Published in final edited form as:
Mol Cells. 2009 October 31; 28(4): 315–320. doi:10.1007/s10059-009-0143-7.

Non-Redundancy within the *RAS* Oncogene Family: Insights into Mutational Disparities in Cancer

Ken S. Lau and **Kevin M. Haigis***
Molecular Pathology Unit and Center for Cancer Research, Massachusetts General Hospital and Department of Pathology, Harvard Medical School, USA

Abstract

The RAS family of oncoproteins has been studied extensively for almost three decades. While we know that activation of RAS represents a key feature of malignant transformation for many cancers, we are only now beginning to understand the complex underpinnings of RAS biology. Here, we will discuss emerging cancer genome sequencing data in the context of what is currently known about RAS function. Taken together, retrospective studies of primary human tissues and prospective studies of experimental models support the notion that the variable mutation frequencies exhibited by the *RAS* oncogenes reflect unique functions of the RAS oncoproteins.

Keywords

cancer; mutation; RAS; signaling

INTRODUCTION

Genes encoding the RAS oncoproteins were originally discovered four decades ago in transforming retroviruses that induce sarcomas in rats (Harvey, 1964; Kirsten et al., 1970). Mammalian homologues of these viral oncogenes (referred to as proto-oncogenes) were identified a decade later (Chang et al., 1982). Shortly thereafter, mutationally activated forms of Harvey-*RAS* (*HRAS*) and Kirsten-*RAS* (*KRAS*) were isolated from human bladder and lung cancer cell lines, respectively (Der et al., 1982; Parada et al., 1982; Santos et al., 1982). A third member of the *RAS* oncogene family, *NRAS*, was later isolated from a neuroblastoma cell line (Shimizu et al., 1983).

The three *RAS* proto-oncogenes encode four 21 kDa monomeric GTPases (H-RAS, N-RAS, K-RAS4A, and K-RAS4B) that act as molecular binary switches, being con-formationally activated when they are bound to GTP and subsequently inactivated when they hydrolyze GTP to GDP. Wild-type forms of RAS have low intrinsic GTPase activity, and thus rely on GTPase activating proteins (GAPs) to efficiently hydrolyze GTP. Guanine nucleotide exchange factors (GEFs) facilitate the reloading of GDP-bound RAS with GTP. The regulation of wild-type RAS function by GEFs and GAPs has been reviewed extensively (Henis et al., 2009; Mor and Philips, 2006; Plowman and Hancock, 2005; Quinlan and Settleman, 2009). In this review, we will instead focus on (1) the biological consequences of mutational activation of RAS, (2) insights from emerging large scale sequencing projects, and (3) existing evidence for functional differences among the RAS family members.

*Correspondence: khaigis@partners.org.

RAS signaling in general terms

Cancer-associated activating mutations in RAS render it resistant to GAPs, essentially eliminating its catalytic activity and freezing the oncoprotein into its signal propagating structural conformation. This mode of oncogenic activation - disabling the enzymatic activity - distinguishes RAS from oncogenic kinases, for example EGFR or B-RAF, which are typically mutated to produce a hyperactive enzyme.

When bound to GTP, RAS directly binds to, and activates, several so-called effector proteins. The first RAS effector identified, and perhaps the most thoroughly studied, was the RAF serine/threonine kinase (Moodie et al., 1993; Vojtek et al., 1993; Warne et al., 1993; Zhang et al., 1993). GTP-bound RAS acts as a scaffold to bring RAF to the plasma membrane, where it is phosphorylated by membrane-bound kinases (Wellbrock et al., 2004). Activation of RAF initiates a phosphorylation cascade that progresses through MEK and ERK (p42/p44 MAPK), which has many downstream targets that regulate cell proliferation, survival, and differentiation (Seger and Krebs, 1995). A second RAS effector thought to be important for its oncogenic function is the p110 catalytic subunit of the phosphatidylinositol 3-kinase (PI3K) (Rodriguez-Viciana et al., 1994). Phosphorylation of phosphatidylinositol by PI3K brings the AKT serine/threonine kinase to the plasma membrane, where it becomes activated and transmits downstream signals to regulate cell survival, protein synthesis, and metabolism (Engelman et al., 2006). Although RAS is known to engage many different effector pathways, RAF and PI3K are the only effector pathways that are also commonly mutated in human cancers, suggesting that these proteins might be the primary oncogenic effectors of RAS signaling.

