ANTICANCER DRUGS

A double strike at kinases

A new study published in *PNAS* has shown that a dual kinase inhibitor targeting platelet-derived growth factor receptor-β (PDGFRβ) and B-RAF produces potent anti-angiogenic effects, which are not seen with the inhibition of either target alone. Compared with currently used multi-target kinase inhibitors, specific dual inhibitors might offer more efficacious anticancer activity with minimal side effects.

Cheresh and colleagues proposed that a compound that inhibited B-RAF and PDGFRβ would have a potent anti-angiogenic effect by targeting endothelial cells and pericytes, the two cell types involved in angiogenesis and vascular remodelling, respectively.

The authors used computational docking studies to design an amino-triazole scaffold that fitted into the allosteric site in the inactive conformation of PDGFRβ and B-RAF, as well as of other kinases while avoiding the ATP-binding pocket. The docking studies were based on the binding of the multi-target inhibitors sorafenib (Nexavar; Bayer/Onyx) and imatinib (Glivec/Gleevec; Novartis), which act, in part, to stabilize the inactive conformation of these kinases and have some allosteric activity against them. Nine amino-triazole-based compounds were designed *in silico*, then chemically synthesized and screened for activity against PDGFRβ in cell-based assays. The compounds were also screened for their capacity to inhibit the phosphorylation of endothelial cell mitogen-activated protein kinase kinase (MEK) and extracellular signal-regulated kinase (ERK), which are downstream of B-RAF signalling. Active compounds were then analysed for anti-angiogenic activity in developing zebrafish.

One compound — known as compound 6 — that inhibited both PDGFRβ activity and B-RAF heterodimer formation, and had cytotoxic activity was chosen for further study. Compared with imatinib and sorafenib, compound 6 had improved selectivity against 70 diverse kinases. In zebrafish embryos treated with compound 6, endothelial cells failed to form mature blood vessels. Moreover, the compound disrupted a late step in the formation of blood vessel lumens, which was followed by the induction of apoptosis. To produce the anti-angiogenic effect, dual inhibition of PDGFRβ and B-RAF was required, as both a B-RAF inhibitor and a PDGFRβ inhibitor were needed to produce a similar response to compound 6; either inhibitor alone did not produce this effect.

The authors next examined the anti-angiogenic properties of compound 6 in mice. Injection of the compound to mice with induced neovascularization blocked angiogenesis and inhibited signalling mediated by B-RAF and PDGFRβ. In an orthotopic pancreatic carcinoma model, tumour growth was suppressed in mice systemically treated with the compound — with treated tumours showing less vascularization. In addition, oral administration of compound 6 suppressed tumour growth in an orthotopic renal cell carcinoma model.

A unique feature of compound 6 is that it is the first strictly allosteric inhibitor of B-RAF that avoids the ATP-binding pocket of the kinase. Indeed, three recent papers (see page 271) have highlighted that B-RAF inhibitors that target the ATP-binding pocket cause B-RAF dimerization and increased activation of MEK and ERK, which leads to tumour cell proliferation. So, allosteric inhibitors — such as compound 6, which disrupts B-RAF dimerization — could offer the anticancer benefits seen with other B-RAF inhibitors while avoiding the problems associated with ATP mimetics of this target.

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ORIGINAL RESEARCH PAPER Murphy, E. A. *et al.* Disruption of angiogenesis and tumor growth with an orally active drug that stabilzes the inactive state of PDGFRβ/B-RAF. *Proc. Natl Acad. Sci. USA* **107**, 4299–4304 (2010)