IN BRIEF

ANTIVIRAL DRUGS

An anti-CD45RO immunotoxin kills HIV-latently infected cells from individuals on HAART with little effect on CD8 memory.

Saavedra-Lozano, J. *et al. Proc. Natl Acad. Sci. USA* **101**, 2494–2499 (2004).

Although highly active antiretroviral therapy (HAART) can successfully control HIV infection for prolonged periods, reservoirs of latently infected T cells remain, representing a major obstacle to curing the disease. The authors tested an immunotoxin targeted to an antigen on latently infected T cells, and found that it could kill such cells from individuals receiving HAART, while sparing another type of T cell that is thought to have a key role in containing HIV infection.

NEUROLOGICAL DISEASE

Cytosolic phospholipase A_2 plays a key role in the pathogenesis of multiple sclerosis-like disease.

Kalyvas, A. & David, S. *Neuron* **41**, 323–335 (2004).

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory, demyelinating disease that has been widely used as an animal model of multiple sclerosis (MS). Kalyvas and David provide evidence that cytosolic phospholipase A_2 ($cPLA_2$) is highly expressed in EAE lesions and show that inhibiting this enzyme with a small molecule markedly affects the onset and progression of EAE, suggesting that $cPLA_2$ could be an attractive therapeutic target for MS.

ANTICANCER DRUGS

Small-molecule antagonists of the oncogenic Tcf/β-catenin protein complex.

Lepourcelet, M. *et al. Cancer Cell* **5**, 91–102 (2004).

Protein–protein interactions have traditionally been viewed as highly challenging drug targets, but significant progress has been made in this field recently, as highlighted by this paper. Lepourclet *et al.* developed a high-throughput assay for immuno-enzymatic detection of the interaction between β-catenin and the transcription factor Tcf4, which is important in colorectal cancer. Several diverse compounds that disrupt the Tcf4–β-catenin association were identified, and their activity confirmed in a range of independent assays.

KINASES

The structural basis for autoinhibition of FLT3 by the juxtamembrane domain.

Griffith, J. *et al. Mol. Cell* **13**, 169–178 (2004).

Mutations in the type III receptor tyrosine kinase (RTK) FLT3 that lead to its constitutive activation have been strongly implicated in acute myelogenous leukaemia. This paper presents the crystal structure of the inactive form of FLT3, which sheds light on how it could be activated by mutations, and which should aid in the design of drugs that target FLT3 and other type III RTKs.

RNA INTERFERENCE

Eight steps to silence

Knowing how best to design small interfering RNAs (siRNAs) for RNA interference (RNAi) studies makes the difference between effectively silencing genes and merely creating a gentle and ineffective hush. How exactly this is achieved has been something of a mystery, but now, in *Nature Biotechnology*, one of the leading players in the siRNA design market, Dharmacon, provides proof-of-principle for eight of the criteria it identified and developed for rational siRNA design.

The criteria have been developed from a systematic analysis of 180 siRNAs that targeted every other position across two 197-nucleotide regions of firefly luciferase and human cyclophilin B mRNA. The authors found that the efficiency of siRNAs is determined by the siRNA sequence itself, rather than the mRNA content. This differs from gene knockdown by antisense methods, in which silencing is determined by the local mRNA conformation.

The small proportion of siRNAs that produced highly potent (>95%) gene silencing — 24.4% of luciferase siRNAs and 11.1% cyclophilin B siRNAs — confirms the necessity for rational siRNA design. In no order of importance, the eight criteria determined for effective siRNA design are: low G/C nucleotide content (30–52% G/C); three or more A/U nucleotides at the 3'-terminus of the sense strand (that is, the same sequence as mRNA target), in other words a bias towards low internal stability in this region; a lack of internal repeats that can form secondary structures; and sequence-specific preferences at the following positions on the sense strand — an A at position 19, an A at position 3, a U at position 10, and an absence of a G or C at position 19 and a G at position 13.

The number of criteria reflect the many steps involved in the RNAi process: the assembly of the siRNA with the RNA-induced silencing complex (RISC); activation of RISC; target mRNA recognition and target mRNA cleavage. Each criterion alone is therefore not sufficient to ensure efficient gene silencing. The greater the number of steps incorporated into siRNA design the greater chance of success — incorporating all eight into a single multi-component algorithm was more than 96% effective in predicting functional siRNAs.

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References and links

ORIGINAL RESEARCH PAPER Reynolds, A. *et al.* Rational design for RNA interference. *Nature Biotechnol.* 1 February 2004 (DOI:10.1038/nbt936)
FURTHER READING Nature Reviews Journals web focus on RNA interference http://www.nature.com/focus/rnai