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RAS oncogenes: weaving a tumorigenic web

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Abstract

RAS proteins are essential components of signalling pathways that emanate from cell surface receptors. Oncogenic activation of these proteins owing to missense mutations is frequently detected in several types of cancer. A wealth of biochemical and genetic studies indicates that RAS proteins control a complex molecular circuitry that consists of a wide array of interconnecting pathways. In this Review, we describe how RAS oncogenes exploit their extensive signalling reach to affect multiple cellular processes that drive tumorigenesis.

The realization in the late 1970s that RAS harboured transforming properties that were bestowed by gain-of-function mutations shaped our view of the molecular biology of cancer. These studies spearheaded the discovery of many more genes the functions of which were altered in tumours, and gave rise to the concept that the progressive transition from a normal to a malignant phenotype reflects the successive accumulation of genetic alterations that each confer a unique capability that cancer cells need to acquire in order to evade homeostatic barriers. These capabilities, which more than a decade ago were dubbed by Hanahan and Weinberg as the hallmarks of cancer, encompass self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis[1]. To accommodate the emergence of additional cancer-associated pathologies this list has been increased to include additional hallmarks such as metabolic fitness and genomic plasticity[2]. Within this paradigm, the transforming function of oncogenic RAS has been initially attributed to its capacity to endow cells with sufficiency in growth signals. As our understanding of the tumorigenic process and its underlying mechanisms is evolving, it is becoming clear that the net cast by oncogenic RAS is much wider and captures many of the original, as well as some of the newly established, hallmarks of cancer. This Review discusses the ensemble of oncogenic RAS functions that fuel the tumorigenic process.

Oncogenic activation – themes and variations

In humans, three RAS genes encode four distinct but highly homologous ~21 kDa RAS proteins: HRAS, NRAS, KRAS4A and KRAS4B (KRAS4A and KRAS4B are alternative splice variants of the *KRAS* gene). Serving as transducers that couple cell surface receptors to intracellular effector pathways, RAS proteins cycle between ‘on’ and ‘off ’ conformations that are conferred by the binding of GTP and GDP, respectively. Under physiological conditions, the transition between these two states is regulated by guanine nucleotide exchange factors (GEFs), which promote the activation of RAS proteins by stimulating GDP

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for GTP exchange, and by GTPase-activating proteins (GAPs), which accelerate RAS-mediated GTP hydrolysis. This inactivation of RAS activity by GAPs is the predominant target of the most common somatic mutations that are found in the oncogenic variants of RAS alleles. Accordingly, oncogenic mutations of Q61 impair the GTP hydrolysis reaction by interfering with the coordination of a water molecule that is required for the nucleophilic attack on the γ-phosphate[3,4]. Similarly, oncogenic substitutions in residues G12 and G13 prevent the formation of van der Waals bonds between RAS and the GAP through steric hindrance and so perturb the proper orientation of the catalytic glutamine (Q61) in RAS, which results in the pronounced attenuation of GTP hydrolysis[5]. The outcome of these substitutions is the persistence of the GTP-bound state of RAS and, as a consequence, the incessant activation of a multitude of RAS-dependent downstream effector pathways.

Although not all RAS mutants are created equal, the extent to which specific mutations affect the biological behaviour of RAS remains to be established (FIG. 1). Studies carried out in patients with leukaemia and bladder cancers failed to identify a correlation between the occurrence of specific RAS mutations and the aggressiveness of the disease, suggesting that the different RAS mutations may lead to a common pathophysiological end point[6,7]. Likewise, differences in the range of *KRAS* mutations observed in human tumours of the gastrointestinal tract seemingly reflect variations in the aetiology of RAS mutations as opposed to specific mutation-dependent disease characteristics[8–10]. By contrast, in colorectal and lung cancers, $KRAS^{G12V}$ mutations have been associated with a worse prognosis than $KRAS^{G12D}$ mutations, raising the possibility that particular amino acid substitutions might dictate specific transforming characteristics of oncogenic RAS alleles[11,12]. In support of this idea, $HRAS^{G12V}$ exhibits weaker GTPase activity and stronger binding to GTP than $HRAS^{G12D}$ (REFS 13,14), and it is also more potent in cell culture-based transformation assays[15]. A new perspective on the issue of the contributions of particular amino acid substitutions to the *in vivo* transforming capabilities of RAS has been added by recent findings that have documented previously uncharacterized RAS mutations in colorectal tumours and leukaemias[16,17]. In addition, certain RAS mutations may be associated with an altered response to therapy[18]. It thus seems that much remains to be learned about the link between sequence permutations and functional alterations of oncogenic forms of RAS.

Another unresolved question concerning the molecular principles of the oncogenic activation of RAS pertains to whether mutations in a particular RAS isoform dictate unique oncogenic outputs. This question has been predominantly instigated by the well-recognized non-random distribution pattern of activated isoforms of RAS among the range of cancer types[19,20]. Thus, *KRAS* mutations are most frequently detected in colorectal tumours, lung carcinomas (mostly non-small-cell lung cancer (NSCLC)) and in pancreatic carcinomas; *HRAS* mutations are associated with tumours of the skin and of the head and neck; and *NRAS* mutations are common in haematopoietic malignancies (TABLE 1). Earlier attempts to delineate the functional diversity of different oncogenic RAS isoforms mainly relied on the use of ectopic overexpression approaches[21]. However, as it became apparent that the expression levels of oncogenic RAS impinge on the phenotypic outcome, more recent attempts have explored the possible functional importance of tissue type-dependent isoform segregation using knockout and knock-in mouse models of oncogenic RAS alleles[22–27] (TABLE 2). In a model where either *Kras4b* or *Hras* was expressed at endogenous levels from the *Kras* promoter, To *et al.*[25] demonstrated that $HRAS^{Q61L}$ was fully capable of substituting for $KRAS^{Q61L}$ in carcinogen-induced lung tumorigenesis. Furthermore, carcinogen-induced oncogenic mutations were found to target the *Hras* allele knocked into the *Kras* locus in a lung tumorigenesis model, as well as the endogenous *Hras* allele in a skin tumorigenesis model. These observations suggest that the selective predisposition of RAS isoforms to mutagenesis in a particular tissue reflects gene-specific regulatory elements

rather than distinct functional outputs. Examining the roles of $KRAS^{G12D}$ and $NRAS^{G12D}$ in colonic tumorigenesis and haematological malignancies, Haigis *et al.*[26] and Li *et al.*[27] have reached a somewhat different conclusion. Using knock-in mouse models that do not rely on chemical carcinogenesis and that feature intact endogenous regulatory elements, they have found that, of the two isoforms, only $KRAS^{G12D}$ was capable of inducing hyperproliferation and enhanced tumorigenesis when expressed in the crypts of the colonic epithelium and that it induced an aggressive as opposed to an indolent myeloproliferative disorder when expressed in haematopoietic cells. In addition, germline mutations in *KRAS* and *HRAS* are associated with the acquisition of distinct developmental disorders (*KRAS* is associated with Noonan syndrome and cardio-facio-cutaneous syndrome, and *HRAS* is associated with Costello syndrome)[28,29], thus suggesting that mutations in specific isoforms might be selected in accordance with their capacity to deregulate cellular homeostasis *in vivo*. Notably, a substitution of *HRAS* for *KRAS* in a knock-in model rescues the embryonic lethality that is caused by the loss of *KRAS4B*, thus implying a functional overlap between the two isoforms when expressed from the wild-type allele[30].

