. Another assay screened a small chemical compound library by monitoring the interaction of the GTPase with an effector in the presence of a co-expressed GEF[138]. This 'yeast 3-hybrid assay' identified several inhibitors of RhoG activation by TRIO. One of these, ITX3, was specific and active in cell-based assays[139]. Screening using a fluorescence polarization guanine nucleotide-binding assay also identified small molecule inhibitors of ARHGEF12 (LARG)-stimulated RhoA nucleotide binding *in vitro*[140]. Although the inhibitors and aptamers discovered in these screens were of low potency, they support the potential for identifying GEF-targeted inhibitors.

Another related example of a way to target GTPase activity is through targeting the surface of GTPases that is required for GEF activation. Through computational screening of the surface of Rac1 known to interact with GEFs, the small molecule NSC23766 was discovered, which inhibited activation of Rac1 by the Rac-specific GEFs Trio and Tiam1, but not GEF activation of RhoA or Cdc42 *in vitro* and in cells[141]. Using a similar strategy, and utilizing structural information from NSC23766 in complex with Rac1, five additional small molecules structurally unrelated to NSC23766 were discovered that could specifically block Rac activation by GEFs[142]. These molecules do not directly target GEFs, and are likely to lack GEF specificity since they would block the surface of GTPases and thus activation by a variety of GEFs. They could nonetheless provide an interesting approach to block GEF activation of Rho or other small GTPases important in cancer.

GAP-targeted therapies

RasGAPs stimulate the intrinsic GTPase activity of Ras by up to 10^5-fold, but have virtually no effect oncogenic Ras mutants[143]. Therefore, one strategy has been to identify small

molecules that restore the ability of RasGAPs to work on mutant Ras. However, despite great effort, this was unsuccessful, likely because oncogenic mutations disturb the active site of Ras, preventing the proper transition state that is needed for GAP-mediated hydrolysis[144]. Thus, even if the GAP activity of RasGAPs was increased by small molecules, Ras will likely still be refractory to the higher activity. The involvement of GAPs in cancer is most commonly associated with loss-of-function and hence they exhibit properties of tumor suppressors, although as listed in Supplementary Table S2, some GAPs may have oncogenic properties and could thus be drug targets. Since it is traditionally easier to develop small molecule antagonists rather than agonists, GAPs are less attractive targets. Instead, since loss of GAP function leads to GTPase activation, most efforts are focused on blocking the persistent GTPase effector signaling that occurs.

There is limited but promising evidence that small molecule modulators of Ras superfamily GAPs may be possible to develop. High throughput screening identified small molecule inhibitors of RGS domains, which are GAPs for heterotrimeric G proteins[145]. Despite their low structural homology to RasGAPs, they share a similar enzymatic transition state[144], suggesting that this could be a starting point for the design of Ras superfamily GAP inhibitors. One class of RhoGAPs, the Rac-selective chimaerins (CHN) possess C1 zinc finger domains that bind diacyglycerol, a cofactor for their activity[26]. Therefore, small molecules that bind C1 domains may activate their GAP activities, causing downregulation of Rac GTPase activity[26]. While such a therapeutic approach will be complicated by the existence of other proteins with C1 domains (e.g., RasGRP), there is evidence that C1 binding molecules can have some degree of selectivity for a subset of C1-containing proteins. This approach may be a therapeutic option for cancer where there is RacGEF-mediated activation of Rac.

Future Directions

We have highlighted key evidence for the role of aberrant expression and function of GEFs and GAPs of Ras superfamily small GTPases in cancer, with an emphasis on the two key early steps in cancer drug discovery, target validation and druggability. With the continued application of genome-wide analyses of cancer cells, additional correlative evidence for aberrant GEF and GAP expression and function is expected to continue at a rapid rate although validation of their functional importance in cancer will be a rate-limiting factor. Even the current body of experimental evidence validating GEFs and GAPs will require more rigorous validation. While RNAi-based analyses have contributed critical validation, the multi-domain and multi-functional nature of GEFs and GAPs emphasizes that caution must be exercised in simply concluding that any phenotypic alterations are due solely to their roles in regulating small GTPase GDP-GTP cycling. For example, RalGDS can activate AKT independent of its RalGEF function[146]. Is the impaired *HRAS*-driven skin tumor formation due to ablation of RalGDS expression due to loss of Ral or AKT activation, or both? Rescue experiments with carefully designed GEF or GAP domain-impaired mutants are needed to access possible GEF/GAP-independent functions of these regulatory proteins.

Furthermore, while mouse model analyses where a deficiency in a GEF or GAP is achieved at the onset of tumor formation provide important validation, these studies validate more the preventative value rather than the therapeutic value with a pre-existing tumor. For example, TIAM1 was shown to be necessary for initial growth of *HRAS*-induced skin tumors, but mice lacking it had more aggressive tumors when they did arise[67]. Finally, genetic ablation of a target is not equivalent to pharmacologic inhibition of a target. This is demonstrated dramatically with studies that showed that preventing Ras binding to PI3K but not

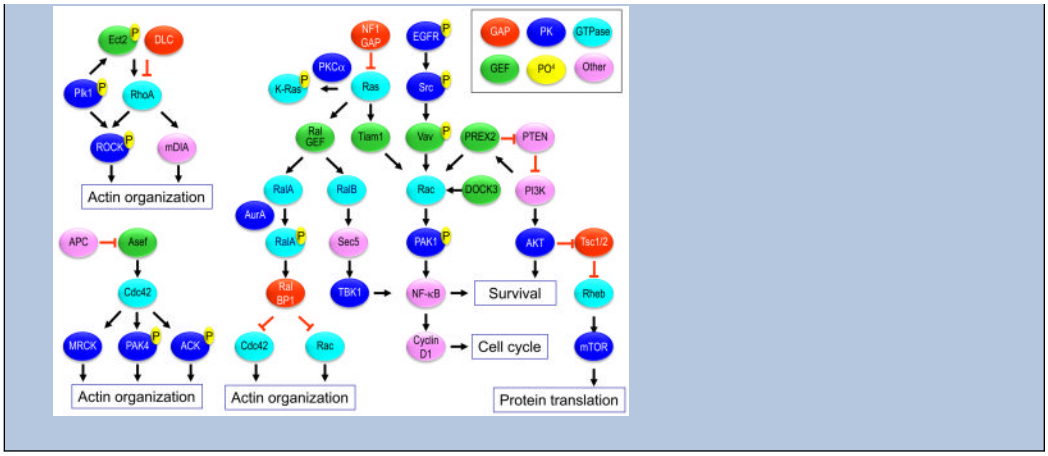
pharmacologic inhibition of PI3K was effective in preventing mutant *KRAS*-induced lung tumor formation[147].

Regarding druggability, it is still very early days in this phase of drug discovery, with the current body of evidence more proof-of-concept and less one of identifying promising leads for clinical evaluation. The lessons learned from BFA currently provide the best evidence for the tractability of GEFs. Perhaps chemical libraries based on such natural products will be a more fruitful direction than the traditional use of libraries based on chemical structures based on past success with enzymes and GPCRs. As the processes and paradigms of drug discovery continue to evolve, so will the definition of druggability. With advances in the use of structural information in virtual screening, structure-based design, fragment-based library screening, coupled with functional screens focused on protein complexes rather than isolated proteins, perhaps GEFs and GAPs can be rendered druggable. That protein kinases, currently the "low hanging fruit" of anti-cancer drug discovery, may serve as key regulators of GEFs and GAPs and their downstream signalling pathways, suggests that more conventional directions for GEF and GAP drug discovery are also promising directions (BOX 4).

Box 4

Signaling networks regulated by Ras and Rho family GTPases in cancer

In contrast to Ras, the specific downstream effectors that mediate the cancer cell phenotype, proliferation and survival, invasion and metastasis of other Ras and Rho family GTPases remain poorly understood. In this figure, we highlight protein kinases as effectors or regulators of Ras and Rho family GTPase oncogenesis. First, analogous to the role of Raf in Ras function, protein kinases have been implicated as downstream effectors of GTPase-mediated oncogenesis. In particular, there is evidence that the ROCK[153–155], MRCK[156], PAK[157–160] and ACK[161, 162] protein kinase effectors can promote oncogenesis. Much of the evidence for ROCK involvement in cancer is based on studies with ROCK inhibitors[163]. However, since these inhibitors have considerable off-target activities, it is unclear if ROCK inhibition alone accounts for the anti-tumor activities of ROCK inhibitors. There is emerging evidence that protein phosphorylation is an important mechanism for regulation of small GTPase function, often by controlling subcellular localization and interaction with other proteins. PKCα phosphorylation causes K-Ras4B translocation from the plasma membrane to the mitochondria, where K-Ras4B association with Bad results in apoptosis[164], suggesting that agonists of PKCα may act as K-Ras-directed therapies. Similarly, Aurora-A phosphorylation of RalA is essential for RalA promotion of pancreatic cancer cell line tumorigenic growth[165]. Additional effectors of Rho GTPases that regulate actin organization (e.g., mDIA) may influence cell motility, and hence, be important mediators of Rho GTPase induction of tumor cell invasion and metastasis[166]. A second theme is the signaling crosstalk that can occur between different members of the Ras and Rho families. For example, the RalBP1/RLIP76 effector of Ral functions as a RhoGAP for Rac and Cdc42 inactivation associated with transformation[165]. Ras activation of mTOR can involve AKT activation, leading to inactivation of Tsc2, causing Rheb activation.



Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors' research on GEFs and GAPs is supported in part by grants number CA042978, CA129610, CA127152, CA67771 and CA106991 from the US National Institutes of Health (C.J.D), from the American Cancer Society (D.V. and K.L.R.), and from the Centre National de la Recherche Scientifique (J.C.), and by grants from the Association pour la Recherche Contre le Cancer (J.C.) and the Agence National de la Recherche (J.C.). We thank L. DeGraffenreid for outstanding assistance in the preparation of this manuscript.

Glossary

ACK1	Activated Cdc42-associated kinase 1 is an intracellular tyrosine kinase that binds activated Cdc42 and inhibits both the intrinsic and GTPase-activating protein (GAP)-stimulated GTPase activity of Cdc42Hs
Alternative splicing	A mechanism by which different forms of mature mRNAs are generated from the same gene, leading to the production of more than one related protein, or isoform
Aptamer	A double stranded DNA, single-stranded RNA molecule or peptide that binds to specific molecular targets, such as a protein or metabolite. Aptamers are usually selected from large libraries of synthesized molecules
Arf GTPases	Regulate membrane trafficking and intracellular transport
C1 zinc finger domain	Protein kinase C conserved region 1 domains are approximately 50 amino acid phospholipid binding domains. They typically bind membrane-bound phorbol esters or diacylglycerol, to promote membrane localization
CAAX motif	C-terminal tetrapeptide sequence comprised of a cysteine, followed by two aliphatic amino acids and a terminal × residue that dictates specificity for farnesyltransferase or geranylgeranyltransferase-I catalized addition of a C15 farnesyl or C20 geranylgeranyl isoprenoid lipid

CDC25 homology domain	RasGEF catalytic domain, named after the first protein it was identified in: CDC25 in *S. cerevisiae*
Cholangiocarcinoma	An adenocarcinoma of the intrahepatic bile ducts
Dbl homology domain	The RhoGEF catalytic domain, named after the first protein it was identified in, the Dbl protein encoded by a transforming gene identified from an NIH/3T3 focus formation assay using genomic DNA from a human diffuse B-cell lymphoma
Druggability	The likelihood of being able to modulate the activity of a target protein with a small molecule drug
Fluorescence polarization	Fluorescence polarization is a technique specially applied to study molecular interactions. When fluorescent molecules in solution are excited with plane-polarized light, they will rotate and tumble, and the planes into which light is emitted can be very different from the plane used for initial excitation
Farnesyltransferase	One of three human prenyltransferase enzymes, catalyzes addition of a 15-carbon farnesyl group to proteins terminating with a CAAX tetrapeptide motif at the carboxyl terminus of a subset of Ras and Rho family proteins
RGS domains	Regulator of G protein Signalling domains function as GTPase-activating proteins that accelerate the intrinsic GTP hydrolysis activity of heterotrimeric G protein alpha subunits, causing inactivation of G protein-coupled receptor signalling
ROCK	The Rho-associated, coiled-coil containing protein kinases I and II are serine/threonine kinases (also called ROKβ and α) and effectors of RhoA and C and phosphorylate proteins that regulate actin stress fiber formation and focal adhesion assembly
Invadopodia	Actin-rich subcellular protrusions with associated proteases used by carcinoma cells to degrade extracellular matrix to promote invasion
Matrigel	Matrigel is the trade name for a gelatinous protein mixture secreted by mouse tumor cells. This mixture resembles the complex extracellular environment found in many tissues and is used commonly as a three-dimentional matrix substrate for cell culture-based in vitro migration and invasion assays
mTOR	The mammalian target of rapamycin (also known as FK506 binding protein 12-rapamycin associated protein 1; FRAP1), is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and gene transcription
Myeloproliferative disease	A group of diseases of the bone marrow in which excess cells are produced
MRCK	The myotonic dystrophy kinase-related Cdc42-binding kinases (α and β) are serine/threonine kinases that bind preferentially to activated Cdc42 and phosphorylate proteins that regulate actin reorganization

Noonan syndrome	This syndrome is characterized by short stature, characteristic facies, learning problems and a predisposition to develop leukaemia and other cancers, including myeloproliferative disease and neuroblastoma
Neurofibromatosis type 1	Patients with this autosomal dominant familial tumor syndrome are at increased risk of developing tumors of the peripheral and central nervous system, including neurofibromas, plexiform neurofibromas, malignant peripheral nerve sheath tumors, and low-grade gliomas
Organotypic	Resembling an organ *in vivo*, morphologically, functionally or both
PAK	21-activated kinases comprise a group of six structurally similar human serine/threonine kinases that can function as effectors of Rac (PAK1-3) or Cdc42 (PAK1-6)
Philadelphia chromosome	The chromosome abnormality that causes chronic myeloid leukemia. It is formed by a translocation between chromosomes 9 and 22, causing formation of the chimeric BCR-Abl tyrosine kinase
Pleckstrin homology domain	A sequence of approximately 100 amino acids that is present in many signalling molecules and that commonly binds to phospholipids and proteins
Rab GTPases	Regulate membrane trafficking and intracellular transport
Ran GTPase	Regulates nucleocytoplasmic transport of macromolecules and the organization of the spindle apparatus during mitosis
Ras exchange motif	This domain is found in several a subset of RasGEFs and lies N-terminal to the CDC25 catalytic domain
Ras GTPases	Key regulators of extracellular signal-regulated cytoplasmic signaling networks that control cell growth, survival and differentiation
Rho GTPases	Share similar roles in signal transduction to RasGTPases and are best-characterized for their regulation of actin cytoskeletal organization and cell shape, movement and polarity
Sec7 domain	ArfGEF catalytic domain, named after the first protein that it was identified in, the S. cerevisiae SEC7 gene product
Sterile alpha motif	An ~70 amino acid domain involved in protein-protein interactions and is found in a wide variety of proteins involved in many biological processes
StAR-related lipid transfer domain	The steroidogenic acute regulatory protein (StAR) related lipid transfer (START) domain is an ~ 200 amino acid motif initially identified as a lipid binding domain