Fifteen years of biochemical studies have revealed RAS to be a pleiotropic signaling molecule that can engage downstream biochemical pathways to regulate diverse cellular processes, thus laying a firm mechanistic foundation for its function as an oncoprotein. Nevertheless, most of what we know about RAS signaling has been gleaned from analyses of cultured cells (most commonly fibroblasts) that over-express a mutationally activated form of RAS (usually H-RAS). It remains unclear how relevant the information garnered from these studies is to human cancer, where endogenous levels of activated K-RAS promote tumorigenesis primarily in epithelial cells. For example, while activation of K-RAS at endogenous levels enhances cellular proliferation, over-expression of activated K-RAS causes cell cycle arrest and premature senescence (Tuveson et al., 2004), indicating that the phenotypic effects of RAS activation are dependent upon expression level. Moreover, endogenous activation of RAS *in vivo*, in hematopoetic and epithelial tissues, results in significantly attenuated signaling compared to over-expression of RAS *in vitro* (Braun et al., 2004; Haigis et al., 2008; Guerra et al., 2003; Tuveson, Shaw et al., 2004). In sum, although the past thirty years have produced a wealth of knowledge relating to RAS biology, our knowledge remains incomplete.

Broad insights from mutation studies

Thanks to large-scale genome sequencing efforts over the past several years, we now have a fairly comprehensive view of the *RAS* mutational spectrum in human cancers (Supplemental Table 1). Overall, activating *RAS* mutations are found in 15% of cancers. It is important to note that the classical *RAS* activating mutations consist of missense substitutions at codons 12, 13, and 61. Other, non-classical, mutations can also activate RAS, however. For example, codon 146 mutations in *KRAS* are more common in colon cancers than are mutations in codon 61 (Edkins et al., 2006). Thus, the incidence of *RAS* gene mutation in cancer might actually be much higher than 15%. In the cancers that contribute most heavily to worldwide mortality, *RAS* mutations are extremely common (Fig. 1).

Several facets of biological insight can be gleaned from the currently available sequencing data. First, the frequency of *RAS* mutations varies among tumors of differing origin. For example, *RAS* mutations are common in certain cancers (e.g. pancreas and colon), but extremely rare in others (e.g. bone and kidney) (Supplemental Table 1). Second, even within a common organ site, *RAS* mutations often occur in specific cancer subtypes. For example, activating mutations in *RAS* are common in non-small cell lung cancer (NSCLC), but uncommon in small cell lung cancer (SCLC). Finally, even in cancers where *RAS* mutations are common, mutation of one *RAS* gene is typically selected over another (Table 1). For example, 70% of pancreatic ductal cancers express mutationally activated *KRAS*, but mutations in *NRAS* and *HRAS* are rare. In total, greater than 85% of all *RAS* mutations are found in *KRAS*, 12% are in *NRAS*, and 2% arise in *HRAS* (Supplemental Table 1).

Insights into RAS signaling can also be garnered from mutation studies. Activating mutations in B-RAF, one of the three RAF kinases, are common in many cancers, and these mutations are mutually exclusive with mutations in RAS (Sensi et al., 2006; Simi et al., 2008). One explanation for the mutual exclusivity of RAS and RAF mutations is that these events are functionally equivalent. Nevertheless, the observation that mutation of a single RAS effector can substitute for mutation of RAS itself does not fit with the notion of RAS as a pleiotropic signal transducer. Mutations in *PIK3CA*, which encodes the p110α catalytic subunit of PI3K are also common. In contrast to *BRAF*, however, *PIK3CA* mutations are not mutually exclusive with *KRAS* mutations in cancer, suggesting that endogenous levels of activated RAS do not efficiently activate PI3K signaling.

Biochemical studies have failed to identify any obvious differences between the RAS family members. If all *RAS* proteins are functionally equal, why do specific cancers select for mutation of one over another? There are likely multiple causes for the *RAS* mutation selection observed in human cancers. First and foremost, the expression of the different family members within a given tumor-initiating cell could affect the choice of mutation for a corresponding cancer (Leon et al., 1987). While *HRAS* mutations are not found in human lung cancers, studies utilizing mouse models have recently demonstrated that mutant H-Ras can efficiently drive chemically-induced lung tumorigenesis if it is expressed from the *Kras* locus, suggesting that the lack of *HRAS* mutations in NSCLC is a result of lack of expression in the cell type that gives rise to lung cancer (To et al., 2008). A second explanation for mutational selection is that each RAS family member performs a unique function, perhaps by activating different effectors, and that certain cancers require the activation of specific oncogenic signaling pathways.

Cell type-specific effects of RAS activation

While the vast majority of cancers select for mutations in *KRAS*, certain cancers clearly prefer to activate N-RAS or H-RAS. For example, *KRAS* mutations are most common in cancers derived from colon, pancreas and biliary tract, *NRAS* mutations are more common in hematopoetic malignancies, and cancers arising in the salivary and pituitary glands select for H-RAS activation (Supplemental Table 1), suggesting that certain tissues are sensitive to mutational activation of specific RAS family members. This hypothesis, founded upon retrospective mutational analyses, is strongly supported by prospective experimental studies. Consistent with the observation that *NRAS* mutations are common in leukemia, Maher and colleagues demonstrated that hematopoetic cells were more readily transformed by mutant N-RAS, but that fibroblasts were more readily transformed by activated H-RAS (Maher et al., 1995). In similar experiments, and again consistent with mutation data, only activated N-RAS was able to efficiently transform immortalized melanocytes, while colonic epithelial cells were especially sensitive to activation of K-RAS (Keller et al., 2007a; Whitwam et al., 2007).

