PERSPECTIVE

Lung premalignancy induced by mutant B-Raf, what is thy fate? To senesce or not to senesce, that is the question

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For more than 25 years, the Ras proteins have been widely accepted as central players in the malignant transformation of a variety of different tumor types. The ability of mutationally activated Ras to provide a powerful oncogenic stimulus lies in its capacity to signal through a multitude of downstream effector pathways that regulate a diverse set of cellular processes (for a recent review, see Downward 2006). It is only in the past several years that activating mutations in these Ras effector pathways have also been identified in human cancers. For example, mutations in *PIK3CA* are common in colon, gastric, and brain cancers (Samuels et al. 2004), while *BRAF* mutations occur at high frequency in melanomas, colon cancers, and ovarian cancers (Davies et al. 2002). In this issue of *Genes & Development*, Dankort et al. (2007) provide the first formal demonstration that mutationally activated B-Raf can act as the initiating event for lung tumorigenesis, further establishing B-Raf as a bona fide oncoprotein. But unlike the story with conventional oncogenes, like Ras, the B-Raf story has an interesting twist.

Activating mutations in B-Raf are found in 3% of non-small-cell lung cancers (NSCLC) (Brose et al. 2002; Davies et al. 2002; Naoki et al. 2002). Dankort et al. (2007) have used gene targeting to create the first mouse model of B-Raf-induced lung tumorigenesis. In the absence of Cre recombinase, the engineered *Braf* allele (*Braf*^{CA}, for Cre-activated) expresses a chimeric mRNA composed of exons 1–14 of the mouse gene and exons 15–18 of the wild-type *BRAF* gene from humans (Dankort et al. 2007). After Cre-mediated recombination, the human exons are removed and the mouse *Braf* is expressed in its entirety, including the V600E-activating mutation in exon 15 (Dankort et al. 2007). As with other mouse models of lung cancer, tumors were initiated in this new model by infecting the lung epithelium with Adenovirus carrying the gene for Cre recombinase (AdCre), which allows one to titrate the levels of Cre activity and also to temporally regulate Cre expression (Jackson et al. 2001). Importantly, the mutant form of B-Raf (B-Raf^{V600E}) is expressed from its endogenous locus in this model, mimicking what occurs in human cancers. Within 2–4 wk after infection with AdCre, lungs of B-Raf^{CA} mice exhibited hyperplastic epithelium that progressed to papillary adenomas within 6–8 wk (Dankort et al. 2007). A striking feature of the B-Raf-mutant lung tumors from these animals was that they failed to progress to carcinoma, and instead, exhibited growth arrest and senescence-like features. This senescence-like phenotype could be overcome through concomitant mutation of p53 or p16^{Ink4a}/p19^{Arf}, which allowed the tumors to progress to full-blown adenocarcinoma. Interestingly, B-Raf-induced senescence has also been observed in vivo in benign nevi, the precursor lesion to melanoma, although the role that p53 and p16^{Ink4a} play in this process is not completely clear (Michaloglou et al. 2005).

Several mouse models of NSCLC have been generated recently. Those relying on mutationally activated K-Ras as the initiating event most closely resemble the B-Raf model of Dankort et al. (2007). In two independent models, expression of the mutant form of K-Ras (K-Ras^{G12D} or K-Ras^{G12V}) from its endogenous promoter leads to the development of lung tumors that progress to full-blown adenocarcinoma (Jackson et al. 2001; Guerra et al. 2003), a stark contrast from the phenotype of expressing mutant B-Raf in the lung. Nevertheless, in their earliest stages of development, tumors initiated by K-Ras^{G12D} or B-Raf^{V600E} are similar in their histology and in their expression of markers for alveolar type 2 (AT2) cells. Thus, the early stages of tumorigenesis induced by B-Raf^{V600E} appear to phenocopy the early stages of K-Ras^{G12D}-induced tumorigenesis in the lung. Indeed, sequencing analysis of human tumors indicates that Ras and B-Raf mutations are mutually exclusive, suggesting that signaling through B-Raf is key to the oncogenic properties

