ANNUAL REVIEWS

Annual Review of Cancer Biology

Targeting KRAS Directly

Frank McCormick[1,2]

[1]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, California 94143, USA; email: frank.mccormick@ucsf.edu

[2]Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702, USA

Annu. Rev. Cancer Biol. 2018. 2:81–90

The *Annual Review of Cancer Biology* is online at cancerbio.annualreviews.org

https://doi.org/10.1146/annurev-cancerbio-050216-122010

Keywords

KRAS, small GTPase, targeted therapy, oncogene, GTP-binding protein, precision medicine

Abstract

RAS proteins play a major, causal role in many human cancers. No therapies have been developed for these cancers because the RAS protein has been considered undruggable given that it has no accessible pocket to which a drug could bind with high affinity, and the mutant proteins that cause cancer are virtually identical to their essential, wild-type counterparts. New technologies in drug development, such as nuclear magnetic resonance–based fragment screening and covalent tethering, and new insights into RAS structure and function have changed this perception and facilitated the development of several drug candidates.

INTRODUCTION

RAS proteins play a direct role in many human cancers, including pancreatic cancers, lung adenocarcinomas, and colorectal cancers (Stephen et al. 2014). *KRAS* is mutated most frequently, followed by *NRAS* and *HRAS* (Prior et al. 2012). However, cancers driven by mutant RAS proteins are refractory to most therapies, and no drugs have been approved that act on RAS proteins directly. In this review, I discuss prospects for targeting RAS proteins directly. To date, most efforts to treat cancers with *RAS* mutations have focused on targets downstream of mutant RAS, such as RAF, MEK, or PI-3 kinase, each of which are druggable. However, these approaches have not yet proven successful. Therefore, attention has refocused on finding drugs that interact directly with RAS proteins to block their function (Ostrem & Shokat 2016).

RAS STRUCTURE AND FUNCTION

RAS proteins consist of a highly conserved GDP/GTP-binding domain (the G domain) (**Figure 1**) and a C-terminal region that is responsible for membrane localization. This region is often referred to as the hypervariable region (HVR) because it differs among RAS family members. KRAS has two splice variants, KRAS4A and KRAS4B, which share the same G domain but have different HVRs (**Figure 1**). The G domain is conserved among a large superfamily of proteins that includes elongation factors, heterotrimeric G proteins, and small GTPases (Bourne et al. 1991). These proteins use GDP or GTP to maintain them in inactive or active states, respectively, and bind these nucleotides with picomolar affinities. Many of the residues that contact GDP or GTP directly are identical among this large family of proteins. These considerations, along with the high intracellular concentrations of GTP, have discouraged attempts to find nucleotide analogs that might compete for binding and somehow inactivate RAS function. In contrast to protein kinases, RAS proteins do not have an active site that is accessible to chemical inhibitors.

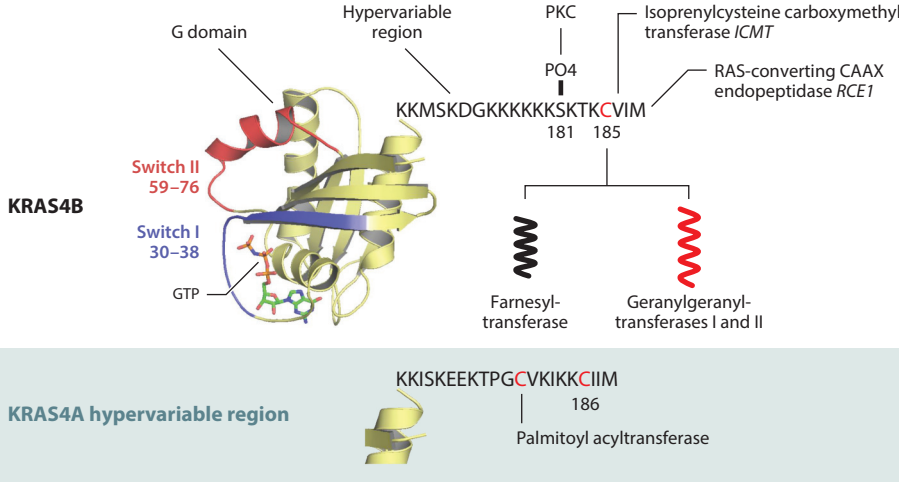

Figure 1

KRAS4A and -4B. The G domain is identical between KRAS4A and -4B. Switches I and II change in formation when GDP switches to GTP. The hypervariable regions, encoded in exon 4A or 4B, are shown as linear sequences, as there are no structures of these regions available. Enzymes involved in processing the CAAX sequences at the C termini are shown, as well as enzymes that modify KRAS4A (palmitoyl acyltransferase) and KRAS4B [protein kinase C (PKC)].

