DRUG DISCOVERY

Inhibitors that activate

Karen Cichowski and Pasi A. Jänne

Inhibitors of RAF enzymes can suppress or activate the same signalling pathway. The details of how this happens provide a cautionary note for those targeting the pathway for anticancer drug discovery.

Over the past year, there has been considerable excitement following the positive therapeutic effects of the compound PLX4032 in early clinical trials in patients who have metastatic melanoma[1]. The compound is thought to selectively inhibit $BRAF^{V600E}$, a mutant, cancer-associated form of the kinase enzyme BRAF. The positive clinical effects of PLX4032 contrast with clinical responses to inhibitors of MEK, another kinase directly downstream of BRAF in the RAS–RAF–MEK–ERK signalling cascade. MEK inhibitors have been less effective than PLX4032 in clinical trials[2], despite the fact that they — like the BRAF inhibitor — selectively killed melanoma cells that carry $BRAF^{V600E}$ mutations in preclinical studies[3].

But despite the antitumour effects of PLX4032 on melanomas, about 25% of patients treated with the drug unexpectedly developed hyperproliferative skin lesions (keratoacanthomas) and, in some cases, squamous-cell carcinomas, which disappeared following drug withdrawal[1]. Adding to this paradox, although most cancer-causing (oncogenic) *BRAF* mutations — such as $BRAF^{V600E}$ — enhance the kinase's activity, some patients with melanoma harbour BRAF-inactivating mutations[4]. Three studies, two in this issue[5,6] and another in *Cell*[7], begin to provide an explanation for these unexpected findings.

All three studies[5–7] started with the same intriguing observation: whereas selective BRAF inhibitors potently suppress RAF–MEK–ERK signalling in *BRAF*-mutant melanoma cells, the same inhibitors unexpectedly activate this pathway in a different type of tumour cell — those that carry an oncogenic mutation in the gene *KRAS*, but which possess a wild-type *BRAF* gene (Fig. 1). Heidorn *et al.*[7] report that the selective BRAF inhibitor 885-A binds to wild-type BRAF in *KRAS*-mutant cells and promotes the formation of BRAF–CRAF complexes, in which CRAF is a RAF kinase related to BRAF in this signalling pathway. Formation of the complex activates CRAF, and consequently the MEK–ERK pathway.

Heidorn *et al.* also demonstrate that a 'kinase-dead' mutant of BRAF (which behaves like an inhibited BRAF enzyme) similarly binds to CRAF and cooperates with a mutant form of the *RAS* gene to promote MEK–ERK activation, thus inducing melanomas in genetically engineered mice (Fig. 1). The authors therefore provide a unifying explanation for the oncogenic effect of kinase-dead BRAF proteins, and for the pro-tumorigenic effects of BRAF inhibitors in some settings (such as in cells that lack *BRAF*-activating mutations).

Hatzivassiliou and colleagues' studies[6] (page 431) are thematically similar, but their focus is on working out the molecular mechanisms by which two selective, structurally unrelated BRAF inhibitors — GDC-0879 and PLX4720 — activate the RAF–MEK–ERK pathway in *RAS*-mutant cells. The authors' findings suggest that GDC-0879 promotes the formation of dimeric complexes of RAF-type kinases, specifically BRAF–CRAF, BRAF–ARAF and CRAF–CRAF complexes. The authors also obtained a crystal structure of a close analogue of GDC-0879 bound to CRAF. This revealed that drug binding causes a conformational change in the kinase that promotes the formation of dimeric RAF complexes.

Although PLX4720 also induces activation of the RAF–MEK–ERK pathway, Hatzivassiliou *et al.* found that it actually prevents the formation of BRAF–CRAF complexes. Their models suggest that this is because the inhibitor induces a different conformational change in BRAF, which specifically impairs BRAF binding to other RAF kinases. It therefore seems that PLX4720 mediates its effects by promoting the formation of CRAF–CRAF dimers (Fig. 1).

