The RAS proteins are small GTP-binding proteins that integrate extracellular signals and activate several downstream pathways to control cell proliferation, differentiation, and survival. There are three RAS genes in humans (HRAS, KRAS, NRAS) and gain-of-function mutations occur in these genes in 15% to 30% of human cancers. Despite this prevalence, oncogenic RAS has so far proven to be an intractable therapeutic target, and thus drug discovery efforts have largely focused on inhibiting the downstream pathways (1). The RAF-MEK-ERK pathway in particular has attracted a great deal of attention because RAF, MEK, and ERK are protein kinases that form a 3-tiered cascade that drives cell proliferation downstream of RAS (Fig. 1A), and furthermore, protein kinases are known to be tractable therapeutic targets. Both RAF and MEK inhibitors are currently in late-stage clinical development (2, 3). However, there are three RAFs (ARAF, BRAF, and CRAF), two MEKs (MEK1, MEK2), and two ERKs (ERK1, ERK2), and to date the role of these individual proteins in cancer cell signaling has not been fully explored.

To address the role of these individual proteins, Karreth et al. (ref. 4; in this issue of Cancer Discovery) and Blasco et al. (5) used Cre-recombinase/loxP technology to selectively delete the individual genes for components of this pathway in the lungs of mice bearing cancer driven by oncogenic Kras. Karreth et al. (4) show that Braf depletion did not affect tumor burden, whereas Craf depletion significantly reduced tumor formation. Blasco et al. (5) made similar observations: Braf depletion had little effect, whereas Craf depletion caused a significant reduction in tumor burden. Blasco et al. (5) went on to demonstrate that depletion of Mek1, Mek2, Erk1, or Erk2 alone did not affect tumor development, but when Mek1 and Mek2, or Erk1 and Erk2, were concomitantly deleted, as with Craf, there was again a significant reduction in tumor formation. Importantly, in both studies, any tumors that did arise in the Craf, Mek1/Mek2, or Erk1/Erk2 deleted mice were “escapers” in which the target gene(s) had not been deleted.

These data demonstrate that whereas Mek1 and Mek2, and Erk1 and Erk2, can compensate for each other’s loss (in this model at least), Braf is unable to compensate for the loss of Craf. This shows that Craf is responsible for transmitting signals from oncogenic Ras to Mek and that Braf is not required (Fig 1B). In agreement with this, Blasco et al. (5) demonstrate that cells expressing oncogenic Kras but lacking Craf do not display increased levels of apoptosis or senescence, suggesting that when Craf is lost, cells simply do not register the presence of the oncogenic Kras.

Taken together, these data show that in this lung model, signaling through Craf, Mek, and Erk is essential for tumor initiation by oncogenic Kras. It should be noted that these data fall a little short of validating Craf and the Mek/Erk pathway as a therapeutic target because oncogenic Kras was expressed at the same time as Craf, Mek1/2, or Erk1/2 were deleted. Thus, it is not possible to determine if the deleted proteins merely play a role in tumor initiation (in which case they are unlikely to be effective therapeutic targets) or...
if they are also required for tumor maintenance (necessary for therapeutic effect). However, we have previously established that oncogenic NRAS and KRAS signal exclusively through CRAF to MEK in human melanoma cell lines (6), and it has been shown that Crab is required for tumor maintenance in a mouse skin carcinogenesis model (7). Thus, although the new data do not fully validate Crab, overall the case for targeting this pathway in general and CRAF in particular—either alone or in combination with targeting other proteins/pathways—in RAS-mutant tumors is becoming increasingly compelling. Of course, the effectiveness of CRAF-selective drugs will need to be carefully judged. It has recently been shown that in the presence of oncogenic RAS, BRAF-selective and pan-RAF inhibitors drive paradoxical activation of the MEK/ERK pathway through induction of BRAF-CRAF and CRAF-CRAF dimers that then lead to CRAF hyperactivation (8–10). Clearly, it will be important to determine if CRAF-selective inhibitors also drive this response.

A neat twist in the Blasco et al. (5) study was the demonstration that global deletion of Mek1 and Mek2 or Erk1 and Erk2 in the animals led to death within a few weeks due to multiple organ failure. These data show that this pathway is essential for the general fitness of the animals. Of course, it is unlikely that ATP-competitive drugs will be as efficient as gene deletion at inhibiting individual kinases, and complete protein loss may have the same effects as its inhibition, but this observation does suggest that complete blockade of this pathway cannot be tolerated, which will need to be borne in mind in the clinical setting. In contrast to Mek1/2 and Erk1/2, concomitant deletion of Braf and Crab did not lead to death of the animals, suggesting that loss of these proteins does not result in complete blockade of the pathway and that other kinases must sustain Mek/Erk signaling sufficiently for survival. Araf is of course a clear candidate for this role, but other kinases that can phosphorylate Mek, such as Csk/Tpl2, may also perform this function. It is also possible that KSR (a distant relative of RAF that was originally thought to be a pseudokinase, but was recently shown to phosphorylate MEK) may also play a role (11).

The results presented by Karreth et al. (4) in this issue of Cancer Discovery and Blasco et al. (5) provide unequivocal evidence that Crab is responsible for coupling oncogenic Ras to Mek in vivo, at least in some settings (Fig. 1B). These data support the need for the development of CRAF-selective drugs, both as therapeutic agents and as research tools. They also raise some interesting questions. For example, why does oncogenic Ras not signal through Braf? Is this because Braf is not available, or because signaling by oncogenic Ras through Braf is incompatible with tumor progression? What is the role of Araf? And finally, can targeting Crab inhibit tumor growth in humans? These studies also serve as a timely reminder that even after all the years of study, we still appear to have much to learn about the subtleties of signaling through this cascade.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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New Insight Puts CRAF in Sight as a Therapeutic Target

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