In summary, it seems that despite the remarkable progress made over the past three decades in understanding the mechanisms and outcomes of oncogenic RAS activation, fundamental issues concerning the role of distinct oncogenic RAS mutations and the contribution of different RAS isoforms to cancer aetiology remain. The recent emergence of sophisticated experimental tools to probe and to model oncogenic RAS-driven cancers clearly presents new opportunities to revisit these issues.

RAS and the transformed phenotype

Promotion of proliferation

The continual expansion of cancer cells relies on the erosion of the mechanisms that obligate cells to respond appropriately to mitogenic and anti-mitogenic factors that are present within the extracellular milieu. As RAS proteins are immediate arbitrators of mitogenic stimuli, it is perhaps not surprising that constitutive activation of RAS fuels cell proliferation (FIG. 2). Indeed, soon after the identification of RAS, pioneering studies revealed that overexpression of oncogenic HRAS is sufficient for driving the entry of G0 phase-arrested cells into the cell cycle in the absence of growth factors[31,32]. Adding to its function as a transducer of growth factor signalling, active RAS can also enhance the proliferative capacity of cells by inducing the transcriptional upregulation of growth factors such as heparin-binding epidermal growth factor-like growth factor (HBEGF), transforming growth factor-α (TGFα) and amphiregulin (AREG)[33–35]. Another output of oncogenic RAS signalling that contributes to the aberrant proliferation is the alteration in expression of growth factor receptors[33,34]. Additionally, oncogenic $HRAS^{G12V}$ induces the upregulation of integrins that promote proliferation while downregulating the integrin subunits that could maintain cellular quiescence[36,37]. On the opposing side of the proliferative balance, oncogenic RAS can directly interfere with anti-proliferative signals by inhibiting signalling from TGFβ[38–43].

The panoply of proliferative signals generated by oncogenic RAS culminates with the upregulation of several transcription factors that are required for cell cycle entry and progression, including FOS, serum response factor (SRF), the leucine zipper protein JUN, the ETS domain-containing transcription factor ELK1, activating transcription factor 2 (ATF2) and nuclear factor-κB (NF-κB)[44–49]. In turn, these factors trigger the expression of the G1 cyclin, cyclin D1 (REFS 50–52). Although initial studies attributed the stimulation of cyclin D1 transcription by oncogenic RAS to the activation of the RAF–MAPK pathway, it has become evident that additional levels of control are executed through RAS activation of PI3Ks, the RHO family GTPase RAC1, and the RAL-guanine nucleotide dissociation stimulator (GDS) family of GEFs[50,51,53–57]. In addition to stimulating cyclin D1 gene

transcription, oncogenic $HRAS^{G12V}$ also regulates the metabolic stability of the cyclin D1 protein through PI3K-dependent inhibition of glycogen synthase kinase 3β (GSK3β), which is the kinase that is responsible for the phosphorylation and the consequent ubiquitylation and proteasomal degradation of cyclin D1 (REF. 58).

Given the central role of cyclin D1 in RAS-induced self-sufficiency in growth signals it is perhaps not surprising that cyclin D1 is a crucial determinant of RAS-induced transformation. As such, cyclin D1-deficient mice are resistant to developing mammary cancers and squamous tumours that are induced by the *HRAS* oncogene[59,60]. Notably, dependency on cyclin D1 seems to be a unique property of RAS-induced tumours, as the absence of cyclin D1 had no effect on MYC- or WNT1-induced tumours. However, overexpression of cyclin D1 is not sufficient to transform cells without the activity of a cooperating oncogene, pointing to additional RAS-induced mechanisms to provoke a state of proliferative overdrive[61,62]. Indeed, oncogenic RAS can promote cell cycle progression by deregulating anti-growth signalling pathways through the suppression of cyclin-dependent kinase inhibitors (CKIs), such as p27 and p21, which would otherwise associate with and inhibit cyclin-dependent kinases (CDKs). This suppressive effect is mediated by multiple RAS effector pathways and is exerted at the transcriptional, translational and post-translational levels[42,63–66].

An inevitable consequence of the persistent mitogenic stimulation that is imposed by RAS is the initiation of replicative stress, which is marked by increased numbers of active DNA replication origins and collapsed replication forks, which ultimately leads to DNA damage and the activation of the DNA damage response (DDR)[67–70]. Indeed, the presence of DNA damage in precancerous lesions has been reported in several cancers that harbour oncogenic RAS, such as cancers of the pancreas, colon and lung. The outcome of overworking the replicative machinery can vary depending on cellular and genetic context. In a cell that possesses the full complement of functional DNA damage checkpoints, the engagement of the DDR by oncogenic $HRAS^{G12V}$ leads to an irreversible cell cycle arrest that is known as oncogene-induced senescence (OIS)[67]. Although OIS is a tumour-constraining response, its induction could contribute to the tumorigenic process by exerting a strong selective pressure in favour of cells in which crucial components of the DNA damage checkpoint have been lost (such as p53 and ARF (also known as p19))[71]. Conversely, in cells that are deficient in DNA damage checkpoints (through the loss of p53, for example) deregulated DNA replicative activity that is induced by oncogenic RAS results in the generation of chromosome aberrations such as dicentric chromosomes, acentric chromosomes and double-minute chromosomes, leading to the improper segregation of chromosomes and the consequent exclusion of chromosomes from daughter nuclei[22,72–75]. The documented capacity of oncogenic $HRAS^{G12V}$ to induce accelerated transition through G2/M, to inhibit the activation of the G2 DNA damage checkpoint, as well as to induce defects in mitotic spindle checkpoints[76], may also contribute to the genomic instability that is observed in RAS-driven cancers. With our understanding of genomic stress and its consequences in cancer initiation and progression still evolving, the contribution of oncogenic RAS to these processes will undoubtedly be an exciting avenue of cancer research in the coming years.

