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## RNA INTERFERENCE

# Silencing the sceptics

We've all heard of the promise of RNA interference (RNAi) as a potential approach to treating disease, but how close are we to delivering on this promise? As described in *Nature*, Vornlocher and colleagues have now taken us a step closer to the goal of selectively modifying gene activity for a clinical benefit.

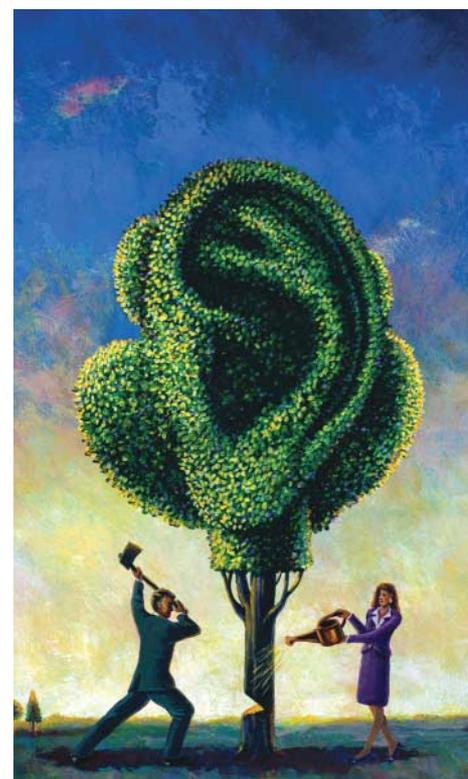
Vornlocher and colleagues have demonstrated the *in vivo* silencing of an endogenous gene that encodes a protein of therapeutic relevance, apolipoprotein B (APOB), which is essential for the formation of low-density lipoprotein (LDL) and therefore implicated in a range of cardiovascular disorders. Although RNAi has been demonstrated *in vivo* before, previous studies have either not targeted an endogenous gene or have used a route of administration that is not applicable to the human therapeutic setting. In this study, small interfering RNA (siRNA) molecules, which induce RNAi of genes with complementary sequences, were administered to mice by tail-vein injection, a route that can be readily translated to human patients.

Just as gene therapy and antisense approaches have been held back by problems of delivery, so too has RNAi. In the past, viral vectors have been used, among other delivery vehicles, to get siRNA to the target tissue. This study used chemical modifications to render the siRNA molecules more drug-like and stable within the body, and more likely to be taken up by cells. The conjugation of cholesterol to

the 3' end of siRNA molecules significantly improved their *in vivo* pharmacological properties, such as cellular delivery and half-life. Such improvements lend credence to the idea that further chemical modifications could improve the prospects of siRNA-based therapeutics.

To explore the *in vivo* effect of these APOB-targeting, cholesterol-modified siRNAs, they were injected into mice that were fed a normal diet. Levels of the target mRNA were then measured in the liver and jejunum, key sites of APOB expression. The modified siRNA caused a significant reduction of APOB mRNA in both tissues, which was reflected as a diminution of APOB protein levels. Desirable effects were seen at the physiological level too: siRNA treatment resulted in a 25% reduction in high-density lipoprotein levels, and a 40% reduction in LDL levels.

A key concern with studies of RNAi is that the observed results might be caused by nonspecific 'off target' effects or the interferon response. Vornlocher and colleagues were able to eliminate this possibility, because two APOB-specific siRNAs that target different regions of the APOB mRNA resulted in similar effects. Furthermore, control siRNA, although present in the liver and jejunum at levels comparable to those of therapeutic siRNA, had no effect. The authors were also able to establish, through an analysis of APOB degradation products, that an RNAi



mechanism of action was responsible for the experimental findings.

With the first demonstration of the *in vivo* silencing by siRNA of an endogenous gene through an RNAi mechanism — and using an administration route that could readily be applied to humans — now provided, RNAi looks set to gain further prominence as a research tool and potential therapeutic.

Daniel Jones

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## NEURODEGENERATIVE DISEASE

# Body-building for small molecules



Alzheimer's disease is the most common cause of dementia in the elderly, and, at present, there are no effective disease-modifying treatments. A key pathological feature of Alzheimer's disease is the build up of  $\beta$ -amyloid (A $\beta$ ) in the early phase, making this process a promising target for therapeutic intervention. However, developing small-molecule drugs that prevent the aggregation of A $\beta$  is highly challenging, because the binding energy that drives protein-protein interactions involved in such processes is often distributed over a large surface area. Reporting in *Science*, Graef and colleagues now describe an innovative strategy for creating molecules that overcome these difficulties.