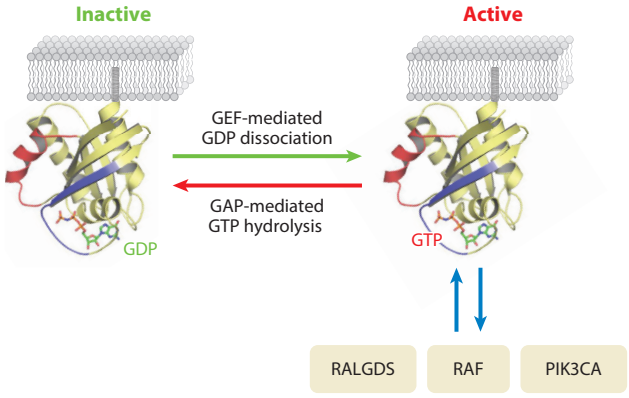

Figure 2

RAS proteins are localized in the membrane through their farnesylated C-terminal regions. Inactive, GDP-bound forms are switched to active GTP-bound forms in response to cellular signals that recruit guanine nucleotide exchange factors (GEFs) to the plasma membrane. These GEFs include SOS, RASGRP, and RASGRF family members (Bos et al. 2007). The GTP-bound forms recruit and activate effectors such as RAF kinases (ARAF, BRAF, and CRAF), as well as PI-3′ kinases, RALGDS proteins, and potentially others (Stephen et al. 2014). Active RAS is converted to the inactive form by GTP-activating proteins (GAPs), including NF1, RASA, RASA2, SYNGAP, and RASAL.

The proportion of RAS proteins in the active GTP-bound state or the inactive GDP state is determined by large, multidomain regulatory proteins that are recruited to RAS in the plasma membrane in response to multiple signals (Bos et al. 2007). Guanine nucleotide exchange factors bind to RAS proteins in response to growth factor signals, resulting in a reduced affinity for nucleotides (**Figure 2**). GDP dissociates and is replaced by GTP, because GTP is in excess relative to GDP in cells. RAS.GTP binds and activates effectors, as described below. Alternatively, RAS.GTP can bind to GTP-activating proteins (GAPs), resulting in GTP hydrolysis. The binding of GAPs to RAS proteins creates an active site for GTP hydrolysis (Scheffzek et al. 1997). In the absence of GAPs, RAS proteins hydrolyze GTP very slowly, with a half-life of about 30 min (Hunter et al. 2015). GAP stimulates GTP hydrolysis by up to 100,000-fold, depending on the local concentration of GAP proteins in the plasma membrane.

Most oncogenic *RAS* mutations make them resistant to GAPs. Codon-12 and -13 mutations replace glycine with other residues, whose bulkier side chains prevent a critical arginine residue in GAPs (the arginine finger) from triggering GTP hydrolysis. Glutamine 61 in normal RAS proteins is a key part of the GTP hydrolysis mechanism: Replacing this by mutations therefore prevents GTP hydrolysis. Loss of GAP-mediated GTPase activity results in the accumulation of RAS in the GTP state and persistent downstream signaling. Glutamine 61 mutants have extremely low rates of intrinsic GTPase activity in addition to their complete insensitivity to GAP. For these mutants, the levels of GTP-bound forms are close to 100%, reflecting the ratio of GTP to GDP in the cell. However, codon-12 mutants retain levels of intrinsic GTPase activity that, in some cases, are similar to wild-type RAS (Hunter et al. 2015). The G12C mutant, as discussed below, retains wild-type levels of intrinsic GTPase activity. Yet even this mutant is about 75% GTP-bound at steady state (Patricelli et al. 2016). This is because the GDP off rate is much faster than the GTPase rate: When GDP dissociates, it is replaced by GTP. Growth factors can increase this level of binding through engagement of SOS and further acceleration of GDP dissociation, but the effects on levels of G12C RAS.GTP are not dramatically increased. In contrast, wild-type RAS is almost exclusively GDP-bound in the absence of growth factors because GAP activity

dominates under these conditions. From these considerations, it seems that drugs that block SOS activity are unlikely to have a significant effect on oncogenic mutants but will dramatically inhibit wild-type RAS activity.

