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Oncogenic Ras Isoforms Signaling Specificity at the Membrane

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Abstract

How do Ras isoforms attain oncogenic specificity at the membrane? Oncogenic KRas, HRas and NRas (K-Ras, H-Ras, N-Ras) differentially populate distinct cancers. How they selectively activate effectors and why is KRas4B the most prevalent are highly significant questions. Here we consider determinants that may bias isoform-specific effector activation and signaling at the membrane. We merge functional data with a conformational view to provide mechanistic insight. Cell-specific expression levels, pathway cross-talk and distinct interactions are the key; but conformational trends can modulate selectivity. There are two major pathways in oncogenic Ras-driven proliferation: MAPK (Raf/MEK/ERK) and PI3K α /Akt/mTOR. All membrane-anchored, proximally-located, oncogenic Ras isoforms can promote Raf dimerization and fully activate MAPK signaling. So why the differential statistics of oncogenic isoforms in distinct cancers and what makes KRas so highly oncogenic? Many cell-specific factors may be at play, including higher *KRAS* mRNA levels. As a key factor, we suggest that because only KRas4B binds calmodulin, only KRas can *fully* activate PI3K α /Akt signaling. We propose that *full* activation of both MAPK and PI3K α /Akt proliferative pathways by *oncogenic* KRas4B – but not by HRas or NRas – may help explain why the KRas4B isoform is especially highly populated in certain cancers. We further discuss pharmacological implications.

Keywords

K-RAS; H-RAS; N-RAS; K-RAS4A; K-RAS4B; KRas4A; small molecule drug

Introduction

A question which has been at the center stage of cancer biology is *why the differential statistics of Ras oncogenic isoforms in distinct cancers*. Here we argue that even though conformational heterogeneity at the membrane will influence signaling preferences – it will

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not fully explain them. The more likely reasons are certain determinants of cell specificity. Below, we review possible cell-specific factors differentiating KRas4B signaling from HRas and NRas, and suggest that these may help unravel Ras isoforms oncogenic specificity.

HRas, NRas, and alternatively spliced KRas4A and KRas4B control key cell proliferation pathways (1–3). They bind a common set of activators and effectors (4, 5), but are not functionally redundant and certain oncogenic isoforms are more commonly observed in distinct cancer types (4, 6–8). Interest has largely focused on oncogenic mutants of KRas4B because of its relatively high frequency in cancers, particularly adenocarcinomas in pancreas, colorectal and lung cancers (9), although early (10–12) and more recent work (13–15) affirmed that KRas4A can also be widely expressed in human cancer cell lines, and that the relationship between KRas4A/KRas4B may be the reason for the higher frequency of mutations observed in KRas4A (10).

Ras isoforms signal effectively when anchored and enriched at the plasma membrane (PM) (16–21). Signaling pathways include mitogen-activated protein kinase (MAPK), phosphoinositide-3-kinase (PI3K), Ras-Ral guanine nucleotide dissociation stimulator (RalGDS), phospholipase C ϵ (PLC ϵ), Cdc42/Rac, Ras association domain family 5 (RASSF5, also known as NORE1A) mediating mammalian sterile 20-like kinase 1/2 (MST1/2) Hippo pathway (albeit with limited direct experimental data supporting RASSF5 repressing Yes-associated protein 1 (YAP1) activity (22)), as well as the emerging pathway cross-talk (23).

Insight into *how differential isoform signaling at the membrane is achieved* is among the most coveted aims of Ras cancer biology (1, 6, 15). Ras proteins consist of the soluble catalytic G-domain and the hypervariable region (HVR). The sequences and structures of the catalytic domains of the isoforms are highly similar, in contrast to their disordered C-terminal HVRs that attach them to the PM (24–26). Nature stapled the HVRs with distinct combinations of lipid post-translational modifications (PTMs) – farnesyl (or geranylgeranyl) and palmitoyl. Membrane attachment can also be regulated by cell-specific mechanisms, such as monoubiquitination (27). PM lipids segregate laterally into microdomains with distinct composition and organization (28–30) which appear to be differentially preferred by Ras isoforms, resulting in homogeneous dimerization and nanoclustering. The microdomain environments can influence Ras structure and orientation at the membrane, which influences Ras isoforms accessibility, thus association states with its effectors (31). Recent saturation mutagenesis of the catalytic domain unraveled the selection pressures to retain (or prevent loss of) Ras function (32). The similarity of the catalytic domains and heterogeneity of the HVRs along with their combinations of lipid PTMs speak of encoding unique functional roles (33). A key question is thus how the HVRs assist in biasing isoform signaling selectivity, and how they partner with the conserved catalytic domains to execute function. To date, attention has largely focused on revealing the interplay between Ras isoform-specific lipidation motifs and the membrane (34). Here we discuss how they can also help to accomplish Ras isoform-specific signaling. Insight into these mechanisms may help tumor-specific drug discovery.

