Kinase inhibitors: Vice becomes virtue

In this issue of *Cancer Cell*, Fan and coworkers describe a novel inhibitor of PI3 kinase (PI3K) that potently interferes with the growth of glioma cells. They show that the efficacy of this inhibitor results from dual, synergistic activity against the p110α subunit of PI3K and against TOR. Although p110α and TOR belong to the same signaling pathway, they both must be inactivated because of the need to silence the regulatory feedback loop that remains unaffected by monospecific inhibitors. The new PI3K inhibitor achieves the effects of combination therapy as a single agent by fortuitously hitting two critical targets.

PI3K as a cancer target

The family of PI3 kinases (PI3Ks) has now joined protein kinases as a class of attractive and promising targets for small molecule inhibitors that have therapeutic potential in cancer. Of particular interest are the four isoforms of class 1 PI3K, p110α, -β, -γ, and -δ. They generate phosphatidylinositol-3,4,5-*tris*phosphate (PIP_3), an important second messenger molecule that sets in motion complex growth-promoting signaling chains in the cell. Gain of function in PI3K-dependent signaling is common in cancer. It has three principal causes: amplification of the *PIK3CA* gene, which codes for the catalytic subunit p110α of class 1 PI3K; point mutations in that same gene; or loss of function in PTEN, the lipid phosphatase catalyzing the conversion of PIP_3 into PIP_2 (Bader et al., 2005; Vivanco and Sawyers, 2002). Increased PI3K activity can be a significant and even critical determinant of the oncogenic cellular phenotype, suggesting that PI3K could be an important cancer target. When fused to a sequence that mediates membrane localization, p110α functions as a retroviral oncoprotein, inducing transformation in cell culture and tumors in the animal (Chang et al., 1997). Gain-of-function mutations in p110α that are derived from human cancer confer oncogenicity onto the protein in vitro and in vivo (Vogt et al., 2006). Inhibition of the downstream effector of PI3K, the protein kinase TOR, interferes with PI3K-induced oncogenic transformation in cell culture. In animal model systems, inhibition of TOR can impede the growth of tumors that show a gain of function in PI3K signaling (Bader et al., 2006; Neshat et al., 2001; Podsypanina et al., 2001). These observations document dependence of oncogenic properties on PI3K signaling. Other reasons for the attractiveness of PI3K as a cancer target include the facts that PI3K is an enzyme and hence can readily be manipulated with small molecules, and that it shows gain of function in cancer, a condition that is easier to correct than loss of function. However, the identification of small molecule inhibitors for PI3K has encountered some of the same problems that are known from inhibitors of protein kinases.

Lessons from inhibitors of protein kinases

Protein kinases are important cancer targets. Clinical experience with Gleevec in chronic myelogenous leukemia (CML) and Iressa and Tarceva in non-small cell lung cancer has shown that small molecule kinase inhibitors can be therapeutically highly effective (Druker, 2004). Yet these clinically successful compounds constitute an exception. The majority of protein kinase inhibitors lack clinical utility. One reason for this limited success lies in the mechanism by which most kinase inhibitors work: they compete with ATP by targeting the ATP binding site of the kinase. Since ATP pockets of different kinases are structurally similar, these inhibitors typically not only affect the intended target molecule but also indiscriminately inhibit the activities of several other kinases as well (Fabian et al., 2005). Such broad reactivity is generally perceived as an undesirable feature, as it can lead to side effects that disqualify the compound as a safe and effective drug. Yet there are situations where vice can turn into virtue and where lack of narrow target specificity has advantages. Hitting multiple kinases can expand clinical utility to diseases for which the drug was not originally intended. Broad reactivity could also be an important factor determining drug efficacy. Gleevec is the now classic example of a protein kinase inhibitor with several targets (ABL, KIT, PDGFR) (Fabian et al., 2005). Initially developed to target BCR-ABL, the kinase driving CML, Gleevec has gained a second important application in the treatment of gastrointestinal stromal tumors because of its additional activity against KIT. Even in CML, Gleevec may suppress other signaling pathways besides BCR-ABL.

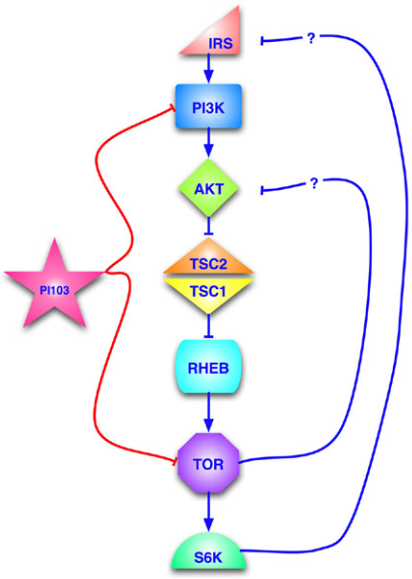

Figure 1. Compound PI-103 inhibits PI3K and TOR

This target combination is essential for the ability of PI-103 to arrest the growth of glioma cells and may reflect the need to interfere with both the forward signal from PI3K and the negative feedback from TOR for an effective shutdown of the signaling chain. As indicated by the question marks, there is still uncertainty about the precise upstream targets and their relative importance for the negative feedback loop. IRS, insulin receptor substrate; PI3K, phosphoinositide 3 kinase; AKT, serine-threonine kinase Akt; TSC, tuberous sclerosis complex; RHEB, Ras homolog enriched in brain; TOR, target of rapamycin; S6K, S6 protein kinase.

