

IN THE SPOTLIGHT

RAF/MEK Dependence of KRAS-Mutant Pancreatic Ductal Adenocarcinomas

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Summary: Studies using genetically engineered mouse models indicate that RAF activation is sufficient to induce pancreatic intraepithelial neoplasms, suggesting that mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitor-based combination approaches may have clinical use in patients with pancreatic ductal adenocarcinomas. *Cancer Discov*; 2(8); 666-9. ©2012 AACR.

Commentary on Collisson et al., p. 685 (1).

Thirty years have passed since missense mutations in RAS were first identified as the transforming factors in the Harvey and Kirsten strains of the mouse sarcoma virus. Somatic mutations of the 3 RAS genes have since been shown to be among the most prevalent somatic alterations in human cancer. Studies using genetically engineered mouse models (GEMM) of pancreatic and lung cancer, among others, have confirmed that mutant RAS contributes to cancer initiation and maintenance of the transformed phenotype even in the setting of established, metastatic disease. These results have prompted intensive academic- and industry-led efforts to identify direct inhibitors of oncogenic RAS. These efforts have failed to date likely due to the high affinity of the RAS-GTP interaction, as have efforts to selectively inhibit the posttranslational modifications required for RAS activation. The latter approach was ineffective in KRAS- and NRAS-mutant tumors as geranylgeranyl modification can substitute for farnesylation in targeting KRAS and NRAS to the plasma membrane.

An alternate approach is to target the effector pathways responsible for RAS-mediated transformation. Biochemical studies have identified more than 20 distinct RAS effector molecules, the best characterized of which include the RAF proteins, the phosphatidylinositol 3-kinases (PI3K), and the RAL exchange factors. Our understanding of the contribution of individual RAS effectors to transformation remains incomplete, but is likely influenced by the spatial/temporal availability of effectors, the presence or absence of extracellular stimuli, and the pattern of coincident muta-

tional events. In this issue of *Cancer Discovery*, Collisson and colleagues (1) set out to investigate which of the various RAS effectors are required for tumor initiation and progression in pancreatic ductal adenocarcinoma (PDA). An in-depth focus on PDA is justified by the high rate of KRAS mutation in this disease (>90%) and the urgent need to develop effective therapies for this common and almost universally lethal cancer.

Prior studies using GEMMs have shown that expression of mutant *Kras* leads to the formation of multifocal pancreatic intraepithelial neoplasms (PanIN; ref. 2). Furthermore, coincident loss of *Ink4a/Arf* or *Trp53* function results in the development of invasive pancreatic adenocarcinomas that phenocopy the human disease (3, 4). To determine whether RAF activation is sufficient to initiate pancreatic tumor formation, the authors generated mice with constitutive or conditional expression of *Braf*^{V600E} in pancreatic cells. The V600E mutation accounts for more than 90% of the BRAF mutations found in human tumors and locks the kinase into a constitutively active conformation. In the *Braf*^{CA} mouse model generated by Dankort and colleagues (5), the *Braf*^{CA} allele contains an insert that includes a floxed cassette containing exons 15 to 18 of human wild-type *Braf* cDNA upstream of a modified exon 15, which harbors the V600E mutation. The wild-type *Braf* allele is expressed before Cre-mediated recombination, but upon expression of Cre, the wild-type exon 15 to 18 insert is excised, and the expression of *Braf*^{V600E} is initiated under the control of the endogenous *Braf* promoter. Targeted expression of *Braf*^{V600E} using this model in the mouse lung leads to the development of benign lung tumors that progress to adenocarcinoma in the setting of concomitant loss of *Trp53* or *Ink4A/Arf* (5). Similarly, conditional melanocyte-specific expression of *Braf*^{V600E} in mice using this model results in benign melanocytic hyperplasia, which in the setting of coincident *Pten* loss progresses to invasive melanoma (6).

In the current study, Collisson and colleagues (1) express *Braf*^{V600E} in the mouse pancreas by crossing *Braf*^{CA} mice

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with mice that express Cre recombinase under the control of the *p48/Ptf1* gene (*p48^{Cre}; Braf^{CA/+}*), a pancreas-specific transcription factor expressed at embryonic day 9.5. None of the progeny survived past weaning, which indicates that expression of *Braf^{V600E}* is toxic for normal pancreatic development, a result which contrasts with the viability of *p48^{Cre}; Kras^{LSL-G12D}* mice. To bypass the embryonic lethality observed in the *p48^{Cre}; Braf^{CA/+}* model, the authors generated mice (*Pdx1::CreERT²; Braf^{CA/+}*) in which conditional expression of *Braf^{V600E}* is activated postnatally via tamoxifen-induced Cre recombinase activity driven by the *Pdx-1/Ipf1* promoter. In parallel, the authors used this system to generate mice expressing physiologic levels of activated *Kras^{G12D}* in the pancreas (*Pdx1::CreERT²; Kras^{LSL-G12D}*). Notably, constitutive expression of activated *Braf^{V600E}* in the adult pancreas phenocopied oncogenic activation of *Kras^{G12D}*, including the development of PanIN lesions lacking primary cilia in the exocrine pancreas, upregulation of the ductal marker cytokeratin 19, increased proliferation, and increased expression of nuclear phosphorylated extracellular signal-regulated kinase (ERK)1/2. Moreover, concomitant expression of mutant p53 (*Pdx1::CreERT²; Braf^{CA/+}; Trp53^{LSL-R270H/+}*) resulted in the development of PDA with abundant stroma and desmoplasia similar to that seen in *Kras/tp53*-mutant PDA mouse models and human PDA.