Poulikakos and colleagues[5] (page 427) also investigated the biochemical effects of RAF inhibitors. They found that these agents activate MEK and ERK not only in tumour cells that harbour a mutant version of *RAS*, but also in cells in which the RAS–RAF–MEK–ERK pathway is activated by other oncogenes, such as *HER2*. Consistent with Hatzivassiliou and colleagues' report[6], Poulikakos *et al.* suggest that PLX4720 functions by promoting CRAF–CRAF formation (Fig. 1). In addition, they make the intriguing observation that, at low concentrations, compounds that are thought to inhibit all RAFs (pan-RAF inhibitors) stimulate the RAF–MEK–ERK pathway. Presumably this is because, at low concentrations, the inhibitors bind to only a fraction of the available RAF kinases, promoting the formation of complexes of the drug-bound kinases with non-drug-bound RAFs. Because only one kinase within a given complex is drug-bound (inhibited), such complexation induces the activation of the non-drug-bound kinase, thus promoting MEK–ERK signalling.

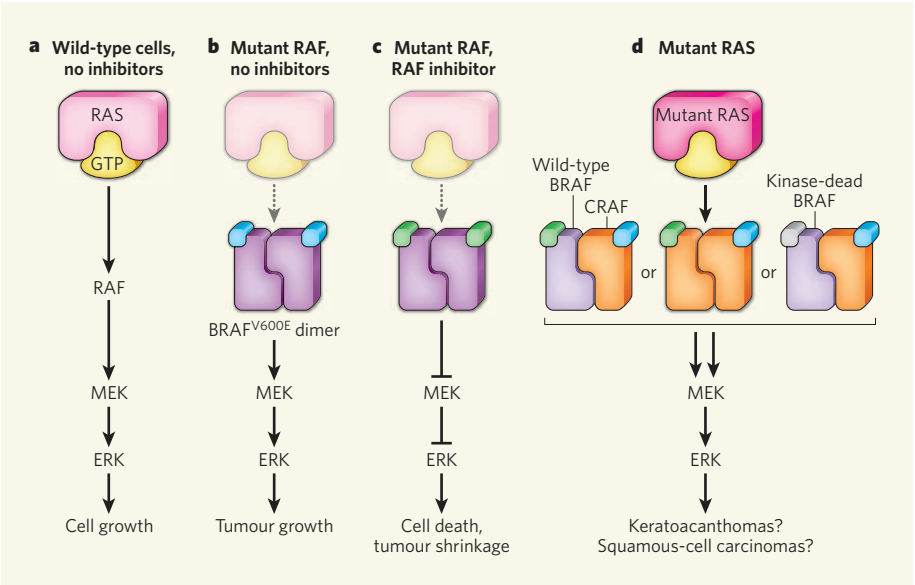

Figure 1 | The effects of RAF inhibitors in mutant and wild-type cells. **a**, Activation of the RAS–RAF–MEK–ERK signalling pathway promotes cell growth, but excessive activation is associated with cancer. RAS enzymes activate signalling when bound to the nucleotide GTP. **b**, $BRAF^{V600E}$ is a mutant, oncogenic version of a RAF kinase enzyme found in the pathway. In tumour cells that harbour $BRAF^{V600E}$, RAS–GTP activation is low, and ERK signalling is predominantly activated by dimers of the mutant kinase. Phosphate groups (blue) are present on the activated kinase. **c**, In the presence of a BRAF inhibitor that blocks phosphorylation (green), MEK–ERK signalling in $BRAF^{V600E}$ mutant cells is suppressed, causing cell death and tumour shrinkage. **d**, In *RAS* mutant cells that contain wild-type BRAF, RAF inhibitors block phosphorylation of RAF monomers (BRAF or CRAF), which go on to form dimeric complexes[5–7] such as BRAF–CRAF or CRAF–CRAF. Similarly, a 'kinase-dead' BRAF that cannot phosphorylate (grey block) forms a complex with CRAF[7]. The formation of these RAF complexes causes excessive signalling, stimulated by the mutant RAS enzyme. This might explain the formation of skin lesions (keratoacanthomas) and squamous-cell carcinomas observed in melanoma patients given RAF inhibitors during clinical trials[1].

