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An Update on the Biology of RAS/RAF Mutations in Colorectal Cancer

Mandayam O. Nandan · Vincent W. Yang

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Abstract Deaths caused by colorectal cancer (CRC) are among the leading causes of cancer-related death in the United States and around the world. Approximately 150,000 Americans are diagnosed with CRC each year and around 50,000 will die from it. Mutations in many key genes have been identified that are important to the pathogenesis of CRC. Among the genes mutated in CRC, RAS and RAF mutations are common events. Both RAS and RAF are critical mediators of the mitogen-activated protein kinase (MAPK) pathway that is involved in regulating cellular homeostasis, including proliferation, survival, and differentiation. In this review, we provide a historical perspective and update on RAS/RAF mutations as related to colorectal cancer. Additionally, we will review recent mouse models of RAS and RAF mutations that have an impact on CRC research.

Keywords RAS signaling pathway · Oncogene · Colorectal cancer · KRAS and B-RAF mutations · Mouse models

Introduction

The high incidence and mortality rate makes colorectal cancer (CRC) a major health care concern in the US and around the world. Research pertaining to the pathogenesis and treatment of CRC is therefore of utmost importance. As with other cancers, CRC is a genetic disease involving many known mutations, which are classified as tumor suppressor genes or oncogenes. Tumor suppressors act as checkpoints in the cell cycle to keep cells under strict physiological control. Mutations in genes encoding tumor suppressors result in hyperproliferation and deregulated cell cycle control. The other major mutational events in cancers are those that occur in oncogenes. These are often activating mutations in genes that are also involved in cellular homeostasis. In particular, the most common activating mutation in CRC occurs in the RAS gene. This change transpires in about 50% of most CRCs [1••]. In addition, mutations in RAF that is downstream from RAS, are gaining importance in the pathogenesis of CRC. Here we review the recent progress on the roles of RAS and RAF in CRC.

M. O. Nandan · V. W. Yang (✉)
Division of Digestive Diseases, Department of Medicine,
Emory University School of Medicine,
Atlanta, GA 30322, USA
e-mail: vyang@emory.edu

M. O. Nandan
e-mail: mnandan@emory.edu

V. W. Yang
Department of Hematology and Medical Oncology,
Emory University School of Medicine,
Atlanta, GA 30322, USA

Mitogen-Activated Protein Kinase Signaling Cascade

Mitogen-activated protein kinase (MAPK) signaling is essential for maintenance of the normal physiological processes including proliferation and differentiation. This intracellular MAPK signaling network is complex and involves a large number of intermediates. An important component of this network is the RAS signaling pathway. The canonical RAS pathway includes the key mediators RAS and RAF proteins along with the intracellular signal kinases, mitogen extracellular kinase (MEK) and extracellular signal-related kinase (ERK).

The RAS family of proteins were initially isolated and identified as Rat Sarcoma factors. This family credits its origin as the first oncogene discovered. The RAS super-

family consists of several members including Ras, Rab, Rho, Arf, and Ran sub-families. The RAS family of proteins includes KRAS, HRAS, NRAS, ERAS, MRAS, and RRAS, all of which have been characterized. The RAS proteins are monomeric 21-kDa proteins that have a high degree of homology, especially in their amino-terminal and carboxyl-terminal domains, while still maintaining unique functions [2]. These proteins function as GTP/GDP-binding signal transducing molecules from the cell surface to the nucleus. They fluctuate between inactive GDP-bound and active GTP-bound states to achieve cell signaling. These proteins eventually help in the regulation of essential cell physiological functions such as proliferation and survival [2, 3].

RAS is normally present in the inactive GDP-bound form, which can be activated by external stimuli, such as the presence of mitogens, cytokines, and growth factors. Binding of ligands to receptor tyrosine kinases (RTKs) results in the dimerization of the receptor. This activated receptor then binds to Grb2 protein either directly or through other mediator proteins. Intracellular Sos protein is then recruited to the cell surface through interaction with Grb2. Sos displays GEF (guanine nucleotide exchange factor) activity as part of the RTK-Grb2-Sos complex. This complex then binds to RAS-GDP, resulting in the dissociation of GDP. Nucleotide-free RAS preferentially binds to GTP to activate a downstream signaling cascade [4]. GTP-bound RAS activates a whole spectrum of kinases that carry the signal from the membrane toward the nucleus. On the contrary, another class of proteins, RAS-GAPs (RAS-GTPase-activating proteins), deactivates this signaling pathway by removing GTP from the activated RAS molecule.

