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Why Does K-Ras Display Oncogenic Specificity?

Ras GTPases regulate cell proliferation pathways, making them important molecules in oncogenesis and cancer cell migration and invasion[1-4]. The four isoforms of Ras, H-Ras, N-Ras, K-Ras4A, and K-Ras4B (due to alternative splicing), were identified over 30 years ago for their oncogenic activation in human tumors[1,2]. Activating Ras mutations are single amino acid substitutions (e.g., G12C, G12V, G12D) and have been identified in approximately 30% of all human cancers[5-7].

The same signaling pathways activate all Ras isoforms via guanine exchange factor (GEF)-mediated exchange of GDP for GTP, followed by binding to the same effector proteins. However, Ras oncogenic isoforms are differentially expressed aacross different cancers with oncogenic specificity significantly favoring K-Ras[2,8-11]. Indeed, K-Ras is the most common mutated Ras isoform (86% of all Ras mutations) and is correlated with over 21% of human cancers[5]. In particular, K-Ras is the predominant or exclusive Ras gene mutated in three of the top four cancers with the highest mortality rates in the US: lung, colon, and pancreatic cancers[5]. In most instances, the K-Ras4B is the primary isoform mutated in K-Ras-associated cancers[8]. This newsletter discusses potential explanations for the biological basis of K-Ras's oncogenic specificity.

Membrane subdomain localization of Ras

For GEF-mediated activation of Ras and subsequent interaction with downstream effectors, Ras first must be trafficked, inserted, and anchored to different subdomains on the inner surface of the plasma membrane (termed nanoclustering). Localization is isoform-specific and determined by each isoform's particular lipid post-translational modifications (PTMs; e.g., palmitoyl, farnesyl, geranylgeranyl) within the C-terminus region known as the hypervariable region (HVR)[2,6,10-12] (Fig. 1). Palmitoylation favors membrane insertion into lipid microdomains such as lipid rafts or liquid-ordered phase membranes. Farnesylation and geranylgeranylation primarily favor membrane insertion into liquid-ordered phase membranes. H-Ras has one farnesyl and two palmitoyls lipid attachments, while N-Ras and K-Ras4A have only one palmitoyl. In contrast, K-Ras4B has only one farnesyl and a charged polybasic HVR (Fig. 1). K-Ras's unique lipid PTM profile favors acid membranes that are disordered in opposition to the membrane composition that H- and N-Ras favor[2,6,10-12]. Differences in PTMs and the resulting conformations within the membrane can affect effector binding affinity. The same catalytic domain surface that interacts with the effector protein can also engage with the plasma membrane[12]. Even in the GTP-bound state, if the catalytic domain is facing the membrane, effector binding affinity can be compromised[13].

K-Ras is also uniquely regulated by ubiquitin and phosphorylation PTMs. At steady state, the deubiquitinating enzyme USP17 inhibits wild-type and mutant H- and N-Ras functional membrane localization, but spares K-Ras, both under steady state and epidermal growth factor (EGF)-stimulated conditions[14]. Phosphorylation also negatively regulates K-Ras4B membrane binding and clustering[2,15-17].

Effector protein binding and downstream pathways

The two predominant physiological and oncological signaling cascades downstream of active Ras are the mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways[12]. Active Ras isoforms anchored to the plasma membrane promote Raf-1 dimerization and activate MAPK. Activated K- and H-Ras nanoclusters recruit Raf-1, but it is only retained within the K-Ras nanoclusters, making K-Ras a more potent activator of Raf-1 than H-Ras[18,19]. With respect to PI3K, K-Ras is a weaker activator than the other isoforms[18], but has the potential to activate PI3K in the absence of receptor tyrosine kinase (RTK) stimulation[12,20,21].

K-Ras4B is also specifically required for PDGF-mediated activation of Akt and subsequent enhanced cell migration[22]. This pathway also requires calmodulin (CaM) and CaM's role in growth factor-mediated activation of Akt requires K-Ras4B, likely involving the PDGF-stimulated increase in K-Ras/CaM-Ca^{2+} complex formation[22].