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of mutationally activated Ras. Nevertheless, several lines of evidence point to differences between the B-Raf^{V600E} and K-Ras^{G12D} phenotypes. For example, while B-Raf^{V600E} adenomas undergo growth arrest and fail to progress to cancer, K-ras^{G12D} tumors routinely progress, and appear to do so in the absence of activation of the mitogen-activated protein kinase (MAPK) pathway (Jackson et al. 2001; Lee et al. 2002; Tuveson et al. 2004). In this Perspective, we focus on this important observation and address a major question that arises from the current study of Dankort et al. (2007): What is the mechanistic explanation for the phenotypic differences between lung tumors expressing mutationally activated K-Ras or B-Raf? Here we explore three possibilities (Fig. 1). First, do the phenotypic differences result from the ability of B-Raf^{V600E} to actively promote growth arrest? Conversely, do K-Ras^{G12D} and B-Raf^{V600E} target unique cell types within the lung epithelium that possess inherent differences in replicative potential? And finally, might K-Ras^{G12D} and not B-Raf^{V600E} differentially affect the ability of lung stem cells to maintain their self-renewal capability?

The active B-Raf hypothesis: negative feedback on PI3K signaling

A provocative new mechanism for Ras- and Raf-induced cellular senescence has recently been proposed. In this "revised model of oncogene-induced senescence," increased flux through the Raf → Mek → Erk signaling cascade (induced by suppressing Nf1 expression or by activating B-Raf) activates negative feedback signals to suppress the GTP-binding status of Ras (Fig. 1A; Courtois-Cox et al. 2006). This diminution of GTP-bound Ras leads to reduced flux through the PI3K → Akt signaling cascade and this, Courtois-Cox et al. (2006) argue, accounts for the senescence associated with loss of Nf1 function or mutational activation of B-Raf. Based on the observations of Dankort et al. (2007), both the up-regulation of p16 via MAPK and the up-regulation of p53 via PI3K attenuation would appear to be important for inducing senescence in tumors (Dankort et al. 2007). Such a tumor suppressor mechanism would not be expected to operate in cells expressing K-Ras^{G12D} for several reasons. First, mutant Ras is insensitive to feedback mechanisms