An added level of selectivity is among oncogenic mutants of the same isoform (24). As documented for *KRAS*, specific mutations can be more frequent in distinct adenocarcinomas (9), leading to questions like how mutations in the catalytic domain can bias the conformational ensemble, and whether distinct mutations may promote oncogenic signaling specificity via the farnesylated HVR behavior. Allosteric effects elicited by mutations can affect the conformation of the effector binding site (35, 36) as well as its exposure (14, 37); they can affect regulatory sites (38), enhance nanoclustering (39), and be mediated by the HVR at the PM (33, 40). Mutant Ras molecules are also greatly affected by cell-specific environments (14) and genetics (41, 42).

Here we address the long-standing question of how Ras isoforms (and their distinct mutants) acquire oncogenic specificity despite their high structural homology. Our model integrates functional cell biology data with conformational theory. Our key underlying hypotheses are that (i) the conformational behavior of Ras at the membrane is important in modulating specificity but is unable to explain isoform and oncogenic mutant signaling preferences in specific cancers which we believe is determined by the cell-specific environment; and (ii) even though all adjacent membrane-anchored oncogenic Ras isoforms can stimulate Raf's dimerization and fully activate MAPK, only oncogenic KRas4B can fully activate PI3K α /Akt signaling. This may explain why KRas4B (and KRas4A) – rather than HRas or NRas – is particularly oncogenic. Perceiving differential isoform signaling at the membrane requires a grasp of Ras conformational landscape. Below we provide the basis, and then discuss isoform selectivity from this standpoint.

The Importance of a Structural View

Numerous pathway databases exist for Ras and its signaling pathways (43). The mammoth task of assembling cellular, biochemical, clinical and high resolution structural data results in an extremely useful overall picture; however, it still falls short. An organized and informative cartoon still cannot decipher the relationship between distinct isoforms and their preferred signaling pathways; nor are compilations able to explain the root causes of signaling trends which can be correlated with specific cancers. Compilations are critical; but insufficient. A conformational view of the data can build a structural map of Ras pathways, and consider concepts that underlie such isoform signaling enigmas.

Among these concepts, is the idea of the energy landscape (44); that is, that biomolecules are not static sculptures. Molecules continuously interconvert between different states, and the statistics of these states reflect their relative populations. These depend on mutational events, membrane composition, post-translational modifications, ion concentrations, and more. Thus, the most populated state of KRas^{G12D} differs from that of the KRas^{G12V} mutant (45, 46); and the most populated state of KRas4B at the membrane differs from that of NRas because they favor altered interactions with membrane phospholipids (14, 24, 37). Ras molecules constantly fluctuate, and the fluctuations depend on their specific sequence and the molecules that they bind, e.g. lipids, proteins, ions and water. The fluctuations are critical for the preferred orientational states with respect to the membrane as well as the time spent in these states, and the consequent exposure of the effector binding site. Forecasting them calls for deciphering atomic-scale interactions and conformational distributions, which in

principle may be achieved by molecular dynamics simulations. Current simulations are handicapped by their affordable timescales, by the idealized membrane systems whose kinetics may differ from those of complex lipid mixtures found in nature, inaccurate forcefields, and simulations of monomers rather than the functional states.

