described here opens new therapeutic possibilities for ATL treatment. Targeting Polycomb activity, restoring the tumor suppressor miR-31, or inhibiting NIK are all attractive potential strategies for eliminating ATL tumor cells. Furthermore, the described involvement of miR-31 in breast cancer cells (Valastyan et al., 2009) raises the question of whether miR-31 silencing through PRC2 occurs in other type of tumors and whether these pathways could be also be targets for molecular therapies in those malignancies. Finally, from a basic biology viewpoint, the novel mechanism described by Yamagishi et al. (2012) might have a wider role in normal cells, given the ubiquitous roles of NF-κB, Polycomb, and miRNAs in several tissues.

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How to Fool a Wonder Drug: Truncate and Dimerize

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In a recent paper, Poulikakos et al. describe a new and potentially common mechanism whereby melanomas develop resistance to the BRAF inhibitor vemurafenib by expressing truncated forms of BRAF(V600E) that can dimerize in the absence of activated RAS. Will it be possible to block this with improved BRAF inhibitor design?

Metastatic melanoma has long been renowned for being extremely difficult to treat effectively. However, the last few years have witnessed dramatic changes to the landscape of this disease. In 2002, it was discovered that over 50% of melanomas harbor activating mutations, most commonly V600E, in the gene encoding the protein kinase BRAF, which lead to constitutive activation of the RAF/MEK/ERK pro-proliferative signaling pathway (Davies et al., 2002). Within a few years, the first selective BRAF inhibitor was in clinical trials producing highly encouraging results. In a phase I clinical trial, the BRAF(V600E) selective inhibitor vemurafenib (PLX4032) resulted in complete or partial regression in the majority of melanoma patients harboring the BRAF(V600E) mutation (Flaherty et al., 2010). However, the excitement from this spectacular result was soon tempered as resistance to the therapy quickly developed, resulting in response durations of only 2 to 18 months.

Vemurafenib is only effective in BRAF mutant cells. In normal tissues and in cells where the RAF/MEK/ERK pathway is activated by mutation of the upstream RAS signaling proteins, vemurafenib actually enhances signaling. Key to understanding this surprising result is the fact that RAF isoforms BRAF and CRAF normally homo- or heterodimerize following activation of RAS proteins. RAF inhibitor binding appears to cause a conformational change that promotes the formation of BRAF-CRAF or CRAF-CRAF dimers in which the drug-inactivated molecule is able to induce activation of its drug-free partner within the dimer. On the other hand, in cells harboring BRAF(V600E), the levels of activated RAS (GTP bound) are insufficient to induce dimer formation, so BRAF(V600E) signals only as a monomer and the inhibitor can completely block its kinase activity (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010) (Figure 1).

This model suggests that molecular lesions that enhance RAF dimerization in tumor cells will increase RAF activity upon drug treatment and promote tumor resistance. Poulikakos et al. 2011 have now found evidence for the operation of just such a mechanism in vemurafenib-resistant, BRAF(V600E) mutant melanoma cell lines, and patient samples. The authors generated resistant cell lines by exposing a BRAF(V600E) melanoma

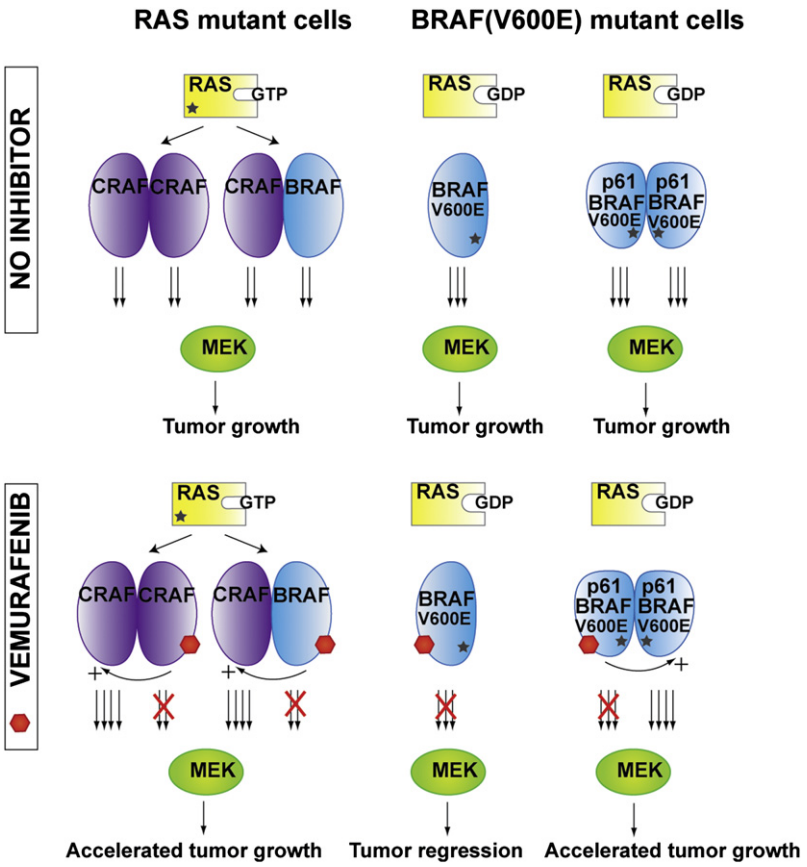

Figure 1. Effects of RAF Inhibitors on Cells with BRAF or RAS Mutations
Active GTP-bound RAS promotes the formation of homo- and heterodimers, but BRAF(V600E) signals primarily as a monomer in BRAF(V600E) mutant cells because the levels of RAS-GTP are low. In contrast, the BRAF(V600E) splice variant p61BRAF(V600E) lacks domains necessary for RAS interaction and for preventing RAS-independent dimerization. When melanoma cells are treated with a BRAF inhibitor, BRAF(V600E) is inhibited, leading to tumor regression. However, when RAF isoforms form dimers, either due to RAS mutation or BRAF truncation, the drug-inactivated kinase subunit induces the transactivation of the drug-free kinase subunit in the dimmer, resulting in continued activation of downstream pro-proliferative signaling.

