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.

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References and links

ORIGINAL RESEARCH PAPER Hajduk, P. J., Huth, J. R. & Fesik, S. W. Druggability indices for protein targets derived from NMR-based screening data. *J. Med. Chem.* 24 Feb 2005 (doi:10.1021/jm049131r)

FURTHER READING Rees, D. C. *et al.* Fragment-based lead discovery. *Nature Rev. Drug Discov.* **3**, 660–672 (2004) | Pellecchia, M. *et al.* NMR in drug discovery. *Nature Rev. Drug Discov.* **1**, 211–219 (2002)