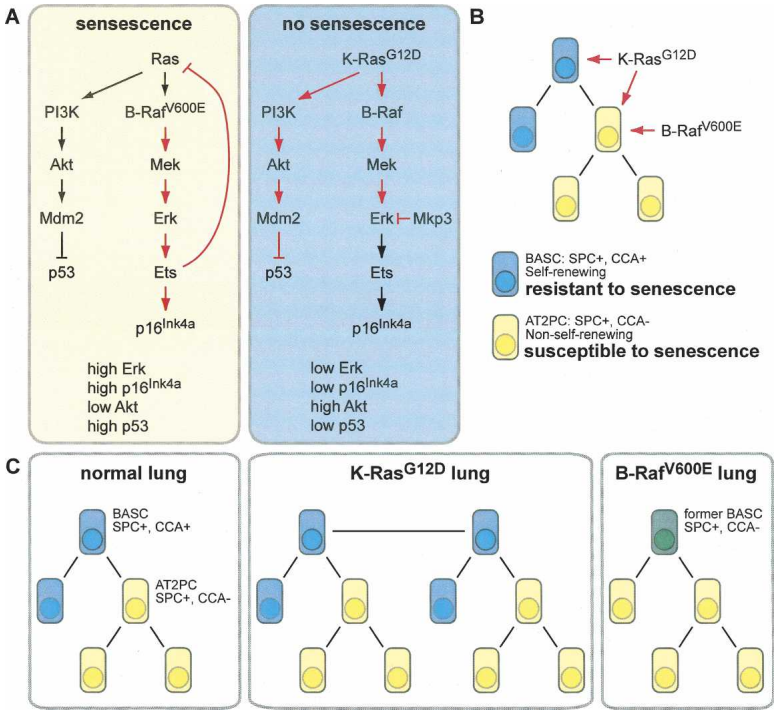

Figure 1. Models to account for the difference in malignant potential between K-Ras^{G12D} and B-Raf^{V600E} lung tumors. (*A*) In the first model, mutationally activated B-Raf actively promotes senescence by signaling through the canonical Raf → Mek → Erk pathway. The increased Erk signaling activates transcription factors, like Ets, to up-regulate molecules that promote senescence, like p16^{Ink4a}. In addition, the expression of molecules that negatively regulate Ras activity is increased, leading to a down-regulation of Ras activity and reduced flux through its downstream effector pathways, like PI3K → Akt. This attenuation of Akt activity leads to activation of p53, which also promotes senescence. This model was recently proposed (Courtois-Cox et al. 2006) and is consistent with the fact that eliminating p53 or p16^{Ink4a} in B-Raf-mutant tumors overcomes senescence (Dankort et al. 2007). This senescence mechanism would not operate in K-Ras^{G12D} tumors because of attenuated Erk signaling and insensitivity of mutant K-Ras to negative feedback. (*B*) In the second model, the senescence phenotype associated with mutant B-Raf derives from the cell of origin of the lung tumors. In normal lung, the CCA- and SPC-positive bronchioalveolar stem cell (BASC) can self-renew and also give rise to other, differentiated cell types (Kim et al. 2005). As in other tissues, a hierarchy of progenitor cells likely exists. We hypothesize the existence of a committed progenitor cell, termed the alveolar type 2 progenitor cell (AT2PC), which cannot self-renew, divides symmetrically, and has a defined replicative life span. Consequently, these AT2PC cells are inherently susceptible to senescence-like growth arrest. This model predicts that mutant K-Ras can target both the BASC and AT2PC to generate tumors that can progress to carcinoma (derived from the BASC) and tumors that undergo growth arrest (derived from the AT2PC). In contrast, mutant B-Raf targets only the AT2PC and produces tumors that undergo growth arrest unless p53 or p16^{Ink4a} is mutated. (*C*) The third model, a variation on the first two models, predicts that mutant K-Ras and B-Raf actively alter the kinetic properties of the tumor's cell of origin (i.e., the BASC). Homeostasis in the normal lung epithelium is maintained by the ability of the BASC to self-renew and to generate progenitors to the fully differentiated cell types of the lung. Mutant K-Ras, through as-yet-uncharacterized pathways, can promote the expansion and maintenance of BASCs, making it a potent lung oncoprotein (Kim et al. 2005). In contrast, B-Raf may be incompatible with a BASC-like state. Thus, even if B-Raf becomes activated in a BASC, this mutant cell (represented in green) may lose its ability to self-renew.

such as the up-regulation of GTPase-activating proteins (GAPs) or the down-regulation of guanine nucleotide exchange factors (GEFs) (Fig. 1A; Bos 1989). Second, lung tumors expressing K-Ras^{G12D} have attenuated MAPK signaling, probably due to the up-regulation of Erk-specific phosphatases such as Mkp3 (Jackson et al. 2001; Lee et al. 2002; Sweet-Cordero et al. 2005). Thus, tumors expressing B-Raf^{V600E} would be predicted to undergo senescence via this mechanism, while those expressing K-Ras^{G12D} would not. Whether this mechanism is operating in the context of an autochthonous lung tumor will require further analysis and tackling of several central questions. For example, what are the relative levels of activation of the PI3K and MAPK pathways in B-Raf-mutant lung tumors versus K-Ras-mutant lung tumors? Moreover, could tumors expressing K-Ras^{G12D} be forced into the senescence pathway by treatment with PI3K inhibitors and would this be overcome by mutating p53 or Ink4a/Arf? And alternately, could activation of the PI3K pathways, perhaps by mutating PTEN, overcome senescence in B-Raf^{V600E} tumors in the same way as does loss of p53 or Ink4a/Arf?

Although this new model of oncogene-induced senescence provides a somewhat satisfying explanation for the difference in malignant potential between B-Raf and K-Ras mutant lung tumors, there remain several confounding issues. For example, B-Raf-mutant lung tumor cells grow for 15–20 cell divisions before undergoing senescence-like growth arrest. This effect is similar to what is seen in benign human nevi and differs markedly from the effect of expressing mutant B-Raf in cultured fibroblasts, which senesce within days of oncogene expression (Mercer et al. 2005; Michaloglou et al. 2005). In addition, the McMahon group had demonstrated previously that activation of PI3K could overcome B-Raf-induced senescence (Mirza et al. 2004). Moreover, in many different cell types (e.g., MEFs and human colorectal cancer cells), mutationally activated K-Ras does suppress PI3K/Akt signaling, but does not induce senescence (Tuveson et al. 2004; Pollock et al. 2005). Does mutant K-Ras activate PI3K- and MAPK-independent pathways that suppress senescence? Alternatively, are the pathways regulating the senescence program intrinsic to certain cell types, but not to others? Indeed, while the study of Courtois-Cox et al. (2006) was performed predominantly in primary human fibroblasts, which underwent senescence in response to loss of Nf1 or activation of B-Raf, neither activation of B-Raf nor loss of Nf1 induces senescence in mouse embryonic fibroblasts (MEFs) (Tuveson et al. 2004; Mercer et al. 2005).