Studies utilizing transgenic mice also support the notion of tissue-specific effects of RAS mutations. When expressed in transplanted bone marrow cells, mutant forms of K-Ras, H-Ras, and N-Ras elicited unique leukemic phenotypes (Parikh et al., 2007). Consistent with cancer genome sequencing data, mutant N-Ras induced AML and CMML and mutant K-Ras induced only CMML. Interestingly, ectopic expression of activated H-Ras in bone marrow cells led to the development of AML, even though *HRAS* mutations are never found in human AML. This observation provides additional support to the study of To and colleagues, who surmised that the lack of *HRAS* mutations in certain cancers (for example AML and NSCLC) results from a lack of *HRAS* expression in the corresponding tumor initiating cell (To et al., 2008). In experiments utilizing mice genetically engineered to expressing mutant forms of Ras from their endogenous loci, we demonstrated that activated K-Ras, but not N-Ras, was able to cooperate with loss of the Adenomatous polyposis coli (Apc) tumor suppressor to promote the development of malignant colon cancers (Haigis et al., 2008). This observation is again consistent with the human mutation data linking K-RAS activation with colon cancer progression. Taken together, *in vitro* and *in vivo* studies indicate that the biological outcome of RAS mutation is highly dependent upon cellular context. With this in mind, what are the underlying molecular mechanisms that account for context dependence of RAS-mediated oncogenicity?

RAS family members exhibit unique subcellular localizations

To understand the molecular mechanisms underlying potential functional diversity within the RAS family of proteins, it is critical to explore the structural relationships among the family members. The four family members are highly similar in size and primary amino acid sequence. They are over 90% identical over the first 166 residues, which includes the G-domain that binds guanine nucleotides, the switch 1 and switch 2 loops that undergo conformational change upon GTP binding, and the binding surfaces for downstream effectors, GEFs, and GAPs. Because the RAS isoforms are essentially identical over the first 90% of the proteins, it is unlikely that the mutational selectivity seen in cancers results simply from differences in nucleotide binding or relative sensitivity to GEFs and GAPs.

The final 24-25 amino acids, referred to as the hypervariable region (HVR), of the RAS proteins are poorly conserved. For all of the RAS family members, this region undergoes a series of post-translational modifications that culminate in the prenylation, by farnesyltransferase (FT) or geranylgeranyltransferase (GT), of a conserved C-terminal cysteine. Additionally, isoform-specific, post-translational modifications occur in the HVR as well, including palmitoylation and phosphorylation (Bivona et al., 2006). Ultimately, these post-translational modifications regulate the trafficking and localization of the RAS family members; while primarily K-RAS4B localizes almost exclusively to the plasma membrane, K-RAS4A, H-RAS, and N-RAS signal extensively from endomembranes (e.g. the Golgi). Again, this aspect of RAS biology has been reviewed extensively (Hancock., 2003; Mor and Philips, 2006; Philips, 2005; Plowman and Hancock., 2005; Prior and Hancock., 2001). Here, we will explore the emerging data supporting functional uniqueness within the RAS family, with the underlying hypothesis that any functional differences between family members result, at least in part, from their disparate subcellular localizations.

Differential binding and activation of effectors

That each RAS family member is functionally unique is evidenced, not only by the mutation selection seen in human cancers, but also by the fact that the mutant forms elicit unique changes in genes and protein expression (Keller et al., 2007a; Zuber et al., 2000). The differential molecular outputs of each mutant family member likely result from differential

engagement of effector pathways, a concept that is again supported by experimental observations. For example, N-RAS binds RAF-1 more effectively in transformed fibroblasts than does H-RAS and RAF-1 mediates the oncogenic properties of mutant N-RAS in melanomas (Dumaz et al., 2006; Hamilton and Wolfman, 1998). In kidney epithelial cells, mutant K-RAS preferentially activates RAF, but mutant H-RAS preferentially activates PI3K (Yan et al., 1998). Similarly, K-RAS activates RAC signaling more efficiently than does H-RAS (Walsh and Bar-Sagi, 2001). In sum, there is evidence, albeit still incomplete, that each mutant RAS family members can signal through distinct downstream effector pathways. Further studies are required to fully characterize the common and unique effectors that are engaged by each family member, taking into account the context dependence of these interactions.

How do certain family members engage specific effector pathways? One way that the RAS family members could engage a specific downstream pathway would be for the effector to exhibit a subcellular localization that matches one of the family members, but not the others. Although many of the canonical RAS effectors are generally cytoplasmic, mutant H-RAS associated with the endoplasmic reticulum (ER) activates RAF and PI3K, while Golgi-associated H-RAS activates JNK (Chiu et al., 2002).