Suppression of apoptosis—Apoptotic cell death functions as a crucial defence mechanism against malignancy, and the corruption of the apoptotic machinery is a defining signature of cancer cells. A complex process, apoptosis is the result of balanced molecular actions that are initiated by a diverse range of signals, and it is regulated at several levels by both positive and negative modulators. Apoptotic cell death can be initiated extrinsically whereby extracellular cues such as growth factor withdrawal or matrix detachment induce the stimulation of death receptors, or it can be initiated intrinsically through the mitochondrion-mediated pathway, which is activated by cues such as DNA damage and

nutrient deprivation. Although both the intrinsic and extrinsic signalling cascades converge at the point of caspase 3 activation, they are subject to different regulatory mechanisms, including the pro-apoptotic and anti-apoptotic members of the BCL-2 family acting on the mitochondrion-mediated pathway, the anti-apoptotic FADD-like molecule FLIP (also known as CFLAR) acting on the extrinsic pathway and the inhibitors of apoptosis (IAPs) acting on both pathways[77]. Oncogenic RAS-driven erosion of the apoptotic pathways and its contribution to cancer has been well documented[77] (FIG. 3). For example, the elimination of inducible oncogenic $HRAS^{G12V}$ expression results in the regression of melanomas accompanied by massive tumour cell apoptosis[78]. Additionally, withdrawal of doxycycline-inducible oncogenic $KRAS^{G12D}$ expression from type II pneumocytes causes apoptosis and regression of both early proliferative lesions and lung cancers[79].

Mechanistically, the PI3K and RAF pathways activated by oncogenic RAS can downregulate pro-apoptotic mediators or upregulate anti-apoptotic molecules. Thus, activation of PI3K leads to the downregulation of the pro-apoptotic protein BCL-2-homologous antagonist/killer 1 (BAK1), and augments levels of IAPs through the activation of NF-κB[80–83]. RAF contributes to RAS-induced suppression of apoptosis by the downregulation of the pro-apoptotic transcriptional repressor prostate apoptosis response 4 (PAR4; also known as PAWR)[84,85], and the upregulation of the anti-apoptotic proteins BCL-2 and apoptosis repressor with caspase recruitment domain (ARC; also known as NOL3)[86,87]. Additionally, both the RAS–PI3K–AKT and the RAS– RAF pathways have been shown to mediate the phosphorylation of the pro-apoptotic BCL-2 family member BCL-2-associated agonist of cell death (BAD) on serine 136 and serine 122. Phosphorylation of BAD in either site results in the preferential formation of an inactive complex with 14-3-3, and thereby prevents the heterodimerization with, and subsequent inactivation of, BCL-2 and BCL-XL[88,89]. Recent findings also implicate oncogenic RAS-induced epigenetic silencing of the pro-apoptotic *CD95* (also known as *TNFRSF6*) gene as a potential anti-apoptotic mechanism that is induced by RAS[90,91]. The effector molecules operating downstream of RAS in this process remain to be elucidated.

In addition to its pro-survival function, cell type and context-specific oncogenic RAS signalling can also promote pro-apoptotic programmes[92,93]. For example, the preferential activation of the RAS–RAF–MAPKK–MAPK pathway exacerbates apoptosis[94], and $HRAS^{Q61L}$-mediated activation of JUN N-terminal kinase (JNK) signalling has also been associated with a pro-apoptotic role[95,96]. Phosphorylation of oncogenic KRAS by protein kinase C (PKC) promotes translocation to the mitochondria and induces apoptosis in a BCL-XL-dependent manner[97]. Additional RAS-regulated molecules with a role in pro-apoptotic programmes include RASSF1 and NORE1 (also known as RASSF5)[98]. On overexpression, these proteins are found in a pre-existing complex with mammalian STE20-like protein kinase 1 (MST1; also known as STK4), which is an enhancer of caspase 3 activation, and co-transfection of oncogenic RAS results in increased binding of active RAS to this complex and increased apoptosis[99,100]. There is an indication that such a pro-apoptotic function of RAS might be selected against in cancer, as the expression of either *RASSF1* or *RASSF5* in tumours is frequently suppressed either owing to promoter hypermethylation or — in the case of *RASSF1* — owing to the deletion of the chromosomal region that contains the gene[98,101–105]. Thus, although the context in which the pro-apoptotic capabilities of RAS are enacted remains unknown, it is the balance of pro-survival and pro-apoptotic signals that would ultimately determine whether the RAS-transformed cell will shift towards life or towards death. The sheer prevalence of oncogenic RAS in cancer is an indication that the pro-survival axis has a dominant role.

Metabolism—As a result of their high proliferative rates, cancer cells are crucially dependent on metabolic pathways that generate the building blocks that are needed to

produce a new cell[106]. These unique metabolic needs were first described in the 1920s by Otto Warburg and are typified by an increase in glucose uptake and a shift from mitochondrial oxidative phosphorylation to aerobic glycolysis[107,108]. Although the full ramifications of this metabolic phenotype are yet to be deciphered, it has been postulated that — although it is less efficient in generating ATP — the catabolism of glucose through glycolysis is highly effective in providing the macromolecular precursors that are necessary for the replication of biomass (such as nucleotides, amino acids and lipids)[109]. Oncogenic RAS impinges on the metabolic reprogramming of cancer cells predominantly through the upregulation of hypoxia-inducible factor 1α (HIF1α), which forms the HIF transcription factor when bound to HIF1β (also known as ARNT), and is well recognized for its ability to stimulate a glycolytic shift. This is achieved through oncogenic RAS-induced concurrent activation of MAPK and PI3K effector pathways leading to the stimulation of mTOR activity and mTOR-mediated cap-dependent translation of HIF1α[110,111]. RAS-dependent upregulation of HIF1α has been implicated in enhancing both the transport and the glycolytic capture of glucose, as well as its processing to biosynthetic intermediates. With respect to the transport and capture of glucose, oncogenic RAS increases the transcription of the glucose transporter GLUT1 (also known as SLC2A1), thus conferring cells with an increased capacity to take up glucose[112–117]. With respect to the processing of biosynthetic intermediates , oncogenic RAS leads to an increase in the levels of key glycolytic enzymes, such as hexokinase, phosphofructokinase and lactate dehydrogenase[117–121]. Hence, oncogenic RAS directly contributes to metabolic reactions that promote the use of glucose as an anabolic substrate in generating building material for cellular growth[106] (FIG. 4). Whether this contribution is solely attributable to the upregulation of HIF1α by oncogenic RAS or whether it might involve other RAS targets remains to be established.

Another avenue through which oncogenic RAS interfaces with cellular metabolism is by affecting autophagy — a process of self-consumption that generates energy, as well as building blocks that are necessary for cellular survival, and that supports organelle homeostasis[122]. Although autophagy has been shown to have both tumour-suppressive and tumour-promoting qualities, recent studies have implicated oncogenic RAS in the upregulation of the autophagic processes, resulting in the upkeep of mitochondria, an increase in glycolytic rate and cellular viability, and ultimately supporting tumour growth *in vivo*[123–125]. Such dependency requires essential autophagy genes such as *ATG5* and *ATG7*, as well as the autophagy cargo receptor p62 (REF. 125). With the rapid expansion of the field of cancer metabolism, we are likely to encounter an increasing number of molecular interactions that link metabolic nodes to oncogenic RAS signalling.