line to a high dose of vemurafenib in vitro. In three of five resistant clones obtained, they detected a smaller BRAF transcript that contained both the V600E mutation and an in-frame deletion of exons 4–8, resulting in expression of a BRAF variant lacking domains necessary for interaction with RAS. This deletion also removes sequences that inhibit BRAF dimerization in the absence of RAS binding, allowing dimerization of this variant in a RAS-independent manner. Thus, this truncation results in a constitutively activated BRAF(V600E) dimer rather than the BRAF(V600E) monomer found in the parental cells. The dimer displays the transactivation of the drug-free subunit by the drug-bound subunit that has been observed for other RAF dimers (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010), reducing sensitivity to vemurafenib by 100-fold.

Acknowledging that generation of resistant cell lines by drug exposure in vitro may have its limitations, Poulikakos et al. 2011 went on to demonstrate the importance of this resistance mechanism in the clinic. The authors analyzed tumors from 19 BRAF(V600E) mutant melanoma patients with acquired resistance to vemurafenib and identified a total of four shorter BRAF transcript variants in 6 of them. All these splicing variants lack minimally exons 4–8, one of which was identical to that seen in the cell lines. As yet, it is unclear how these variants were generated: could they be caused by mutations at the splice junctions or perhaps epigenetic changes?

This BRAF inhibitor resistance mechanism is the first identified that involves a structural change in BRAF itself, bringing BRAF more in line with resistance mechanisms commonly seen when pharmacologically targeting other oncogenes, such as activated EGFR or BCR-ABL. Several other mechanisms of resistance to RAF inhibitors have been previously reported, but in each study, only a very small group of tumor samples has been analyzed, making it hard to assess their relative importance in the clinic. Poulikakos et al. 2011 have analyzed the largest cohort of tumor samples thus far, with 19 patients, of which 6 had BRAF splice variants and 4 had mutations in NRAS, suggesting that both BRAF and NRAS mutations are likely to play major roles in the development of resistance. A mutation in NRAS had previously been identified as a resistance mechanism (Nazarian et al., 2010). Other possibly rarer molecular events reported previously that may also reactivate RAF/MEK/ERK signaling include enhancement of MAP3K8 (Cot1/Tpl2) mRNA levels (Johannessen et al., 2010) and an activating mutation in MEK1 (Wagle et al., 2011). Alterations that activate PI3K pathway signaling, including increased expression of PDGFRβ or IGF-1R levels or deletion of PTEN, have also been detected (Nazarian et al., 2010).

Drug resistance is arguably the biggest challenge blocking progress toward better outcomes in cancer treatment. Obviously, the importance of identifying drug resistance mechanisms lies in the possibility of developing better drugs or drug combinations to overcome resistance. For BRAF mutant melanoma, many resistance mechanisms result in ERK pathway reactivation, suggesting that inhibition of the pathway downstream of RAF using MEK inhibitors could overcome acquired resistance and might also have value in combination with vemurafenib to limit the development of resistance. However, MEK inhibitors have yet to prove their worth in the clinic. Perhaps a more interesting approach to tackling resistance is the development of new RAF inhibitors. The ideal drug would be one that was specific for oncogenic BRAF but would not transactivate CRAF or truncated BRAF, but achieving this may be quite a challenge for drug developers. Existing BRAF inhibitors also have some activity toward CRAF, and it is possible that

the transactivation has actually been selected in the drug development process because it circumvents potential systemic toxicity associated with pan-RAF inhibition.

Vemurafenib resistance often develops rapidly and multiple resistant tumor nodules usually appear at the same time (Wagle et al., 2011). The efficacy of RAF inhibitors depend on almost complete inhibition of ERK signaling; partial RAF inhibition or small changes that increase pathway activity can produce resistance. One possible reason for the rapid simultaneous appearance of resistant nodules is the existence of minor populations of resistant cells in the original tumors prior to treatment that can overtake the drug-sensitive populations. If this is the case, emergence of a single resistance mechanism should be seen if several different resistant lesions from the same patient are analyzed. More worrying is the possibility that tumor cells can escape destruction via any of a plethora of relatively easily accessed routes, so that each different resistant lesion in a patient is using a different mechanism. It has been suggested that tumor heterogeneity and changes in drug response can be mediated by epigenetic changes (Sharma et al., 2010), leading to changes in expression of some genes and potentially splicing alterations. This suggests that combination treatment with epigenetic modulators such as histone deacetylase inhibitors could be tested to overcome RAF inhibitor-mediated resistance.

The extremely rapid progress in understanding BRAF inhibitor resistance mechanisms raises hopes that the partial success of targeted agents like vemurafenib may soon lead to more lasting patient benefit. However, the complexity seen in the BRAF signaling network response to these drugs and the ease with which tumors develop resistance to them suggests that there will be many more unexpected twists to this story before metastatic melanoma can be considered beaten.

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