The Chemistry of the Farnesyl and Palmitoyl Biases Signaling

Ras signaling initiates at the membrane. Thus, the heterogeneity of HVR sequences across the isoforms and the distinct combinations of the lipid PTMs (Figure 1) should be considered within this framework (15). The palmitoyl is a 16-carbon saturated fatty acid attached to a cysteine residue via thioester linkage (S-palmitoylation) (47). Saturation coupled with chain length makes it a major driving force for insertion into lipid microdomains, including lipid rafts or liquid ordered phase (48–52). The thioester linkage can be hydrolyzed by palmitoyl protein thioesterases (PTEs) (53). By contrast, the thioether-linked 15-carbon farnesyl (and 20-carbon geranylgeranyl) isoprenoid group prefers to insert into liquid ordered phase membranes rich in unsaturated fatty acids (54, 55). Its hydrocarbon chain contains *cis* double bonds, making farnesyl (and geranylgeranyl) insertion reversible. In the absence of palmitoyl in KRas4B (or in depalmitoylated KRas4A), the HVR's strong positive charge stabilizes membrane anchorage (56). The PTMs' favored membrane environments coupled with their combinatorial codes and HVR properties point to distinct favored orientations of the Ras isoforms. The HVRs of HRas and NRas are electrostatically similar (13, 15). However, HRas has a farnesyl and two palmitoyls and NRas one. Like NRas, KRas4A has a farnesyl and a single palmitoyl; however, its positive charge may result in altered favored orientations (14, 15). With only a farnesyl and charged HVR, KRas4B may prefer different orientations on the anionic membranes. Why are the distinct HVR-PM states important for isoform-specific signaling at the membrane? Binding affinity reflects the strength of the interaction between the molecules. Because all isoforms bind the same set of effectors, even slight differences in the surfaces that they expose to the cytoplasm – largely due to the differential occlusion by the membrane – may alter their affinities to their cognate receptors, thus bias their effector selectivity (56–58). Hydrolysis of the palmitoyl linkage will disengage HRas and NRas whose HVR-membrane interaction is weak, and result in shuttling to the Golgi for re-palmitoylation (16). KRas4A also anchors during its palmitoylation/depalmitoylation cycle (13). Calmodulin (CaM) binding (59) or Ser181 phosphorylation by protein kinase C (PKC) or cyclic GMP (cGMP)-dependent protein kinase (PKG) (60) disengage, or reduce the interaction of KRas4B with the membrane; nonetheless, phosphorylated molecules are still able to form nanoclusters and their lateral membrane organization is unaffected (61).

The functional importance (62, 63) and different chemical properties and linkages of the palmitoyl and the farnesyl evolved varied means for their transportation (33, 64); vesicular for HRas, NRas and palmitoylated KRas4A (65), and phosphodiesterase- δ (PDE δ) for KRas4B (13, 66). Farnesyl contributes dominantly to the PDE δ –Ras interaction (66). Experimental data indicated that PDE δ can extract from the PM NRas – but not KRas4A; modeling suggested that the stronger interaction with the PM of KRas4A versus NRas can explain these extraction data (67).

Accessibility of the Effector Binding Site on the Membrane

Experiments and simulations suggest that the same catalytic domain surface can be engaged in binding protein effectors and in facing – albeit not securely interacting with – the membrane (37, 40, 58, 68). The interaction of the catalytic domain with the PM is weak. Instead, evolution has endowed proteins such as Ras and G protein subunit α_{i1} (69) with hydrophobic PTM-decorated termini (C- or N-), which are disordered when in the free, uncomplexed state (26). This elegant solution also permits multiple states, creates space for large complexes and facilitates lateral mobility (70) and nanocluster formation. Accessibility can be defined in terms of the residence time of the ensemble in an ‘open state’; occlusion denotes conformations with the binding site facing the PM and fluctuating against it, or against the HVR. Even if GTP-bound, occlusion of this site may hamper signaling of weak-binding effectors (14). Thus, whereas the classical definition of an active Ras state is based on its nucleotide (GTP/GDP) binding status, conformational reasoning argues that on its own, GTP-loading may not be sufficient for downstream effector activation from the PM (58).

In vitro and *in silico*, albeit not tested *in vivo* when competing with the membrane or PDE δ , the HVR can directly interact with the effector binding site of inactive KRas4B, and this interaction is almost 100-fold tighter than that of the active state (68). Oncogenic mutations away from this site can allosterically amplify this exposed state to facilitate effector binding (71). *In vivo*, KRas has a high degree of specificity for phosphatidylserine (PS), particularly with certain amino acid side-chain/lipid interactions (17). *In vitro*, the HVRs of GDP-bound KRas4B^{G12V/G12D} mutants interact selectively with phosphatidylinositol 3-phosphate (PI3P), phosphatidylinositol 4-phosphate (PI4P), phosphatidylinositol 5-phosphate (PI5P) and phosphatidic acid (PA), suggesting that mutations in the catalytic domain can allosterically modulate the HVR-phospholipid binding specificity, tuning KRas4B selectivity (33, 40).