References

1. Colicelli J. Human RAS superfamily proteins and related GTPases. Sci STKE. 2004; 2004:RE13. [PubMed: 15367757]

2. Wennerberg K, Rossman KL, Der CJ. The Ras superfamily at a glance. J Cell Sci. 2005; 118:843–6. [PubMed: 15731001]
3. Tcherkezian J, Lamarche-Vane N. Current knowledge of the large RhoGAP family of proteins. Biol Cell. 2007; 99:67–86. [PubMed: 17222083]
4. Bos JL, Rehmann H, Wittinghofer A. GEFs and GAPs: critical elements in the control of small G proteins. Cell. 2007; 129:865–77. [PubMed: 17540168]
5. Cox AD, Der CJ. Ras history: the saga continues. Small GTPases. 2010 in press.
6. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. Nat Rev Mol Cell Biol. 2008; 9:517–31. [PubMed: 18568040]
7. Ma WW, Adjei AA. Novel agents on the horizon for cancer therapy. CA Cancer J Clin. 2009; 59:111–37. [PubMed: 19278961]
8. Feuerstein J, Kalbitzer HR, John J, Goody RS, Wittinghofer A. Characterisation of the metal-ion-GDP complex at the active sites of transforming and nontransforming p21 proteins by observation of the 17O-Mn superhyperfine coupling and by kinetic methods. Eur J Biochem. 1987; 162:49–55. [PubMed: 3028791]
9. Blum R, Cox AD, Kloog Y. Inhibitors of chronically active ras: potential for treatment of human malignancies. Recent Pat Anticancer Drug Discov. 2008; 3:31–47. [PubMed: 18289122]
10. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene. 2007; 26:3291–310. [PubMed: 17496923]
11. Ha VL, Luo R, Nie Z, Randazzo PA. Contribution of AZAP-Type Arf GAPs to cancer cell migration and invasion. Adv Cancer Res. 2008; 101:1–28. [PubMed: 19055940]
12. D'Souza-Schorey C, Chavrier P. ARF proteins: roles in membrane traffic and beyond. Nat Rev Mol Cell Biol. 2006; 7:347–58. [PubMed: 16633337]
13. Sjoblom T, et al. The consensus coding sequences of human breast and colorectal cancers. Science. 2006; 314:268–74. [PubMed: 16959974]
14. Wood LD, et al. The genomic landscapes of human breast and colorectal cancers. Science. 2007; 318:1108–13. [PubMed: 17932254]
15. Parsons DW, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008; 321:1807–12. [PubMed: 18772396]
16. Jones S, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008; 321:1801–6. [PubMed: 18772397]
17. Srivastava SK, Wheelock RH, Aaronson SA, Eva A. Identification of the protein encoded by the human diffuse B-cell lymphoma (dbl) oncogene. Proc Natl Acad Sci U S A. 1986; 83:8868–72. [PubMed: 3491366]
18. Katzav S, Martin-Zanca D, Barbacid M. vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. EMBO J. 1989; 8:2283–90. [PubMed: 2477241]
19. Miki T, Smith CL, Long JE, Eva A, Fleming TP. Oncogene ect2 is related to regulators of small GTP-binding proteins. Nature. 1993; 362:462–5. [PubMed: 8464478]
20. Whitehead I, Kirk H, Kay R. Expression cloning of oncogenes by retroviral transfer of cDNA libraries. Mol Cell Biol. 1995; 15:704–10. [PubMed: 7823939]
21. Ebinu JO, et al. RasGRP, a Ras guanyl nucleotide- releasing protein with calcium- and diacylglycerol-binding motifs. Science. 1998; 280:1082–6. This study provided an important demonstration of an alternative mode of RasGEF regulation, by diacylglyerol-mediated recruitment to the plasma membrane. Since phospholipase C isoforms function downstream of cell surface receptors aberrantly activated in cancer, this provided another example of how Ras may be activated through the actions of a GEF. [PubMed: 9582122]
22. Tognon CE, et al. Regulation of RasGRP via a phorbol ester-responsive C1 domain. Mol Cell Biol. 1998; 18:6995–7008. [PubMed: 9819387]
23. Reuther GW, et al. RasGRP4 is a novel Ras activator isolated from acute myeloid leukemia. J Biol Chem. 2002; 277:30508–14. [PubMed: 11880369]
24. Whitehead IP, Campbell S, Rossman KL, Der CJ. Dbl family proteins. Biochim Biophys Acta. 1997; 1332:F1–23. [PubMed: 9061011]

25. Prenen H, Tejpar S, Cutsem EV. New Strategies for Treatment of KRAS Mutant Metastatic Colorectal Cancer. Clin Cancer Res.
26. Blumberg PM, et al. Wealth of opportunity - the C1 domain as a target for drug development. Curr Drug Targets. 2008; 9:641–52. [PubMed: 18691011]
27. Margarit SM, et al. Structural evidence for feedback activation by Ras.GTP of the Ras-specific nucleotide exchange factor SOS. Cell. 2003; 112:685–95. This study provided structural evidence for a feed forward mechanism where activated Ras can then activate a RasGEF. [PubMed: 12628188]
28. Roberts AE, et al. Germline gain-of-function mutations in SOS1 cause Noonan syndrome. Nat Genet. 2007; 39:70–4. This study and the following one below identified mutationally activated SOS1 RasGEF in a developmental disorder where other components of Ras signaling are also mutationally activated. [PubMed: 17143285]
29. Tartaglia M, et al. Gain-of-function SOS1 mutations cause a distinctive form of Noonan syndrome. Nat Genet. 2007; 39:75–9. [PubMed: 17143282]
30. Swanson KD, et al. SOS1 mutations are rare in human malignancies: implications for Noonan Syndrome patients. Genes Chromosomes Cancer. 2008; 47:253–9. [PubMed: 18064648]
31. Tidyman WE, Rauen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. Curr Opin Genet Dev. 2009; 19:230–6. [PubMed: 19467855]
32. Lopez I, Mak EC, Ding J, Hamm HE, Lomasney JW. A novel bifunctional phospholipase c that is regulated by Galpha 12 and stimulates the Ras/mitogen-activated protein kinase pathway. J Biol Chem. 2001; 276:2758–65. [PubMed: 11022047]
33. Song C, et al. Regulation of a novel human phospholipase C, PLCepsilon, through membrane targeting by Ras. J Biol Chem. 2001; 276:2752–7. [PubMed: 11022048]
34. Kelley GG, Reks SE, Ondrako JM, Smrcka AV. Phospholipase C(epsilon): a novel Ras effector. EMBO J. 2001; 20:743–54. [PubMed: 11179219]
35. Ikuta S, Edamatsu H, Li M, Hu L, Kataoka T. Crucial role of phospholipase C epsilon in skin inflammation induced by tumor-promoting phorbol ester. Cancer Res. 2008; 68:64–72. [PubMed: 18172297]
36. Song C, et al. Differential roles of Ras and Rap1 in growth factor-dependent activation of phospholipase C epsilon. Oncogene. 2002; 21:8105–13. [PubMed: 12444546]
37. Bodemann BO, White MA. Ral GTPases and cancer: linchpin support of the tumorigenic platform. Nat Rev Cancer. 2008; 8:133–40. [PubMed: 18219307]
38. Gonzalez-Garcia A, et al. RalGDS is required for tumor formation in a model of skin carcinogenesis. Cancer Cell. 2005; 7:219–26. This paper demonstrated a crucial role for a RalGEF in Ras-induced skin cancer in a mouse model. [PubMed: 15766660]
39. Vigil D, et al. Aberrant overexpression of the RGL2 ral small GTPase-specific guanine nucleotide exchange factor promotes pancreatic cancer growth through ral-dependent and -independent mechanisms. J Biol Chem. August 27.2010 Epub ahead of print.
40. Chien Y, White MA. RAL GTPases are linchpin modulators of human tumour-cell proliferation and survival. EMBO Rep. 2003; 4:800–6. This study showed that the two highly related substrates of RalGEFs may have highly divergent roles in normal and neoplastic cell growth and survival. [PubMed: 12856001]
41. Yin J, et al. Activation of the RalGEF/Ral pathway promotes prostate cancer metastasis to bone. Mol Cell Biol. 2007; 27:7538–50. [PubMed: 17709381]
42. Oxford G, et al. RalA and RalB: antagonistic relatives in cancer cell migration. Cancer Res. 2005; 65:7111–20. [PubMed: 16103060]
43. Lim KH, et al. Divergent roles for RalA and RalB in malignant growth of human pancreatic carcinoma cells. Curr Biol. 2006; 16:2385–94. This study showed that a RalGEF effector pathway may be a critical mechanism for Ras-mediated oncogenesis, in particular, in pancreatic cancer. [PubMed: 17174914]
44. Vega FM, Ridley AJ. Rho GTPases in cancer cell biology. FEBS Lett. 2008; 582:2093–101. [PubMed: 18460342]
45. Ellenbroek SI, Collard JG. Rho GTPases: functions and association with cancer. Clin Exp Metastasis. 2007; 24:657–72. [PubMed: 18000759]