Although the precise mechanisms by which different RAF inhibitors activate the RAF–MEK–ERK pathway may differ, all three studies[5–7] demonstrate that inhibitors thought to be selective for mutant BRAF can activate CRAF through the formation of dimeric RAF complexes — a process that is enhanced by the presence of an oncogenic *RAS* mutation. Consequently, these studies support the same fundamental conclusion: mutant-specific BRAF inhibitors should be used for treating cancers caused by *BRAF* mutants (such as $BRAF^{V600E}$-associated melanomas), but should be avoided in cancers caused by *RAS* mutants (including other kinds of melanoma). This underscores the need to select patients who have *BRAF*-mutant cancers for current and future clinical studies of BRAF inhibitors.

These findings[5–7] may also help to explain the side effects of RAF inhibitors in patients with melanoma, and they raise additional therapeutic considerations. Although it remains to be formally proved, it is tempting to speculate that the keratoacanthomas and squamous-cell carcinomas observed in clinical trials[8] of RAF inhibitors are attributable to the activation of ERK signalling by one or more of the proposed mechanisms[5–7] (Fig. 1). Consistent with this possibility, Hatzivassiliou *et al.*[6] report that GDC-0879 causes hyperactivation of ERK and hyperproliferation of skin cells in mice. Moreover, *RAS* mutations are found in up to 20% of squamous-cell carcinomas. *RAS* mutations are also present[7,9] in a subset of actinic keratoses — thick, scaly patches of skin that sometimes precede keratoacanthomas — supporting the notion that *RAS* mutations may enhance the pro-tumorigenic side effects of RAF inhibitors in patients. Although this particular lesion is easily removed, it could be that other, less visible pre-malignant lesions associated with *RAS* mutations might be induced by RAF inhibitors, such as those in the airways of smokers. This suggests that patients treated with RAF inhibitors should be monitored carefully for side effects. Nevertheless, the success of BRAF inhibitors in patients with metastatic melanoma[1], a group of patients who until recently had a poor prognosis, suggests that clinical development of these compounds should continue.

The trio of studies[5–7] might also help us to understand the clinical differences observed when melanomas are treated with MEK or BRAF inhibitors, and may provide insight into which drugs should be selected to treat genetically distinct tumours. The reports suggest that BRAF inhibitors do not inhibit (and may even potentiate) ERK signalling in normal tissues, such as skin. This effect might actually provide a greater therapeutic window for anticancer treatment, allowing BRAF inhibitors to be administered at sufficient doses to fully inhibit ERK signalling in *BRAF*-mutant tumours, while sparing normal tissue. In contrast, MEK inhibitors block ERK signalling in both tumour and normal tissue, so that the therapeutically acceptable doses of these compounds are limited by the side effects associated with ERK inhibition in normal tissue, such as intolerable skin rashes[2]. It is currently not known whether the greater success of PLX4032 in clinical trials, compared with MEK inhibitors, is solely the result of a greater therapeutic window, or whether this success also (or instead) stems from the selectivity of PLX4032 for $BRAF^{V600E}$. The answer to this question is important for future drug development, as BRAF inhibitors that do not activate CRAF might ultimately cause side effects similar to those caused by MEK inhibitors. Thus, a compound that inhibits $BRAF^{V600E}$ alone, if it is possible to develop such an agent, might ultimately be the best drug for treating $BRAF^{V600E}$-mutant melanomas.

