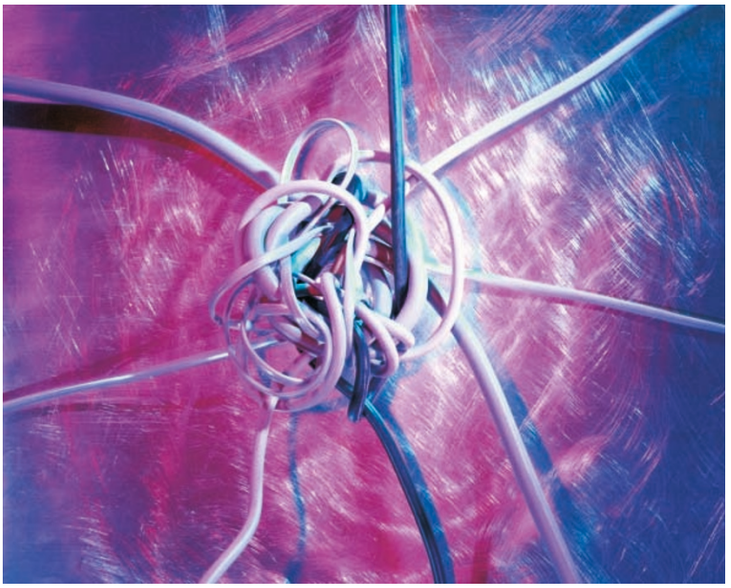
NEURODEGENERATIVE DISEASES

Tying up loose ends



It's always a bonus for researchers when apparently separate pathways for pathogenetic mechanisms in fact conveniently and neatly intertwine. A report by Stuart Lipton and colleagues in *Science* shows how two proposed mechanisms for nerve-cell death are intimately linked, and should therefore help to direct therapies aimed at treating neurological disorders.

It had been known that the nerve-cell damage that triggers their demise during stroke, Alzheimer's disease and other neurodegenerative diseases not only occurs from inside the cell but externally too. The best-characterized nerve-cell-death pathways occur inside cells, but in terms of external mechanisms, two observations had previously been made — levels of matrix metalloproteinases (particularly MMP9) are elevated in neurodegenerative disorders, and nitric oxide (NO) can modulate the activity of proteins (in a manner that can be described as being analogous to phosphorylation) by reacting with cysteine thiols to form an *S*-nitrosylated derivative.

Now, Lipton and colleagues show that these two events are linked. They found that NO switches on the overexpression of MMP enzymes, which, in turn, chew up the environment that surrounds nerve cells.

Initial *in vitro* analysis showed that NO can directly activate MMP9 and induce neuronal apoptosis. To confirm their results *in vivo*, Lipton and colleagues used mass spectrometry to characterize the events during focal ischaemia (the lack of blood supply owing to the occlusion of an artery) and reperfusion (the return of blood to an ischaemic region, which is also accompanied by tissue damage). This showed that MMP9 is activated by *S*-nitrosylation of a cysteine residue followed by further oxidation to a sulphinic- or sulphonic-acid derivative. This latter step is particularly interesting, as it is irreversible, which would explain the permanent pathophysiological activation of

THERAPEUTIC PROTEINS

High affinity good, lower affinity better

When proteins bind their cognate receptors on the cell surface, signalling cascades are set in motion. The cytokine granulocyte colony-stimulating factor (GCSF) stimulates the proliferation of certain immune cells and is used to treat cancer patients who have low levels of white blood cells as a result of cytotoxic drugs. Unfortunately, the value of such therapeutic proteins is limited because of rapid clearance by receptor-mediated endocytosis and consequent protein degradation. In the September issue of *Nature Biotechnology*, scientists from the Massachussetts Institute of Technology (MIT) and Amgen used computer modelling and protein chemistry to engineer GCSF variants with lower receptor affinity that are as biologically potent as wild-type GCSF, but remain active for longer.

Activation of a cytokine receptor causes internalization of the receptor–ligand complex by the cell and results in delivery of the complex to acidified endosomal compartments. At this point, the cytokine ligand is either sent to lysosomal organelles, where the protein is degraded, or it is routed to recycling vesicles, which transport the cytokine out of the cell. What determines the fate of the cytokine–receptor complex in the endosome? Evidence indicates that when the receptor–ligand complex dissociates in the endosome, both the ligand and receptor are more likely to be recycled to the cell surface. Recycling preserves the structural integrity and biological activity of the ligand, and leads to an increase in the amplitude of the signal from a given concentration of ligand.

Sarkar *et al.* postulated that if they could increase the recycling of GCSF and its receptor without disrupting the ligand–receptor complex at the cell surface, the efficacy and potency of GCSF could be improved. Exploiting the pH difference in the endosome compared with the cell surface, the authors set out to create a cytokine that might have lower receptor affinity at a more acidic pH.

Using the solved crystal structure of the interactions between GCSF and its receptor, a series of sites were identified at the ligand–receptor interface where the presence of a positive charge would destabilize the complex. Introducing histidine residues at these sites by site-directed mutagenesis resulted in a neutral charge at the cell surface and a positive charge in the endosome. This allowed pH-dependent modulation of the affinity of the complex, so that the GCSF variants bound to the receptor at the cell surface with equal affinity to that of the wild type, but with much lower affinity at the lower endosomal pH. As the authors had predicted, *in vitro* assays showed that the variants were depleted less quickly from the growth medium and were more effective in promoting cell proliferation.

It is not yet clear whether the surface histidine mutations alter the antigenic effects of the protein, or its stability, and these engineered GCSF variants still need to be tested in an animal model. However, these variants show the validity of the approach, which could be widely applied to other protein ligands, whose efficacy is enhanced by increasing recycling back to the cell surface.