In contrast, mice expressing mutant PI3K- α in the mouse pancreas (*Pdx1::CreERT²; Pik3ca^{lat-H1047R}*) had no apparent phenotype. These results suggest that RAF but not PI3K activation is sufficient to induce PanIN development. Limitations of this study include the possibility that oncogenic RAS does not exclusively activate p110 α PI3K, and that the spectrum of downstream effectors activated by the kinase domain mutant may differ from that regulated by an activated wild-type allele. Furthermore, in light of recent findings that the class IB PI3K p110 γ isoform is overexpressed in PDA (7) and that deletion of *Pten* in mouse pancreatic centroacinar cells leads to ductal malignancy (8), the current data do not fully exclude the possibility that activation of PI3K signaling by other mechanisms may be sufficient to induce PanIN formation.

Overall, the results imply that inhibition of RAF signaling may be an effective therapeutic approach in patients with *KRAS*-mutant pancreatic tumors (Fig. 1). Highly selective RAF inhibitors were recently shown to prolong the survival of patients with *BRAF^{V600E}* melanoma. These agents, however, inhibit RAF activation in a mutant-selective manner and are thus ineffective in tumors that express activated RAS (9). Highly selective, allosteric inhibitors of mitogen-activated protein (MAP)/ERK kinase (MEK) have also shown promising activity in *BRAF*-mutant melanoma and provide an alternative approach to inhibiting ERK pathway activity in *KRAS*-mutant tumors (10). To determine whether MEK inhibitors could inhibit ERK signaling at a nontoxic dose, Collisson and colleagues (1) treated *Kras^{LSL-G12D}; Trp53^{LSL-R270H/+}; p48^{Cre}* mice with PD0325901, an allosteric inhibitor of MEK1 and MEK2. Treatment with PD032901 potentially downregulated ERK activity, as measured by a

decrease in phosphorylated ERK expression, indicating that sufficient intratumoral levels of the MEK inhibitor could be achieved at nontoxic doses to potently inhibit ERK pathway activation. This result is notable, as resistance of pancreatic tumors to systemic cytotoxic therapies has been attributed to limited drug exposure resulting from poor intratumoral perfusion. To determine whether sufficient ERK pathway inhibition could be maintained to induce meaningful antitumor effects, the authors turned to an orthotopic, syngeneic model of PDA. In this model, treatment with the MEK inhibitor was associated with downregulation of phosphorylated ERK expression and an improvement in survival. In sum, the results provide strong rationale for clinical trials of MEK inhibitors in patients with advanced pancreatic cancer but also highlight the logistical challenges associated with the use of GEMMs for preclinical drug development.

Recently, the MEK inhibitor trametinib (GSK1120212) was shown to improve survival as compared with chemotherapy in a randomized trial of patients with metastatic melanoma whose tumors harbored *BRAF^{V600E/K}* mutations (10). On the basis of these results, U.S. Food and Drug Administration approval for the use of trametinib in patients with *BRAF*-mutant melanoma is anticipated. Notably, 22 patients with pancreatic cancer were treated with trametinib within the context of the phase I trial of this agent (11). One patient achieved a partial response and several additional patients were noted to have minor responses or stable disease. Although these results are disappointing in light of the GEMM studies reported by Collisson and colleagues (1), they are consistent with studies of human cancer cell lines conducted by this group and others showing that in contrast to *BRAF*-mutant cell lines, which are with rare exception sensitive to MEK inhibition, *KRAS*-mutant cell lines exhibit variable sensitivity to MEK inhibitors. The basis for this heterogeneity of MEK dependence in *KRAS*-mutant cell lines has been explored in colorectal cancer cell lines and in this context can be attributed, in part, to the presence of *PIK3CA* co-mutation in some models (12). While *PIK3CA* mutations are rarely observed in pancreatic cancers, Collisson and colleagues (1) show that MEK inhibition in *KRAS*-mutant PDA cell lines is associated with a reciprocal increase in the expression of phosphorylated AKT and that cotreatment with a selective inhibitor of AKT is associated with synergy in many, but not all, models.

In sum, the results reported by Collisson and colleagues (1) in concert with the clinical experience to date indicate that despite the sufficiency of RAF activation for PanIN development, MEK inhibitor-based combination approaches will be needed to induce durable tumor regressions in most patients with *KRAS*-mutant PDA. Future laboratory studies will be needed to define the molecular basis for the variable response of *KRAS*-mutant PDA tumors to MEK inhibition, as such studies would aid in the development of rational MEK inhibitor-based combination strategies.

