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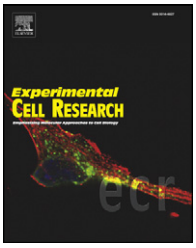

Review Article

Regulation of homeostasis and oncogenesis in the intestinal epithelium by Ras

Sergia Velho[a,b,c], *Kevin M. Haigis*[a,b,*]

[a]*Molecular Pathology Unit, Center for Cancer Research, and Center for Systems Biology, Massachusetts General Hospital, Charlestown, MA, USA*
[b]*Department of Pathology, Harvard Medical School, Boston, MA, USA*
[c]*Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal*

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ABSTRACT

Much of our current state of knowledge pertaining to the mechanisms controlling intestinal epithelial homeostasis derives from epidemiological, molecular genetic, cell biological, and biochemical studies of signaling pathways that are dysregulated during the process of colorectal tumorigenesis. Activating mutations in members of the RAS oncoprotein family play an important role in the progression of colorectal cancer (CRC) and, by extension, intestinal epithelial homeostasis. Mutations in K-RAS account for 90% of the RAS mutations found in CRC. As such, the study of RAS protein function in the intestinal epithelium is largely encompassed by the study of K-RAS function in CRC. In this review, we summarize the data available from genetically defined *in vitro* and *in vivo* models of CRC that aim to characterize the oncogenic properties of mutationally activated K-RAS. These studies paint a complex picture of a multi-functional oncoprotein that engages an array of downstream signaling pathways to influence cellular behaviors that are both pro- and anti-tumorigenic. While the complexity of K-RAS biology has thus far prevented a comprehensive understanding of its oncogenic properties, the work to date lays a foundation for the development of new therapeutic strategies to treat K-RAS mutant CRC.

Contents

* *Corresponding author at:* Molecular Pathology Unit, Massachusetts General Hospital, 149 13th Street, Charlestown, MA 02129, USA.
E-mail address: khaigis@partners.org (K.M. Haigis).

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Introduction

Among the vast array of mutations present in cancers, activating mutations in the RAS family of small, monomeric GTPases (i.e., K-, N-, and H-RAS) are both common and widespread. K-RAS activation is most common, with an incidence of approximately 15% across all human tumor types [1]. RAS family members normally cycle between active GTP-bound and inactive GDP-bound states and function at cellular membranes to transmit signals originating from extracellular stimuli to influence cell growth, proliferation, differentiation, and survival [2]. Oncogenic missense mutations at codons 12, 13, 61, and 146 strongly attenuate GTPase activity and cause RAS to accumulate in the GTP-bound state, resulting in sustained activation of downstream signaling pathways [3]. The downstream "effector" pathways engaged by GTP-bound RAS are numerous and include both phosphorylation cascades (*e.g.* RAF → MEK → ERK, PI3K → AKT, PLCε → PKC) and secondary GTPase pathways (*e.g.* RAC and RAL) [4]. In theory, these effectors constitute potential therapeutic targets for RAS-mutant cancers, yet activation of individual pathways is context-dependent, making it difficult to predict which would be an effective target in a given cancer.

In the context of colorectal cancer (CRC), K-RAS mutations far outnumber those in N-RAS and H-RAS, arising in approximately 40% of cases [5,6]. N-RAS mutations occur in 3–5% of cases and H–RAS mutations have not been identified [6–8]. Interestingly, while codon 12/13 mutations account for the majority of activating mutations in K-RAS (G12D is the most common mutation in CRC), weak activating mutations at codon 146 significantly outnumber strong activating mutations at codon 61 [8]. Moreover, codon 146 mutations appear to be specific for CRC, as they are not found in other cancers that commonly express mutant K-RAS (*e.g.* non-small cell lung cancer) [9]. While molecular and pathologic characterization of pre-neoplastic and neoplastic colonic lesions confirms that mutant K-RAS plays an early and broad oncogenic role in the process of colorectal carcinogenesis [6,10–12], epidemiological data provides a more sinister view of K-RAS with respect to CRC outcomes. For example, multiple studies have identified a connection between K-RAS mutation and poor clinical outcome [8,13]. More recently, K-RAS activating mutations have been found to be strongly predictive for the failure of colorectal cancers to respond to therapies targeting the Epidermal Growth Factor Receptor (EGFR) [14–19].

