NUCLEAR RECEPTORS

Choosing the right path

A compound that acts via the oestrogen receptor (ER) to selectively inhibit nuclear factor-κB (NF-κB) without displaying classical oestrogenic side effects could be used to treat chronic inflammatory disorders such as inflammatory bowel disease (IBD), according to a recent paper in the *Proceedings of the National Academy of Sciences*. Oestrogens have a wide variety of biological effects and although many of them are beneficial, oestrogen therapy has been associated with side effects such as uterine bleeding, increased risk of endometrial cancer and venous thrombosis. This has led to a search for ER ligands that retain beneficial properties but without the side effects.

Non-selective oestrogens (those that bind both ERα and ERβ with equal affinity) are known to have anti-inflammatory activity that is thought to result from interference with the NF-κB signalling pathway. Douglas C. Harnish and colleagues therefore set out to identify ER ligands that inhibited NF-κB without displaying any conventional oestrogenic functions and identified a first-in-class pathway-selective group of ER ligands.

The authors used cells transiently expressing ERα or ERβ and an NF-κB–luciferase reporter gene that was induced by interleukin-1β (IL-1β) to identify compounds that inhibited NF-κB activity. The first compound they discovered was WAY-169916, a non-steroidal, orally active molecule that inhibited 50% of IL-1β-stimulated NF-κB activity, without affecting a marker of conventional oestrogenic activity. They then studied the pathway-selectivity of this compound *in vivo* using a high-fat-diet mouse model in which NF-κB and other inflammatory genes are upregulated in the liver. Daily treatment of ovariectomized mice with either 17α-ethynyloestradiol (EE) or WAY-169916 significantly inhibited the high-fat-diet-induced expression of these genes, but, significantly, WAY-169916 did so without the accompanying oestrogenic effects observed with EE.

The potential of WAY-169916 as an anti-inflammatory therapy for IBD was then demonstrated using the HLA-B27 transgenic rat model.

SCREENING

Hitting the hot spots

A computational approach that allows a quantitative assessment of the ability of a given binding site of a protein to bind small 'leadlike' molecules has been reported by Hadjuk and colleagues in the *Journal of Medicinal Chemistry*. Their studies, which are based on nuclear magnetic resonance (NMR) screening data, also illustrate how 'hot spots' on protein surfaces — regions that make major contributions to the binding energy in protein–ligand complexes — can be identified. Such approaches should be particularly useful for novel genomics-derived targets, for which information on small-molecule-binding sites is typically limited.

NMR-based screening methods such as that described by Hadjuk *et al.* have a number of strengths. First, the perturbations to NMR signals that are indicative of small-molecule binding can be monitored for the whole protein, and so small-molecule binding to any region can in principle be detected. Consequently, both the affinity and the site of small-molecule–protein interactions can be characterized. Second, NMR-based screening approaches can detect small-molecule ligands that have considerably weaker affinities than would be detectable in standard high-throughput screens. These advantages make such approaches ideal for identifying and characterizing hot spots on protein surfaces.

To investigate both the capacity of proteins to bind small molecules and the nature of the binding sites, Hadjuk and colleagues analysed NMR-based screening data from 23 diverse protein targets. Across all targets, the vast majority of the hits identified in the screens bind to a known small-molecule-binding site, demonstrating the selectivity of protein surfaces to bind to ligands at only very specific locations. Interestingly, a small number of novel potential hot spots were also identified. Furthermore, their data indicated that NMR-based screens can be used as a reliable guide to the 'druggability' of a given protein target before investing in the development of complex biochemical assays, or even before the function of the protein is known.

As an alternative to performing an NMR-based screen against every potential protein target, it would be of great value to be able to confidently predict that high-affinity leads can be identified for a particular target. With this in mind, the authors analysed the protein pockets and NMR screening data to try to understand the factors that influence the observed hit rate. Using this analysis, they developed a simple computational model that included parameters describing the protein binding pocket, such as surface complexity, that could be used to predict with high accuracy the druggability of protein targets not used to construct the model.

As the authors conclude, the relationships derived between hit rate and binding-pocket parameters have important implications for the understanding of fundamental principles of molecular recognition, and should facilitate quantitative comparative analyses of binding pockets for use in target assessment and validation, virtual screening and structure-based drug design.

Peter Kirkpatrick

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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

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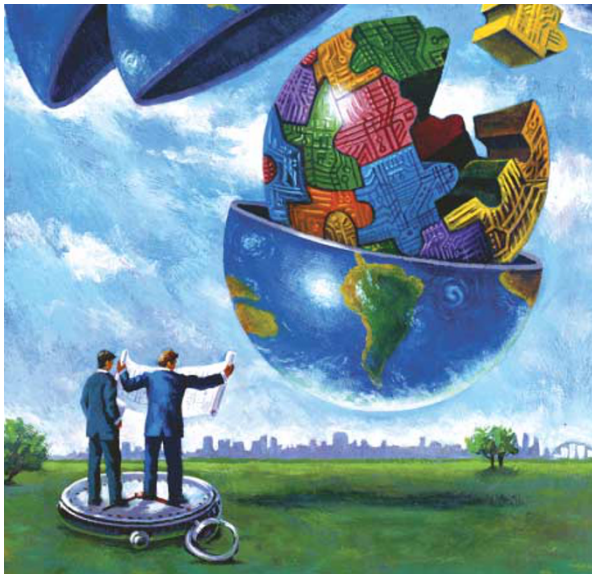
KINASE INHIBITORS

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

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