Remodeling the microenvironment—For decades RAS has been the prime example of a potent cell-autonomous oncogene. It is, however, becoming increasingly evident that the effects of oncogenic RAS stretch further to include non-cell-autonomous changes in the cellular microenvironment that have essential roles in tumour initiation and progression. A prime example of such an effect is the induction of the outgrowth of new blood vessels, or angiogenesis, which allows cells within the evolving tumour to accommodate the physiological need for an adequate supply of oxygen and nutrients[126,127]. The mechanisms by which RAS activation initiates and sustains pro-angiogenic processes are complex and impinge on the modulation of levels of endothelial growth factors and also increase local inflammation and stromal remodelling[128–130] (FIG. 5).

Vascular endothelial growth factor A (VEGFA), which is a key player in the induction of endothelial cell proliferation and the sprouting of new blood vessels, is a well-recognized target of oncogenic RAS. In fact, the disruption of either $KRAS^{G12V}$ or $KRAS^{G13D}$ expression and the subsequent reduction in VEGFA levels leads to diminished tumour growth[127,131]. Oncogenic RAS-mediated upregulation of VEGFA involves the activation of

multiple signalling cascades that eventually culminate in the stabilization of the pro-angiogenic transcription factor HIF1α, boosting its transactivation potential at the *VEGFA* promoter[132–135]. There is also another pathway by which RAS can upregulate VEGFA: via the pro-angiogenic enzyme cyclooxygenase 2 (COX2), which, through the production of prostaglandins, leads to the enhancement of the cyclic AMP-dependent transcription of *VEGFA*[129]. COX2 can also increase the levels of a plethora of other endothelial growth factors, such as basic fibroblast growth factor (bFGF; also known as FGF2) and platelet-derived growth factor (PDGF), and is required for integrin-mediated endothelial cell spreading and migration[136,137].

Oncogenic $HRAS^{G12V}$-mediated production of pro-inflammatory cytokines has emerged as another contributor to the induction of angiogenesis[130]. Several cytokines have been implicated in this response, including interleukin-8 (IL-8), IL-6 and GRO1 (also known as CXCL1), and their RAS-dependent upregulation is predominantly mediated by the activation of signalling pathways that impinge on the transcriptional machinery controlling their expression[130,138]. Once produced, the pro-inflammatory cytokines recruit immune cells, such as neutrophils and macrophages, which produce angiogenic growth factors[138–140].

Cancer cells and the pro-angiogenic growth factors that they produce are often physically trapped by the extensive network of the extracellular matrix (ECM). Hence, the modification of the surrounding ECM is necessary both for the growth factors to reach the target endothelium and for the migration of the newly generated endothelium into the tumour[129,141,142]. Oncogenic $HRAS^{G12V}$-mediated upregulation of matrix metalloproteinase 2 (MMP2), MMP9 and urokinase-type plasminogen activator (uPA) has been shown to be instrumental in removing the physical confines of the basement membrane, with upregulation of uPA especially important in potentiating endothelial cell migration and vessel sprouting[129,143–148]. Oncogenic RAS can also promote the angiogenic process by restricting the expression of negative regulators of neo-vascularization, such as thrombospondin 1 (TSP1; also known as THBS1) and TSP2 (also known as THBS2), in tumour cells[149,150]. These extracellular glycoproteins interact with components of the ECM to restrict the availability of endothelial growth factors and chemokines to the vascular system. In addition, they directly affect the viability of endothelial cells[151,152]. Overall, the role of oncogenic RAS in the development of tumour vasculature represents a compendium of cell-autonomous and non-cell-autonomous functions that are engaged together to activate the pro-angiogenic programme.

Evasion of the immune response—The emergence of a tumour in spite of an immune system that in principle should be able to recognize it as a foreign entity raises the question of how a cancer cell evades such surveillance. Thus far, two mechanisms by which oncogenic RAS can subvert antitumour immunity have surfaced.

First, oncogenic activation of RAS reduces the surface expression of antigen-presenting major histocompatibility complexes (MHC) on cancer cells, and such downregulation results in decreased immunogenicity of the RAS-transformed cells[153–157]. The oncogenic RAS-mediated downregulation of MHC was shown to be independent of the promoter activity at MHC loci, suggesting that defects in the components of antigen-processing machinery could be responsible for the compromised antigenic peptide transport and loading[154,158,159]. Indeed, transformation by RAS reduced the levels and functionality of the antigen peptide transporters TAP1 and TAP2 and proteasome subunits LMP2 (also known as PSMB9) and LMP7 (also known as PSMB8), resulting in decreased MHC expression[160,161].

Second, data from human cancers and transgenic mouse models indicate that RAS-driven cancers possess the capacity to overcome host-protecting adaptive immune responses[162]. Thus, although T cells specific for the mutated RAS antigens can be found in patients with melanoma, pancreatic and colon cancers, they are often anergic and so inactive towards the tumour[158,163–166]. This concept is supported by recent experimental evidence from a mouse model of oncogenic $KRAS^{G12D}$-induced lung cancer, demonstrating that the initial immune response becomes substantially attenuated, eventually leading to a full escape from immune surveillance[167]. One possible mechanism by which oncogenic RAS expression may lead to a compromised antitumour immune response is through the recruitment of immunosuppressive regulatory T cells (TRegs) and myeloid-derived suppressor cells (MDSCs) to the tumour site[168,169]. The potential importance of this mode of immune modulation for the tumorigenic process is suggested by the observation that TRegs are required for cancer formation in the mouse model of $KRAS^{G12V}$-initiated lung tumorigenesis[170]. Whether RAS-transformed cells influence the immune response through direct recruitment of immunosuppressive cells or in conjunction with the induction of an inflammatory response remains to be elucidated[171,172].

Metastasis—Among the most threatening aspects of an evolving tumour is the acquisition of metastatic properties, whereby the cancer cells spread to the surrounding and distant organs. Many metastatic tumours (such as lung, pancreas and colon tumours) contain RAS mutations. This fact, along with the demonstration that oncogenic mutants of RAS could confer metastatic properties to mouse cells in culture, served as a foundation for a large body of work that aimed to understand the role of oncogenic RAS in metastatic tumour spread. Although far from complete, the picture that has emerged so far implicates RAS in multiple cellular processes that endow cells with metastatic potential.