The effector cell hypothesis

A putative epithelial stem cell referred to as the bronchioalveolar stem cell (BASC) has been postulated as the tumor cell of origin for the K-Ras^{G12D} lung tumor model (Kim et al. 2005). The hallmark of BASCs is their concomitant staining both for markers of AT2 cells, like Surfactant Protein C (SPC), and markers of Clara cells, such as Clara Cell Antigen (CCA). BASCs reside at the junction of the bronchiolar and alveolar duct structures, and they probably expand outward toward the alveolar structures as they develop into atypical adenomatous hyperplasias (AAH), the precursors to lung adenomas and adenocarcinomas in K-Ras^{G12D} mice (Kim et al. 2005). Although the phenotypic similarities in the early lesions induced by K-Ras^{G12D} and B-Raf^{V600E} suggest a common cell of origin, phenotypic differences later in tumor development leave open the possibility that these unique tumor types have different cells of origin (Fig. 1B). That cell of origin can have a major effect on tumor phenotype has been known for some time. In a seminal study by Brown and Balmain (Brown et al. 1998), the malignant potential of H-Ras-induced skin tumors was shown to depend upon the cell type in which the mutant allele of H-Ras was expressed. Is it possible that the B-Raf-mutant tumors derive from a different type of progenitor cell not yet identified? If so, is the limited growth potential of tumors expressing B-Raf^{V600E} a reflection of proliferative properties intrinsic to these cells? Indeed, although B-Raf is believed to operate directly downstream from K-Ras in MAPK signaling, it is not known whether K-Ras and B-Raf are simultaneously expressed in the same cell types in the lung epithelium in vivo.

Adenocarcinomas that arise after activation of K-Ras most likely originate from the BASCs in the terminal bronchiole, and these are cells that have been shown to possess self-renewal capability (Kim et al. 2005). It is conceivable that BASCs expressing K-Ras^{G12D} become tumor stem cells and that their ability to self renew prevents the early lesions as a whole from undergoing growth arrest. Nevertheless, there is some evidence to suggest that unique subsets of tumors are initiated by K-Ras^{G12D}; when individual tumors in a given mouse are followed over time via noninvasive imaging, some lesions continue to grow and develop into adenocarcinoma, while other undergo growth arrest and do not progress (Cody et al. 2005). The fact that certain K-Ras^{G12D} tumors experience growth arrest is, of course, reminiscent of tumors expressing B-Raf^{V600E}. Perhaps mutationally activated B-Raf exclusively targets symmetrically dividing progenitor cells (hypothetically termed alveolar type 2 progenitor cells [AT2PCs]) (Fig. 1B) and these cells have a defined replicative life span (e.g., 15–20 cell divisions). Current evidence points to the existence of multiple stem cell niches within the lung epithelium; however, little is known about the similarities and differences between the progenitor cells within these niches (Otto 2002). In the intestine, for example, a three-tiered hierarchy of progenitor cells exists, and each subclass of progenitor cells has unique growth properties and regenerative capabilities (Potten 1998). Further characterization of progenitor populations throughout the lung is required to fully investigate whether unique cell types can serve as the effector cell for lung tumorigenesis. Importantly, a better understanding of these unique niches may allow for the mutational activation of K-Ras or B-Raf specifically within subsets of progenitor cells.

The active K-Ras hypothesis: maintenance of stem cell properties

An alternative to the effector cell hypothesis is that the same stem cells (i.e., BASCs) are the target for both B-Raf^{V600E} and K-Ras^{G12D} mutations, but that these distinct genetic changes differ in their abilities to maintain the self-renewal capabilities of the stem cell population (Fig. 1C). Kim et al. (2005) demonstrated that mutation of K-Ras led to an almost immediate expansion of BASCs within the bronchioalveolar junction and that BASC-like cells persisted in fully developed adenomas and adenocarcinomas. Clearly, expression of K-Ras^{G12D} is compatible with the maintenance of stem cell properties, such as self-renewal. Perhaps B-Raf^{V600E} does not promote the expansion of the BASC population and rather promotes differentiation into committed progenitor cells with no self-renewal capability and a defined replicative potential (i.e., AT2PCs). The ability of K-Ras^{G12D} to suppress differentiation and maintain stem-like properties of progenitor cells may be a tissue-specific phenomenon and may account for the ability of mutant K-Ras to act as the initiating event in some tumor types (e.g., lung) but not in others (i.e., colon). Again, a more complete understanding of the stem cell niches and hierarchies within the lung epithelium is needed in order to address this hypothesis.