TARGETING RAS PROCESSING

The first attempts to target RAS proteins were based on the discovery that they are farnesylated (Hancock et al. 1989) and that this posttranslational modification is essential for RAS activity (Willumsen et al. 1984). Several potent farnesyltransferase inhibitors (FTIs) were discovered and developed, and early preclinical data looked promising. Unfortunately, FTIs failed to show clinical efficacy (Cox et al. 2015). These disappointing results have been attributed to the fact that KRAS and NRAS can also be prenylated by geranylgeranyltransferase, which can take over from farnesyltransferase in the presence of FTIs. In hindsight, it was unlucky that most of the preclinical work on FTIs was, for historical reasons, performed on cells transformed by HRAS rather than KRAS or NRAS. HRAS is not subject to alternative prenylation by geranylgeranyltransferase, and tumors driven by *HRAS* mutations responded well to FTIs (Cox et al. 2015). Furthermore, unlike KRas, HRas is dispensable in normal mice, suggesting that FTIs might have an excellent therapeutic window. Another concern with FTIs relates to the large number of other proteins in cells that depend on farnesyltransferase. However, the lack of activity against KRAS- and NRAS-driven tumors made these concerns irrelevant.

Advances in sequencing technology have made it possible and practical to analyze DNA from cancer patients so that they can be assigned appropriate targeted therapies. Patients with tumors driven by oncogenic HRAS could benefit from FTIs, according to preclinical data from the early days of FTI development. Five percent of bladder cancers and thyroid cancers harbor *HRAS* mutations, as do squamous cell carcinomas of the head and neck. A clinical trial sponsored by Kura Oncology is now underway to determine whether these patients will indeed respond to FTI therapy.

Farnesylated RAS proteins are further processed by the proteolytic removal of the AAX peptides (**Figure 1**) by RAS-converting CAAX endopeptidase 1 (RCE1) followed by the carboxymethylation of the C-terminal, farnesylated cysteine by isoprenylcysteine carboxymethyl transferase (ICMT). The potential value of targeting these enzymes is not clear: Conflicting data on the effects of genetic ablation of these enzymes have not been resolved (Cox et al. 2015). Indeed, the precise roles of these secondary modifications to the C-terminus are not clear. However, a recent unbiased screen for genes essential for proliferation of *RAS*-mutant acute myeloid leukemia (AML) cells, relative to AML with wild-type *RAS*, revealed a strong dependence of mutant *RAS* on RCE1 and ICMT (Wang et al. 2017). Ablation of these genes was as effective as ablation of CRAF, the major effector of RAS signaling.

KRAS4A, NRAS, and HRAS are palmitoylated: These modifications are essential for specific association with the plasma membrane and are catalyzed by palmitoyl acyltransferases. In contrast, KRAS4B associates specifically with the plasma membrane through the polybasic sequence, shown in **Figure 1**. In both cases, however, association with the plasma membrane is a dynamic, reversible process. Depalmitoylation of KRAS4B, NRAS, and HRAS is catalyzed by acyl protein thioesterase (APT1, also referred to as LYPLA1). Association of KRAS4B with the plasma membrane is regulated by calmodulin in response to Ca^{2+} flux and by the chaperone protein PDEδ, which binds selectively to KRAS4B through association with C-terminal amino acids, the farnesyl (or geranylgeranyl) lipid, and the C-terminal carboxymethyl group, which is essential for high-affinity binding. Compounds that block the association of KRAS4B with PDEδ are being developed as potential KRAS inhibitors (Zimmermann et al. 2013).