Organization, Dynamics, Mobility and Signaling at the Membrane

Ras molecules favor distinct organizations in the PM, diffuse, and can dimerize. Unlike HRas^{G12V} in 2-dimyristoylglycero-3-phosphocholine (DMPC), the catalytic domains in GTP- and GDP-bound KRas4B adopt similar membrane orientations (57). Whereas HRas-GTP (but not HRas-GDP) orientations are stabilized by helix α_4 , KRas4B^{G12V} appears stabilized by the HVR. KRas4A (in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine/1,2-palmitoyl-oleoyl-sn-glycero-3-phosphoserine (POPC/POPS)) resembles KRas4B (14, 37, 58). This different behavior of HRas (and presumably NRas as well), is in line with the larger sequence differences and preferred orientations observed in dimer interfaces at the membrane for KRas4B versus HRas (72). Catalytic domain orientations are also influenced by interactions with the PM (33, 73), suggesting that they partner with HVR-phospholipid binding to accomplish signaling (74). KRas4B-GTP dimers interacting through the allosteric lobe favor interfaces involving helices α_3 and α_4 ; those of HRas-GTP may populate α_4 and α_5 . Effector lobe dimer interfaces are mostly β -sheet extension with additional species through β -sandwich (72). Raf's and RASSF5 (NORE1A) activations involve the helical dimer interface. Oncogenic mutations may alter this picture.

The lateral diffusion of Ras is as fast as lipid probes and significantly faster than a typical membrane protein (75–77). However, Ras dimerization/nanoclustering, and its assemblies including high-affinity (but not low-affinity) effectors/regulators and scaffolding protein platforms, is likely to constrain its lateral dynamics and mobility in the PM. Two main factors are at play. The first is the sheer increase in size and molecular weight. The second involves additional membrane anchorage by some of those other molecules. A KRas4B dimer will have two anchorages. If a membrane-anchored Raf is bound to each Ras molecule, anchoring of the two Raf molecules will also be involved. RASSF5 tumor suppressor presents a similar scenario (78). In the complex of KRas4B–PI3Kα both species will be anchored; binding to CaM, which does not interact with the PM, will increase the assembly size (Figure 2). Signaling complexes involving e.g. galectin-3, apoptosis-stimulating of p53 protein 2 (ASPP2) (79–81), or isoleucine-glutamine GTP-activating protein 1 (IQGAP1) (82), and its associated kinases such as Akt, and even actin (83), will slow lateral mobility and increase residence times of certain states.

Most effectors function as single entities. For these, Ras dimerization or proximity status appears irrelevant. For those whose function involves dimerization, such as Raf (84), or for mediating auto-cross-phosphorylation, such as RASSF5 (mediating MST) kinase activation (78, 85, 86)), Ras dimerization (or productive nanocluster organization which leads to similar outcomes (20, 21, 39, 87)), is vital (Figure 2). Multiple mechanisms modulate C-Raf kinase activity including phosphorylation (by Src family kinases and PAK) of Ser338, dephosphorylation of Ser259 (by serine phosphatases), interactions with 14-3-3, with lipids (such as PS and PA), and more. Single molecule fluorescence resonance energy transfer (FRET) data suggested that Raf is recruited to immobile sites on the PM (88, 89). All data point to Raf's activation requiring membrane anchorage which is assisted by Raf's cysteine-rich domain (CRD). Ras membrane-anchored nanoclusters (or dimers) increase the chance of Raf's dimerization, and from the structural standpoint, activation has been fully explained by kinase domain dimerization (84, 90). Ras nanoclusters are homogeneous, segregated by isoform and nucleotide type, likely reflecting distinct preferred local membrane composition. Blocking Ras dimerization blocks nanocluster formation resulting in loss Ras nanoclusters and MAPK signaling (91). The rate of diffusion, or mobility, of Ras monomers is a key factor in Ras dimerization (or nanoclustering). Higher mobility of Ras monomers leads to faster dimerization, promoting Raf's dimerization, thus activation and MAPK signaling. Slower mobility delays dimerization, slowing down MAPK response times. Notably, if Ras is overexpressed, MAPK signaling response times will be fast irrespective of the diffusion rate. By contrast, Ras dimerization is not required for PI3K activation. This is because its activation does not require Ras dimers. PI3K effectively acts a monomeric unit. Under physiological conditions it is activated by a single Ras molecule binding to the p110 subunit, and a phosphorylated RTK C-terminal motif which binds the SH2 domains of the p85 subunit (92). In line with this, microdomains were observed to regulate HRas-driven MAPK signaling - but not PI3K signaling (93).