The initial step in the metastatic cascade is the establishment of local tumour cell invasion, a process that relies on the ability of tumour cells to break away from the primary tumour. Oncogenic RAS contributes to this process by inducing alterations in cell–cell and cell–matrix interactions and the acquisition of a migratory phenotype. The perturbation of cell–cell contacts by oncogenic RAS is accomplished through the targeting of the molecular machinery that maintains intercellular adhesion junctions, which includes the calcium-dependent E-cadherin receptor and its associated cytoplasmic protein β-catenin[173–176]. Thus, the expression of oncogenic RAS reduces the levels of E-cadherin through the upregulation of the E-cadherin transcriptional repressors SNAIL (also known as SNAI1) and SLUG (also known as SNAI2), the stimulation of E-cadherin proteolytic degradation and the induction of E-cadherin promoter methylation[177–179]. RAS activation has also been shown to induce the destabilization of E-cadherin–β-catenin complexes and the relocalization of β-catenin[174,180–182]. Along with the weakening of cell–cell interactions, oncogenic RAS expression reduces attachments to the ECM by downregulating integrin subunits (such as integrin α5β1) that facilitate the maintenance of stable adhesion complexes[183–187]. Finally, oncogenic RAS directly contributes to the enhanced motility of cancer cells by affecting pronounced changes in the polymerization, organization and contraction of actin; the polymerization and/or stability of microtubules; and the transcriptional regulation of mitogenic gene products[188]. Collectively, these changes establish the front–rear asymmetry that is required for cell migration.

Progression through the metastatic process requires the cancer cell to leave the confines of the primary tumour and to enter the blood or lymphatic system (intravasation). Crucial for the execution of this step is the capacity to invade through the physical barrier that is imposed by the basement membrane. The link between oncogenic RAS expression and the acquisition of the invasive phenotype has been attributed to alterations in cellular activities that control ECM degradation. Specifically, signalling pathways that are downstream of

constitutively activated RAS can increase the expression and/or activity of various ECM proteases and in parallel can decrease the expression of protease inhibitors. Oncogenic RAS is also thought to contribute to the capacity of tumour cells to migrate through the circulatory system by protecting them from matrix deprivation-induced apoptosis, or anoikis[77,80,127,174,189–191].

Given the multitude of cellular activities on which tumour metastasis relies, it is not surprising that oncogenic RAS promotes this aspect of the transformed phenotype by engaging a diverse and broad platform of effector mechanisms. RAS-dependent signalling pathways that have been demonstrated to have an essential role in metastatic progression include the RAS–MAPK, RAS–PI3K, RAS–RAL GTPase and RAS–RHO GTPase pathways[182,188]. Each of these pathways can promote the metastatic process at multiple steps. For example, the activation of RHO GTPases leads to concurrent alterations in cell adhesion and cell motility. In addition, the identity of RAS-dependent signals that promote metastasis has been shown to vary substantially depending on tissue type and genetic background[192–196]. Furthermore, in some settings, the induction of metastasis is the product of cooperation between oncogenic RAS and other metastasis-promoting pathways, such as the TGFβ pathway[177,197]. Thus, defining the precise modes by which RAS-responsive pathways affect metastatic capacity awaits an improved understanding of the context-dependent outcome of their coordinated activation.

The road ahead

As research on oncogenic RAS is entering its fourth decade, the information it has generated thus far serves as a rich and instructive backdrop for the challenges and opportunities that lie ahead. A unifying concept that emerges from the large number of genetic, biochemical and cell biological studies is that the oncogenic potential of RAS manifests in a context-dependent manner. Thus, the subcellular, cellular and tissue environments within which oncogenic RAS operates crucially determine its functional output. In addition, depending on the genetic landscape of an individual cell, different RAS-dependent oncogenic activities might become more or less important during tumour evolution. Although the task of developing a mechanistic understanding of how these determinants dictate a specific pathological outcome may seem daunting, the outpouring over the past few years of highly refined experimental tools to address these questions holds promise for considerable advances.

At the subcellular level, RAS proteins have been shown to reside in distinct compartments within the cell, with each compartment eliciting differential signalling outputs that may control various aspects of oncogenic transformation[198,199]. Recent advancements in the development of multi-parameter fluorescent reporters and biosensors, along with improved access to high-sensitivity real-time imaging techniques, should provide important insights into the spatiotemporal coordination of oncogenic RAS signalling in live cells. At the cellular and tissue level, our capacity to probe the *in vivo* ramifications of the expression of oncogenic RAS has been continuously improving owing to an ever-growing collection of sophisticated genetically engineered mouse models that feature activating mutations in RAS. By affording tissue- and cell-specific expression in a time-controlled and reversible manner, these models often recapitulate the genetic and biological evolution of human cancers. As such, their future use could not only augment the understanding of the crucial mediators of RAS-driven oncogenesis but could also be instrumental in testing and developing novel targeting strategies directed at RAS. Finally, signalling networks that are triggered by oncogenic RAS within the cell are complex and highly dynamic. Computational approaches that are designed to model how the integration of multi-pathway networks determines their biological output will clearly be an area of intense investigation in the years to come.

Equipped with these tools we might be in a unique position to translate major advances in basic research on the RAS oncogenes into meaningful clinical benefits.

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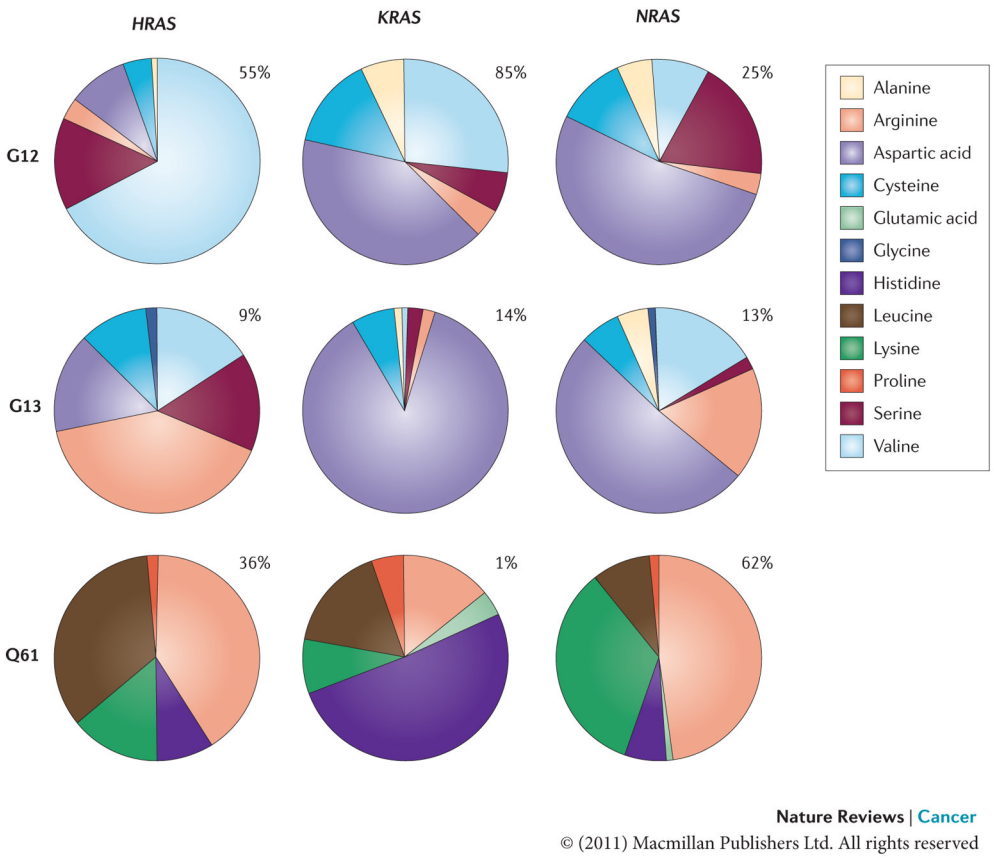

Figure 1. Frequency of mutations at G12, G13 and Q61 in RAS isoforms

The frequency of mutational substitution at G12, G13 or Q61 for a particular amino acid has been represented using pie charts. Percentages indicate the frequency with which a given residue is mutated within a particular isoform. Primary data source is the COSMIC database (see Further information).