The secret of this new strategy lies in the synthesis of small molecules that have dual functions. One end of the bifunctional molecule — which is small enough to reach its biological target — binds tightly to A $\beta$ ; the other end binds to a protein chaperone, thereby providing the necessary bulk

to prevent A $\beta$  aggregation. Graef and colleagues generated a compound that they dubbed SLF-CR, using Congo Red (CR), a small molecule that is known to function as an amyloid ligand, and a synthetic ligand for the FK506-binding protein (FKBP) chaperone family.

Several measures of inhibition showed that this molecule successfully blocked A $\beta$  accumulation when combined with FKBP, in contrast to SLF-CR alone or CR/FKBP, indicating that recruitment of the chaperone is crucial for inhibition of toxicity. More importantly, the authors showed that this molecule prevented neurotoxicity of aggregated A $\beta$  *in vitro*: A $\beta$  samples treated with SLF-CR/FKBP were considerably less toxic than untreated samples or those treated with CR/FKBP.

Morphological examination of cultured hippocampal neurons that were treated with SLF-CR/FKBP showed that this combination not only blocked the formation of aggregates, but also prevented changes in

## INFLAMMATION

# Nicotine goes into septic shock

Sepsis is the third leading cause of death in the developed world and accounts for 9% of the overall deaths in the United States every year. Well known for being a mild stimulant, nicotine is able to improve survival in mice with established sepsis, according to research published in *Nature Medicine*.

Local inflammation is an important defensive response to infection or injury. The process normally leads to recovery from infection and to healing. However, if targeted destruction and assisted repair are not properly co-ordinated, inflammation can lead to persistent tissue damage. The control of inflammation is complex, involving positive and negative feedback, and is sensitive to context. When an infection spreads to the bloodstream, inflammatory mediators that effectively contain local infections lead to an overwhelming systemic inflammatory response — sepsis — that causes multiple organ failure.

The central nervous system is able to rapidly and directly modulate the activity

of the immune system. This physiological mechanism is known as the neuronal anti-inflammatory system, and is mediated primarily by nicotinic acetylcholine receptors expressed on macrophages. Sensory fibres within the vagus nerve detect systemic inflammation; the brain responds with anti-inflammatory signals carried through efferent fibres of the vagus nerve, which terminates in most critical organs.

The high-mobility group box 1 (HMGB1) protein has recently been identified as a late-stage mediator of lethal systemic inflammation in sepsis, causing further stimulation of inflammatory mediators. In the current work, Ulloa and collaborators showed that acetylcholine, the principal neurotransmitter of the vagus, targets macrophages and other immune cells to inhibit the release of inflammatory mediators, such as tumour-necrosis factor- $\alpha$  and HMGB1.

Blocking the production of HMGB1 from macrophages could be a useful strategy to

combat sepsis. The authors demonstrate that nicotine, a more selective cholinergic agonist than acetylcholine, suppresses HMGB1 release from macrophages *in vitro*. Nicotine also reduces HMGB1 levels in a mouse model of sepsis. Most importantly, the chances of survival are improved in established sepsis even when treatment is initiated after the onset of disease. Nicotine exerts its anti-inflammatory effect via a 'nicotinic anti-inflammatory pathway' by signalling through the  $\alpha 7$  nicotinic acetylcholine receptor.

Nicotine has several side effects that make it unsuitable as a therapeutic, and so the authors are currently designing more specific nicotinic agonists to overcome these problems. The 'nicotinic anti-inflammatory pathway' could explain the therapeutic effect reported in clinical trials using nicotine for the treatment of ulcerative colitis, and suggests therapeutic potential for other inflammatory disorders.

Melanie Brazil

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cell morphology and cell death induced by A $\beta$  toxicity. Finally, the generation of a series of bifunctional molecules with the aim of improving potency led to the discovery of an inhibitor that had an IC<sub>50</sub> of ~50 nM — lower than that of CR by a factor of ~40, and of SLF-CR by a factor of ~6.

The development of further small molecules on the basis of this strategy might therefore lead to promising therapeutics for targeting an early, pre-symptomatic, pathological process in Alzheimer's disease. Moreover, this methodology might be generally applicable in other diseases in which it is desirable to target protein–protein interactions.