The linkage between the lipid content and KRas binding has also been investigated. The inner PM leaflet is often enriched with anionic phospholipids such as PS, and cell signaling lipid molecules like phosphatidylinositols (PIs). KRas activation level in cancer cells has been linked to PS content (94) and the membrane potential influences the organization of the

phospholipids in the PM, which in turn regulates the localization and activity of KRas4B (20, 95). HVR amino acid side-chains may favor specific lipid head groups; one example is PA and KRas4B (33); another is the non-equivalency of lysine and arginine despite both being positively charged (17). Thus, taken together, mobility, and PM composition and localization may modulate Ras action (33, 96–98), substantiating the role of PM microdomains as signaling platforms (99) (Figure 1). Further substantiating this notion, relationships between inhibitory specificities of Ras isoforms and microlocalization (100) and between NRas clustering and activation patterns (101) were also observed.

Additional factors that govern membrane localization and Ras signaling include e.g., HVR motifs (102, 103), the influence of domain structure (104), PM scaffolding proteins such as galectins (105–108), partitioning of membrane molecules between raft and non-raft domains (109), and nanoclusters segregation within these (110).

Mechanisms Extracting KRas4B from the Membrane and Their Distinct Functions

Complex mechanisms coevolved to accomplish isoform-specific signaling at the membrane. At their core is the distinct chemical nature of the HVR sequences and PTMs. It is thus not surprising that the three main mechanisms extracting KRas4B, but not HRas or NRas, from the PM to block Raf's activation and MAPK signaling, also involve the distinct properties of the KRas4B HVR (16, 92, 111–113).

Phosphorylation

Modeling suggests that the strong electrostatic interaction of the phosphoryl with the HVR's lysine side-chains bends the HVR and collapses the C-terminal residues around it (56). This results in the phosphoryl releasing the farnesyl-PM interaction and increasing the farnesyl-water and farnesyl-peptide interactions. The phosphoryl also generates electrostatic repulsion between the HVR and the phospholipid headgroups. Phosphorylation reduces but does not inhibit membrane binding and clustering of KRas4B (60, 61). Electrostatic repulsion may however operate when KRas attempts to rebind the membrane after removal (56).

Ca²⁺-CaM

Ca²⁺-CaM has numerous roles in oncogenic signaling (114, 115). KRas4B's positively charged HVR – but not HRas or NRas – binds tightly to Ca²⁺-CaM, and its farnesyl docks into a CaM pocket (116). CaM's binding extracts KRas4B from the PM (59); CaM also interacts with PI3K α and activates it (117, 118). Under normal physiological conditions, a receptor tyrosine kinase (RTK) phosphorylated C-terminal peptide motif couples with KRas4B (as well as other Ras isoforms, such as HRas (119)) to fully activate PI3K α . Because the affinity of Ras to the Ras binding domain (RBD) of PI3K α is low (in the high micromolar range), PI3K α 's activation requires that Ras is also attached to the membrane, which increases the proximity and stabilizes the Ras–PI3K α interaction. In cancer, KRas4B–CaM is the driving force in Akt activation and PI3K/Akt pathway (120, 121). Oncogenic KRas4B–CaM–PI3K α ternary complex (121), where CaM replaces the RTK

motif, can explain how PI3K α gets *fully* activated by oncogenic KRas4B in the absence of a signaling cue (112). CaM's binding to the SH2 domains of the p85 subunit of PI3K α (117) releases its autoinhibition and together with KRas4B promotes its *full* activation, KRas4B/PI3K α /Akt/mTOR signaling and cell proliferation (59, 112) (Figure 2). Even though non-phosphorylated CaM molecules can bind, CaM can be phosphorylated including at Tyr99 (114, 115), and the affinity of phosphorylated CaM is higher (92, 118, 122). We expect that other, still-to-be discovered, proteins help PI3K α activation by oncogenic HRas and NRas.