46. Fernandez-Zapico ME, et al. Ectopic expression of VAV1 reveals an unexpected role in pancreatic cancer tumorigenesis. Cancer Cell. 2005; 7:39–49. This study found a crucial role for a Rac GEF which is overexpressed in pancreatic cancer and activated by the epidermal growth factor receptor. [PubMed: 15652748]
47. Patel V, et al. Persistent activation of Rac1 in squamous carcinomas of the head and neck: evidence for an EGFR/Vav2 signaling axis involved in cell invasion. Carcinogenesis. 2007; 28:1145–52. [PubMed: 17234718]
48. Salhia B, et al. The guanine nucleotide exchange factors trio, Ect2, and Vav3 mediate the invasive behavior of glioblastoma. Am J Pathol. 2008; 173:1828–38. [PubMed: 19008376]
49. Brantley-Sieders DM, et al. Host deficiency in Vav2/3 guanine nucleotide exchange factors impairs tumor growth, survival, and angiogenesis in vivo. Mol Cancer Res. 2009; 7:615–23. The study found that a deficiency of Vav2 and Vav3 in normal mouse tissue impaired the growth of transplanted cancer cells, demonstrating a crucial for RhoGEFs in the tumor microenvironment. [PubMed: 19435813]
50. Qin J, et al. Upregulation of PIP3-dependent Rac exchanger 1 (P-Rex1) promotes prostate cancer metastasis. Oncogene. 2009; 28:1853–63. [PubMed: 19305425]
51. Karlsson R, Pedersen ED, Wang Z, Brakebusch C. Rho GTPase function in tumorigenesis. Biochim Biophys Acta. 2009; 1796:91–8. [PubMed: 19327386]
52. Shields JM, et al. Lack of extracellular signal-regulated kinase mitogen-activated protein kinase signaling shows a new type of melanoma. Cancer Res. 2007; 67:1502–12. [PubMed: 17308088]
53. Fine B, et al. Activation of the PI3K pathway in cancer through inhibition of PTEN by exchange factor P-REX2a. Science. 2009; 325:1261–5. This paper identified a function for a RacGEF that is distinct from its activation of Rac, where direct association with the PTEN tumor suppression caused activation of PI3K signaling. [PubMed: 19729658]
54. Tatsumoto T, Xie X, Blumenthal R, Okamoto I, Miki T. Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. J Cell Biol. 1999; 147:921–8. [PubMed: 10579713]
55. Solski PA, et al. Requirement for C-terminal sequences in regulation of Ect2 guanine nucleotide exchange specificity and transformation. J Biol Chem. 2004; 279:25226–33. [PubMed: 15073184]
56. Saito S, et al. Rho exchange factor ECT2 is induced by growth factors and regulates cytokinesis through the N-terminal cell cycle regulator-related domains. J Cell Biochem. 2003; 90:819–36. [PubMed: 14587037]
57. Sano M, et al. Expression level of ECT2 proto-oncogene correlates with prognosis in glioma patients. Oncol Rep. 2006; 16:1093–8. [PubMed: 17016598]
58. Hirata D, et al. Involvement of epithelial cell transforming sequence-2 oncoantigen in lung and esophageal cancer progression. Clin Cancer Res. 2009; 15:256–66. [PubMed: 19118053]
59. Zhang ML, Lu S, Zhou L, Zheng SS. Correlation between ECT2 gene expression and methylation change of ECT2 promoter region in pancreatic cancer. Hepatobiliary Pancreat Dis Int. 2008; 7:533–8. [PubMed: 18842503]
60. Justilien V, Fields AP. Ect2 links the PKCiota-Par6alpha complex to Rac1 activation and cellular transformation. Oncogene. 2009
61. Kourlas PJ, et al. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia. Proc Natl Acad Sci U S A. 2000; 97:2145–50. This paper identified a rearranged gene in an acute myelogenous leukemia that encoded a chimeric protein that altered the structure of a RhoGEF. [PubMed: 10681437]
62. Kelly P, et al. The G12 family of heterotrimeric G proteins promotes breast cancer invasion and metastasis. Proc Natl Acad Sci U S A. 2006; 103:8173–8. [PubMed: 16705036]
63. Kelly P, et al. A role for the G12 family of heterotrimeric G proteins in prostate cancer invasion. J Biol Chem. 2006; 281:26483–90. [PubMed: 16787920]
64. Sahay S, et al. The RhoGEF domain of p210 Bcr-Abl activates RhoA and is required for transformation. Oncogene. 2008; 27:2064–71. This paper found that the BCR-ABL oncoprotein may promote oncogenesis, in part, through its RhoGEF domain and activation of Rho GTPase function. [PubMed: 17922031]

65. Yoshizuka N, et al. An alternative transcript derived from the trio locus encodes a guanosine nucleotide exchange factor with mouse cell-transforming potential. J Biol Chem. 2004; 279:43998–4004. [PubMed: 15308664]
66. Lambert JM, et al. Tiam1 mediates Ras activation of Rac by a PI(3)K-independent mechanism. Nat Cell Biol. 2002; 4:621–5. [PubMed: 12134164]
67. Malliri A, et al. Mice deficient in the Rac activator Tiam1 are resistant to Ras-induced skin tumours. Nature. 2002; 417:867–71. This study found that loss of the Tiam1 RacGEF impeded mutant Ras-induced skin tumor formation, but promoted later-stage malignant conversion, demonstrating that GEFs may possess stage-specific oncogenic or tumor suppressive functions. [PubMed: 12075356]
68. Malliri A, et al. The rac activator Tiam1 is a Wnt-responsive gene that modifies intestinal tumor development. J Biol Chem. 2006; 281:543–8. [PubMed: 16249175]
69. Strumane K, Rygiel T, van der Valk M, Collard JG. Tiam1-deficiency impairs mammary tumor formation in MMTV-c-neu but not in MMTV-c-myc mice. J Cancer Res Clin Oncol. 2009; 135:69–80. [PubMed: 18592271]
70. Stebel A, Brachetti C, Kunkel M, Schmidt M, Fritz G. Progression of breast tumors is accompanied by a decrease in expression of the Rho guanine exchange factor Tiam1. Oncol Rep. 2009; 21:217–22. [PubMed: 19082465]
71. Cote JF, Vuori K. Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange activity. J Cell Sci. 2002; 115:4901–13. [PubMed: 12432077]
72. Meller N, Merlot S, Guda C. CZH proteins: a new family of Rho-GEFs. J Cell Sci. 2005; 118:4937–46. [PubMed: 16254241]
73. Yang J, Zhang Z, Roe SM, Marshall CJ, Barford D. Activation of Rho GTPases by DOCK exchange factors is mediated by a nucleotide sensor. Science. 2009; 325:1398–402. [PubMed: 19745154]
74. Jarzynka MJ, et al. ELMO1 and Dock180, a bipartite Rac1 guanine nucleotide exchange factor, promote human glioma cell invasion. Cancer Res. 2007; 67:7203–11. [PubMed: 17671188]
75. Gadea G, Sanz-Moreno V, Self A, Godi A, Marshall CJ. DOCK10-mediated Cdc42 activation is necessary for amoeboid invasion of melanoma cells. Curr Biol. 2008; 18:1456–65. [PubMed: 18835169]
76. Sanz-Moreno V, et al. Rac activation and inactivation control plasticity of tumor cell movement. Cell. 2008; 135:510–23. This study showed that mesenchymal tumor cell motility is promoted by the DOCK10 GEF activation of Rac whereas amoeboid tumor cell movement involves activation of the ARHGAP22 RacGAP to inactivate Rac. [PubMed: 18984162]
77. Boulay PL, Cotton M, Melancon P, Claing A. ADP-ribosylation factor 1 controls the activation of the phosphatidylinositol 3-kinase pathway to regulate epidermal growth factor-dependent growth and migration of breast cancer cells. J Biol Chem. 2008; 283:36425–34. [PubMed: 18990689]
78. Muralidharan-Chari V, et al. ADP-ribosylation factor 6 regulates tumorigenic and invasive properties in vivo. Cancer Res. 2009; 69:2201–9. [PubMed: 19276388]
79. Ahn JY, Hu Y, Kroll TG, Allard P, Ye K. PIKE-A is amplified in human cancers and prevents apoptosis by up-regulating Akt. Proc Natl Acad Sci U S A. 2004; 101:6993–8. [PubMed: 15118108]
80. Sabe H, et al. The EGFR-GEP100-Arf6-AMAP1 signaling pathway specific to breast cancer invasion and metastasis. Traffic. 2009; 10:982–93. [PubMed: 19416474]
81. Morishige M, et al. GEP100 links epidermal growth factor receptor signalling to Arf6 activation to induce breast cancer invasion. Nat Cell Biol. 2008; 10:85–92. This study found a clear role for a specific ArfGEF in breast cancer invasion and metastasis, and this ArfGEF directly links EGFR activation to Arf signaling. [PubMed: 18084281]
82. Hashimoto S, et al. Requirement for Arf6 in breast cancer invasive activities. Proc Natl Acad Sci U S A. 2004; 101:6647–52. [PubMed: 15087504]
83. Li M, et al. EFA6A enhances glioma cell invasion through ADP ribosylation factor 6/extracellular signal-regulated kinase signaling. Cancer Res. 2006; 66:1583–90. [PubMed: 16452216]