A second way for RAS family members to engage specific effector pathways is through the formation of distinct signaling complexes. One such complex involves the specific binding of calmodulin to the hypervariable region of K-RAS4B (Abraham et al., 2009; Sidhu et al., 2003; Villalonga et al., 2001). The KRAS4B/calmodulin interaction links RAS function to calcium signaling and is important for the ability of GTP-bound K-RAS4B to activate AKT in response to mitogenic stimuli such as platelet-derived growth factor (PDGF) (Liao et al., 2006).

Another example of distinct signaling clusters involves a group of proteins called galectins. Galectins are a family of proteins that are characterized by their ability to bind β-galactoside. Mutant H-RAS binds galectin-1 more efficiently than does mutant K-RAS and this interaction stimulates RAF activation at the expense of PI3K activation (Elad-Sfadia et al., 2002). Conversely, activated K-RAS binds more efficiently to galectin-3, resulting in prolonged activation of RAF and attenuation of PI3K and RAL signaling (Elad-Sfadia et al., 2004; Shalom-Feuerstein et al., 2005). Given the ability of known RAS binding proteins, like calmodulin and galectin, to modulate the signaling output of specific RAS family members, it is conceivable that cell type specific RAS binding proteins exist and, in fact, underlie the mutational selection seen in human cancers.

Differential effects on differentiation and apoptosis

RAS activation impinges upon processes that regulate cellular homeostasis: proliferation, differentiation, apoptosis, *et cetera*. Ultimately, the selection for particular RAS mutations in certain cancers may derive from the ability of each RAS family member to influence specific cellular processes. For example, while both activated K-RAS and H-RAS enhance proliferation, only mutant K-RAS inhibits the *in vitro* differentiation of colorectal adenocarcinoma cells (Yan et al., 1997). Similarly, mutant K-Ras suppresses differentiation in autochthonous mouse colonic tumors, but mutant N-Ras does not (Haigis et al., 2008). These observations are consistent with the prevalence of *KRAS* mutations in primary colon cancers (Table 1). In another study, mutant K-RAS was found to inhibit retinoic acid (RA) induced differentiation of embryonal carcinoma cells into endoderm (Quinlan et al., 2008). And while mutant N-RAS had no effect on this process, mutant H-RAS actually promoted differentiation in the absence of RA (Quinlan et al., 2008). These observations are consistent

with the preponderance of *KRAS* activation mutations in cancers arising from endodermal tissues (Table 1).

Functional divergence within the RAS family is perhaps most extensively associated with apoptosis (Cox and Der, 2003). Mutant K-RAS is pro-apoptotic, while mutant N-RAS suppresses apoptosis (Jansen et al., 1997; Haigis et al., 2008; Klampfer et al., 2004; Wolfman and Wolfman, 2000). The proapoptotic function of K-RAS is related, at least in part, to its phosphorylation by PKCα, which causes K-RAS4B to translocate from the plasma membrane to the mitochondrial outer membrane (Bivona et al., 2006). K-RAS4B is phosphorylated on serine 181, which is not conserved in any of the other RAS family members. The consequences of the K-RAS4B phosphorylation and re-localization are two-fold. First, K-RAS4B is removed from the plasma membrane, where it would otherwise recruit and activate RAF to promote proliferation and survival. Second, phosphorylated K-RAS4B associates with the outer mitochondrial membrane, where it interacts with Bcl-xL and promotes apoptosis (Bivona et al., 2006).

In parallel to its mitochondrial pro-apoptotic function, K-RAS is unique in its ability to promote apoptosis through a direct interaction with the RASSF1/NORE1/MST1 protein complex (Khokhlatchev et al., 2002). Similar to the mode of activation for other RAS effectors, RASSF1/NORE1/MST1 is recruited to the membrane through its binding to GTP-bound K-RAS, and it is within this subcellular compartment that the complex is activated (Khokhlatchev et al., 2002). MST1 is a pro-apoptotic serine/threonine kinase that is cleaved by activated caspase and then shuttled to the nucleus where it regulates chromatin condensation (Radu and Chernoff, 2009).

In addition to its interactions with Bcl-xL and RASSF1, mutant K-RAS actively suppresses the pro-survival function of NRAS (Keller et al., 2007b). The mechanisms underlying the anti-apoptotic effects of mutant N-RAS are not clear, but mutant N-RAS is reported to up-regulate the anti-apoptotic protein Bcl-2 (Borner et al., 1999) and to inactivate the pro-apoptotic protein Bad (Wolfman and Wolfman, 2000). The fact that K-RAS and N-RAS play opposing roles in the regulation of apoptosis highlights the complex molecular mechanisms underlying their oncogenic functions.