Activated, GTP-bound RAS protein directly binds and interacts with RAF, which belongs to a family of kinases comprising of three main isoforms: A-RAF, B-RAF, and C-RAF (RAF-1). Unlike the RAS family molecules, the RAF proteins can dimerize. These are cytosolic, serine/threonine protein kinases that lie dormant in the inactive form by binding to 14-3-3 proteins. The RAF molecules are then recruited to the cell surface upon activation of RAS-GTP. Upon interaction of RAF with the RAS-GTP molecule, a series of phosphorylation events releases the inhibition of RAF by 14-3-3. Activation of RAF leads to heterodimerization among the RAF isomers. The active RAF heterodimer then recruits, binds, and activates a scaffolding protein called KSR1 (kinase suppressor of RAS1). Initially, the KSR1 protein is also bound to 14-3-3 in the cytoplasm and maintained in an inactive state. Upon RAS/RAF activation, inhibition of KSR1 is removed and it is mobilized to interact with RAF heterodimers. MEK and ERK are downstream substrates/activators of the RAS signaling pathway. Both MEK and ERK are recruited to interact with the KSR1 scaffolding protein. RAF exerts its kinase activity by phosphorylating MEK, which in turn is activated and phosphorylates ERK. Phosphorylated ERK is then translocated to the nucleus where it activates a host of proliferative and survival signals [4].

The normal function of the MAPK signaling pathway is to maintain cellular homeostasis. Mutations in components of the pathway lead to signal imbalance and can potentially result in cancer formation. Activating mutations in the MAPK pathway occur in about 30% of all cancers. Among those mutations, a majority (~50%) occur in the RAS gene. A comprehensive review that describes mutational changes and pathway modifications in various cancers has been recently published [5••].

RAS Oncogene

RAS proteins were the first to be isolated from cancers and subsequently characterized as oncogenes. As previously described, the RAS family is comprised of many members sharing a high degree of homology. The members that have been well characterized include KRAS, HRAS, NRAS, ERAS, MRAS, and RRAS.

The most interesting paradox within RAS mutational research is the physiological disparity upon its expression in primary cells when compared with immortalized cells. Immortalized cells are defined as those that do not respond to the Hayflick limit, which is the number of times a diploid cell can duplicate before it ceases to divide and undergoes senescence, or cell cycle arrest [6]. The senescence event was later shown to be due to telomere shortening as cells age [7]. This holds true in the case of primary and pre-cancerous cells cultured in vitro and is known as replicative senescence [8].

Earlier reports suggested that cellular immortality was essential for complete transformation by the RAS oncogene, specifically HRAS [9]. Oncogenic RAS expression in primary human and rodent cells results in G_1 arrest in the cell cycle [10]. The studies were performed using human, mouse, and rat fibroblasts retrovirally transfected using the oncogenic H-RAS^{V12} gene. The primary fibroblasts developed a flattened, enlarged phenotype and overexpressed p53, p21, and $p16^{INK4a}$. Transfecting the primary fibroblasts with E1A was sufficient to rescue them from RAS-induced G_1 arrest [10]. It was later shown that both constitutive MEK/MAPK signals were essential to trigger this premature senescence [11, 12]. Recent reports have suggested that expression levels of RAS can determine the outcome of senescence. Expression of RAS from the endogenous promoter did not induce senescence, but overexpression led to low-grade tumors with enhanced senescence [13••].

The Hayflick limit can be overcome by immortalizing cells with introduction of foreign DNA, including but not restricted to specific oncogenes [8]. Cancer cells have attained mechanisms to avoid telomere shortening, including telomerase overexpression and alternative lengthening of telomeres (ALT) [14]. Recent studies have suggested that tumors with induced oncogenic RAS levels are suspended at a low grade until mutations in other genes such as CDKN2A or p53 [13••].

Mutations in RAS Family in Colorectal Cancers

KRAS Gene Mutations

RAS signaling pathway mutations are common in colorectal cancer. However, among several members of this pathway, KRAS and B-RAF seem to be mutated in more than 60% of such cancers [15].

The c-KRAS (Kirsten Rat Sarcoma cellular) gene maps to chromosome 12p12.1 in the human genome. It is the cellular analog of the oncogenic KRAS gene, which is represented as v-KRAS (Kirsten Rat Sarcoma viral homolog) [16]. v-KRAS was subsequently shown to possess the ability to transform mouse fibroblasts cells (NIH3T3) [17]. The v-KRAS gene was also isolated from cDNA clones in human libraries and identified as two isoforms, KRAS1 and KRAS2 [18]. KRAS2 was also identified in a human colorectal carcinoma cell line, SW480, and subsequently several other cancer cell lines [19]. The KRAS1 gene was found to be a pseudogene, which was derived from KRAS2 mRNA processing. KRAS2 was initially reported to contain four exons, with the fourth exon having two forms: KRAS4A and KRAS4B [20]. The gene has since then been updated and found to contain six exons, with exon 5 alternatively spliced to generate two splice variants, KRASA and KRASB [21]. Targeted homozygous deletion of the mouse KRAS gene resulted in embryonic lethality between E12.5 and term [22]. In contrast, homozygous deletions in mouse HRAS or NRAS genes did not result in any significant phenotypic or viability changes [23].

