The transgenic rat expresses human proteins that provoke a misdirected immune response, the first stage of which is chronic intestinal inflammation. This phenotype serves as a model of IBD in which low doses of EE have been shown to be effective, and so lends itself to studies of WAY-169916.

Treatment with WAY-169916 had a positive outcome on the disease phenotype: it rapidly halted chronic diarrhoea and significantly reduced all histological parameters of intestinal inflammation to an extent comparable with EE. Co-administration of an ER antagonist showed that these effects resulted from ER activity. In addition to its potential development as an anti-inflammatory drug, WAY-169916 could provide insight into the molecular mechanism of the divergent roles of the ER.

Joanna Owens

References and links

ORIGINAL RESEARCH PAPER
Chadwick, C. C. *et al*. Identification of pathway-selective estrogen receptor ligands that inhibit NF-κB transcriptional activity. *Proc. Natl Acad. Sci. USA* **102**, 2543–2548 (2005)

FURTHER READING
Gronemeyer, H. *et al*. Principles for modulation of the steroid hormone receptor superfamily. *Nature Rev. Drug Discov.* **3**, 950–964 (2004)

KINASE INHIBITORS

Surveying the kinome

Sequencing of the human genome has so far revealed more than 500 genes encoding protein kinases. Many of these enzymes are directly involved in diseases such as cancer and inflammation, making them excellent targets for drug development. In the March issue of *Nature Biotechnology*, Lockhart, Zarrinkar and colleagues go into uncharted protein kinase territory by developing a new type of assay to determine the specificity of a number of kinase inhibitors against a panel of 119 protein kinases.

Kinase inhibitors are an important new class of anticancer drugs, and have clinical activity in tumours in which the target kinase is activated by mutation, such as the mutant kinase BCR–ABL in chronic myeloid leukaemia (CML). The success of small-molecule inhibitors such as imatinib (Gleevec; Novartis) to treat CML, and gefitinib (Iressa; AstraZeneca) and erlotinib (Tarceva; Genentech/OSIP), both of which target the epidermal growth factor receptor (EGFR) to treat lung cancer, has demonstrated clear proof-of-principle that this strategy is effective.

Most kinase inhibitors in clinical development target the ATP-binding site common to all kinases, and bind multiple kinases. However, it is not possible to predict binding specificity and affinity on the basis of available sequence or structural information. Conventional profiling methods that use the measurement of *in vitro* activity are limited by the difficulty of building and running large numbers of kinase activity assays. Such information is very valuable, both for finding new clinical uses for inhibitors and for predicting or explaining toxicity.

Fabian *et al.* have developed a quantitative competitive assay for measuring the binding of small molecules to the ATP-binding site of kinases. Human kinase domains are first expressed as a fusion attached to T7 bacteriophage capsid protein. Then, the test compound in solution competes with an immobilized 'bait' ligand to bind the phage expressing the kinase domain. The amount of phage bound to the bait is quantified to determine the affinity of the test compound for each kinase. If the free test compound binds the kinase and directly or indirectly blocks the ATP site, fewer protein molecules bind to the immobilized bait; if the free test compound does not bind to the kinase, the fusion proteins are able to bind to the immobilized bait.

For most of the test compounds, the tightest interaction is with the kinase that the drug was optimized to inhibit, but the difference in affinity between the primary target and other kinases varies substantially. For the inhibitors BIRB-796, VX-745, erlotinib, GW-2016 and SU11248 there is at least a tenfold difference in affinity between the intended target and off-targets, whereas for SP600125, EKB-569 and ZD-6474 there is less than a twofold difference. This indicates that optimization efforts are generally successful, but there is room for improved discrimination if necessary.

A number of CML patients develop resistance to imatinib. The authors developed similar assays for imatinib-resistant ABL kinases. The most dramatic finding is that the p38 inhibitor BIRB-796 binds tightly to an imatinib-resistant ABL mutant. The authors also uncovered a new target for imatinib, the SRC-family tyrosine kinase LCK, to which imatinib binds tightly. Finally, the authors showed that clinically observed mutations in EGFR do not affect the binding affinity of gefitinib or erlotinib.

The authors show that their kinase-binding assay provides results consistent with more standard *in vitro* results, although whether this reflects the capability of a compound to inhibit a kinase in a cell remains to be determined. Certainly, this assay is a useful tool and it will probably accelerate drug discovery and development efforts for kinase inhibitors.

Melanie Brazil

References and links

ORIGINAL RESEARCH PAPER Fabian, M. A. *et al*. A small molecule–kinase interaction map for clinical kinase inhibitors. *Nature Biotechnol.* 13 Feb 2005 (doi:10.1038/nbt1068)

FURTHER READING Daub, H., Specht, S. & Ullrich, A. Strategies to overcome resistance to targeted protein kinase inhibitors. *Nature Rev. Drug Discov.* **3**, 1001–1010 (2004) | Yingling, J. M., Blanchard, K. L. & Sawyer, J. S. Development of TGF-β signalling inhibitors for cancer therapy. *Nature Rev. Drug Discov.* **3**, 1011–1022 (2004)