84. Yano H, et al. Fbx8 makes Arf6 refractory to function via ubiquitination. Mol Biol Cell. 2008; 19:822–32. [PubMed: 18094045]
85. McClatchey AI. Neurofibromatosis. Annu Rev Pathol. 2007; 2:191–216. [PubMed: 18039098]
86. Brems H, Beert E, de Ravel T, Legius E. Mechanisms in the pathogenesis of malignant tumours in neurofibromatosis type 1. Lancet Oncol. 2009; 10:508–15. [PubMed: 19410195]
87. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008; 455:1061–8. [PubMed: 18772890]
88. McGillicuddy LT, et al. Proteasomal and genetic inactivation of the NF1 tumor suppressor in gliomagenesis. Cancer Cell. 2009; 16:44–54. This paper described proteosomal degradation of the neurofibromin RasGAP leading to Ras hyperactivation in cancer. [PubMed: 19573811]
89. Aspuria PJ, Tamanoi F. The Rheb family of GTP-binding proteins. Cell Signal. 2004; 16:1105–12. [PubMed: 15240005]
90. Napolioni V, Curatolo P. Genetics and molecular biology of tuberous sclerosis complex. Curr Genomics. 2008; 9:475–87. [PubMed: 19506736]
91. Inoki K, Guan KL. Tuberous sclerosis complex, implication from a rare genetic disease to common cancer treatment. Hum Mol Genet. 2009; 18:R94–100. [PubMed: 19297407]
92. Yeung RS, et al. Predisposition to renal carcinoma in the Eker rat is determined by germ-line mutation of the tuberous sclerosis 2 (TSC2) gene. Proc Natl Acad Sci U S A. 1994; 91:11413–6. [PubMed: 7972075]
93. Kobayashi T, Hirayama Y, Kobayashi E, Kubo Y, Hino O. A germline insertion in the tuberous sclerosis (Tsc2) gene gives rise to the Eker rat model of dominantly inherited cancer. Nat Genet. 1995; 9:70–4. [PubMed: 7704028]
94. Kwiatkowski DJ, et al. A mouse model of TSC1 reveals sex-dependent lethality from liver hemangiomas, and up-regulation of p70S6 kinase activity in Tsc1 null cells. Hum Mol Genet. 2002; 11:525–34. [PubMed: 11875047]
95. Zhou X, et al. DLC1 suppresses distant dissemination of human hepatocellular carcinoma cells in nude mice through reduction of RhoA GTPase activity, actin cytoskeletal disruption and down-regulation of genes involved in metastasis. Int J Oncol. 2008; 32:1285–91. [PubMed: 18497990]
96. Lahoz A, Hall A. DLC1: a significant GAP in the cancer genome. Genes Dev. 2008; 22:1724–30. [PubMed: 18593873]
97. Yuan BZ, et al. Cloning, characterization, and chromosomal localization of a gene frequently deleted in human liver cancer (DLC-1) homologous to rat RhoGAP. Cancer Res. 1998; 58:2196–9. [PubMed: 9605766]
98. Xue W, et al. DLC1 is a chromosome 8p tumor suppressor whose loss promotes hepatocellular carcinoma. Genes Dev. 2008; 22:1439–44. This study found that the rate of genetic loss of the DLC-1 RhoGAP approaches that of the p53 tumor suppressor in some cancers, and it demonstrated a clear role for RhoGAP loss in tumorigenesis in a mouse model of liver cancer. [PubMed: 18519636]
99. Ching YP, et al. Deleted in liver cancer (DLC) 2 encodes a RhoGAP protein with growth suppressor function and is underexpressed in hepatocellular carcinoma. J Biol Chem. 2003; 278:10824–30. [PubMed: 12531887]
100. Nagaraja GM, Kandpal RP. Chromosome 13q12 encoded Rho GTPase activating protein suppresses growth of breast carcinoma cells, and yeast two-hybrid screen shows its interaction with several proteins. Biochem Biophys Res Commun. 2004; 313:654–65. [PubMed: 14697242]
101. Durkin ME, Ullmannova V, Guan M, Popescu NC. Deleted in liver cancer 3 (DLC-3), a novel Rho GTPase-activating protein, is downregulated in cancer and inhibits tumor cell growth. Oncogene. 2007; 26:4580–9. [PubMed: 17297465]
102. Scholz RP, et al. DLC1 interacts with 14–3–3 proteins to inhibit RhoGAP activity and block nucleocytoplasmic shuttling. J Cell Sci. 2009; 122:92–102. [PubMed: 19066281]
103. Yang XY, et al. p120Ras-GAP binds the DLC1 Rho-GAP tumor suppressor protein and inhibits its RhoA GTPase and growth-suppressing activities. Oncogene. 2009; 28:1401–9. [PubMed: 19151751]

104. Zhou X, Thorgeirsson SS, Popescu NC. Restoration of DLC-1 gene expression induces apoptosis and inhibits both cell growth and tumorigenicity in human hepatocellular carcinoma cells. Oncogene. 2004; 23:1308–13. [PubMed: 14647417]

105. Durkin ME, et al. DLC-1:a Rho GTPase-activating protein and tumour suppressor. J Cell Mol Med. 2007; 11:1185–207. [PubMed: 17979893]

106. Liao YC, Lo SH. Deleted in liver cancer-1 (DLC-1): a tumor suppressor not just for liver. Int J Biochem Cell Biol. 2008; 40:843–7. [PubMed: 17521951]

107. Healy KD, et al. DLC-1 suppresses non-small cell lung cancer growth and invasion by RhoGAP-dependent and independent mechanisms. Mol Carcinog. 2008; 47:326–37. [PubMed: 17932950]

108. Goodison S, et al. The RhoGAP protein DLC-1 functions as a metastasis suppressor in breast cancer cells. Cancer Res. 2005; 65:6042–53. [PubMed: 16024604]

109. Sabe H, Onodera Y, Mazaki Y, Hashimoto S. ArfGAP family proteins in cell adhesion, migration and tumor invasion. Curr Opin Cell Biol. 2006; 18:558–64. [PubMed: 16904307]

110. Ahn JY, et al. PIKE (phosphatidylinositol 3-kinase enhancer)-A GTPase stimulates Akt activity and mediates cellular invasion. J Biol Chem. 2004; 279:16441–51. [PubMed: 14761976]

111. Cai Y, et al. GGAP2/PIKE-a directly activates both the Akt and nuclear factor-kappaB pathways and promotes prostate cancer progression. Cancer Res. 2009; 69:819–27. [PubMed: 19176382]

112. Liu X, Hu Y, Hao C, Rempel SA, Ye K. PIKE-A is a proto-oncogene promoting cell growth, transformation and invasion. Oncogene. 2007; 26:4918–27. [PubMed: 17297440]

113. Soundararajan M, Yang X, Elkins JM, Sobott F, Doyle DA. The centaurin gamma-1 GTPase-like domain functions as an NTPase. Biochem J. 2007; 401:679–88. [PubMed: 17037982]

114. Ehlers JP, Worley L, Onken MD, Harbour JW. DDEF1 is located in an amplified region of chromosome 8q and is overexpressed in uveal melanoma. Clin Cancer Res. 2005; 11:3609–13. [PubMed: 15897555]

115. Lin D, et al. ASAP1, a gene at 8q24, is associated with prostate cancer metastasis. Cancer Res. 2008; 68:4352–9. [PubMed: 18519696]

116. Onodera Y, et al. Expression of AMAP1, an ArfGAP, provides novel targets to inhibit breast cancer invasive activities. EMBO J. 2005; 24:963–73. [PubMed: 15719014]

117. Hashimoto S, et al. Targeting AMAP1 and cortactin binding bearing an atypical src homology 3/proline interface for prevention of breast cancer invasion and metastasis. Proc Natl Acad Sci U S A. 2006; 103:7036–41. [PubMed: 16636290]

118. Okabe H, et al. Isolation of development and differentiation enhancing factor-like 1 (DDEFL1) as a drug target for hepatocellular carcinomas. Int J Oncol. 2004; 24:43–8. [PubMed: 14654939]

119. Ha VL, et al. ASAP3 is a focal adhesion-associated Arf GAP that functions in cell migration and invasion. J Biol Chem. 2008; 283:14915–26. [PubMed: 18400762]

120. Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. Nature. 2007; 450:1001–9. [PubMed: 18075579]

121. Goldberg J. Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. Cell. 1998; 95:237–48. [PubMed: 9790530]

122. Boriack-Sjodin PA, Margarit SM, Bar-Sagi D, Kuriyan J. The structural basis of the activation of Ras by Sos. Nature. 1998; 394:337–43. [PubMed: 9690470]

123. Worthylake DK, Rossman KL, Sondek J. Crystal structure of Rac1 in complex with the guanine nucleotide exchange region of Tiam1. Nature. 2000; 408:682–8. [PubMed: 11130063]

124. Singleton VL, Bohonos N, Ullstrup AJ. Decumbin, a new compound from a species of Penicillium. Nature. 1958; 181:1072–3. [PubMed: 13541371]

125. Donaldson JG, Finazzi D, Klausner RD. Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein. Nature. 1992; 360:350–2. [PubMed: 1448151]