Mutations in the KRAS gene are the most common among the RAS mutations in cancer. Reddy et al. [24] and Taparowsky et al. [25] were among the first to describe codon 12-point mutation in the HRAS gene in bladder cancer cells. Similarly, codon 61 mutations in the HRAS gene were described in lung cancer cells [26]. Feig et al. [27] initially found that KRAS was mutated in tumor cells of a human patient with ovarian cancer but not in normal ovarian cells. It was subsequently found that similar mutations in codon 12 [28, 29] and codon 61 [29] were present in the KRAS gene. Another mutation that is prevalent among the RAS genes occurs in codon 13. This was first shown in the NRAS gene in leukemia [30]. Additional mutations in the KRAS gene have been described. Mutations in codons 14, 58, and 153 have been identified in patients with Noonan syndrome [31], characterized by short stature, short neck with webbing, cardiac defects, and hypertelorism. Among these mutations, changes in codons 14 and 58 showed hyperactive RAS phenotypes, including decreased GTPase activity and increased sensitivity to growth factors [31].

The most prevalent KRAS mutations in colorectal cancer occur at codons 12 and 13, with mutations in codon 61 occurring at a lower frequency. A clinical analysis in matched tumor and normal samples from 160 untreated patients examined KRAS mutational changes by sequencing [32]. KRAS mutations were observed in 46% of the total colorectal carcinomas, out of which 54% contained mutations in codon 12 and 42% in codon 13. The study classified codon 12 mutations as occurring in mucinous types of colorectal cancer while codon 13 mutations resulted in more aggressive tumors [32].

Epidermal growth factor receptor (EGFR) is an important molecule involved in cancer biology and therapy. It is a growth factor–dependent receptor tyrosine kinase that is involved in a host of critical cellular responses, including growth, survival, and proliferation [33, 34•]. Disorders in the EGFR gene are common in many types of cancers. Both increased EGFR expression and elevated gene copy numbers are seen in colorectal cancers [33]. EGFR-mediated signaling is mainly routed through two pathways: RAS-RAF and phosphatidylinositol 3-kinase (PI3K). The PI3K pathway involves AKT-mediated cellular responses [34•]. Several cancer therapies are based on EGFR-targeted antibodies in conjunction with radiation or chemotherapy. Recent clinical studies have suggested the importance of maintaining the normal (wild-type) KRAS status when treating patients with antibodies against EGFR [35, 36]. Yen et al. [36] reported reduced progression-free survival and overall survival in patients having an activating mutation in KRAS compared to those having wild-type or nonactivating mutations in KRAS. Arnado et al. [35] reported that a human EGFR-specific antibody, panitumumab, was more efficacious when in patients with wild-type KRAS tumors compared to those with activating KRAS mutations.

An accompanying review paper in this issue of *Current Colorectal Cancer Reports* provides excellent details on the advances made in the diagnosis and treatment of colorectal cancer. The authors have also presented an exhaustive analysis on current colon cancer prognostic and predictive biomarkers [37]. This review contains recent reports published on the prognostic status of KRAS and B-RAF mutations in CRC with relevance to drug treatment [37].

Mouse Models of KRAS-Mediated Colorectal Cancer

The first mouse model for transgenic KRAS activation was developed using a fusion gene approach. A mutated KRAS gene ($KRAS^{G12V}$; glycine to valine change in codon 12) was fused to the rat thyroglobulin promoter to direct expression in the thyroid gland of the transgenic mice [38]. Tissue-specific expression of mutated KRAS was confirmed using a chloramphenicol acetyl transferase (CAT) reporter gene. Upon expression of the mutated KRAS gene, the mice developed thyroid abnormalities or lesions around 12 months of age. However, the very low incidence of these thyroid lesions in addition to the long latency period suggested that KRAS mutations were capable but not sufficient to drive thyroid malignancies. However, the number of lesions was significantly enhanced when the animals were treated with an agonist to elevate hormonal secretion [38].