Epidemiological studies underscore the importance of K-RAS mutations in the progression and treatment of CRC, and therefore the urgent need for therapies targeting the pathway, but their retrospective nature makes them largely uninformative at the mechanistic level. In this review, we survey data from *in vitro* and *in vivo* model systems that provide insight into the mechanisms underlying the contribution of mutant K-RAS to colorectal cancer.

Oncogenic properties of K-RAS in CRC cell lines

Much of what we know about the oncogenic properties of mutationally activated RAS is derived from *in vitro* transformation assays, which measure an oncogene's ability to influence cellular properties that are integral to tumorigenesis, for example replicative potential, clonogenic survival, or anchorage-independent growth. A large body of work establishes the pro-tumorigenic properties of activated RAS, but the development of a unifying model of RAS function has been hindered by the fact that the phenotypes associated with oncogenic RAS are highly dependent upon which family member is mutated, the level of expression of the mutant protein, and the cellular context in which the oncoprotein is expressed. For K-RAS in particular, over-expression studies are complicated by the fact that the endogenous gene is alternatively spliced to produce two distinct proteins, K-RAS4A and K-RAS4B, which differ only in their extreme C-termini. Endogenous activating point mutations affect both splice forms, but over-expression studies (both *in vitro* and *in vivo*) typically measure the transforming activity of a single K-RAS isoform. As a result of these caveats, the molecular mechanisms underlying the contribution of mutationally activated K-RAS to CRC progression are poorly characterized.

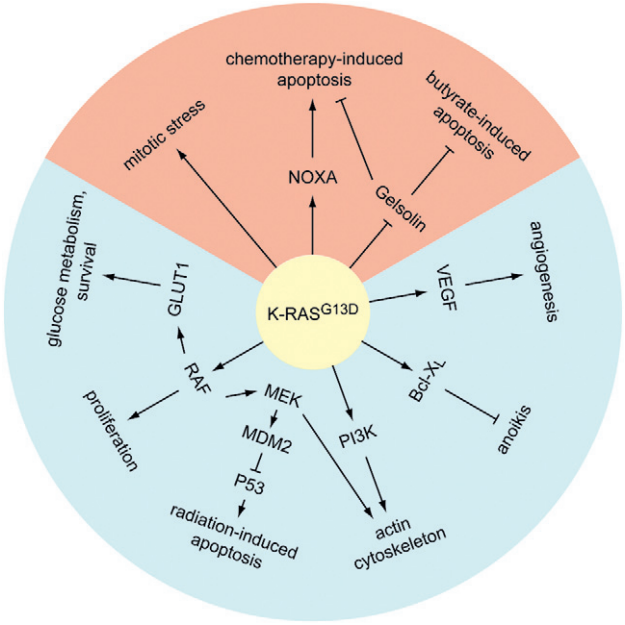

Fig. 1 – Properties of mutant K-RAS revealed by analysis of isogenic human CRC cell line pairs. Oncogenic K-RAS engages both pro-tumorigenic pathways (shaded in blue) and, paradoxically, anti-tumorigenic pathways (shaded in red). Arrows represent both direct and indirect interactions.

One powerful approach that has been utilized to characterize the contribution of oncogenic K-RAS to CRC utilizes somatic cell gene targeting to remove the endogenous mutant allele of *KRAS* from CRC cell lines by homologous recombination, creating a derivative cell line that is essentially isogenic with the exception of the K-RAS mutational status [20]. While this system has limitations (*e.g.* the derivative cell lines carry one wild-type and one null allele of *KRAS*), its major advantage in comparison to RAS over-expression strategies is that it allows for the study of endogenous mutations affecting both K-RAS4A and 4B in the cell type relevant for CRC. A summary of the phenotypes associated with mutant K-RAS, as revealed by the use of isogenic pairs, is shown in Fig. 1.

Deletion of the mutant *KRAS* allele from HCT-116 and DLD-1 CRC cells, which both harbor a single endogenous G13D activating mutation, results in a morphologic change, a decrease in proliferation, abrogation of anchorage-independent growth, and a complete loss of *in vivo* tumorigenic potential [20]. The signaling pathways connecting K-RAS^{G13D} to each of these phenotypes have been studied in more detail. The ability of mutant K-RAS to affect cellular morphology may