126. Helms JB, Rothman JE. Inhibition by brefeldin A of a Golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF. Nature. 1992; 360:352–4. [PubMed: 1448152]

127. Peyroche A, et al. Brefeldin A acts to stabilize an abortive ARF-GDP-Sec7 domain protein complex: involvement of specific residues of the Sec7 domain. Mol Cell. 1999; 3:275–85. This study and the one below provide an important structural snapshot of how a GEF inhibitor inhibits

nucleotide exchange and provides a basis for discovery of novel GEF inhibitors. [PubMed: 10198630]

128. Renault L, Guibert B, Cherfils J. Structural snapshots of the mechanism and inhibition of a guanine nucleotide exchange factor. Nature. 2003; 426:525–30. [PubMed: 14654833]
129. Mossessova E, Corpina RA, Goldberg J. Crystal structure of ARF1*Sec7 complexed with Brefeldin A and its implications for the guanine nucleotide exchange mechanism. Mol Cell. 2003; 12:1403–11. [PubMed: 14690595]
130. Robineau S, Chabre M, Antonny B. Binding site of brefeldin A at the interface between the small G protein ADP-ribosylation factor 1 (ARF1) and the nucleotide-exchange factor Sec7 domain. Proc Natl Acad Sci U S A. 2000; 97:9913–8. [PubMed: 10954741]
131. Viaud J, et al. Structure-based discovery of an inhibitor of Arf activation by Sec7 domains through targeting of protein-protein complexes. Proc Natl Acad Sci U S A. 2007; 104:10370–5. [PubMed: 17563369]
132. Pommier Y, Cherfils J. Interfacial inhibition of macromolecular interactions: nature's paradigm for drug discovery. Trends Pharmacol Sci. 2005; 26:138–45. [PubMed: 15749159]
133. Mayer G, et al. Controlling small guanine-nucleotide-exchange factor function through cytoplasmic RNA intramers. Proc Natl Acad Sci U S A. 2001; 98:4961–5. [PubMed: 11320245]
134. Hafner M, et al. Inhibition of cytohesins by SecinH3 leads to hepatic insulin resistance. Nature. 2006; 444:941–4. [PubMed: 17167487]
135. Saenz JB, et al. Golgicide A reveals essential roles for GBF1 in Golgi assembly and function. Nat Chem Biol. 2009; 5:157–65. [PubMed: 19182783]
136. Schmidt S, Diriong S, Mery J, Fabbrizio E, Debant A. Identification of the first Rho-GEF inhibitor, TRIPalpha, which targets the RhoA-specific GEF domain of Trio. FEBS Lett. 2002; 523:35–42. [PubMed: 12123800]
137. Bouquier N, et al. Aptamer-derived peptides as potent inhibitors of the oncogenic RhoGEF Tgat. Chem Biol. 2009; 16:391–400. [PubMed: 19389625]
138. Blangy A, et al. Identification of TRIO-GEFD1 chemical inhibitors using the yeast exchange assay. Biol Cell. 2006; 98:511–22. [PubMed: 16686599]
139. Bouquier N, et al. A cell active chemical GEF inhibitor selectively targets the Trio/RhoG/Rac1 signaling pathway. Chem Biol. 2009; 16:657–66. [PubMed: 19549603]
140. Evelyn CR, et al. High-throughput screening for small-molecule inhibitors of LARG-stimulated RhoA nucleotide binding via a novel fluorescence polarization assay. J Biomol Screen. 2009; 14:161–72. [PubMed: 19196702]
141. Gao Y, Dickerson JB, Guo F, Zheng J, Zheng Y. Rational design and characterization of a Rac GTPase-specific small molecule inhibitor. Proc Natl Acad Sci U S A. 2004; 101:7618–23. [PubMed: 15128949]
142. Ferri N, Corsini A, Bottino P, Clerici F, Contini A. Virtual screening approach for the identification of new Rac1 inhibitors. J Med Chem. 2009; 52:4087–90. [PubMed: 19527032]
143. Gibbs JB, Schaber MD, Allard WJ, Sigal IS, Scolnick EM. Purification of ras GTPase activating protein from bovine brain. Proc Natl Acad Sci U S A. 1988; 85:5026–30. [PubMed: 3293047]
144. Scheffzek K, et al. The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. Science. 1997; 277:333–8. [PubMed: 9219684]
145. Roman DL, et al. Identification of small-molecule inhibitors of RGS4 using a high-throughput flow cytometry protein interaction assay. Mol Pharmacol. 2007; 71:169–75. [PubMed: 17012620]
146. Hao Y, Wong R, Feig LA. RalGDS couples growth factor signaling to Akt activation. Mol Cell Biol. 2008; 28:2851–9. [PubMed: 18285454]
147. Gupta S, et al. Binding of ras to phosphoinositide 3-kinase p110alpha is required for ras-driven tumorigenesis in mice. Cell. 2007; 129:957–68. [PubMed: 17540175]
148. Vetter IR, Wittinghofer A. The guanine nucleotide-binding switch in three dimensions. Science. 2001; 294:1299–304. [PubMed: 11701921]
149. Mitin N, Rossman KL, Der CJ. Signaling interplay in Ras superfamily function. Curr Biol. 2005; 15:R563–74. [PubMed: 16051167]

150. Buday L, Downward J. Many faces of Ras activation. Biochim Biophys Acta. 2008; 1786:178–87. [PubMed: 18541156]

151. Rossman KL, Der CJ, Sondek J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. Nat Rev Mol Cell Biol. 2005; 6:167–80. [PubMed: 15688002]

152. Kahn RA, et al. Consensus nomenclature for the human ArfGAP domain-containing proteins. J Cell Biol. 2008; 182:1039–44. [PubMed: 18809720]

153. Sahai E, Ishizaki T, Narumiya S, Treisman R. Transformation mediated by RhoA requires activity of ROCK kinases. Curr Biol. 1999; 9:136–45. [PubMed: 10021386]

154. Sahai E, Marshall CJ. Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. Nat Cell Biol. 2003; 5:711–9. [PubMed: 12844144]

155. Croft DR, et al. Conditional ROCK activation in vivo induces tumor cell dissemination and angiogenesis. Cancer Res. 2004; 64:8994–9001. [PubMed: 15604264]

156. Wilkinson S, Paterson HF, Marshall CJ. Cdc42-MRCK and Rho-ROCK signalling cooperate in myosin phosphorylation and cell invasion. Nat Cell Biol. 2005; 7:255–61. [PubMed: 15723050]

157. Qu J, et al. Activated PAK4 regulates cell adhesion and anchorage-independent growth. Mol Cell Biol. 2001; 21:3523–33. [PubMed: 11313478]

158. Callow MG, et al. Requirement for PAK4 in the anchorage-independent growth of human cancer cell lines. J Biol Chem. 2002; 277:550–8. [PubMed: 11668177]

159. Chow BJ, et al. Prognostic value of 64-slice cardiac computed tomography severity of coronary artery disease, coronary atherosclerosis, and left ventricular ejection fraction. J Am Coll Cardiol. 55:1017–28. [PubMed: 20202518]

160. Kimmelman AC, et al. Genomic alterations link Rho family of GTPases to the highly invasive phenotype of pancreas cancer. Proc Natl Acad Sci U S A. 2008; 105:19372–7. [PubMed: 19050074]

161. van der Horst EH, et al. Metastatic properties and genomic amplification of the tyrosine kinase gene ACK1. Proc Natl Acad Sci U S A. 2005; 102:15901–6. [PubMed: 16247015]

162. Mahajan NP, Whang YE, Mohler JL, Earp HS. Activated tyrosine kinase Ack1 promotes prostate tumorigenesis: role of Ack1 in polyubiquitination of tumor suppressor Wwox. Cancer Res. 2005; 65:10514–23. [PubMed: 16288044]

163. Olson MF. Applications for ROCK kinase inhibition. Curr Opin Cell Biol. 2008; 20:242–8. [PubMed: 18282695]

164. Bivona TG, et al. PKC regulates a farnesyl-electrostatic switch on K-Ras that promotes its association with Bcl-XL on mitochondria and induces apoptosis. Mol Cell. 2006; 21:481–93. [PubMed: 16483930]

165. Lim KH, et al. Aurora-A phosphorylates, activates, and relocalizes the small GTPase RalA. Mol Cell Biol. 30:508–23. [PubMed: 19901077]

166. Narumiya S, Tanji M, Ishizaki T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. Cancer Metastasis Rev. 2009; 28:65–76. [PubMed: 19160018]

167. Aghazadeh B, Lowry WE, Huang XY, Rosen MK. Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogene Vav by tyrosine phosphorylation. Cell. 2000; 102:625–33. This paper described the activation mechanism for a RhoGEF by tyrosine phosphorylation, suggesting that protein kinase inhibitors may be one approach to block the activation of some RhoGEFs. [PubMed: 11007481]