Expression of $KRAS^{G12V}$ (also referred to as $KRAS^{V12}$) in the gut epithelium did not result in any significant abnormalities [39]. Specifically, mice that expressed mutated KRAS from the intestinal fatty acid binding protein (Fabpi) gene promoter (which drives specific expression in the postmitotic enterocytes along the villus region of the small intestine) did not show differences in gut proliferation or differentiation. In contrast, significant intestinal dysplasia was observed when the SV40 T-antigen was co-expressed along with KRAS directed by the Fabpi promoter [39].

Targeted insertion of oncogenic $KRAS^{G12D}$ (glycine to aspartic acid substitution in codon 12) mutation was carried out using homologous recombination in mouse embryonic stem cells [40]. The mutant mice had decreased survival rates along with increased tumor burden, mainly in the lungs. The tumor burden and size steadily increased with age, eventually resulting in death due to respiratory distress. Other areas of tumorigenesis included thymus, skin, and kidney but not colon or pancreas. The authors of the study suggested that the differential tumor spectrum was potentially due to varying frequencies of recombination, sensitivity of tissues, or lack of effect on certain tissues [40].

Tissue-specific expression of exogenous genes has been achieved using the Cre-LoxP system. An earlier study expressed the SV40 large T-antigen gene specifically from the mouse αA-crystallin promoter that led to the formation of lens tumors [41]. Since then, several groups have had enormous success in recapitulating the Cre-LoxP system for specific expression in the intestinal epithelium. Three models have been widely cited, Fabpl-Cre, Villin-Cre, and Ah-Cre [1••].

The Fabpl-Cre mouse comprises of promoter elements of the rat liver fatty acid binding protein (Fabpl) gene preceding the Cre recombinase [42]. This mouse displayed small intestinal, colonic, bladder, and ureter epithelial Cre-recombinase expression beginning from embryonic day 13.5. The Villin-Cre mouse model was independently developed by two groups. Cre recombinase expression in intestinal epithelial cells was driven by the 9-kb regulatory region of the mouse villin gene [43]. Cre expression was turned on in the intestinal epithelial cells from embryonic 12.5 dpc (days post coitus). Recombinase expression was also located in the proximal kidney epithelial cells. Subsequently, another group developed a Villin-Cre mouse that was driven by the 12.4-kb mouse villin promoter [44]. This mouse was reported to recapitulate the endogenous expression of villin in the intestinal epithelial cells. Lastly, the cytochrome p4501A1 (CYP1A1) promoter element was used to control Cre expression in the AhCre transgenic model [45]. Cre recombinase expression from this mouse was detected in the intestine, liver, pancreas, gall bladder, and stomach. The cytochrome p450 promoter was normally transcriptionally silent but was induced in response to treatment with lipophilic compounds, such as β-napthoflavone [45]. Inducible tissue-specific Cre expression has also been developed using the other two previously discussed promoters. A reverse tetracycline-regulated transactivator (rtTA) was used to generate inducible Fabpl-Cre mice [42]. A tamoxifen-activated estrogen receptor (ER) driven inducible villin-Cre expression was developed [46]. All three models have been used to drive oncogenic KRAS expression in intestinal epithelial cells.

As discussed earlier, targeted oncogenic KRAS expression in mice did not display small intestinal or colonic adenomas [40]. These mice did show early signs of dysplasia, mainly in the form of aberrant crypt foci (ACF) [40]. A recent study observed a muted effect of adenomatous polyposis upon oncogenic KRAS overexpression targeted to the intestinal epithelium [47]. Villin-controlled expression of oncogenic $KRAS^{G12V}$ led to increased MAPK activity in the intestinal epithelial cells [47]. A majority of these mice developed intestinal lesions ranging from ACFs to adenomas. The lesions were few in number and the mice were able to survive over 9 months of age [47].

The Lox-Stop-Lox (LSL)-$KRAS^{G12D}$ conditional mutant strain has helped to create a more physiologically based approach for transgenic analysis [48]. In this mutant, the mutated KRAS gene is "knocked in" the wild-type KRAS allele but is silenced due to the presence of an upstream stop codon. In the presence of Cre recombinase, the stop codon is removed, resulting in activation of the mutated KRAS allele from the endogenous locus [48]. This approach has been used to express mutant KRAS protein in the intestinal and colonic epithelia using Fabp-Cre [49]. All of the mice that showed expression of the oncogene

displayed diffused hyperplasia and dysplasia visible from 4 weeks of age. These mice also showed increased proliferative cells in the normal epithelia, but no significant changes in the MAPK pathways were observed [49]. A subsequent study involved floxed KRAS mice crossed with the Villin-Cre mouse [50•]. These mice showed the capacity to develop spontaneous ACFs similar to the Fabp-Cre floxed KRAS mice [49]. The authors further delineated the ACFs formed in the proximal and distal colon. They report that the ACFs formed in the proximal colon have the capability to develop into adenomas upon azoxymethane (AOM) treatment, but this characteristic is deemed to be missing among ACFs formed in the distal colon [50•].