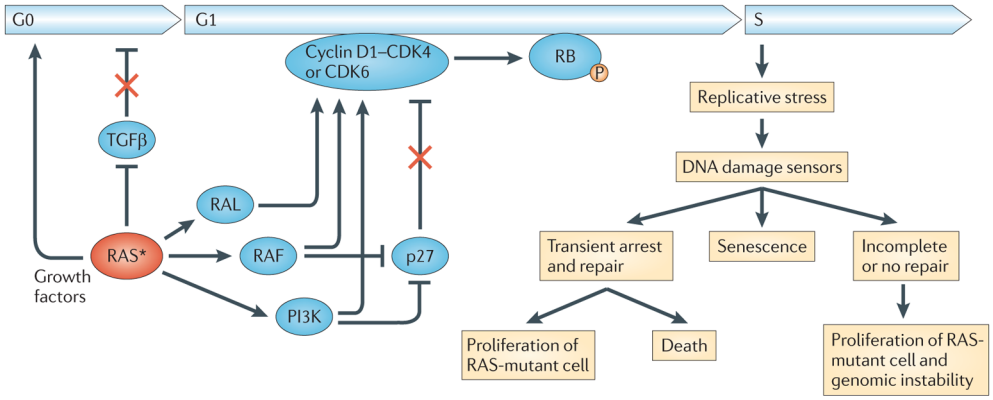

Figure 2. RAS effects on proliferation

Oncogenic RAS establishes independence from extracellular growth factors and growth inhibitors, thereby promoting exit from the G0 phase of the cell cycle, progression through G1 and entry into S phase. RAS induces the transcriptional upregulation of growth factors and interferes with transforming growth factor-β (TGFβ) signalling through inhibition of TGFβ receptor expression or downstream signalling by downregulating the expression of SMAD3, as well as the nuclear accumulation of SMAD2 and SMAD3. RAS also upregulates the levels of cyclin D1 and suppresses the cyclin-dependent kinase inhibitor (CDKI) p27. The newly synthesized cyclin D1 associates with and activates the cyclin-dependent kinases CDK4 and CDK6, leading to the phosphorylation of RB and the subsequent dissolution of the RB–E2F transcription factor complexes. Once released, E2F transcription factors transactivate several genes that are required for cell cycle progression, including cyclin E (*CCNE*) and cyclin A (*CCNA*) that induce transition through the G1/S checkpoint (not shown). Hyperproliferative cues from activation of the RAS oncogene can result in replicative stress leading to DNA damage. In response to DNA damage cells can activate the DNA damage checkpoints to transiently arrest and restore the integrity of the genome, enter a state of irreversible arrest (senescence) or undergo apoptosis. Inaccurate repair of DNA damage can lead to mutations and chromosome aberrations, thereby contributing to tumorigenesis. The asterisk represents the mutational activation of RAS. P, phosphorylation.

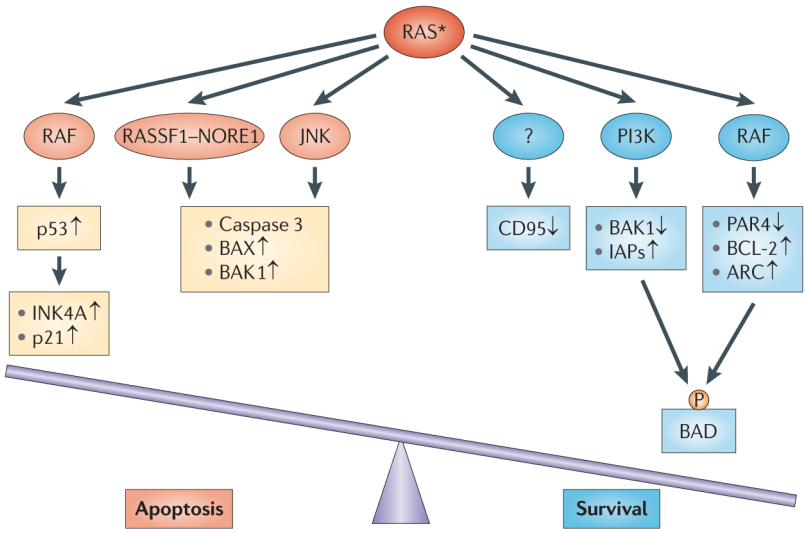

Figure 3. RAS effects on apoptosis

Oncogenic RAS may have both pro-apoptotic and anti-apoptotic functions depending on the status of RAS effector pathways and the apoptotic machinery. In many cases oncogenic RAS signalling through the RAF pathway engages an apoptotic response that is mediated by p53. Also, signalling through the RAS effectors RASSF1, NORE1, mammalian STE20-like protein kinase 1 (MST1) and JUN N-terminal kinase (JNK) can lead to apoptotic death via the activation of caspase 3 and the pro-apoptotic proteins BCL-2-associated X protein (BAX) and BCL-2-homologous antagonist/killer 1 (BAK1). Acquisition of a tumorigenic phenotype is marked by the suppression of such mediators of RAS-induced apoptosis. In this context, the anti-apoptotic activity of RAS takes a stronghold. The anti-apoptotic function of oncogenic RAS is mediated by several effector pathways, including the RAS–PI3K effector pathway, which regulates the levels of pro-apoptotic protein BAK1 and inhibitors of apoptosis (IAPs), and the RAS–RAF pathway, which downregulates the pro-apoptotic transcriptional repressor prostate apoptosis response 4 (PAR4) while upregulating the anti-apoptotic proteins BCL-2 and apoptosis repressor with caspase recruitment domain (ARC). Both pathways have been implicated in phosphorylating and inactivating the pro-apoptotic protein BCL-2-associated agonist of cell death (BAD). The mechanism through which RAS induces the epigenetic silencing of the pro-apoptotic *CD95* gene remains to be uncovered. The asterisk represents the mutational activation of RAS protein. P, phosphorylation.