168. Llorca O, Arias-Palomo E, Zugaza JL, Bustelo XR. Global conformational rearrangements during the activation of the GDP/GTP exchange factor Vav3. EMBO J. 2005; 24:1330–40. [PubMed: 15775967]

169. Yohe ME, et al. Auto-inhibition of the Dbl family protein Tim by an N-terminal helical motif. J Biol Chem. 2007; 282:13813–23. [PubMed: 17337446]

170. Yohe ME, Rossman K, Sondek J. Role of the C-terminal SH3 domain and N-terminal tyrosine phosphorylation in regulation of Tim and related Dbl-family proteins. Biochemistry. 2008; 47:6827–39. [PubMed: 18537266]

171. Hart MJ, et al. Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13. Science. 1998; 280:2112–4. This study identified a RhoGEF downstream of G

protein-coupled receptor signaling through G alpha 13, and established another indirect mechanism for Rho GTPase activation in cancer. [PubMed: 9641916]

172. Booden MA, Siderovski DP, Der CJ. Leukemia-associated Rho guanine nucleotide exchange factor promotes G alpha q-coupled activation of RhoA. Mol Cell Biol. 2002; 22:4053–61. [PubMed: 12024019]
173. Suzuki N, et al. Activation of leukemia-associated RhoGEF by Galpha13 with significant conformational rearrangements in the interface. J Biol Chem. 2009; 284:5000–9. [PubMed: 19074425]
174. Kawasaki Y, et al. Asef, a link between the tumor suppressor APC and G-protein signaling. Science. 2000; 289:1194–7. [PubMed: 10947987]
175. Murayama K, et al. Crystal structure of the rac activator, Asef, reveals its autoinhibitory mechanism. J Biol Chem. 2007; 282:4238–42. [PubMed: 17190834]
176. Mitin N, et al. Release of autoinhibition of ASEF by APC leads to CDC42 activation and tumor suppression. Nat Struct Mol Biol. 2007; 14:814–23. [PubMed: 17704816]
177. van Dam TJ, Rehmann H, Bos JL, Snel B. Phylogeny of the CDC25 homology domain reveals rapid differentiation of Ras pathways between early animals and fungi. Cell Signal. 2009; 21:1579–85. [PubMed: 19567266]
178. Raaijmakers JH, Bos JL. Specificity in Ras and Rap signaling. J Biol Chem. 2009; 284:10995–9. [PubMed: 19091745]

Biographies

Dominico Vigil

Dominico Vigil completed his Ph.D. at the University of California, San Diego, studying protein kinase A structure, biochemistry and regulation. He is currently a postdoctoral research fellow at at the Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. His research has focused on GEFs and GAPs involved in cancer.

Jacqueline Cherfils

Jacqueline Cherfils is Head of the Enzymology and Structural Biochemistry Laboratory, Centre National de la Recherche Scientifique (CNRS), Gif-sur-Yvette, France. Her main research interest is structural biology of small GTP-binding proteins and their activation by GEFs, and the discovery of small chemicals that inhibit members of these proteins families involved in human diseases.

Kent Rossman

Kent Rossman completed his Ph.D. and postdoctoral research at the University of North Carolina at Chapel Hill. He is currently a Research Assistant Professor of Pharmacology at the Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. His research has focused on the biochemistry, regulation and structure of Dbl family Rho GEFs.

Channing J. Der

Channing J. Der is Sarah G. Kenan Professor of Pharmacology at the Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. His research has focused on dissection of the signaling mechanisms of Ras superfamily small GTPases involved in oncogenesis and on molecularly-targeted therapies based on these mechanisms for cancer treatment.

At a glance

- There is increasing evidence that the aberrant activity of numerous members of the Ras superfamily of small GTPases contribute to cancer growth, invasion and metastasis.
- Unlike the frequent direct mutational activation of the three Ras proteins (33% of human cancers), other Ras superfamily GTPases are deregulated by indirect mechanisms, commonly involving the altered expression or activity of their regulatory proteins.
- Guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) that control the GDP-GTP cycle of specific members of the Ras superfamily have been shown to contribute to cancer by either promoting or suppressing tumor progression and growth.
- GEFs and GAPs are deregulated in cancer by somatic mutation, changes in gene expression and through post-translational mechanisms that include aberrant signaling caused by alterations in upstream oncogene or tumor suppressor function.
- Although GEFs and GAPs are not considered classically druggable targets, there is growing evidence that support the feasibility. For example, nature has provided examples (e.g., Brefeldin A) that provide proof-of-concept of GEF and GAP druggability.
- The multi-domain structures of GEFs and GAPs contribute to their regulation by diverse signaling mechanisms and additionally may identify therapeutic approaches for pharmacologic regulation of GEF and GAP activity in cancer.

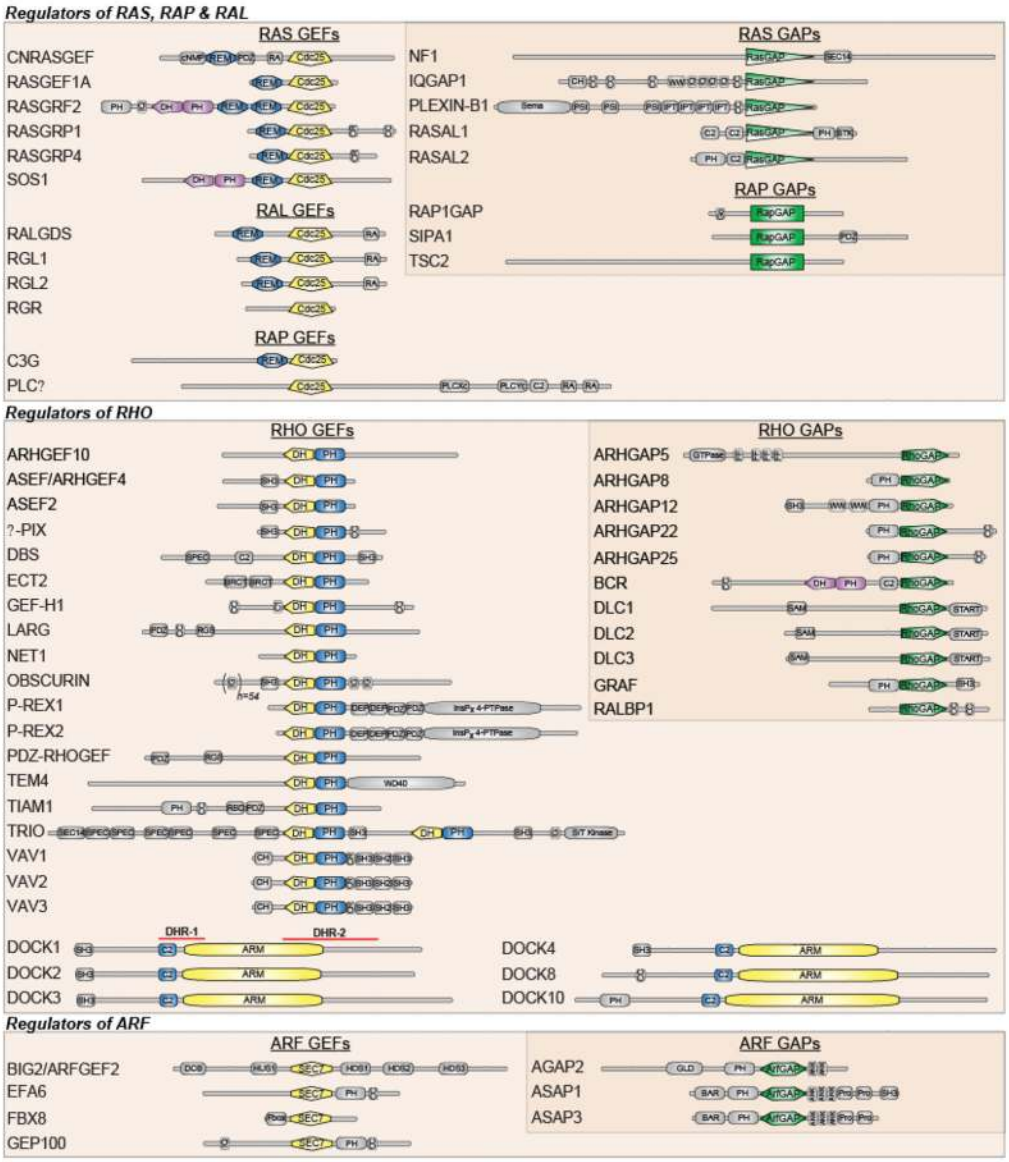


Figure 1. GEFs and GAPs are multi-domain proteins

This figure focuses on those GEFs and GAPs where some degree of validation has been accomplished, and parallels those listed Supplementary Tables S1 and S2. A key point of this figure is to emphasize the complex domain topology of GEFs and GAPs. Beyond their shared catalytic domains, there is significant diversity in the structure of GEFs and GAPs for a specific GTPase. This diversity is especially striking for RhoGEFs[151] and RhoGAPs[3]. These flanking domains or motifs are often involved in promoting their activation by upstream signals (e.g., Ras-binding/association domains). The domains include those that promote protein-protein (e.g., Src homology 2 and 3 domains) or protein-lipid interactions, second messenger binding and protein kinase phosphorylation sites. These interactions may facilitate association with specific subcellular membranes or compartments, regulating spatially-restricted GTPase activation. These interactions may also regulate autoregulatory sequences or allosteric regulation of GAP or GEF catalytic activity. Others may influence the effectors utilized by the GTPases. Some contain additional catalytic domains. For example, some RasGEFs also contain DH-PH domains and can activate Rho GTPases.