PDE δ

Cytosolic PDE δ promotes effective KRas4B signaling by securing its continuous sequestration from endomembranes (16). It shuttles KRas4B to the PM to enrich local KRas4B concentration and enhance signaling of MAPK and Hippo (though only limited direct experimental data for Ras effector RASSF5 (NORE1A) repressing YAP1 activity (22)). PDE δ binding to KRas4B is tight, with high affinity (66, 67). Upon contact with a heterogeneous membrane, KRas4B–PDE δ releases the KRas4B (113). A spatial organizing cycle, facilitated by PDE δ , maintains KRas4B on the PM, countering entropic redistribution to the endomembrane (16). HRas and NRas are extracted through depalmitoylation, followed by trafficking to the Golgi and a new signaling cycle.

Factors Differentiating KRas4B from HRas and NRas Signaling

Why are the differential statistics of oncogenic isoforms in distinct cancers? That is, why is oncogenic KRas the most prevalent isoform in pancreatic cancer (over 95%) whereas NRas is the one in melanoma (15%) and HRas in bladder cancer (5%)? *Further, why is oncogenic KRAS the most abundant overall in Ras-driven cancers? What makes it so highly oncogenic?* To date, these challenging and profound questions – which are the focus of hundreds of laboratories world-wide – are still unresolved. KRas4B is more highly expressed than other isoforms (7); thus, it is not surprising that its oncogenic form is more abundant. Nonetheless, this would not explain these statistics. Cell-specific functions are largely controlled by the cellular environment and genetics (42). Thus, addressing these questions only by assessing the expression levels of the isoforms may not solve them; however, neither would consideration of only cell types, as analyses of patients' variability – even with the same type of cancer – indicate. To decipher these questions, here we ask which factors appear favorable for KRas4B signaling – but not HRas or NRas – viewing some recent observations from a conformational standpoint (Figure 1).

Oncogenic Ras isoforms may not signal equally effectively via the PI3K α /Akt pathway

The two major pathways in oncogenic Ras-driven proliferation are the MAPK (Raf/MEK/ERK) and the PI3K α /Akt/mTOR (123) (Figure 2). PI3K α has two subunits, p85 and p110; their heterodimer acts as a monomeric unit (124–126). By contrast, Raf's activation occurs via kinase domains auto-cross-phosphorylation (84, 90), which argues for dimerization or proximity (nanoclustering) of the respective Ras molecules (127–130). Oncogenic PM-anchored KRas4A, KRas4B, HRas and NRas cluster (and can dimerize) (21, 131); thus, MAPK signaling output is likely to be similar for all. This may not be the case for PI3K α /Akt/mTOR signaling (92, 112). In the absence of the RTK signal, oncogenic Ras

may be unable to *fully* activate PI3K α (124, 132). If available, Ca²⁺-CaM can replace the RTK signal (117) – but only KRas4B (and presumably depalmitoylated KRas4A (15)) can bind CaM (114, 121, 133–135). Thus, *only KRas can fully activate PI3K α /Akt/mTOR signaling* (Figure 2).

PDE δ availability

To signal, Ras isoforms must be translocated to the PM, and the translocation mechanisms vary. PDE δ plays a key role in KRas4B shuttling to the PM. HRas, NRas and palmitoylated KRas4A are shuttled by vesicles. In those cells where PDE δ availability is limited, KRas4B maybe mislocalized (16).

Appropriate membrane composition to accommodate KRas4B

Because KRas, but not other isoforms, favors acidic disordered membranes (involving PS, PIs, and other microdomain components, some of which are detailed above), membrane composition is also an important signaling factor (136).

Oncogenic KRas4B may be less populated in HRas and NRas cancers

Cell-specific factors, including processing enzymes of lipidation motifs (47), isoform-specific scaffolding proteins, localization factors (137) and other cell- and isoform-specific factors (138) may play a role. Comparisons across cell types, e.g. acute myeloid leukemia, melanoma and bladder cancers versus pancreatic cancer, may help in factor identification.