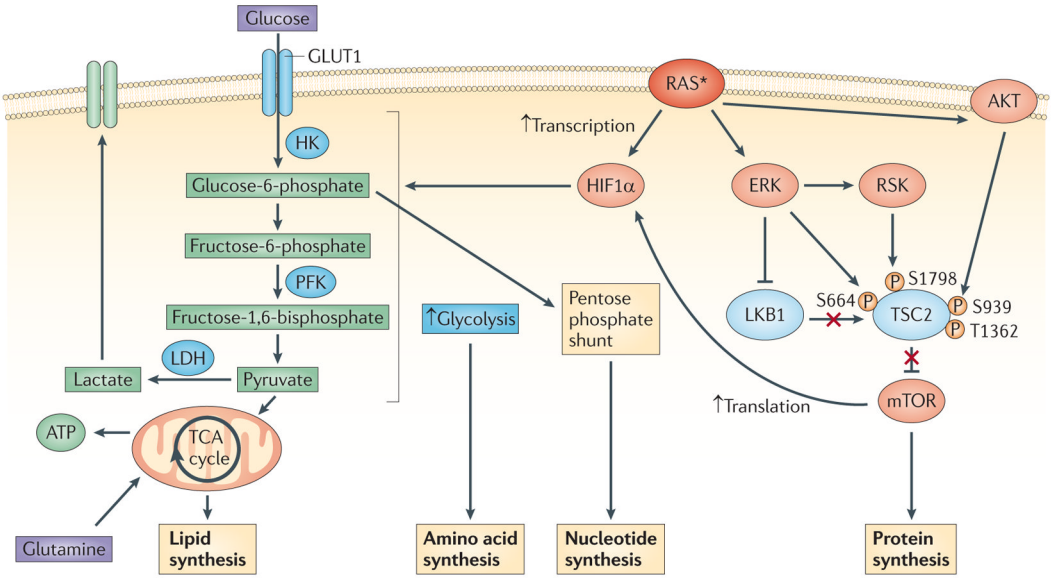

Figure 4. Effect of RAS on energy metabolism in cancer cells: generating macromolecular precursors

ERK and PI3K signalling downstream of oncogenic RAS converge to activate mTOR by inhibiting its negative regulators tuberin (TSC2) and liver kinase B1 (LKB1)–AMP-activated protein kinase (AMPK)[116]. TSC2 can be directly phosphorylated by both ERK and ERK-activated ribosomal protein S6 kinase (RSK) on S664 and S1798, respectively, as well as by AKT (on S939 and T1362), and, likewise, RAF–ERK1 or RAF–ERK2 signalling disrupts the LKB1–AMPK checkpoint[200–204]. This leads to mTOR–eukaryotic translation initiation factor 4 (eIF4)-dependent translation of hypoxia-inducible factor 1α (HIF1α). Activated RAS can also result in the transcriptional upregulation of *HIF1A*. Increased levels of HIF1α augment multiple steps in glycolytic metabolism (shown in blue). The upregulation of hexokinase (HK) facilitates the conversion of glucose to glucose-6-phosphate, a glycolytic intermediate that is used in pentose phosphate pathway-dependent nucleotide synthesis[205]. Higher levels of phosphofructokinase (PFK) lead to an enhanced glycolytic flux and the production of pyruvate, which, in conjunction with the oncogenic RAS-dependent increase in lactose dehydrogenase (LDH) levels, can allow glycolysis to persist by regenerating NAD+, a necessary cofactor for glycolytic reactions[109,120,121]. In addition, some of the pyruvate can enter the tricarboxylic acid (TCA) cycle where its conversion to citrate generates intermediates that are necessary for the synthesis of fatty acids and non-essential amino acids[205]. The asterisk represents the mutational activation of RAS. GLUT1, glucose transporter 1.