Hence, it is likely that GEFs and GAPs will have GEF/GAP-independent functions and be regulated by GTPase-independent mechanisms. Thus caution should be exercised when using RNA interference to suppress their expression and ascribing cellular activities simply to GTPase activity. For descriptions of domain abbreviations and functions, the reader is referred to the SMART website (see the online links box).

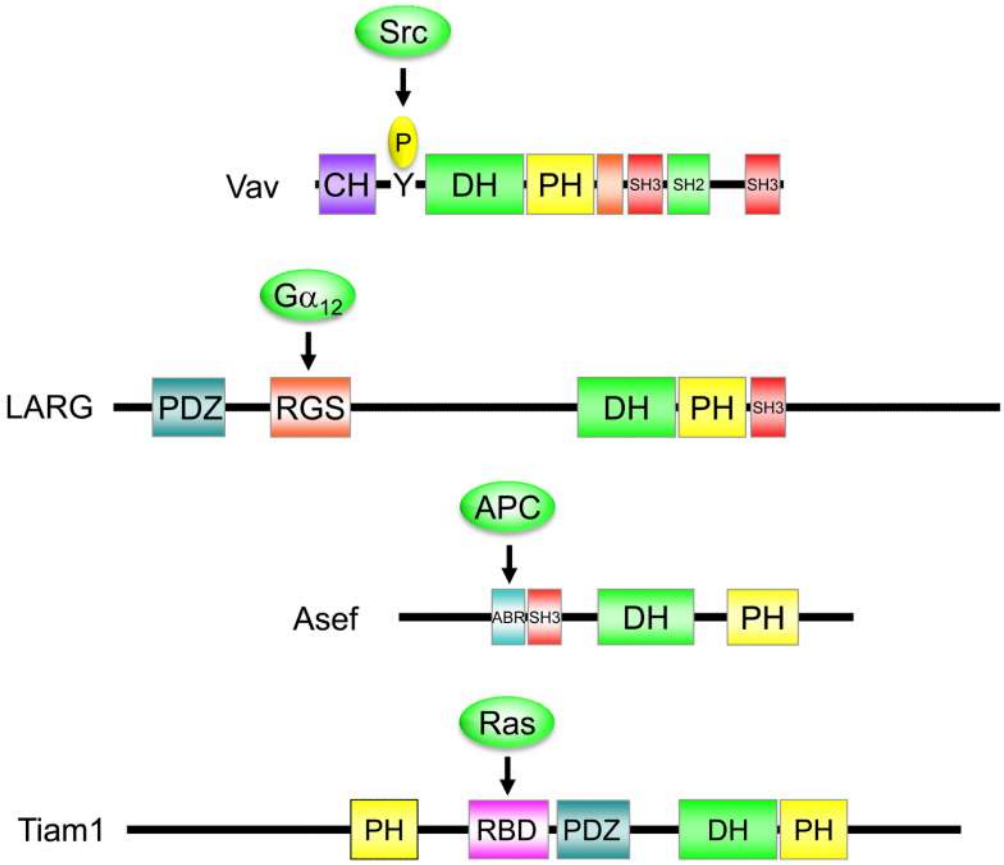


Figure 2. Regulation of RhoGEF activity

For many Dbl family RhoGEFs, N-terminal sequences upstream of the tandem DH-PH domains that catalyze exchange serve as intramolecular, auto-inhibitory sequences. This role is demonstrated by the fact that N-terminal truncations of sequences upstream of the DH-PH domains were responsible for creating the constitutively activated and transforming variants of RhoGEFs identified in transformation or invasion assays. Some RhoGEFs are activated by phosphorylation at an N-terminal motif that relieves the autoinhibitory activity. This is best characterized by Src family protein tyrosine kinase phosphorylation of Vav[167, 168] and other RhoGEFs[169, 170]. Other mechanisms of activation involve protein interaction with N-terminal domains, such G alpha 12/13 interaction with the RGS box-containing RhoGEFs (p115-RhoGEF, Larg and PDZ-RhoGEF)[171–173], Ras interaction with the RBD in Tiam1[66] and APC association with the N-terminus of Asef[174–176]. Thus, these mechanisms of upstream activation identify potential avenues for RhoGEF inhibition in cancer. For example, Vav activation in pancreatic and head and neck cancers involves activation by EGFR-mediated phosphorylation and activation. Therefore, inhibitors of EGFR or the intermediate Src family kinases may be one approach for blocking Vav-mediated oncogenesis.

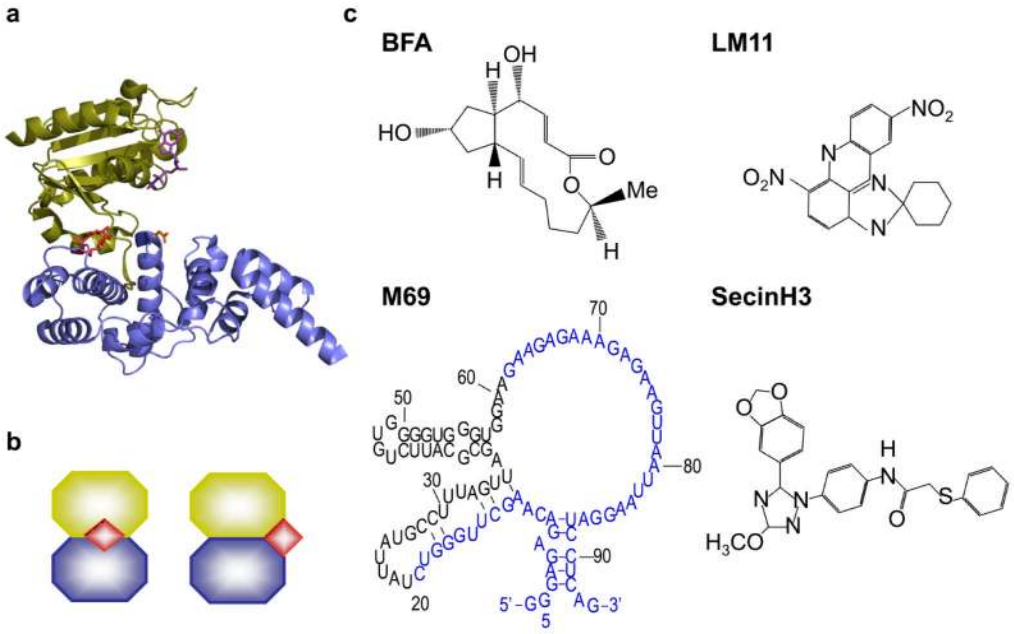


Figure 3. Inhibition of GEFs by Brefeldin A and related molecules

a | The crystallographic structure of Arf-GDP-Sec7 complex inhibited by BFA (modified from[128]). BFA (in red) sneaks in a hydrophobic cavity at the interface between the small G protein Arf (green) and the catalytic domain of its GEF (blue), where it establishes tight hydrophobic and polar contacts with both partners of the complex[128, 129]. Nature probably selected this low affinity intermediate (>100 mM) because its energy is unbalanced. This unbalance triggers the conformational change that secures GTP-bound Arf to membranes in the unperturbed reaction[128], but also yields the conditions for the binding of a small molecule inhibitor. **b |** Interfacial inhibitors trap abortive complexes by binding in (left), or at the periphery of (right), protein-protein interfaces. Molecules that inhibit the Sec7 domain of ArfGEFs: BFA, LM11, SecinH3 and M69. (reproduced from[128, 131, 133, 134])