STRUCTURE-BASED DRUG DESIGN

Workings of a molecular multi-tool

Like many enzymes involved in xenobiotic metabolism, human carboxylesterase 1 (hCE1) is promiscuous, hydrolysing numerous structurally distinct substrates, including fatty acid and cholesterol derivatives, drugs such as cocaine and heroin, and organophosphate toxins such as sarin. Two recent papers describing crystal structures of hCE1 with different substrates shed light on the structural basis of this promiscuity, and might provide new avenues for treating narcotic abuse and cholesterol-related diseases.

hCE1 hydrolyses cocaine to generate its primary urinary metabolite benzoylecognine, and converts heroin to morphine. In the first of the two studies — both of which are from the Redinbo laboratory — the authors investigated the basis of these activities by determining the structures of hCE1 complexed with the cocaine analogue homatropine, and also the heroin analogue naloxone. Their data show that the substrate-binding gorge of hCE1 has both a small, rigid binding pocket and a large, conformationally flexible binding pocket, which allows hCE1 to act on chemically divergent substrates such as cocaine and heroin. The details of the interactions will facilitate the engineering of highly selective forms of the enzyme with improved catalytic activity towards cocaine for treating acute overdoses, and might also aid in engineering hCE1 to protect against organophosphate toxins.

In the second study, the authors determined the structure of hCE1 in complex with tacrine, a potent inhibitor of the related enzyme acetylcholinesterase (AcChE) that is approved for the treatment of Alzheimer's disease. This structure provides a further illustration of how hCEl achieves its ligand-binding promiscuity by using a large, flexible binding site, which allows tacrine to bind in several orientations at once. By contrast, the previously determined structure of an AcChE–tacrine complex shows that tacrine largely fills the smaller AcChE active site, explaining why tacrine is a nanomolar-affinity inhibitor of AcChE, but does not inhibit hCE1 up to concentrations of 100 μM. However, on the basis of the crystal structure of hCE1, the authors identified analogues of tacrine that were selective, low-micromolar inhibitors of hCE1, which might be leads for cardiovascular drug development, as hCE1 seems to be important in cholesterol transport. Furthermore, as hCE1 is also responsible for producing the toxic metabolite cocaethylene that is formed when cocaine and alcohol are abused together, hCE1 inhibitors could be useful in limiting toxicity in this abuse situation.

Peter Kirkpatrick

References and links

ORIGINAL RESEARCH PAPERS Bencharit, S. *et al.* Crystal structure of human carboxylesterase 1 complexed with the Alzheimer's drug tacrine: from binding promiscuity to selective inhibition. *Chem. Biol.* **10**, 341–349 (2003) | Bencharit, S. *et al.* Structural basis of heroin and cocaine metabolism by a promiscuous human drug-processing enzyme. *Nature Struct. Biol.* **10**, 349–356 (2003)

IN BRIEF

BIOTECHNOLOGY

Production of knockout rats using ENU mutagenesis and a yeast-based screening assay.

Zan, Y. *et al. Nature Biotechnol.* **21**, 645–651 (2003)

The rat is often the preferred model in many areas of biomedical research, but its use has been hampered by the inability to produce gene-disrupted knockout rats. This challenge has now been overcome by Zan *et al.*, who have developed a protocol that combines rat germline mutagenesis using ENU with yeast-based assays that screen for functional mutations in selected genes, which they demonstrate by generating rat knockouts for the breast-cancer suppressor genes *Brca1* and *Brca2*.

NEURODEGENERATIVE DISEASE

GSK-3α regulates production of Alzheimer's disease amyloid-β peptides.

Phiel, C. J. *et al. Nature* **423**, 435–439 (2003)

Alzheimer's disease is associated with increased production and aggregation of amyloid-β (Aβ) peptides, which are produced by proteolytic processing of the amyloid precursor protein (APP). The authors show that glycogen synthase kinase-3α (GSK-3α) facilitates APP processing, and that lithium blocks the production of Aβ peptides by inhibiting GSK-3α. Lithium, which is used to treat bipolar disorder, has a narrow therapeutic window and a higher frequency of side effects in older patients, but agents that specifically target GSK-3α might prove to be valuable in the treatment of Alzheimer's disease.

CARDIOPULMONARY DISEASE

Heterozygous deficiency of hypoxia-inducible factor-2α protects mice against pulmonary hypertension and right ventricular dysfunction during prolonged hypoxia.

Brusselmans, K. *et al. J. Clin. Invest.* **111**, 1519–1527 (2003)

Present therapies for pulmonary hypertension — a common complication of chronic obstructive pulmonary disease that often leads to right ventricular hypertrophy and heart failure — have limited success. Brusselmans *et al.* provide evidence that the transcription factor hypoxia-inducible factor-2α (HIF-2α) is important in the pathogenesis of pulmonary hypertension, indicating that inhibition of HIF-2α could be a promising strategy for this disease.

BIOTECHNOLOGY

Expression profiling reveals off-target gene regulation by RNAi.

Jackson, A. L. *et al. Nature Biotechnol.* **21**, 635–637 (2003)

RNA inteference using small interfering RNAs (siRNAs) designed to match specific messenger RNA sequences has been generating considerable excitement as a potent method to suppress gene expression, with its apparent high specificity being a particularly attractive characteristic. However, by using large-scale gene expression profiling, Jackson *et al.* have shown that siRNAs can cross-react with targets of limited sequence similarity, suggesting that this possibility should be carefully considered when designing and interpreting siRNA-based experiments.