RAS proteins appear to cluster in membranes because of specific interactions between their processed C-terminal regions and specific membrane lipids (e.g., see Zhou et al. 2017). Whether RAS proteins bind directly to one another or simply localize to microdomains has been difficult to assess. The G domains do not form dimers in solution, but protein-protein contacts can be seen in crystals, fluorescently tagged RAS proteins certainly appear to dimerize, and, indeed, forced dimerization increases signal output (Nan et al. 2015). From the perspective of therapeutic intervention, a protein-protein interface that is necessary for efficient RAS activity might provide an effective drug target, but more needs to be done to verify (or refute) the existence of such an interface. An encouraging example of such an effort recently proposed a novel interface based on the effects of monobodies that bind directly to RAS proteins in cells and disrupt their membrane distribution (Spencer-Smith et al. 2017).

FRAGMENT-BASED SCREENS FOR RAS BINDERS

In 1996, Stephen Fesik of Abbot Laboratories described a novel process for discovering drugs that bind to proteins lacking deep pockets or active sites. This process bootstraps toward a high-affinity binding compound by first identifying compounds that bind at shallow sites using nuclear magnetic resonance (NMR) as readout and then the chemical linkage of compounds that bind at distinct sites to generate a high-affinity compound with multivalent binding. The process is referred to as SAR (structure-activity relationships) by NMR (Shuker et al. 1996). This approach has not yet been applied successfully to identify RAS binders, but the first step, the identification of compounds that bind directly by NMR, led two research groups (Maurer et al. 2012, Sun et al. 2012) to identify compounds that bind directly to RAS. This pocket is part of the SOS-binding site, and, indeed, compounds that bind here block SOS-mediated GDP/GTP exchange. Unfortunately, blocking SOS is expected to inhibit wild-type RAS but not oncogenic mutants, as the latter are already GTP-loaded because they are refractory to GAPs. Interestingly, other, earlier attempts to identify compounds that bind to RAS resulted in compounds that block RAS-SOS interaction and have not been further developed (summarized in Ostrem & Shokat 2016).

IN SILICO SCREEN FOR RAS BINDERS

Recently, Stockwell and colleagues (Welsch et al. 2017) successfully used computational methods to identify compounds that bind directly to RAS. They chose to focus on a site in switch I that is involved in effector binding and undergoes conformational changes when RAS proteins switch between the GDP- and GTP-bound forms. They screened a library of compounds that might bind at this site using in silico docking technology. This led to the identification of a small number of compounds predicted to bind at this shallow pocket, and, indeed, subsequent biochemical analysis revealed weak binding at this site. They then extended this approach to two or three adjacent predicted sites to obtain multivalent inhibitors. These predicted compounds were synthesized and shown to bind at 4–6-μM affinity and be biologically active in cells. This remarkable undertaking illustrates one elegant solution to the challenge of targeting a protein with no single deep pocket and may well lead to compounds that can soon be tested against RAS cancers.

TARGETING ONCOGENIC MUTANTS SPECIFICALLY

In the mid-1980s, my colleagues and I (Feramisco et al. 1985) showed that antibodies that recognize mutant forms of RAS directly reverse transformation when injected into cancer cells driven by cognate mutant RAS proteins. This proof-of-principle experiment showed that cells transformed

by RAS retain dependence on RAS for their phenotype, a result that has been repeated in many ways, including the use of temperature-sensitive mutants and through the in vivo depletion of RAS using inducible genetic systems. Although our early proof-of-principle experiment had no practical value, it was of interest because the antibodies used, which were raised against peptide sequences corresponding to codon-12 substitutions, were unable to bind to RAS in its native, nucleotide-bound form. This is because the P-loop that contains codon 12 is buried within the RAS protein in its native, nucleotide-bound state. The fact that the antibody binds to this region in cells suggests that the RAS protein cycles through a state in which this region is exposed at a reasonable frequency and that this state could potentially be targeted.