Concluding remarks

The last three decades have produced a wealth of knowledge pertaining to the *RAS* oncogene family. From their high frequency of mutation in a wide variety of cancers, to their complex regulation of molecular processes controlling cellular transformation, it is clear that mutant forms of RAS play a critical role in the initiation and progression of cancer. Nevertheless, this extensive knowledge has not yet translated into effective therapies for cancers expressing mutant forms of RAS. Over the next decade, it will be critical to unravel the complexities of RAS biology to the extent that we can develop therapies to counteract the oncogenic phenotypes associated with mutational activation of the RAS oncoproteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This review is meant to be a broad overview of RAS function as it relates to emerging cancer genome sequencing data. The authors would like to apologize to those investigators whose work we could not discuss in the limited space available. This work was supported by grants from the National Cancer Institute (K01-CA118425, P50-CA127003), the National Institute for General Medical Science (R01-GM088827), and the American Cancer

Society (MGO-114877) and a donation from the Merlino Family Endowment Fund. K.L. is a Robert Black Fellow of the Damon Runyon Cancer Research Foundation.

References

Abraham SJ, Nolet RP, Calvert RJ, Anderson LM, Gaponenko V. The Hypervariable region of K-Ras4B is responsible for its specific interactions with calmodulin. Biochemistry. 2009; 48:7575–7583. [PubMed: 19583261]

Bivona TG, Quatela SE, Bodemann BO, Ahearn IM, Soskis MJ, Mor A, Miura J, Wiener HH, Wright L, Saba SG, 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; 21:481–493. [PubMed: 16483930]

Borner C, Schlagbauer Wadl H, Fellay I, Selzer E, Polterauer P, Jansen B. Mutated N-ras upregulates Bcl-2 in human melanoma in vitro and in SCID mice. Melanoma Res. 1999; 9:347–350. [PubMed: 10504052]

Braun BS, Tuveson DA, Kong N, Le DT, Kogan SC, Rozmus J, Le Beau MM, Jacks TE, Shannon KM. Somatic activation of oncogenic Kras in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. Proc Natl Acad Sci USA. 2004; 101:597–602. [PubMed: 14699048]

Chang EH, Gonda MA, Ellis RW, Scolnick EM, Lowy DR. Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. Proc Natl Acad Sci USA. 1982; 79:4848–4852. [PubMed: 6289320]

Chiu VK, Bivona T, Hach A, Sajous JB, Silletti J, Wiener H, Johnson RL 2nd, Cox AD, Philips MR. Ras signalling on the endoplasmic reticulum and the Golgi. Nat Cell Biol. 2002; 4:343–350. [PubMed: 11988737]

Cox AD, Der CJ. The dark side of Ras: regulation of apoptosis. Oncogene. 2003; 22:8999–9006. [PubMed: 14663478]

Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. Proc Natl Acad Sci USA. 1982; 79:3637–3640. [PubMed: 6285355]

Dumaz N, Hayward R, Martin J, Ogilvie L, Hedley D, Curtin JA, Bastian BC, Springer C, Marais R. In melanoma, RAS mutations are accompanied by switching signaling from BRAF to CRAF and disrupted cyclic AMP signaling. Cancer Res. 2006; 66:9483–9491. [PubMed: 17018604]

Edkins S, O'Meara S, Parker A, Stevens C, Reis M, Jones S, Greenman C, Davies H, Dalgliesh G, Forbes S, et al. Recurrent KRAS codon 146 mutations in human colorectal cancer. Cancer Biol Ther. 2006; 5:928–932. [PubMed: 16969076]

Elad-Sfadia G, Haklai R, Balan E, Kloog Y. Galectin-3 augments K-Ras activation and triggers a Ras signal that attenuates ERK but not phosphoinositide 3-kinase activity. J Biol Chem. 2004; 279:34922–34930. [PubMed: 15205467]

Elad-Sfadia G, Haklai R, Ballan E, Gabius HJ, Kloog Y. Galectin-1 augments Ras activation and diverts Ras signals to Raf-1 at the expense of phosphoinositide 3-kinase. J Biol Chem. 2002; 277:37169–37175. [PubMed: 12149263]

Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet. 2006; 7:606–619. [PubMed: 16847462]

Guerra C, Mijimolle N, Dhawahir A, Dubus P, Barradas M, Serrano M, Campuzano V, Barbacid M. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. Cancer Cell. 2003; 4:111–120. [PubMed: 12957286]

Haigis KM, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, Niwa-Kawakita M, Sweet-Cordero A, Sebolt-Leopold J, Shannon KM, et al. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. Nat Genet. 2008; 40:600–608. [PubMed: 18372904]

Hamilton M, Wolfman A. Ha-ras and N-ras regulate MAPK activity by distinct mechanisms *in vivo*. Oncogene. 1998; 16:1417–1428. [PubMed: 9525741]

Hancock JF. Ras proteins: different signals from different locations. Nat Rev. 2003; 4:373–384.