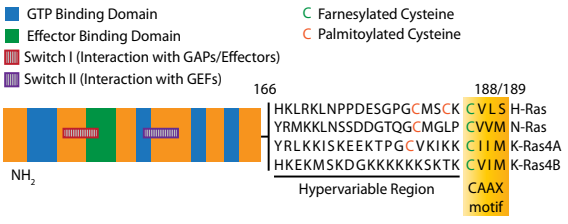

Figure 1. Primary structure of Ras isoforms. Adapted from ref. # 2.

News
Publications
Research Tools

Continued from Page 1

Distinct binding partners

Researchers have posited that at least one unique K-Ras binding partner exists that mediates its unique oncogenic specificity. Two candidates are the CaM-Ca^{2+} complex and PDEδ[12,20,21]. CaM-Ca^{2+} binds only the K-Ras4B isoform with the HVR farnesyl moiety, docking in a pocket on CaM in a Ca^{2+}-dependent manner[20-24]. This binding removes K-Ras4B from the membrane[24,25]. The CaM-Ca^{2+}/K-Ras4B complex also offers a potential pathway by which oncogenic K-Ras4B could activate cell proliferation pathways. Under physiological conditions, Ras-mediated PI3K signaling is only fully engaged in the presence of RTK stimulation. However, CaM-Ca^{2+} can bind and activate PI3K[26], and because only K-Ras4B is capable of binding to CaM-Ca^{2+}, researchers suggest that a K-Ras4B/CaM-Ca^{2+}/PI3K complex can form, which allows the CaM-Ca^{2+} complex to substitute for normal RTK-mediated activation of the Ras-PI3K signaling cascade[12,20,21]. In this scenario, oncogenic K-Ras4B mutants could activate cell proliferation pathways in the absence of physiological RTK stimulation[12,20,21]. In addition, binding of K-Ras to CaM-Ca^{2+} suppresses non-canonical Wnt/Ca^{2+} signaling, resulting in facilitation of K-Ras-mediated tumorigenesis[27].

Another unique K-Ras4B binding partner is PDEδ, which is responsible for trafficking of K-Ras4B to the membrane, while H-, N-, and K-Ras4A rely upon vesicles[12,24,28,29]. Thus, availability of PDEδ can regulate K-Ras trafficking and subsequent membrane localization and function.

Conclusions

The question persists – why is there oncogenic bias toward K-Ras? Besides the differences in conformation, localization, and binding partners discussed above, K-Ras might contribute a unique developmental role as suggested by K-Ras4B knock-out animal studies[2,30,31]. In any case, the clinical oncology data indicate the importance of targeting Ras proteins, and specifically K-Ras4B, to treat the largest percentage of Ras-driven cancers. To better understand the role of all Ras isoforms in physiological and oncological processes, Cytoskeleton offers a variety of Ras-centric proteins and assay kits.

Ras and GEF Proteins

Products	Purity	Cat. #	Amount
H-Ras His Protein, wild-type	>80%	RS01-A RS01-C	1 x 100 µg 3 x 100 µg
NEW **N-Ras Protein, human rec., wild type**	>90%	CS-RS02	1 x 100 µg
NEW **K-Ras4B Protein, human rec., wild-type**	>90%	CS-RS03	1 x 100 µg
NEW **K-Ras4B Protein, human rec., G12V mutant**	>90%	CS-RS04	1 x 100 µg
SOS1 Ras GEF Domain Protein GEF for H-, K- or N-Ras	>90%	CS-GE02 CS-GE02-XL	1 x 100 µg 1 x 1 mg
NEW **Ras-GRF GEF protein Cdc25 domain** Human recomb., MBP tagged	>85%	CS-GE03	1 x 100 µg

Ras Kits and Assays

Products	Cat. #	Amount
Ras Activation Assay Biochem Kit™	BK008-S BK008	20 assays 50 assays
RhoGEF Exchange Assay Biochem Kit™	BK100	60-300 assays
Ras G-LISA® Activation Assay, colorimetric	BK131	96 assays

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