Melanie Brazil

References and links

ORIGINAL RESEARCH PAPER Sarkar, C. A. *et al.* Rational cytokine design for increased lifetime and enhanced potency using pH-activated "histidine switching". *Nature Biotechnol.* **20**, 908–913 (2002)

FURTHER READING Kangas, E. & Tidor, B. Optimizing electrostatic affinity in ligand-receptor binding: theory, computuation, and ligand properties. *J. Chem. Phys.* **109**, 7522–7545 (1998) | Lauffenburger, D. A., Fallon, E. M. & Haugh, J. M. Scratching the (cell) surface: cytokine engineering for improved ligand/receptor trafficking properties. *Chem. Biol.* **5**, R257–R263 (1998)

WEB SITES

Lauffenberger's laboratory: http://web.mit.edu/cbe/dallab/

Tidor's laboratory: http://web.mit.edu/tidor/

MMP9 that has been observed in cerebral ischaemia and reperfusion.

The authors say that this NO-activated MMP mechanism "confers responsiveness of the extracellular matrix to nitrosative and oxidative stress", which are found in several conditions, including cerebral ischaemia and neurodegenerative diseases. The extracellular proteolytic cascades that are triggered by MMPs can disrupt the extracellular matrix, contribute to cell detachment and lead to anoikis (apoptosis due to cell detachment from the substrate). So, the authors conclude that preventing NO-activated MMP activity could be a novel way of tackling neurodegenerative diseases.

Simon Frantz

References and links

ORIGINAL RESEARCH PAPER Lipton, S. A. *et al.* *S*-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. *Science* **297**, 1186–1190 (2002)

FURTHER READING Yong, V. W., Power, C., Forsyth, P. & Edwards, D. R. Metalloproteinases in biology and pathology of the nervous system. *Nature Rev. Neurosci.* **2**, 502–511 (2001)

STRUCTURE-BASED DRUG DESIGN

Reiterating the point

The cyclin-dependent kinases (CDKs) have an important role in controlling the cell cycle. Aberrant CDK activity is a common defect in human tumours, which makes CDKs important targets for therapeutic intervention in cancer, and CDK inhibitors are in clinical trials at present. In the October issue of *Nature Structural Biology*, Davies *et al.* show that structure-based development and iterative biological evaluation can be used to optimize a CDK inhibitor rapidly, resulting in nanomolar potency that is 1,000-fold greater than the parent compound.

Knowledge of the structure of CDK2 has been key in driving the design and development of a large number of ATP competitive inhibitors. The ATP-binding site of CDK2 is located between the two domains of the kinase, and is best described as a hydrophobic 'slot'. Contained within this cleft are various sub-sites, which could be probed by inhibitors, some of which are not explored by ATP itself. The novel ATP-competitive, purine-based inhibitor O^6-cyclohexylmethyl guanine (NU2058) inhibits CDK1 and CDK2, but has no activity against CDK4. To optimize NU2058, the structure of the activated CDK2–cyclin A complex bound to NU2058 was determined. NU2058 forms a triplet of hydrogen bonds between its purine ring and the active site of CDK2. The purine ring also makes several van der Waals interactions and hydrophobic contacts with the ATP-binding cleft of CDK2. The O^6 group sits in the ATP-ribose binding site and forms highly complementary packing and hydrophobic interactions with an apolar pocket in a glycine-rich loop of CDK2.

Structure–activity relationships for the O^6 position show that a cyclic hydrophobic group, such as cyclohexylmethyl, is optimal, so this group was maintained. The binding of CDK inhibitors to CDK2, compared with the natural product indirubin, indicated that the addition of functional groups at the 2-amino (N2) position of NU2058 would increase potency. Groups added at this position would project out of the ATP-binding cleft and contact the 'specificity surface' of CDK2. The greater sequence variation of the specificity surface compared with other parts of the active site (which are highly conserved in all kinases) indicates that targeting it might afford inhibitor specificity as well as potency. NU6094 contained an anilino group at the N2 position, and had a tenfold increase in affinity for CDK2 over the parent, NU2058. To further increase the potency, a sulphonamide group was introduced at the anilino *para* position of NU6094, in an attempt to form an extra hydrogen bond. The resulting compound, NU6102, was a highly potent CDK2 inhibitor.

The crystal structure of activated CDK2–cyclin A complexed with NU6102 shows the interactions formed and explains the tight binding. The purine ring forms the usual triplet of hydrogen bonds with the CDK2 active site. However, although the anilino group packs closely to the specificity surface, the sulphonamide does not form the designed hydrogen bond with CDK2. Instead, the increased potency arises from the formation of two other hydrogen bonds, which facilitate optimum hydrophobic packing of the anilino group with the surface of CDK2.

In cellular studies, NU6102 inhibited growth of a human breast-carcinoma cell line. It also inhibited phosphorylation of downstream CDK target proteins, which is consistent with CDK1 and CDK2 inhibition. This is the first example of using an activated complex to direct iterative synthesis.

Melanie Brazil

References and links

ORIGINAL RESEARCH PAPER Davies, T. G. *et al.* Structure-based design of a potent purine-based cyclin-dependent kinase inhibitor. *Nature Struct. Biol.* 2002 Oct (doi: 10.1038/nsb842)

FURTHER READING Arris, C. E. *et al.* Identification of novel purine and pyrimidine cyclin-dependent kinase inhibitors with distinct molecular interactions and tumor cell growth inhibition profiles. *J. Med. Chem.* **43**, 2797–2804 (2000) | Nabel, E. G. CDKs and CKIs: molecular targets for tissue remodelling. *Nature Rev. Drug Discov.* **1**, 587–598 (2002)

WEB SITE

Jane Endicott's laboratory:
http://biop.ox.ac.uk/www/lj2001/endicott/endicott.html