Selective promiscuity

In this issue of *Cancer Cell*, Fan and coworkers provide an example from the young field of PI3K inhibitors for the unexpected benefits of broad target specificity (Fan et al., 2006). They generated ten structurally distinct inhibitors that show selectivity for one or more of the four class 1 PI3K isoforms and then determined which of these isoforms is critical for the growth of glioma cells in culture. p110α emerged as the relevant driving force, as only the inhibitors selective for this isoform effectively reduced the growth of glioma cells. Blockade of p110α alone, however, was not sufficient to achieve the maximum inhibitory effect. The compound with the most potent growth-arresting activity, PI-103, inhib-

ited not only p110α but also its downstream effector, the protein kinase TOR. Broad inhibitory action was essential for shutting down the growth of glioma cells. However, in order to be effective, the promiscuity of the drug had to be targeted to PI3K and TOR. Then, and only then, did vice become a virtue, with PI-103 achieving the effects of combination therapy as a single agent (Figure 1).

Specificity of target combination

Given the complexity of cellular signaling and the ability of cells to compensate for loss of function, it comes as no surprise that more than one kinase needs to be inhibited to achieve a significant change in the cellular phenotype. The effects on multiple targets need to be complementary and then are synergistic. The action of the new PI3K inhibitor lends support to a model of PI3K signaling that includes as an essential feature a negative feedback loop originating from TOR and targeting an upstream component of the signaling chain (Hay, 2005; Wullschleger et al., 2006). An inhibitor directed to TOR alone weakens this negative feedback and results in activation of the PI3K signaling pathway. Only the dual PI3K-TOR inhibitor can prevent this compensatory effect (Figure 1) (Fan et al., 2006). Among the compounds tested, the inhibitor PI-103 is also the most effective in reducing Akt phosphorylation. A particularly gratifying quality of the dual PI3K-TOR inhibitor PI-103 is its lack of toxicity. This fact allays fears that PI3K inhibitors may induce intolerable side effects on essential cellular activities such as insulin signaling. With the identification of p110α and TOR as a critical target combination in glioma, the stage is set for rapid progress in the field of PI3K inhibitors.

Acknowledgments

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At the gates of death

Apoptosis that proceeds via the mitochondrial pathway involves mitochondrial outer membrane permeabilization (MOMP), responsible for the release of cytochrome c and other proteins of the mitochondrial intermembrane space. This essential step is controlled and mediated by proteins of the Bcl-2 family. The proapoptotic proteins Bax and Bak are required for MOMP, while the antiapoptotic Bcl-2 proteins, including Bcl-2, Bcl-xL, Mcl-1, and others, prevent MOMP. Different proapoptotic BH3-only proteins act to interfere with the function of the antiapoptotic Bcl-2 members and/or activate Bax and Bak. Here, we discuss an emerging view, proposed by Certo et al. in this issue of *Cancer Cell*, on how these interactions result in MOMP and apoptosis.

In his classic film *Rashomon*, Akira Kurosawa told the story of a samurai's death from four distinct points of view and presented his rain-soaked protagonists, and us, with the changing nature of truth. In this issue of *Cancer Cell*, Certo et al. (2006) provide a new perspective on how cells die and provide a possible resolution to a controversy that focuses on the heart of this process, at the gates of death. As in *Rashomon*, we can find four different and perhaps not completely incompatible viewpoints on how an important form of cell death occurs.

Most physiological cell deaths in animals occur through apoptosis, and most apoptosis in mammals proceeds by the mitochondrial pathway, wherein mitochondrial outer membrane permeabilization (MOMP) allows the proteins of the intermembrane space to diffuse into the cytosol (Green, 2005). MOMP is most likely a result of formation of a proteolipid pore, although this has not been visualized. Upon MOMP, holocytochrome c contacts APAF-1, inducing the latter to recruit and activate caspase-9. Caspase-9 in turn cleaves and thereby activates executioner caspases, which then orchestrate apoptosis. Even without downstream caspase activation, however, MOMP appears to be sufficient to commit most cells to die, and death can proceed following MOMP in a caspase-independent manner. Therefore, MOMP is a critical decision point at which cell life and death is determined.