The same LSL-KRAS mouse was also employed using the AhCre model, in the intestinal epithelium [51]. β-Naphthoflavone treatment in the double transgenic mice led to increased expression of oncogenic KRAS in both the intestine and the liver. However, these mice displayed no change in proliferation or apoptosis levels and crypt heights. These mice also showed no major migration or differentiation defects [51].

RAF Gene Mutations and Mouse Models

RAF is a MAP kinase kinase kinase (MAP3K) protein that is phosphorylated and stimulated by activated RAS. Its main purpose is to act as a mediator in the activation of MEK1/2 proteins that are directly upstream of ERK. RAF kinase protein appears as isoforms A-RAF, B-RAF, and C-RAF (RAF-1). C-RAF has two separate phosphorylation sites that are activated by RAS upon recruitment to the cell surface. However, B-RAF phosphorylation appears to be constitutive and independent of RAS activation [52]. B-RAF is a stronger inducer of MEK phosphorylation when compared to A-RAF. A-RAF and C-RAF proteins were both reported to be weakly stimulated by oncogenic RAS as compared to oncogenic Src [52]. These two RAF isoforms require the presence of RAS-GTP on the cell membrane for their activation. In comparison, B-RAF protein only requires the stimulatory signals that activate RAS-GTP instead of the active complex [52].

Similar to RAS, RAF mutations are quite common in cancers, including malignant melanomas, colorectal cancer, and papillary thyroid cancers. It was previously found that mutations in the B-RAF gene were prevalent in several cancers including melanomas, colorectal cancers, and ovarian tumors [53, 54]. Out of these mutations, the T1796A mutation resulting in a valine to glutamic acid change at position 599 (now re-characterized as position 600; V600E) is the most common, occurring more than 80% of the time [53]. Tumors that exhibit V600E mutation in the RAF gene were mutually exclusive from those that display KRAS-activating mutations. Activation of RAF and its targeting for treatment in cancers has been elegantly compiled in two recent reviews [55, 56••]. Other RAF pathway mutations have been observed. A recent report identified over 30 mutations of B-RAF protein in human cancers, with 18 of them showing increased kinase activity [57]. Most of these mutations are clustered in two domains that interact with a C-RAF inhibitor, BAY43-9006. They also showed that four B-RAF mutants (G466E, G466V, G596R, and D594V) had lower activity than wild-type B-RAF [57].

Mouse models of B-RAF activation have been recently created and investigated [58••]. A transgenic mouse that stimulates B-RAF V600E mutation expression has been developed using the Lox-STOP-Lox system. This mouse has a stop codon inserted within the B-RAF gene flanked by two LoxP sites [59]. Upon Cre-recombinase activation, tissue-specific B-RAF V600E expression can be achieved. Widespread activation of mutated B-RAF expression was not tolerated. These mice were found to be embryonic lethal, dying at 7.5 dpc [59]. The authors used an Mx1-Cre mouse line to generate mutations. This promoter is induced by administration of interferons (α and β) or synthetic double-stranded RNA. Interferon-treated Mx1-Cre B-RAF V600E mice developed bone marrow failure and proliferative disorder, leading to death within 4 weeks of age. The mice also presented with evidence of histiocytic nonlymphoid neoplasia [59]. However, the authors reported that there was no change in cellular proliferation among somatic tissues. Another study reported the development of lung adenomas after intranasal administration of adenovirus-Cre [60]. These adenomas were significantly retarded upon pharmacological treatment with drugs that affect MAPK signaling. These adenomas did not progress to adenocarcinomas without further mutational events [60]. A recent study described a mouse model with B-RAF D594A mutation [61•]. This mutation does not have increased kinase activity but has the ability to activate C-RAF. The study reported the mice did not develop tumors but showed increased aneuploidy in the mouse splenocytes and embryonic fibroblasts [61•]. C-RAF defective mice have also been generated. The mice that contained a C-RAF D486A mutation showed decreased kinase activity and increased levels of apoptosis [62].