Finally, the observation[5] that pan-RAF inhibitors might also activate the RAS–RAF–MEK–ERK pathway in *RAS*-mutant cells at low concentrations may suggest that only those inhibitors that potently inhibit CRAF at clinically achievable drug concentrations should be considered as treatments for *RAS*-mutant cancers. Alternatively, MEK inhibitors may be a more appropriate choice of therapeutic agent. Regardless of the agent chosen, it will probably need to be used in combination with another drug to effectively treat *RAS*-mutant tumours.

More fundamentally, these reports[5–7] suggest that, despite 25 years of intensive study, we still do not fully appreciate the complexities of the RAS–RAF–MEK–ERK pathway. The unexpected effects (and, in some cases, lack of effects) of cancer therapies that target it underscore the value of studies aimed at garnering detailed mechanistic insight into both normal and pathogenic signalling mediated by this pathway. Such insight might ultimately lead to the development of effective new anticancer therapies. ■

Karen Cichowski is in the Division of Genetics, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA. Pasi A. Jänne is in the Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA.
e-mails: kcichowski@rics.bwh.harvard.edu; pjanne@partners.org

1. Flaherty, K. *et al. J. Clin. Oncol. (Meet. Abstr.)* **27,** 9000 (2009).
2. Adjei, A. A. *et al. J. Clin. Oncol.* **26,** 2139–2146 (2008).
3. Solit, D. B. *et al. Nature* **439,** 358–362 (2006).
4. Ikenoue, T. *et al. Cancer Res.* **63,** 8132–8137 (2003).
5. Poulikakos, P. I., Zhang, C., Bollag, G., Shokat, K. M. & Rosen, N. *Nature* **464,** 427–430 (2010).
6. Hatzivassiliou, G. *et al. Nature* **464,** 431–435 (2010).
7. Heidorn, S. J. *et al. Cell* **140,** 209–221 (2010).
8. Arnault, J. P. *et al. J. Clin. Oncol.* **27,** e59–e61 (2009).
9. Spencer, J. M., Kahn, S. M., Jiang, W., DeLeo, V. A. & Weinstein, I. B. *Arch. Dermatol.* **131,** 796–800 (1995).

ASTROPHYSICS

First generation of quasars

Giulia Stratta

The discovery of two quasars in the distant Universe that apparently have no hot dust in their environments provides evidence that these systems represent the first generation of their family.

In seeking to understand how, and when, galaxies such as our own formed, astronomers often turn to a type of galaxy called a quasar. Because quasars are extremely bright, they can be seen at much larger distances from Earth than other galaxies, and so allow us to peer into the early history of the Universe. To date, multi-wavelength observations[1–3] have shown that the most distant quasars known are nearly indistinguishable from their closest (lower-redshift) counterparts, suggesting that quasars in the early Universe were already evolved objects. Now Jiang and collaborators[4] (page 380) put this view to the test with the Spitzer Space Telescope's observations of a sample of high-redshift quasars seen at an epoch when the Universe was about one billion years old.

Quasars are a family within a larger class of galaxies called active galactic nuclei. These galaxies host a supermassive black hole (more than a million solar masses) in their nucleus that accretes matter at high rate from a surrounding disk of gas. This accretion process powers the release of intense electromagnetic radiation in the galactic core that has wavelengths spanning from the near-infrared to the X-ray bands of the electromagnetic spectrum. Most of the system's ultraviolet and visible radiation is absorbed by dust close to the accretion disk[5,6] and is re-emitted at infrared wavelengths. The hottest of this dust is directly heated by the central engine and generates near-infrared radiation[7–9].

Observational studies[10,11] have led to the discovery of more than 40 quasars at an epoch when the Universe was about 7% of its present age. These studies have shown that such high-redshift ($z \approx 6$) quasars have very similar properties to their lower-redshift analogues in the rest-frame (not redshifted) ultraviolet, visible and X-ray energy bands[1–3]; light of wavelength λ emitted by a quasar at redshift z is redshifted on its course to Earth by a factor $1+z$ owing to