Alison Rowan

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KINASES 

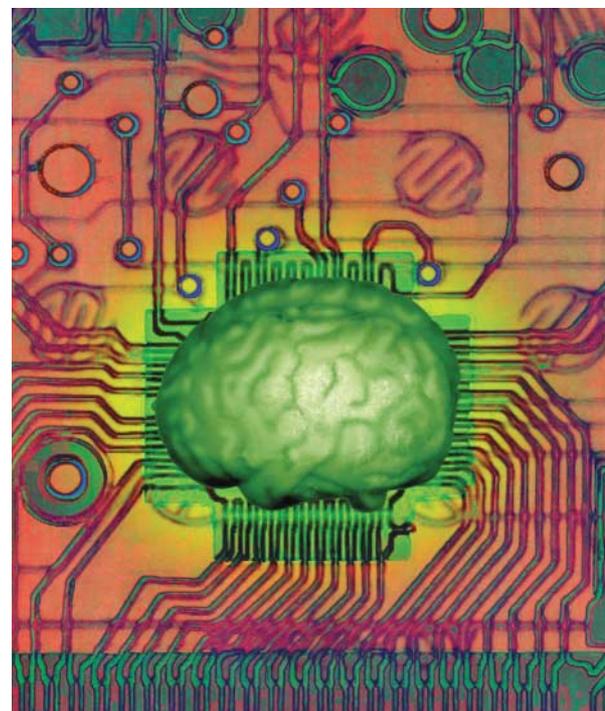
## Making gains on glioma

A small-molecule inhibitor of transforming growth factor- $\beta$  (TGF- $\beta$ ) receptor I kinase could prove to be an effective treatment for human gliomas, according to a recent paper in *Cancer Research*. The investigational drug, SD-208, was shown to antagonize the immunosuppressive and pro-migratory properties that TGF- $\beta$  exerts in cancer, and significantly prolonged the median survival of glioma-bearing mice.

Human glioblastoma is an aggressive form of brain cancer for which treatment options are limited and median patient survival is poor. TGF- $\beta$  has become a key target in the search for more effective drugs because it is one of several immunosuppressive molecules commonly expressed by glioma cells. Although several approaches to inhibit TGF- $\beta$  are being investigated, such as antisense and gene transfer, the identification of a small molecule that is more amenable to development as an oral drug would be desirable. Martin Uhl and colleagues report such a candidate molecule and describe its antagonistic effect on the biological activity of TGF- $\beta$  *in vitro* and *in vivo*.

TGF- $\beta$  exerts different biological effects depending on its cellular context and has roles in growth inhibition, immunosuppression and cell migration. The authors first determined whether SD-208 was an effective antagonist of TGF- $\beta$  *in vitro* by demonstrating that SD-208 was able to reverse TGF- $\beta$ -mediated growth inhibition of lung epithelial cells in a concentration-dependent manner. Moreover, SD-208 was able to neutralize the pro-invasive effect of TGF- $\beta$  observed in assays of cell migratory activity, indicating that SD-208 could possibly prevent the further invasion of gliomas *in vivo*.

Perhaps most remarkable, however, was the ability of SD-208 to restore an immune response against glioma cells in culture. The lytic activity of peripheral blood lymphocytes or purified T cells was enhanced by co-incubation with SD-208 in a similar way to that observed with TGF- $\beta$ -neutralizing antibodies, which confirms that the effect occurs through the inhibition of TGF- $\beta$ . Furthermore, the inhibition of pro-inflammatory cytokine release and natural killer cell activation caused by glioma cells was reversed in the presence of SD-208, whereas release of the immunosuppressive cytokine interleukin-10 was inhibited.



Having established the efficacy of SD-208 *in vitro*, Uhl and colleagues then went on to study its potential anticancer effect in glioma-bearing mice. Measurement of TGF- $\beta$ -dependent SMAD2 phosphorylation in mouse brain and spleen confirmed that SD-208 could inhibit TGF- $\beta$  activity *in vivo*. Moreover, this inhibition correlated with a delayed onset of neurological symptoms in SD-208-treated glioma-bearing mice compared with untreated controls, and also a significantly improved survival rate after 30 days. These results were reflected by histological analysis, which showed a marked increase in immune infiltration in SD-208-treated mice that had smaller tumours compared with those that had larger tumours, indicating that SD-208 could attenuate the immunosuppression caused by TGF- $\beta$  *in vivo*.

The authors conclude that treatment with TGF- $\beta$  receptor-kinase inhibitors could prove useful to treat gliomas either alone or in combination with existing antisense therapies. Furthermore, SD-208 represents a potential new treatment paradigm in which neutralizing the effect of immunosuppressive cytokines secreted from tumours could be successful as an anticancer strategy.

Joanna Owens

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#### WEB SITES

The Hertie Institute for Clinical Brain Research:  
<http://www.neurologie.uni-tuebingen.de>  
Scios, Inc.: <http://www.sciosinc.com/home>

## IN BRIEF

## KINASES

A minimalist approach to fragment-based ligand design using common rings and linkers: application to kinase inhibitors.