A study performed on RAF mutants in human cancers showed that three mutations (G466E, G466V, and G596R) induce the MAPK signaling cascade by activating C-RAF protein [63]. Several recent reports have emerged that have efficiently linked B-RAF and C-RAF activities in relation to oncogenic RAS [61•, 64, 65•]. Heidorn et al. [64] reported a model to support oncogenic RAS-mediated tumorigenesis in the presence of a kinase-dead B-RAF. Treatment with B-RAF inhibitors drove the attachment of B-RAF to C-RAF and subsequent activation of MAPK signaling. This binding of B-RAF and C-RAF took place only in the presence of oncogenic RAS and resulted in the formation of melanomas

[64]. Poulikakos et al. [65•] reported the inhibition of ERK signaling with RAF inhibitor treatment in tumors containing mutant B-RAF. Tumors that had wild-type B-RAF conversely displayed enhanced ERK signaling [65•]. The study reported that ERK activation occurred due to drug binding to the kinase domain of one protomer of RAF dimers. Drug treatment inhibited the kinase domain of one protomer in RAF dimers, leaving the other kinase domain free for downstream activation [65•]. Their model suggested that the enhancement of RAF dimerization by either wild-type RAF or oncogenic RAS led to increased activity in inhibitor-treated mutant B-RAF tumors.

Conclusions

Numerous genetic mutations are involved in CRC. Activating mutations in oncogenes are sufficient to elicit tumorigenic responses. The RAS oncogene is among the first oncogenes to be discovered over 40 years ago. RAS/RAF mutations prevail in over 25% of all tumors, 50% when considering CRC alone. Recent studies have elucidated the mechanisms involved in RAS/RAF activation both during normal cellular homeostasis and during tumorigenesis. Several mouse models of the activated RAS/RAF isoforms and pathway intermediates exist. These models have assisted in understanding the consequences of both amino acid substitution and deletion mutations of RAS/RAF that are present in clinical CRC. In the case of RAS and RAF mutations, most of the mutational changes do not necessarily result in tumorigenesis. Such mutations contribute to cancer by disruption and dysregulation of the normal physiological apparatus. Several recent reports have also suggested a strong cooperative role among RAS and RAF isoforms in regulating drug sensitivity. In conclusion, both animal models and targeted approaches based on RAS/RAF signaling pathways have helped improve clinical management of CRC.

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References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. •• Nandan MO, Yang VW. Genetic and chemical models of colorectal cancer in mice. Curr Colorectal Cancer Rep. 2010;6:51–9. *This review article presents a compilation of different mouse models related to human CRC. Special emphasis is placed on important genetic mutations in human CRC. In addition, chemical models of CRC in mice are also explored*.
2. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer. 2003;3:459–65.
3. Cox AD, Der CJ. Ras family signaling: therapeutic targeting. Cancer Biol Ther. 2002;1:599–606.
4. Mitin N, Rossman KL, Der CJ. Signaling interplay in Ras superfamily function. Curr Biol. 2005;15:R563–74.
5. •• Harris TJ, McCormick F. The molecular pathology of cancer. Nat Rev Clin Oncol. 2010;7:251–65. *The authors review the important clinical prognostic indicators of CRC with respect to genetic mutations. They also analyze the molecular changes in tumors that have helped in tailoring individualized therapies and treatments*.
6. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res. 1965;37:614–36.
7. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990;345:458–60.
8. Shay JW, Wright WE, Werbin H. Defining the molecular mechanisms of human cell immortalization. Biochim Biophys Acta. 1991;1072:1–7.
9. Newbold RF, Overell RW. Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-ras oncogene. Nature. 1983;304:648–51.
10. Serrano M, Lin AW, McCurrach ME, et al. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell. 1997;88:593–602.
11. Lin AW, Barradas M, Stone JC, et al. Premature senescence involving p53 and p16 is activated in response to constitutive MEK/MAPK mitogenic signaling. Genes Dev. 1998;12:3008–19.
12. Zhu J, Woods D, McMahon M, et al. Senescence of human fibroblasts induced by oncogenic Raf. Genes Dev. 1998;12:2997–3007.
13. •• Collado M, Serrano M. Senescence in tumours: evidence from mice and humans. Nat Rev Cancer. 2010;10:51–7. *This is an outstanding review of senescence and its association in tumors. They provide evidence for senescence-deactivated malignant transformation of tumors. They also suggest a role for senescence in future drug therapeutics*.
14. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell. 2007;130:223–33.
15. Forrester K, Almoguera C, Han K, et al. Detection of high incidence of K-ras oncogenes during human colon tumorigenesis. Nature. 1987;327:298–303.
16. Kirsten WH, Mayer LA. Morphologic responses to a murine erythroblastosis virus. J Natl Cancer Inst. 1967;39:311–35.
17. 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;79:3637–40.
18. Chang EH, Gonda MA, Ellis RW, et al. Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. Proc Natl Acad Sci USA. 1982;79:4848–52.
19. McCoy MS, Toole JJ, Cunningham JM, et al. Characterization of a human colon/lung carcinoma oncogene. Nature. 1983;302:79–81.
20. McGrath JP, Capon DJ, Smith DH, et al. Structure and organization of the human Ki-ras proto-oncogene and a related processed pseudogene. Nature. 1983;304:501–6.
21. Carta C, Pantaleoni F, Bocchinfuso G, et al. Germline missense mutations affecting KRAS Isoform B are associated with a severe Noonan syndrome phenotype. Am J Hum Genet. 2006;79:129–35.