In a breakthrough in RAS targeting, Ostrem, Shokat, and colleagues (Ostrem et al. 2013) described an approach for targeting the G12C mutant of KRAS specifically. This approach was based on another novel screening technology, referred to as tethering. Like SAR by NMR, this is a multistep process developed to facilitate the discovery of compounds that bind to shallow pockets. In this case, a covalent bond to a neighboring cysteine compensates for the binding energy associated with a deep pocket. The initial screen is performed in high concentrations of the reducing agent so that stable association with a compound tethered through cysteine requires noncovalent binding to the protein surface. Ostrem, Shokat, and colleagues (Ostrem et al. 2013) applied this concept in a creative way to find compounds that covalently react with the cysteine-12 oncogenic mutation that occurs frequently in lung adenocarcinoma (the G to C substitution is a hallmark of exposure to tobacco smoke). Structural analysis of a hit from this screen tethered to cysteine 12 revealed a noncovalent binding site that had not been previously identified.

The next step in the tethering approach requires the conversion of lead compounds to irreversible electrophiles that are likely to form stable adducts under physiological conditions. This process led to the discovery of compounds that react covalently and specifically with KRAS G12C in cells and inhibit its function. Derivatives of this initial compound are currently in preclinical development.

An unexpected feature of G12C-specific compounds was their specificity for the GDP-bound form of KRAS. At first, this appeared to be a significant weakness to this approach, as the proportion of total KRAS G12C in the inactive, GDP state is only about 25%. Fortunately, however, the intrinsic rate is quite high (the half-life is 30 min) so that the G12C mutant cycles between GDP- and GTP-bound states frequently enough to allow the irreversible inhibitor to react to the GDP-bound form and prevent further cycling (Patricelli et al. 2016). This brilliant approach may lead to G12C-specific compounds that could treat up to 15% of lung adenocarcinomas. It has also encouraged efforts to find compounds specific to other alleles, such as the G12D allele, the most common of all KRAS mutants.

Targeting G12C and analyzing compounds discovered through tethering led to a new strategy for targeting oncogenic RAS: trapping the mutant in the inactive GDP state. In principle, tethering compounds could bind to the GTP state of oncogenic RAS. Such compounds could perturb switch I, directly or allosterically, and so prevent effector engagement. Alternatively, they could bind to the mutant RAS protein but have no effect on its function. If so, analogs could be designed and tested that extend the footprint so that critical regions of the RAS protein would then be affected. They could also be used to promote degradation using PROTAC technology, in which E3 ligases are redirected to target the RAS-drug complex instead of the natural substrate (Lai et al. 2016).

A different approach to inhibiting oncogenic RAS function involves restoring GTP hydrolysis. The loss of GTPase activity, after all, is the basis of oncogenic activation. As a proof of principle, Ahmadian et al. (1999) showed that HRAS G12V is capable of hydrolyzing GTP analogs in which amide functions are covalently attached to the γ-phosphate. This implied that a small molecule that provides the amide function at the same position in the GTPase site could enable GTP

hydrolysis for oncogenic mutants. To date, no structures of oncogenic mutants of KRAS in the GTP state have been published, but molecular modeling suggests that there is insufficient space in the GTPase site for such a molecule to bind, at least for codon-12 and -13 mutants. For codon-Q61 mutants, there may be more opportunities to restore GTPase activity to some extent. A compound that bound in the GTPase site and provided the amide function normally provided by glutamine could, in theory, promote GTP hydrolysis. Further analysis of oncogenic mutants complexed with GAPs should enable a better assessment of these possibilities.

TARGETING KRAS BUT NOT HRAS OR NRAS

Although efforts are underway to find ways of targeting mutant alleles specifically, it should be recognized that direct attack on proteins such as the KRAS G12V or G12D may not be possible and that less-specific approaches may be necessary. Compounds that target KRAS but not NRAS or HRAS may offer sufficient specificity to be safe and effective. Adult mice in which KRas has been ablated live for about one year, whereas mice in which all three *Ras* genes are ablated die rapidly, as expected. Therefore, drugs that target KRAS but spare other RAS proteins seem likely to be effective and safe ways of treating KRAS-driven tumors. However, KRAS proteins exist as two splice variants, KRAS4A and KRAS4B. Of these, KRAS4B is the most abundant and distinctive: Its polybasic C-terminal tail has unique properties not shared by KRAS4A, NRAS, or HRAS. This tail binds calmodulin (Villalonga et al. 2001), is phosphorylated by PKC (Sung et al. 2013), and confers stem-like functions on KRAS4B (Quinlan et al. 2008, Wang et al. 2015). As such, targeting KRAS4B is an attractive therapeutic option. For example, KRAS4B promotes stem-like features in cancer cells that make them more drug resistant and better able to initiate tumors and express stem-like markers on the cell surface. These properties have been attributed to KRAS4B's interaction with calmodulin. Indeed, KRAS4B inhibits CaM kinase and so suppresses noncanonical Ca^{2+}/Wnt signaling. Binding of KRAS4B to calmodulin can be reversed by PKC-dependent phosphorylation at serine 181 (Wang et al. 2015). Agents that increase phosphorylation at serine 181 reverse KRAS4B-mediated stemness, suggesting a novel approach to reversing specific aspects of KRAS signaling.