Importance for clinical therapy

In humans, BRAF mutations occur in cancers associated with significant mortality. Dankort et al. (2007) demonstrated that inhibition of the Mek kinase can prevent B-Raf^{V600E} tumors from ever developing, providing strong evidence that B-Raf is signaling through Mek to promote tumorigenesis. In the Dankort et al. (2007) study, however, it was not determined whether inhibition of Mek could promote the regression of an established tumor or whether tumors mutant for both B-Raf and p53 or p16^{Ink4a}/p19^{Arf} remained sensitive. Other studies have demonstrated, however, that B-Raf-mutant human colon cancer cells are sensitive to Mek inhibition even when p53 is mutated (Sebolt-Leopold et al. 1999; Gayet et al. 2001).

Although the study by Dankort et al. (2007) is the first to demonstrate sensitivity of autochthonous mouse tumors to Mek inhibition, B-Raf mutant cells are generally sensitive to Mek inhibitors in vitro and in xenografts, while cancer cells expressing activated Ras are generally resistant (Sebolt-Leopold et al. 1999; Collisson et al. 2003; Solit et al. 2006). For example, Solit et al. (2006) demonstrated that a panel of melanoma cell lines mutant for B-Raf were invariably growth arrested by exposure to Mek inhibitor, while a companion set of melanoma cell lines mutant for N-Ras did not respond to the drug. A first-generation Mek inhibitor, CI-1040 (the predecessor to PD0325901 used in the study by Dankort et al. [2007]), did poorly in Phase II clinical trials (Rinehart et al. 2004). A review of the patients included in this study reveals that tumors with B-Raf mutations may have been poorly represented—only 20 patients with colon cancer and 18 with NSCLC were included (Rinehart et al. 2004). Nevertheless, previous studies of cultured human cells and the present study of murine lung tumors suggest that Mek inhibitors may be very useful for a subset of cancer patients, specifically those with B-Raf-mutant tumors. That a targeted therapeutic would be efficacious for only a small subset of patients is reminiscent of the recent finding that Egfr inhibitors, like Iressa, have beneficial effects for only certain patients with NSCLC, specifically those with Egfr kinase domain mutations (Lynch et al. 2004; Paez et al. 2004).

There are two important points to highlight with respect to the sensitivity of B-Raf-mutant tumors to inhibition of Mek. First, this is a clear case of where molecular profiling of tumors could be used to guide the clinical care a cancer patient receives. Moreover, the molecular profiling for B-Raf mutations would be relatively facile because the V600E mutation is prominent, accounting for at least 80% of all B-Raf mutations (Davies et al. 2002). Second, even though B-Raf mutations are rare compared with K-Ras mutations, therapies affecting B-Raf-mutant tumors would have a significant impact for a large number of people—5000 lung cancer patients, 38,000 melanoma patients, and 22,000 colon cancer patients per year in the United States.

The future of therapeutic testing in mouse models

The future of the mouse as an experiment platform on which to test novel therapeutics for lung cancer will require the generation of animals expressing all of the combinations of mutant alleles known to exist in human lung cancers. To date, several of the common genetic events in lung cancer have been successfully modeled in the mouse: mutations of K-Ras and B-Raf alone and in combination with p53/p16^{Ink4a} (Jackson et al. 2001, 2005; Guerra et al. 2003; Dankort et al. 2007), overexpression of mutant Egfr (Ji et al. 2006; Politi et al. 2006), and inactivation of p53 together with Rb (Meuwissen et al. 2003). It is now time to better characterize the ability of these lung cancer models to recapitulate subsets of human disease, similar to what other investigators have done with models for breast (Green et al. 2004) and prostate cancer (Ellwood-Yen et al. 2003), by performing global gene expression and DNA copy-number profiling, and analyzing the sensitivity of mice to commonly used chemotherapeutic agents and specific kinase inhibitors. Work in this realm has begun for the K-Ras^{G12D} model (Sweet-Cordero et al. 2005, 2006) and will continue for the other models over the next several years. Once completed, these studies would provide a basis for proceeding with large-scale efforts to screen compounds for activity in the context of specific genetic mutations.

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