Harvey JJ. An unidentified virus which causes the rapid production of tumours in mice. Nature. 1964; 204:1104–1105. [PubMed: 14243400]

Henis YI, Hancock JF, Prior IA. Ras acylation, compartmentalization and signaling nanoclusters (Review). Mol Membr Biol. 2009; 26:80–92. [PubMed: 19115142]

Jansen B, Schlagbauer-Wadl H, Eichler HG, Wolff K, van Elsas A, Schrier PI, Pehamberger H. Activated N-ras contributes to the chemoresistance of human melanoma in severe combined immunodeficiency (SCID). mice by blocking apoptosis. Cancer Res. 1997; 57:362–365. [PubMed: 9012455]

Keller JW, Franklin JL, Graves-Deal R, Friedman DB, Whitwell CW, Coffey RJ. Oncogenic KRAS provides a uniquely powerful and variable oncogenic contribution among RAS family members in the colonic epithelium. J Cell Physiol. 2007a; 210:740–749. [PubMed: 17133351]

Keller JW, Haigis KM, Franklin JL, Whitehead RH, Jacks T, Coffey RJ. Oncogenic K-RAS subverts the antiapoptotic role of N-RAS and alters modulation of the N-RAS:gelsolin complex. Oncogene. 2007b; 26:3051–3059. [PubMed: 17130841]

Khokhlatchev A, Rabizadeh S, Xavier R, Nedwidek M, Chen T, Zhang XF, Seed B, Avruch J. Identification of a novel Ras-regulated proapoptotic pathway. Curr Biol. 2002; 12:253–265. [PubMed: 11864565]

Kirsten WH, Schauf V, McCoy J. Properties of a murine sarcoma virus. Bibl Haematol. 1970; 36:246–249. [PubMed: 5538357]

Klampfer L, Huang J, Sasazuki T, Shirasawa S, Augenlicht L. Oncogenic Ras promotes butyrate-induced apoptosis through inhibition of gelsolin expression. J Biol Chem. 2004; 279:36680–36688. [PubMed: 15213223]

Leon J, Guerrero I, Pellicer A. Differential expression of the ras gene family in mice. Mol Cell Biol. 1987; 7:1535–1540. [PubMed: 3600635]

Liao J, Planchon SM, Wolfman JC, Wolfman A. Growth factor-dependent AKT activation and cell migration requires the function of c-K(B).-Ras versus other cellular ras isoforms. J Biol Chem. 2006; 281:29730–29738. [PubMed: 16908523]

Maher J, Baker DA, Manning M, Dibb NJ, Roberts IA. Evidence for cell-specific differences in transformation by N-, H- and K-ras. Oncogene. 1995; 11:1639–1647. [PubMed: 7478589]

Moodie SA, Willumsen BM, Weber MJ, Wolfman A. Complexes of Ras.GTP with Raf-1 and mitogen-activated protein kinase kinase. Science. 1993; 260:1658–1661. [PubMed: 8503013]

Mor A, Philips MR. Compartmentalized Ras/MAPK signaling. Annu Rev Immunol. 2006; 24:771–800. [PubMed: 16551266]

Parada LF, Tabin CJ, Shih C, Weinberg RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. Nature. 1982; 279:474–478. [PubMed: 6283357]

Parikh C, Subrahmanyam R, Ren R. Oncogenic NRAS, KRAS, and HRAS exhibit different leukemogenic potentials in mice. Cancer Res. 2007; 67:7139–7146. [PubMed: 17671181]

Philips MR. Compartmentalized signalling of Ras. Biochem Soc Trans. 2005; 33:657–661. [PubMed: 16042567]

Plowman SJ, Hancock JF. Ras signaling from plasma membrane and endomembrane microdomains. Biochim Biophys Acta. 2005; 1746:274–283. [PubMed: 16039730]

Prior IA, Hancock JF. Compartmentalization of Ras proteins. J Cell Sci. 2001; 114:1603–1608. [PubMed: 11309191]

Quinlan MP, Quatela SE, Philips MR, Settleman J. Activated Kras, but not Hras or Nras, may initiate tumors of endodermal origin via stem cell expansion. Mol Cell Biol. 2008; 28:2659–2674. [PubMed: 18268007]

Quinlan MP, Settleman J. Isoform-specific ras functions in development and cancer. Future Oncol. 2009; 5:105–116. [PubMed: 19243303]

Radu M, Chernoff J. The DeMSTification of mammalian Ste20 kinases. Curr Biol. 2009; 19:R421–425. [PubMed: 19467213]

Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, Waterfield MD, Downward J. Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature. 1994; 370:527–532. [PubMed: 8052307]

Santos E, Tronick SR, Aaronson SA, Pulciani S, Barbacid M. T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes. Nature. 1982; 298:343–347. [PubMed: 6283384]

Seger R, Krebs EG. The MAPK signaling cascade. FASEB J. 1995; 9:726–735. [PubMed: 7601337]

Sensi M, Nicolini G, Petti C, Bersani I, Lozupone F, Molla A, Vegetti C, Nonaka D, Mortarini R, Parmiani G, et al. Mutually exclusive NRASQ61R and BRAFV600E mutations at the single-cell level in the same human melanoma. Oncogene. 2006; 25:3357–3364. [PubMed: 16462768]