However, cancer cells express both KRAS4A and KRAS4B, and the former is sufficient to initiate and maintain tumors. To target both, we need drugs that target both isoforms. This could be achieved by developing agents that interact specifically with each protein, although the prospects of identifying compounds that block KRAS4A without affecting NRAS and HRAS are daunting. An alternative strategy is to find compounds that target the KRAS G domain, which is shared by both KRAS4A and KRAS4B. Indeed, the KRAS G domain differs from HRAS's G domain by five acids and from that of NRAS by eight amino acids. Whether these differences can be exploited remains to be seen.

PEPTIDES AND PROTEIN-BASED APPROACHES

Efforts to use conventional screening systems to identify small molecules that block RAS binding directly to its effectors, primarily RAF kinases, have not yet been successful because the interface between these proteins is part of an antiparallel β-sheet that offers no pockets to which a small molecule could bind at high affinity (Mott & Owen 2015). Although it is encouraging that single amino acid changes in this region, on either RAS or RAF, completely abrogate binding, the challenge of finding high-affinity binders remains. Some early progress has been made, but to date, no obvious preclinical candidates have emerged.

Peptides have sufficient size and complexity to bind to a target protein specifically. Peptides that block the interaction of RAS proteins with GAPs or of RAF kinase with micromolar potencies have

indeed been described but have not advanced toward the clinic. These have typically been linear peptides based on sequences of the effectors themselves (Clark et al. 1996). Stapled peptides have been described that look more promising as potential drug leads, as they are more stable and potent and have activity in cells. To date, most of these efforts have focused on blocking the interaction of RAS with SOS because the stapled peptides are based on stabilized α-helical structures rather than the antiparallel sheets that compose the RAS-effector binding interface. These compounds do indeed block EGF-activated RAS/MAPK signaling, showing that they block normal RAS function (Leshchiner et al. 2015). Hopefully stapled peptides will soon be identified that specifically block KRAS or oncogenic mutants.

Another creative approach toward using the complexity and specificity of peptides to block RAS-effector interaction was devised and developed by Briesewitz and Pei (Upadhyaya et al. 2015), who sought to find compounds that could bind RAS and recruit FK506BP to suppress RAS activity, just as rapamycin recruits FK506BP to block mTOR activity. After several cycles of optimization and crystallographic analysis, cyclic peptides were identified that bind RAS and block RAF binding, even in the absence of FK506BP (Upadhyaya et al. 2015). One of these compounds, cyclorasin 9A, binds to RAS at a distributed site between switch I and switch II, with a binding constant of 120 nM, and shows activity in cells. Furthermore, it is more effective against RAS in the GTP state and is active in cells. A potential concern might be that the area of RAS to which the compound binds is identical between all RAS isoforms, but the approach and data are encouraging nevertheless.

In the early years of RAS biology, anti-RAS antibodies were used to probe the RAS pathway in living cells, as mentioned above. The monoclonal antibody Y13-259 was used in many of these early microinjection experiments. This antibody was cloned and expressed in cells as a single-chain variable fragment and shown to block cancer cell proliferation when expressed, even in vivo (Tanaka & Rabbitts 2010). More recently, Cetin et al. (2017) developed a protein inhibitor against KRAS based on an engineered domain of human fibronectin. This inhibitor binds to RAS with higher affinity than the RAS-binding domain of RAF. In addition to the protein-based RAS binders that block effector interactions, proteins that inhibit RAS allosterically have been reported: Spencer-Smith et al. (2017) isolated a monobody molecule from a phage library that binds to HRAS and KRAS (but not NRAS, remarkably) with K_D values of 14 and 67 nM, respectively.