22. Koera K, Nakamura K, Nakao K, et al. K-ras is essential for the development of the mouse embryo. Oncogene. 1997;15:1151–9.
23. Esteban LM, Vicario-Abejon C, Fernandez-Salguero P, et al. Targeted genomic disruption of H-ras and N-ras, individually or in combination, reveals the dispensability of both loci for mouse growth and development. Mol Cell Biol. 2001;21: 1444–52.
24. Reddy EP, Reynolds RK, Santos E, et al. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. Nature. 1982;300: 149–52.
25. Taparowsky E, Suard Y, Fasano O, et al. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. Nature. 1982;300:762–5.
26. Yuasa Y, Srivastava SK, Dunn CY, et al. Acquisition of transforming properties by alternative point mutations within c-bas/has human proto-oncogene. Nature. 1983;303:775–9.
27. Feig LA, Bast Jr RC, Knapp RC, et al. Somatic activation of rasK gene in a human ovarian carcinoma. Science. 1984;223:698–701.
28. Shimizu K, Goldfarb M, Suard Y, et al. Three human transforming genes are related to the viral ras oncogenes. Proc Natl Acad Sci USA. 1983;80:2112–6.
29. Winter E, Yamamoto F, Almoguera C, et al. A method to detect and characterize point mutations in transcribed genes: amplification and overexpression of the mutant c-Ki-ras allele in human tumor cells. Proc Natl Acad Sci USA. 1985;82:7575–9.
30. Hirai H, Kobayashi Y, Mano H, et al. A point mutation at codon 13 of the N-ras oncogene in myelodysplastic syndrome. Nature. 1987;327:430–2.
31. Schubbert S, Zenker M, Rowe SL, et al. Germline KRAS mutations cause Noonan syndrome. Nat Genet. 2006;38:331–6.
32. Bazan V, Migliavacca M, Zanna I, et al. Specific codon 13 K-ras mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 K-ras mutations are associated with mucinous histotype. Ann Oncol. 2002;13:1438–46.
33. Heinemann V, Stintzing S, Kirchner T, et al. Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. Cancer Treat Rev. 2009;35:262–71.
34. • Markman B, Javier Ramos F, Capdevila J, et al. EGFR and KRAS in colorectal cancer. Adv Clin Chem. 2010;51:71–119. *This is an excellent review outlining the interplay and relevance between EGFR and KRAS mutations in CRC. It presents studies that have delineated the resistance to EGFR treatment in CRCs with mutated KRAS*.
35. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26:1626–34.
36. Yen LC, Uen YH, Wu DC, et al. Activating KRAS mutations and overexpression of epidermal growth factor receptor as independent predictors in metastatic colorectal cancer patients treated with cetuximab. Ann Surg. 2010;251:254–60.
37. Lee JK, Chan AT. Molecular prognostic and predictive markers in colorectal cancer: current status. Curr Colorectal Cancer Rep. 2011, in press
38. Santelli G, de Franciscis V, Portella G, et al. Production of transgenic mice expressing the Ki-ras oncogene under the control of a thyroglobulin promoter. Cancer Res. 1993;53:5523–7.
39. Kim SH, Roth KA, Coopersmith CM, et al. Expression of wild-type and mutant simian virus 40 large tumor antigens in villus-associated enterocytes of transgenic mice. Proc Natl Acad Sci USA. 1994;91:6914–8.
40. Johnson L, Mercer K, Greenbaum D, et al. Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. Nature. 2001;410:1111–6.
41. Lakso M, Sauer B, Mosinger Jr B, et al. Targeted oncogene activation by site-specific recombination in transgenic mice. Proc Natl Acad Sci USA. 1992;89:6232–6.
42. Saam JR, Gordon JI. Inducible gene knockouts in the small intestinal and colonic epithelium. J Biol Chem. 1999;274:38071–82.
43. Pinto D, Robine S, Jaisser F, et al. Regulatory sequences of the mouse villin gene that efficiently drive transgenic expression in immature and differentiated epithelial cells of small and large intestines. J Biol Chem. 1999;274:6476–82.
44. Madison BB, Dunbar L, Qiao XT, et al. Cis elements of the villin gene control expression in restricted domains of the vertical (crypt) and horizontal (duodenum, cecum) axes of the intestine. J Biol Chem. 2002;277:33275–83.
45. Ireland H, Kemp R, Houghton C, et al. Inducible Cre-mediated control of gene expression in the murine gastrointestinal tract: effect of loss of beta-catenin. Gastroenterology. 2004;126:1236–46.
46. el Marjou F, Janssen KP, Chang BH, et al. Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. Genesis. 2004;39:186–93.
47. Janssen KP, el-Marjou F, Pinto D, et al. Targeted expression of oncogenic K-ras in intestinal epithelium causes spontaneous tumorigenesis in mice. Gastroenterology. 2002;123:492–504.
48. Jackson EL, Willis N, Mercer K, et al. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev. 2001;15:3243–8.
49. Tuveson DA, Shaw AT, Willis NA, et al. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. Cancer Cell. 2004;5:375–87.
50. • Calcagno SR, Li S, Colon M, et al. Oncogenic K-ras promotes early carcinogenesis in the mouse proximal colon. Int J Cancer. 2008;122:2462–70. *This is an important study that presents evidence of ACFs and early carcinogenesis in mice that express oncogenic KRAS in the intestinal epithelium driven by a Villin promoter. They suggest that KRAS mutations provide initiating thrust to neoplastic events in CRCs*.
51. Sansom OJ, Meniel V, Wilkins JA, et al. Loss of Apc allows phenotypic manifestation of the transforming properties of an endogenous K-ras oncogene in vivo. Proc Natl Acad Sci USA. 2006;103:14122–7.
52. Marais R, Light Y, Paterson HF, et al. Differential regulation of Raf-1, A-Raf, and B-Raf by oncogenic ras and tyrosine kinases. J Biol Chem. 1997;272:4378–83.
53. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417:949–54.
54. Rajagopalan H, Bardelli A, Lengauer C, et al. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature. 2002;418:934.
55. Gollob JA, Wilhelm S, Carter C, et al. Role of Raf kinase in cancer: therapeutic potential of targeting the Raf/MEK/ERK signal transduction pathway. Semin Oncol. 2006;33:392–406.
56. •• Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene. 2007;26:3291–310. *The authors provide a thorough analysis of the MAPK pathway starting from Ras. They detail the different mutations prevalent in cancers within this pathway and their inhibitors used in research*.
57. Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004;116:855–67.
58. •• Karreth FA, Tuveson DA. Modelling oncogenic Ras/Raf signalling in the mouse. Curr Opin Genet Dev. 2009;19:4–11. *This excellent review provides detail on mouse models on the MAPK pathway and its mutations. The authors have given special emphasis to oncogenic RAS and RAF models*.
59. Mercer K, Giblett S, Green S, et al. Expression of endogenous oncogenic V600EB-raf induces proliferation and developmental