Shalom-Feuerstein R, Cooks T, Raz A, Kloog Y. Galectin-3 regulates a molecular switch from N-Ras to K-Ras usage in human breast carcinoma cells. Cancer Res. 2005; 65:7292–7300. [PubMed: 16103080]

Shimizu K, Goldfarb M, Suard Y, Perucho M, Li Y, Kamata T, Feramisco J, Stavnezer E, Fogh J, Wigler MH. Three human transforming genes are related to the viral ras oncogenes. Proc Natl Acad Sci USA. 1983; 80:2112–2116. [PubMed: 6572964]

Sidhu RS, Clough RR, Bhullar RP. Ca^{2+}/calmodulin binds and dissociates K-RasB from membrane. Biochem Biophys Res Commun. 2003; 304:655–660. [PubMed: 12727204]

Simi L, Pratesi N, Vignoli M, Sestini R, Cianchi F, Valanzano R, Nobili S, Mini E, Pazzagli M, Orlando C. High-resolution melting analysis for rapid detection of KRAS, BRAF, and PIK3CA gene mutations in colorectal cancer. Am J Clin Pathol. 2008; 130:247–253. [PubMed: 18628094]

To MD, Wong CE, Karnezis AN, Del Rosario R, Di Lauro R, Balmain A. Kras regulatory elements and exon 4A determine mutation specificity in lung cancer. Nat Genet. 2008; 40:1240–1244. [PubMed: 18758463]

Tuveson DA, Shaw AT, Willis NA, Silver DP, Jackson EL, Chang S, Mercer KL, Grochow R, Hock H, Crowley D, et al. Endogenous oncogenic K-ras(G12D). stimulates proliferation and widespread neoplastic and developmental defects. Cancer Cell. 2004; 5:375–387. [PubMed: 15093544]

Villalonga P, Lopez-Alcala C, Bosch M, Chiloeches A, Rocamora N, Gil J, Marais R, Marshall CJ, Bachs O, Agell N. Calmodulin binds to K-Ras, but not to H- or N-Ras, and modulates its downstream signaling. Mol Cell Biol. 2001; 21:7345–7354. [PubMed: 11585916]

Vojtek AB, Hollenberg SM, Cooper JA. Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell. 1993; 74:205–214. [PubMed: 8334704]

Walsh AB, Bar-Sagi D. Differential activation of the Rac pathway by Ha-Ras and K-Ras. The Journal of biological chemistry. 2001; 276:15609–15615. [PubMed: 11278702]

Warne PH, Viciana PR, Downward J. Direct interaction of Ras and the amino-terminal region of Raf-1 *in vitro*. Nature. 1993; 364:352–355. [PubMed: 8332195]

Wellbrock C, Karasarides M, Marais R. The RAF proteins take centre stage. Nat Rev. 2004; 5:875–885.

Whitwam T, Vanbrocklin MW, Russo ME, Haak PT, Bilgili D, Resau JH, Koo HM, Holmen SL. Differential oncogenic potential of activated RAS isoforms in melanocytes. Oncogene. 2007; 26:4563–4570. [PubMed: 17297468]

Wolfman JC, Wolfman A. Endogenous c-N-Ras provides a steady-state anti-apoptotic signal. J Biol Chem. 2000; 275:19315–19323. [PubMed: 10777478]

Yan J, Roy S, Apolloni A, Lane A, Hancock JF. Ras isoforms vary in their ability to activate Raf-1 and phosphoinositide 3-kinase. J Biol Chem. 1998; 273:24052–24056. [PubMed: 9727023]

Yan Z, Chen M, Perucho M, Friedman E. Oncogenic Ki-ras but not oncogenic Ha-ras blocks integrin beta1-chain maturation in colon epithelial cells. J Biol Chem. 1997; 272:30928–30936. [PubMed: 9388239]

Zhang XF, Settleman J, Kyriakis JM, Takeuchi-Suzuki E, Elledge SJ, Marshall MS, Bruder JT, Rapp UR, Avruch J. Normal and oncogenic p21ras proteins bind to the amino-terminal regulatory domain of c-Raf-1. Nature. 1993; 364:308–313. [PubMed: 8332187]

Zuber J, Tchernitsa OI, Hinzmann B, Schmitz AC, Grips M, Hellriegel M, Sers C, Rosenthal A, Schafer R. A genome-wide survey of RAS transformation targets. Nat Genet. 2000; 24:144–152. [PubMed: 10655059]