FUTURE PROSPECTS

Targeting RAS represents a tremendous unmet clinical need that has motivated a new wave of efforts to target RAS proteins directly, including the National Cancer Institute RAS Initiative at the Frederick National Laboratory for Cancer Research and multiple efforts in academia and industry. New technologies such as NMR-based fragment screening, tethering, and in silico drug design have already enabled the discovery of small molecules that bind to RAS. These face the challenges of achieving sufficient potency and specificity. Unconventional approaches including peptides and proteins are also encouraging but face challenges related to delivery. Nevertheless, these challenges do not seem insurmountable and are likely to be solved by continued research and development driven by creative investigators determined to benefit patients suffering from these terrible cancers.

DISCLOSURE STATEMENT

The author is a cofounder of Wellspring Biosciences and Araxes Pharma and a scientific advisor to Kura Oncology.

ACKNOWLEDGMENTS

I wish to thank members of my laboratory at UCSF and at the Frederick National Lab for their brilliant efforts toward solving the RAS problem. I am supported by Daiichi Sankyo, Tokyo, a National Cancer Institute Outstanding Investigator Award (5R35CA197709), the Lustgarten Foundation, and the National Cancer Institute, NIH contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, and the mention of trade names, commercial products, or organizations does not imply endorsement by the US government.

LITERATURE CITED

Ahmadian MR, Zor T, Vogt D, Kabsch W, Selinger Z, et al. 1999. Guanosine triphosphatase stimulation of oncogenic Ras mutants. *PNAS* 96:7065–70

Bos JL, Rehmann H, Wittinghofer A. 2007. GEFs and GAPs: critical elements in the control of small G proteins. *Cell* 129:865–77

Bourne HR, Sanders DA, McCormick F. 1991. The GTPase superfamily: conserved structure and molecular mechanism. *Nature* 349:117–27

Cetin M, Evenson WE, Gross GG, Jalali-Yazdi F, Krieger D, et al. 2017. RasIns: genetically encoded intrabodies of activated Ras proteins. *J. Mol. Biol.* 429:562–73

Clark GJ, Drugan JK, Terrell RS, Bradham C, Der CJ, et al. 1996. Peptides containing a consensus Ras binding sequence from Raf-1 and the GTPase activating protein NF1 inhibit Ras function. *PNAS* 93:1577–81

Cox AD, Der CJ, Philips MR. 2015. Targeting RAS membrane association: back to the future for anti-RAS drug discovery? *Clin. Cancer Res.* 21:1819–27

Feramisco JR, Clark R, Wong G, Arnheim N, Milley R, McCormick F. 1985. Transient reversion of *ras* oncogene-induced cell transformation by antibodies specific for amino acid 12 of *ras* protein. *Nature* 314:639–42

Hancock JF, Magee AI, Childs JE, Marshall CJ. 1989. All *ras* proteins are polyisoprenylated but only some are palmitoylated. *Cell* 57:1167–77

Hunter JC, Manandhar A, Carrasco MA, Gurbani D, Gondi S, Westover KD. 2015. Biochemical and structural analysis of common cancer-associated KRAS mutations. *Mol. Cancer Res.* 13:1325–35

Lai AC, Toure M, Hellerschmied D, Salami J, Jaime-Figueroa S, et al. 2016. Modular PROTAC design for the degradation of oncogenic BCR-ABL. *Angew. Chem. Int. Ed.* 55:807–10

Leshchiner ES, Parkhitko A, Bird GH, Luccarelli J, Bellairs JA, et al. 2015. Direct inhibition of oncogenic KRAS by hydrocarbon-stapled SOS1 helices. *PNAS* 112:1761–66

Maurer T, Garrenton LS, Oh A, Pitts K, Anderson DJ, et al. 2012. Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity. *PNAS* 109:5299–304

Mott HR, Owen D. 2015. Structures of Ras superfamily effector complexes: What have we learnt in two decades? *Crit. Rev. Biochem. Mol. Biol.* 50:85–133