Differential behavior of isoforms of wild-type versus their respective mutants

In an apparent twist, studies of mouse model carcinogenesis as well as patient tumors seemed to indicate that wild-type Ras can act as tumor suppressor of its mutant form, particularly in *KRAS* cancers as compared to *NRAS* (1, 139, 140). KRas is the most abundantly-expressed isoform, and Ras nanoclusters are homogeneous. Thus, we reason that the inhibition of the oncogenic isoform by its respective wild-type (which in the absence of a signaling cue is *inactive*), may simply reflect the reduction in the number of the oncogenic (*active*) Ras molecules in the cluster.

Notably, there is also evidence that wild-type KRas (and HRas and NRas) may also collaborate with oncogenic isoforms to promote cancer (141–143). In these cases, wild-type Ras can be both tumor-suppressing and -promoting, and these effects, as well as clonal fitness, appear context- and tissue-dependent.

Concluding Remarks and Pharmacological Implications

How then to explain Ras isoform and oncogenic mutant signaling preferences in specific cancers and what pharmacology can it suggest? The conformational ensemble of the catalytic domains in solution is broad (24, 35, 45, 144). It is further broadened in full-length Ras on membranes with different local compositions (14, 72, 145). These may reflect distinct orientation preferences of their signaling assemblies. Thus, significant questions are to what extent these determine selectivity and druggability. The mobile nature of Ras molecules at the membrane, the fleeting residence times, and the absence of clear dominant

states – at least in the time scales and PM composition that current molecular dynamics simulations of these huge systems can afford – question the efficacy of targeting preferred membrane interaction states. By contrast, productive cell-specific signaling pathways and cross-talk (23, 146, 147) appear more promising candidates; however, ferreting the complexes and poses to drug presents an even more daunting challenge. Lower drug toxicity calls for specificity; yet, working out the key factors that differentiate among isoforms is demanding. Along these lines, we proposed a ternary complex of KRas4B–CaM–PI3K α (148). Among the recent promising isoform-specific efforts are the palmitoylation/depalmitoylation cycle (149), specific Gal-1 – an HRas scaffolding protein inhibitors (87, 150, 151), monoclonal antibody targeting the α 4- β 6- α 5 dimeric interface, recently shown to reduce the interaction of oncogenic KRas, but not HRas, with Raf, as well as KRas PM localization (152) and PDE δ (153). Past efforts have shown that the paths are treacherous; here we foraged for a merged conformational and cellular view which we believe may better reflect Ras behavior, and thus help in addressing the pharmacology challenge. Still, the ultimate challenge resides in the inevitable emergence of drug resistance, which we believe calls for preorganized combinatorial drug regimens aiming at parallel pathways (123, 154–156).

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Figure 1.

Domain structure of Ras. The catalytic domain (effector lobe: residues 1–86, allosteric lobe: residues 87–166) sequence is highly homologous and the structure is identical among Ras isoforms with five helices and six β -strands connected by ten loops, and the G1-loop (or P-loop) to G5-loop enclosing the nucleotide. Ras isoforms differ in their flexible C-terminal HVRs (residues 167–188/189), which undergo distinct posttranslational modification (PTM) events including prenylation, methylation, and acylation (hydrophobic, polar/glycine, positively charged, and negatively charged residues are colored black, green, blue, and red, respectively; farnesylated cysteine residues are denoted by yellow, and palmitoylated cysteine residues are denoted by orange. The hollowed letters denote “AAX” motif). Ras mutations data were collected from the literature (4, 9). The preferred membrane lodging is also indicated, with ‘lipid rafts’ described as membrane domains whose lipid components are in a liquid-ordered (L_o) state as distinct from their coexisting liquid-disordered (L_d) ‘non-raft’ regions of the bilayer (109).

Figure 2.

Ras signaling pathways. Under physiological conditions, Ras forms nanoclusters and activates the Raf/MEK/ERK (MAPK) pathway, leading to cell proliferation. In the PI3K/Akt pathway, both Ras and RTK activate PI3K, recruiting it to the plasma membrane, leading to cell growth. Under oncogenic conditions, a constitutively active Ras (KRas, HRas or NRas) cluster boosts Raf/MEK/ERK signaling. In adenocarcinoma of *KRAS*-driven cancer, CaM specifically recruits oncogenic KRas4B (and possibly KRas4A, but not HRas or NRas) from the membrane to PI3Kα and substitutes for the missing RTK signal, consequently *fully* activating the PI3Kα/Akt/mTOR pathway.