defects in mice and transformation of primary fibroblasts. Cancer Res. 2005;65:11493–500.
60. Dankort D, Filenova E, Collado M, et al. A new mouse model to explore the initiation, progression, and therapy of BRAFV600E-induced lung tumors. Genes Dev. 2007;21:379–84.
61. • Kamata T, Hussain J, Giblett S, et al. BRAF inactivation drives aneuploidy by deregulating CRAF. Cancer Res. 2010;70:8475–86. *This study relates mutation in B-RAF gene with the activation of C-RAF, along with MEK/ERK, and subsequently aneuploidy.*
62. Noble C, Mercer K, Hussain J, et al. CRAF autophosphorylation of serine 621 is required to prevent its proteasome-mediated degradation. Mol Cell. 2008;31:862–72.
63. Garnett MJ, Rana S, Paterson H, et al. Wild-type and mutant B-RAF activate C-RAF through distinct mechanisms involving heterodimerization. Mol Cell. 2005;20:963–9.
64. Heidorn SJ, Milagre C, Whittaker S, et al. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell. 2010;140:209–21.
65. • Poulikakos PI, Zhang C, Bollag G, et al. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. Nature. 2010;464:427–30. *This exceptional study reports the activation of one protomer of the RAF hetero/homodimers upon deactivation of the other protomer due to drug binding. They suggest a model for RAF/RAS activation in tumors in the presence of a RAF inhibitor.*