Nan X, Tamguney TM, Collisson EA, Lin LJ, Pitt C, et al. 2015. Ras-GTP dimers activate the Mitogen-Activated Protein Kinase (MAPK) pathway. *PNAS* 112:7996–8001

Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. 2013. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 503:548–51

Ostrem JM, Shokat KM. 2016. Direct small-molecule inhibitors of KRAS: from structural insights to mechanism-based design. *Nat. Rev. Drug. Discov.* 15:771–85

Patricelli MP, Janes MR, Li LS, Hansen R, Peters U, et al. 2016. Selective inhibition of oncogenic KRAS output with small molecules targeting the inactive state. *Cancer Discov.* 6:316–29

Prior IA, Lewis PD, Mattos C. 2012. A comprehensive survey of Ras mutations in cancer. *Cancer Res.* 72:2457–67

Quinlan MP, Quatela SE, Philips MR, Settleman J. 2008. Activated Kras, but not Hras or Nras, may initiate tumors of endodermal origin via stem cell expansion. *Mol. Cell Biol.* 28:2659–74

Scheffzek K, Ahmadian MR, Kabsch W, Wiesmuller L, Lautwein A, et al. 1997. The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science* 277:333–38

Shuker SB, Hajduk PJ, Meadows RP, Fesik SW. 1996. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* 274:1531–34

Spencer-Smith R, Koide A, Zhou Y, Eguchi RR, Sha F, et al. 2017. Inhibition of RAS function through targeting an allosteric regulatory site. *Nat. Chem. Biol.* 13:62–68

Stephen AG, Esposito D, Bagni RK, McCormick F. 2014. Dragging Ras back in the ring. *Cancer Cell* 25:272–81

Sun Q, Burke JP, Phan J, Burns MC, Olejniczak ET, et al. 2012. Discovery of small molecules that bind to K-Ras and inhibit Sos-mediated activation. *Angew. Chem. Int. Ed.* 51:6140–43

Sung PJ, Tsai FD, Vais H, Court H, Yang J, et al. 2013. Phosphorylated K-Ras limits cell survival by blocking Bcl-xL sensitization of inositol trisphosphate receptors. *PNAS* 110:20593–98

Tanaka T, Rabbitts TH. 2010. Interfering with RAS–effector protein interactions prevent RAS-dependent tumour initiation and causes stop–start control of cancer growth. *Oncogene* 29:6064–70

Upadhyaya P, Qian Z, Selner NG, Clippinger SR, Wu Z, et al. 2015. Inhibition of Ras signaling by blocking Ras–effector interactions with cyclic peptides. *Angew. Chem. Int. Ed.* 54:7602–6

Villalonga P, Lopez-Alcala C, Bosch M, Chiloeches A, Rocamora N, et al. 2001. Calmodulin binds to K-Ras, but not to H- or N-Ras, and modulates its downstream signaling. *Mol. Cell Biol.* 21:7345–54

Wang MT, Holderfield M, Galeas J, Delrosario R, To MD, et al. 2015. K-Ras promotes tumorigenicity through suppression of non-canonical Wnt signaling. *Cell* 163:1237–51

Wang T, Yu H, Hughes NW, Liu B, Kendirli A, et al. 2017. Gene essentiality profiling reveals gene networks and synthetic lethal interactions with oncogenic Ras. *Cell* 168:890–903.e815

Welsch ME, Kaplan A, Chambers JM, Stokes ME, Bos PH, et al. 2017. Multivalent small-molecule pan-RAS inhibitors. *Cell* 168:878–89.e829

Willumsen BM, Christensen A, Hubbert NL, Papageorge AG, Lowy DR. 1984. The p21 *ras* C-terminus is required for transformation and membrane association. *Nature* 310:583–86

Zhou Y, Prakash P, Liang H, Cho KJ, Gorfe AA, Hancock JF. 2017. Lipid-sorting specificity encoded in K-Ras membrane anchor regulates signal output. *Cell* 168:239–51.e216

Zimmermann G, Papke B, Ismail S, Vartak N, Chandra A, et al. 2013. Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic KRAS signalling. *Nature* 497:638–42