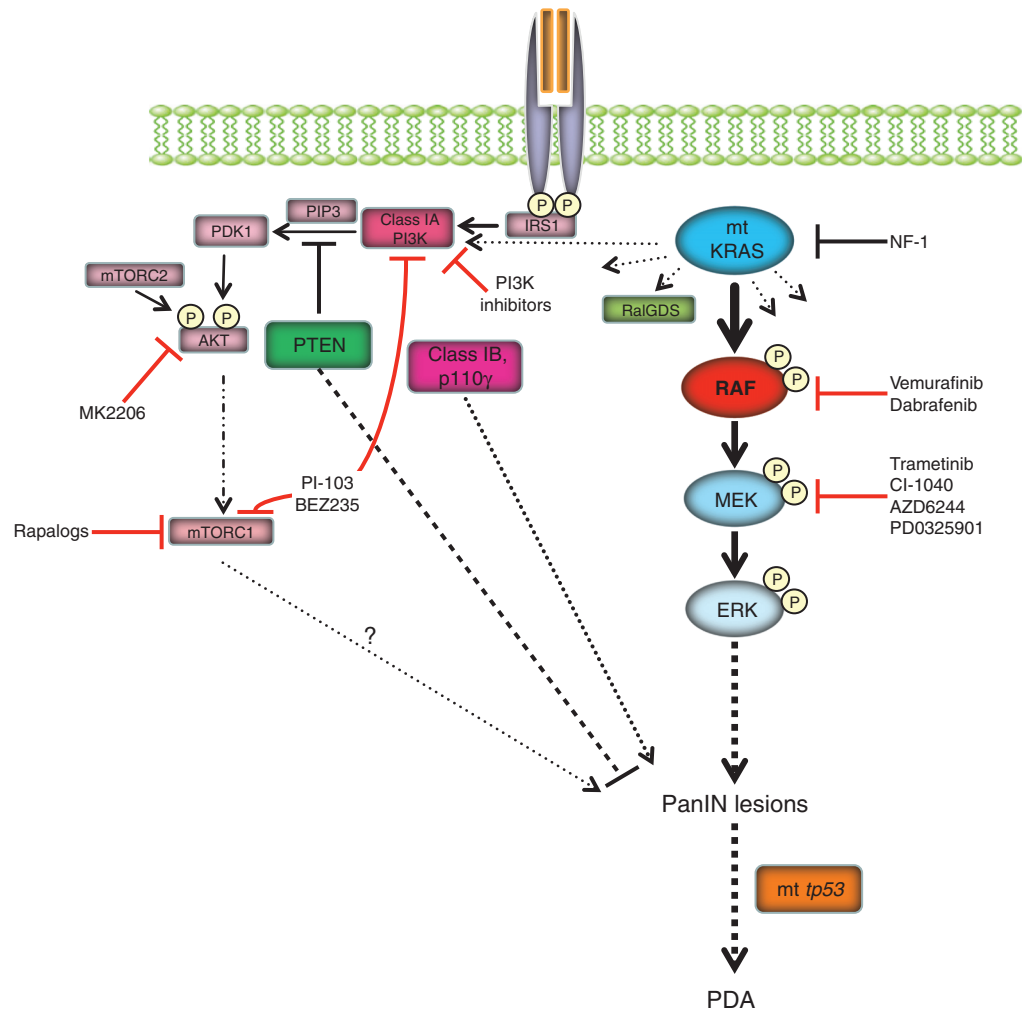


Figure 1. Targeting RAS effectors in PDA. Mutational activation of KRAS is found in more than 90% of PDA and contributes to tumor initiation and progression of the disease. PDA is almost universally fatal due to late stage at diagnosis and intrinsic resistance to conventional chemotherapy and radiation. Data from Collisson and colleagues (1) suggest that activation of BRAF (highlighted in red) is sufficient to recapitulate the tumor initiation and progression seen with activated KRAS in mouse and human PDA. These data imply that targeting RAF/MEK/ERK signaling downstream of KRAS may be of clinical use in PDA. Treatment with MEK inhibitors is associated with a reciprocal increase in AKT activity, and MEK inhibitor-based combinatorial approaches will be needed to induce durable tumor regressions. Selective inhibitors of RAF (vemurafinib, dabrafenib) and MEK (trametinib) have recently been shown to prolong survival in patients with *BRAF*^{V600E}-mutant melanomas. Selective inhibitors of RAF induce a paradoxical activation of ERK signaling in KRAS-mutant tumors whereas MEK inhibitors downregulate ERK pathway activity irrespective of tumor genotype.

Disclosure of Potential Conflicts of Interest

D.B. Solit is a consultant/advisory board member for Roche and GlaxoSmithKline. No potential conflicts of interest were disclosed by the other author.

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