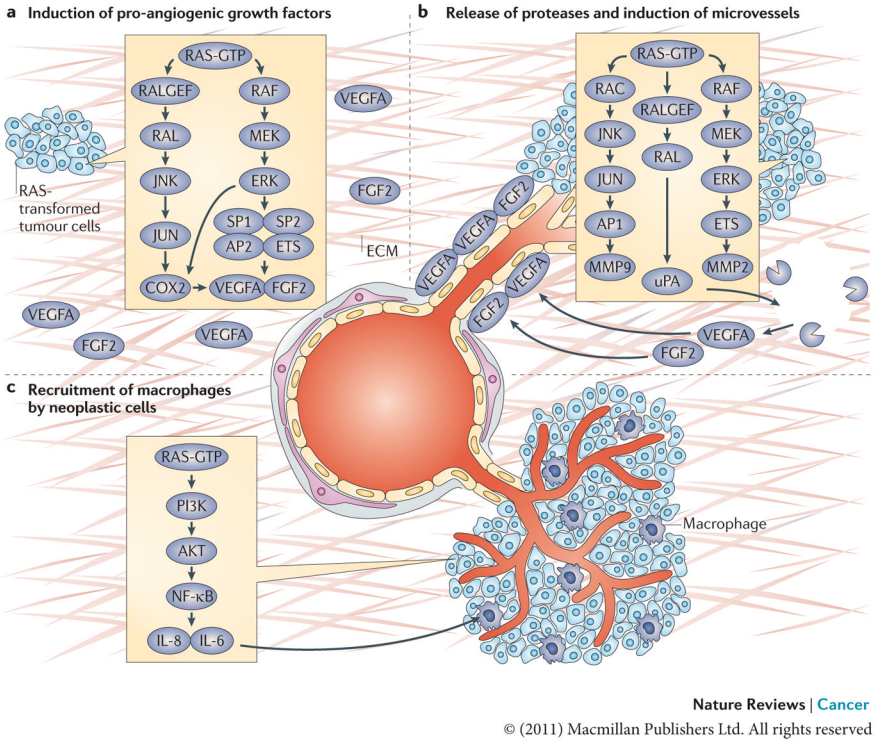

Figure 5. RAS and angiogenesis

a. The induction of pro-angiogenic growth factors (vascular endothelial growth factor A (VEGFA) and fibroblast growth factor 2 (FGF2)) by RAS in neoplastic cells is shown. RAS enhances the transcription of *VEGFA* by recruiting transcription factors such as SP1, SP2, AP2 and ETS to the *VEGFA* promoter. RAS also increases the stability of *VEGFA* mRNA and augments its translation[206–213]. The RAS–JUN N-terminal kinase (JNK) signalling axis is responsible for upregulating the transcription of prostaglandin-endoperoxide synthase 2 (*PTGS2*), which encodes COX2, by activating JUN, a component of the AP1 transcription complex, whereas the RAS–ERK1 and ERK2 pathway contributes to COX2 expression through the phosphorylation of CCAAT/enhancer binding protein-β (C/EBPβ) and ETS transcription factors such as PEA3 (REFS 214–217). Expression of COX2, in turn, increases the levels of VEGFA produced by RAS-transformed cells. **b**. The release of proteases by neoplastic cells cleaves components of the extracellular matrix (ECM) and releases VEGFA and FGF2, which are trapped in the ECM. Expression of proteases urokinase-type plasminogen activator (uPA), matrix metalloproteinase 2 (MMP2) and MMP9 in RAS-transformed cells is increased by the combined effects of ETS transcription factors (activated by the RAF–ERK pathway) and JUN (activated by the RAC–JNK pathway) binding to the promoters of PEA3–AP1 sites, as well as enhanced translation of polysome-associated *MMP9* mRNA[144,218,219]. Stimulation of uPA expression is also dependent on RAS-mediated activation of RAL GTPase[220,221]. This induces neo-proliferation and sprouting of microvessels towards the tumour site. **c**. The recruitment of macrophages by neoplastic cells (through RAS-induced nuclear factor-κB (NF-κB)-dependent production of the cytokines interleukin-6 (IL-6) and IL-8) and subsequent promotion of endothelial proliferation and sprouting by newly recruited macrophages is shown.

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Table 1

Frequency of RAS mutations in human cancer.

Tissue	HRAS*	KRAS*	NRAS*	Incidence Rate‡	Mortality Rate‡
Endocrine	3% (535)	0% (670)	5% (570)	0.7	0.3
Biliary tract	0% (153)	31% (1679)	1% (287)	NA§	NA
Bone	2% (199)	1% (252)	0% (207)	0.9	0.4
Breast	1% (716)	4% (782)	2% (504)	124	24
Central nervous system	0% (964)	1% (1054)	1% (1017)	6.5	4.3
Cervix	9% (264)	7% (637)	2% (132)	8.1	2.4
Endometrium	1% (291)	14% (2251)	0% (314)	23.9	4.1
Eye	0% (33)	4% (90)	1% (106)	0.8	0.1
Haematopoietic and lymphoid tissue	0% (3076)	5% (5978)	10% (8753)	35.2	14.5
Kidney	0% (273)	1% (704)	0% (435)	14.6	4.1
Large intestine	0% (617)	33% (34013)	2% (1570)	47.2	17.6
Liver	0% (270)	5% (461)	3% (310)	7.3	5.2
Lung	0% (2091)	17% (16348)	1% (3081)	62	52.5
Oesophagus	1% (161)	4% (375)	0% (161)	4.5	4.4
Ovary	0% (152)	14% (3181)	5% (191)	12.8	8.6
Pancreas	0% (278)	57% (5329)	2% (305)	12	10.7
Pleura	0% (19)	0% (45)	0% (30)	NA	NA
Prostate	6% (558)	8% (1184)	2% (588)	156	24.7
Salivary gland	15% (161)	3% (170)	0% (45)	NA	NA
Skin	6% (2100)	3% (1462)	18% (4956)	22.7	3.5
Small intestine	0% (5)	20% (316)	0% (5)	2	0.4
Stomach	4% (384)	6% (2793)	2% (215)	7.7	3.8
Testis	4% (130)	4% (432)	3% (283)	5.5	0.2
Thymus	2% (46)	2% (186)	0% (46)	NA	NA
Thyroid	3% (4137)	2% (5166)	8% (4662)	11	0.5
Upper aerodigestive tract	9% (1083)	3% (1582)	3% (836)	14	3.7

Tissue	HRAS*	KRAS*	NRAS*	Incidence Rate‡	Mortality Rate‡
Urinary tract	11% (1765)	5% (1099)	2% (873)	21.1	4.3

NA, not available.

*Data from the COSMIC database (see Further information). Numbers in parentheses indicate total unique samples sequenced.

‡Data from the US National Cancer Institute SEER Cancer Statistics Review (see Further information). Rates are shown as per 100,000 people per year.

§Tumour subtype for which data are unavailable in the SEER database.

Table 2

Mouse models of oncogenic RAS activation

Experimental approach	Targeted tissues and cell types	Tumorigenic phenotype
Conditional endogenous expression of Lox-STOP-Lox- *Kras*^{G12D} or Lox-STOP-Lox- *Nras*^{G12D} cassette	Lung (intranasal administration of adenoviral Cre), pancreas (*Pdx1*-Cre or *Ptf1a*-Cre), colon (*Fabp1*-Cre) and the haematopoietic system (*Mx1*-Cre)	Lung adenocarcinoma, pancreatic intraepithelial neoplasia, colonic hyperplasia (*Kras*^{G12D}), resistance to apoptosis (*Nras*^{G12D}) and aggressive myeloproliferative disorder (*Kras*^{G12D})[26, 27, 222–224]
Endogenous expression of Lox-STOP-Lox-*Kras*^{G12V} – IRES-β-galactosidase cassette	Whole-body (*CMV*-Cre)	Lung hyperplasia, adenocarcinoma and minor sarcoma lesions[22]
Transgenic expression of *Kras*^{G12V}	Gastric and pancreatic epithelium (*Krt19* promoter-driven *Kras*^{G12V})	Gastric cell hyperplasia[225]
Transgenic expression of *Hras*^{Q61L}	Urothelium (*Upk2* promoter-driven rabbit *Hras*^{Q61L})	Bladder tumorigenesis[226]
Inducible transgenic expression of *Kras*^{G12D}	Basal layer of stratified epithelium (*Krt5* promoter-driven tetracycline-responsive *Kras*^{G12D})	Neoplastic squamous epithelium: skin, forestomach and oesophagus[227]
Inducible transgenic expression of *Hras*^{G12V}	Melanocytes (tyrosinase-driven tetracycline-responsive *Hras*^{G12V})	Melanoma[78]
Spontaneous chemical carcinogenesis	Skin (DMBA-TPA-induced mutagenesis of *Hras*) and lung (urethane-induced mutagenesis of *Kras*)	Skin papillomas and lung tumours [25, 228]
Somatic oncogene transfer by RCAS-TVA gene delivery method	Brain (nestin-TVA-targeted expression of *Kras*^{G12D} and *Akt-myr*) and pancreas (Lox-STOP-Lox-*R26tva–LacZ/+*-targeted expression of *Kras*^{G12D})	Glioblastoma and pancreatic intraepithelial neoplasia[229, 230]
Spontaneous recombination that results in expression of activated *Kras*^{G12D}	Whole-body	Lung hyperplasia and carcinoma, thymic lymphoma and skin papilloma[231]

CMV, cytomegalovirus promoter; Fabp1, fatty acid binding protein 1, liver; IRES, internal ribosome entry site; Krt, keratin; Mx1, myxovirus resistance 1; myr, myristoylated; Pdx1, pancreatic and duodenal homeobox 1; Ptfa1, pancreas transcription factor 1, a-subunit; R26, ROSA 26; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; Upk2, uroplakin 2.