Aronov, A. M. & Bemis, G. W. *Proteins* **57**, 36–50 (2004)

Fragment-based approaches to lead discovery, in which low-affinity, low-molecular-mass chemical ‘fragments’ are combined to give high-affinity leads, are becoming increasingly popular. Aronov and Bemis present a novel fragment-based approach to the discovery of kinase inhibitors, which utilizes an analysis of the structures of ~40,000 known kinase inhibitors that indicates that their structures can be largely described by a small number of rings and linkers.

## BIOTECHNOLOGY

Antidote-mediated control of an anticoagulant aptamer *in vivo*.

Rusconi, C. P. *et al. Nature Biotechnol.* 17 Oct 2004 (doi:10.1038/nbt1023)

Current agents used to inhibit pathological blood clotting have to be carefully monitored to reduce the risk of serious bleeding. However, with the exception of heparin, no anticoagulants have a rapidly acting ‘antidote’ that can neutralize such side effects if they occur. Rusconi and colleagues describe an aptamer targeted at coagulation factor IXa, which has anticoagulant effects in animals that can be rapidly reversed using a rationally designed oligonucleotide antidote.

## LUNG DISEASES

Imatinib mesylate inhibits the profibrogenic activity of TGF- $\beta$  and prevents bleomycin-mediated lung fibrosis.

Daniels, C. E. *et al. J. Clin. Invest.* **114**, 1308–1316 (2004)

Profibrotic cytokines, such as transforming growth factor- $\beta$  (TGF- $\beta$ ) and platelet-derived growth factor (PDGF), are thought to be important in the pathogenesis of idiopathic pulmonary fibrosis (IPF), a progressive and fatal fibrotic disease of the lungs. Daniels *et al.* provide data that indicate that fibroblasts respond to TGF- $\beta$  by stimulating c-ABL kinase activity. Furthermore, imatinib (Gleevec; Novartis), which inhibits ABL family kinases and the PDGF receptor tyrosine kinase, was shown to obviate fibrotic changes in a mouse model of pulmonary fibrosis, and so could be a potential therapy for IPF.

## ANTITHROMBOTIC DRUGS

Disruption of protein–membrane binding and identification of small-molecule inhibitors of coagulation factor VIII.

Spiegel, C. P. *et al. Chem. Biol.* **11**, 1413–1422 (2004)

Highly specific interactions between proteins and lipid membranes are essential in many biological pathways and processes; for example, the interaction between factor VIII and the membranes of platelets has a key role in blood coagulation. Spiegel and colleagues have developed an enzyme-linked-immunosorbent-assay (ELISA)-based high-throughput screen, which they used to identify small molecules that inhibit the binding of factor VIII to lipid membranes. These compounds could be leads for the development of novel antithrombotic drugs.



## ANTIVIRAL DRUGS

## Inside the envelope

Drugs that target key enzymes in the life cycle of human immunodeficiency virus (HIV) have revolutionized the treatment of HIV in the past decade. Nevertheless, drug resistance remains a major problem. Writing in *Chemistry and Biology*, Schiffer and colleagues propose a novel structure-based strategy for combating drug resistance, using HIV-1 protease — for which several active-site inhibitors are approved — as an example.

HIV-1 protease cleaves the HIV Gag and Pol precursor proteins in at least nine different locations to allow viral maturation. It was originally speculated that this multiplicity of enzyme substrates would make the development of resistance to protease inhibitors unlikely, because the protease would be unable to accommodate mutations necessary to decrease the affinity of drug binding in its active site without seriously compromising its ability to bind at least one of its substrates. However, this has not turned out to be the case.

So, to understand how drug resistance can emerge while the protease retains its ability to recognise its substrates, the authors determined the crystal structures of an inactive variant of HIV-1 protease in complex with six of its known substrates. Using computational analysis to assess the overlap between the volumes in the active site occupied by these substrates, they identified an ‘envelope’ within the active site that apparently must be made available for substrate binding.

An analogous analysis with HIV-1 protease in complex with eight different inhibitors also revealed an envelope within the active site occupied by inhibitors. However, overlaying the ‘inhibitor envelope’ and the ‘substrate envelope’ revealed several regions where the inhibitor envelope protrudes beyond the substrate envelope. And crucially, many of the residues that have been reported to mutate in drug-resistant HIV-1 strains contact these regions.

It therefore seems that if future drug design focuses on developing inhibitors that interact strongly only with residues within the substrate envelope of HIV-1 protease, the chances of drug resistance developing would be reduced. Indeed, recent inhibitors indicate that this might be possible. Furthermore, this strategy could also be extended to other enzymes important in viral pathogenesis, such as the serine protease of hepatitis C virus.

Peter Kirkpatrick

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