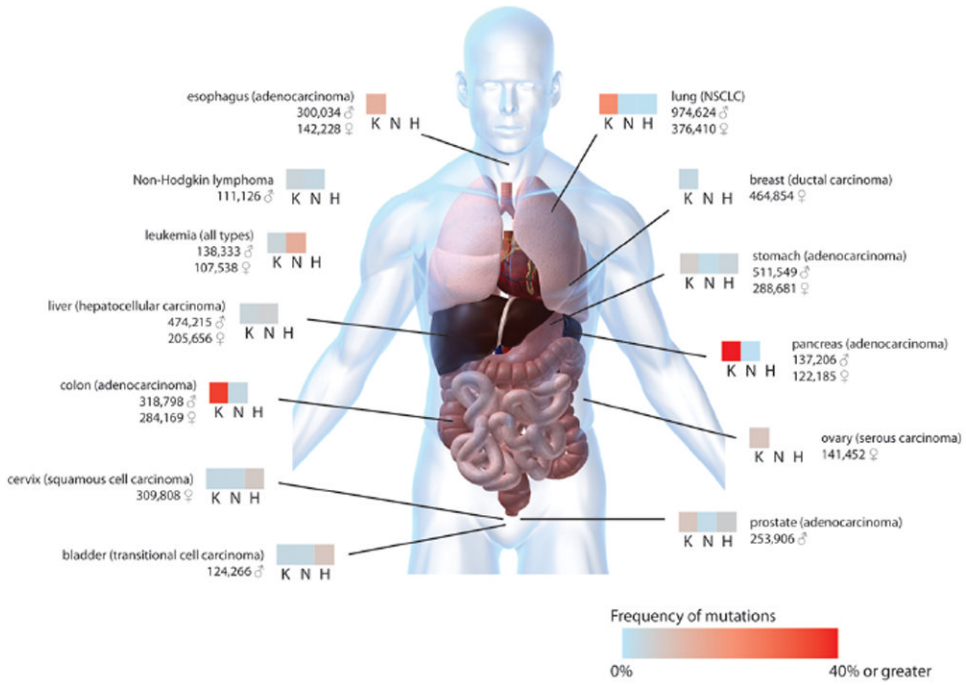

Fig. 1.

Frequency of *RAS* gene mutations in the ten cancers with the highest rates of mortality for men and women worldwide. The specific cancer subtype is listed in parentheses next to the corresponding organ site. For each cancer, the number of worldwide deaths for men and women are listed below the organ site. The frequency of *K-RAS*, *N-RAS*, and *H-RAS* mutation is represented by a color scale. The mortality data was collected by the American Cancer Society (www.cancer.org) for the year 2007.

Table 1

Frequency of RAS gene mutations in human cancers[1]

Organ/Tissue	Tumor type	*KRAS*	*NRAS*	*HRAS*
Autonomic ganglia	Neuroblastoma	3% (75)	7% (118)	0% (75)
Biliary tract	Adenocarcinoma	37% (352)	2% (174)	0% (133)
Bladder	Transitional cell carcinoma	3% (347)	3% (239)	8% (652)
Cervix	Squamous cell carcinoma	2% (275)	3% (65)	7% (164)
Colon	Adenocarcinoma	36% (13326)	3% (257)	0% (331)
Hematopoetic System[2]	AML	4% (1723)	13% (3004)	0% (1219)
	CML	2% (286)	9% (494)	0% (266)
	CMML	12% (84)	18% (152)	1% (118)
	JMML	6% (125)	16% (148)	ND
	Plasma cell myeloma	5% (366)	21% (449)	1% (148)
Liver	Hepatocellular carcinoma	4% (312)	5% (208)	0% (167)
Lung	Carcinoid-endocrine tumor	7% (101)	0% (51)	0% (30)
	Large cell carcinoma	22% (178)	7% (55)	4% (56)
	Non-small cell carcinoma	20% (8420)	1% (1715)	<1% (1223)
	Squamous cell carcinoma	6% (1329)	0% (365)	1% (266)
Pancreas	Ductal carcinoma	71% (3502)	1% (109)	0% (108)
Prostate	Adenocarcinoma	8% (898)	2% (519)	6% (493)
Skin	Basal cell carcinoma	5% (132)	2% (132)	7% (165)
	Melanoma	2% (961)	21% (2965)	1% (887)
	Squamous cell carcinoma	5% (95)	8% (95)	9% (225)
Soft tissue	Malignant fibrous histiocytoma	17% (117)	2% (44)	17% (104)
	Rhabdomyosarcoma	3% (100)	8% (89)	4% (96)
	Synovial sarcoma	0% (51)	0% (1)	6% (50)
Stomach	Carcinoma	6% (1823)	2% (206)	4% (367)
Testis	Germinoma	7% (107)	8% (85)	0% (56)
Thyroid	Anaplastic carcinoma	15% (197)	13% (188)	4% (190)
	Follicular carcinoma	5% (337)	16% (365)	6% (355)
	Papillary carcinoma	2% (1259)	4% (1610)	2% (1212)

[1] Data is collated from the COSMIC database, Wellcome Trust Sanger Institute (www.sanger.ac.uk/genetics/CGP/cosmic/). Only those cancers in which one or more *RAS* family member is mutated at a frequency higher than 5% are shown. Parentheses indicate the number of cases analyzed. Only cases in which at least 50 samples were analyzed have been shown.

[2] Abbreviations: AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia