INFORMATICS

Exploring pharmacological space

What can we learn from the integration of vast collections of pharmacological data? Can we rationally expand the search for new drugs beyond compounds that affect single targets in the 'druggable genome'? Insight into key questions such as these has been provided by a recent extensive analysis, described in *Nature Biotechnology*, which integrates several large sources of structure–activity relationship (SAR) data from medicinal chemistry efforts.

Although databases containing protein sequence and structure data are well established and widely available, analogous resources for SAR

data are at a much earlier stage, and at present most such data are widely spread among research papers, patents and proprietary screening databases. So, Paolini *et al.* set out to create a single integrated database, including ~4.8 million compounds and ~600,000 SARs (for example, IC_{50} values) for ~3,000 protein targets from published and proprietary sources, with the aim of better understanding 'pharmacological space' — the parts of chemical space that contain biologically active small molecules.

In total, the authors identified 529 proteins encoded in the human genome for which there is at least one compound with a binding affinity <100 nM that also satisfies the Lipinski rule-of-five (the standard criteria for likely oral drug absorption). At least one small-molecule 'chemical tool' (binding affinity <10 μ M, but not necessarily satisfying the rule-of-five) has been identified for 836 human proteins.

Several issues that could have considerable significance for future drug discovery efforts were also investigated by the authors. For example, they show how a large-scale integrated chemogenomics database can be used to build Bayesian models to predict compound activity across a virtual screening bank of more than 600 protein targets. Furthermore, the authors propose a refinement of the concept of druggability beyond Lipinski's rule-of-five, by considering

how the ligand properties of known active compounds for protein targets can be used to derive probabilistic approaches to determining 'degrees' of druggability, relative to oral drug space.

The analysis also highlights the potential for more rational investigation of an issue that has attracted much interest in drug discovery recently: the notion of polypharmacology or 'dirty drugs'. So far, such drugs, whose therapeutic activity is thought to depend on their ability to modulate multiple targets — for example, neurotransmitter receptors in the case of several major antipsychotics — have had a fairly limited amount of rational input in their discovery. However, the mapping of polypharmacology networks as described by Paolini *et al.* should facilitate the development of rational design strategies for selectively promiscuous drugs. And overall, increased application of predictive models based on such analysis in the assessment of other important factors in drug discovery such as druggability and attrition risks could lead to significant improvement in the success rate of the process.

Peter Kirkpatrick

ORIGINAL RESEARCH PAPER Paolini, G. V. *et al.* Global mapping of pharmacological space. *Nature Biotechnol.* **24**, 805–815 (2006)

FURTHER READING Lipinski, C. & Hopkins, A. Navigating chemical space for biology and medicine. *Nature* **432**, 855–861 (2004) | Hopkins, A. L. & Groom, C. R. The druggable genome. *Nature Rev. Drug Discov.* **1**, 727–730 (2002)

RESEARCH HIGHLIGHTS ADVISORS

ERIK DE CLERCQ
Katholieke Universiteit Leuven, Belgium
RODERICK FLOWER
William Harvey Research Institute, QMW,
London, UK
YOSHIMI FUJITA
Clinical Proteome Center, Tokyo Medical
University

F. PETER GUENGERICH
Vanderbilt University Nashville, TN, USA
FRANZ HEFTI
Rinat Neuroscience Corporation, Palo Alto,
CA, USA
JOAN HELLER BROWN
University of California San Diego,
CA, USA

MADS KROGSGAARD THOMSEN
Novo Nordisk, Bagsvaerd, Denmark
HUGO KUBINYI
University of Heidelberg, Germany
ROBERT LANGER Massachusetts Institute of
Technology Cambridge, MA, USA
JULIO LICINIO University of California Los
Angeles, CA, USA

CHRISTOPHER LIPINSKI
Pfizer Global Research and Development,
Groton, CT, USA
TOMI SAWYER
Ariad Pharmaceuticals, Cambridge, MA, USA
JANET WOODCOCK
Food & Drug Administration, Rockville,
MD, USA

NEUROLOGICAL DISORDERS

New neurons repair Parkinson's brain

The idea that endogenous progenitor cells might be harnessed to replace neurons lost in neurodegenerative diseases is popular, but requires the development of methods to stimulate their proliferation and differentiation. Hopes for this approach have now received a boost from a study by Van Kampen and Eckman, showing that activation of a particular dopamine receptor subtype stimulates neurogenesis and functional repair in a Parkinson's disease model.

Parkinson's disease involves the degeneration of dopaminergic neurons in the nigrostriatal tract, which projects from the substantia nigra pars compacta (SNc) in the midbrain to the striatum and is essential for the control of movement. Current treatments that boost striatal dopamine levels have adverse side effects, lose efficacy over time and do not alter the underlying pathology, making cell-replacement strategies desirable. Utilizing endogenous progenitor cells for this purpose could have advantages over invasive transplantation strategies.

Encouragingly, such progenitors have been identified in the SNc, and previous work has demonstrated that activation of D₃ dopamine receptors stimulates neurogenesis in this region in healthy adult rats.

The new study demonstrates the effects of a preferential D₃ agonist, 7-OH-DPAT, in a common rat model of Parkinson's disease, the 6-hydroxydopamine model. By labeling cells undergoing DNA synthesis, the researchers showed that chronic drug treatment increased proliferation in the SNc. Many new-born cells subsequently expressed proteins typically found in mature dopaminergic neurons. To achieve functional recovery and repair of the nigrostriatal tract, these newly generated neurons must generate new projections to the striatum. This was assessed by injecting a fluorescent tracer into the striatum. After retrograde transport of the tracer to the SNc, increased numbers of neurons with axons projecting along this tract were revealed. Finally, two different behavioural tests

KINASE INHIBITORS

Stressed out hearts

Imatinib mesylate (Gleevec) potentially inhibits the kinase activity of the oncogenic fusion protein BCR-ABL and is an effective treatment for chronic myeloid leukaemia. Although imatinib seems to be well tolerated by most patients, more than 60% of patients on imatinib in clinical trials develop peripheral oedema, a possible sign of cardiotoxicity. However, heart function was not assessed in any of the clinical trials with this agent. Thomas Force and colleagues report that 10 patients who previously had normal heart function developed severe heart failure after the initiation of imatinib therapy. They found that imatinib is not directly toxic, but that the inhibition of one of its targets, ABL, triggers the stress response in cardiomyocytes and induces cell death.

Myocardial biopsy samples taken from patients showed prominent membrane whorls in the myocytes, an abnormality that is a characteristic of toxin-induced myopathies. The cells also showed pleomorphic mitochondria

“These findings suggest that clinical trials of new agents that target ABL should prospectively assess left ventricular function.”

and dilated endoplasmic reticulum (ER), which are signs of stress.

To investigate this further, the authors treated healthy mice with imatinib and saw similar structural changes in the mouse hearts. Doses of imatinib given to produce blood concentrations comparable to those in humans led to left ventricular dysfunction. Imatinib seemed to cause necrotic cell death (as seen by the dose-dependent collapse of mitochondrial membrane potential, release of cytochrome *c* and pronounced cytosolic vacuolization) rather than apoptosis. Gene transfer of an imatinib-resistant mutant of ABL inhibited the imatinib-induced release of cytochrome *c* and conferred protection against cell death. This suggests that the inhibition of ABL by imatinib is the mechanism of cardiomyocyte toxicity.

What are the mechanisms that regulate imatinib-induced cardiomyocyte death? Because of the observed ER dilation, Force and colleagues investigated the ER stress-response pathway and found that imatinib activated both the EIF2 α and IRE1 parts of this pathway in imatinib-treated mice. Although this response is initially protective, if the inducing stress to cells is not relieved — as occurs with

prolonged imatinib therapy — IRE1 can signal cell death by activating the pro-death JNK pathway. The authors found that JNK was activated in the hearts of imatinib-treated mice and that this activation was reduced by treatment with salubrinol, a small-molecule inhibitor of EIF2 α dephosphorylation. Furthermore, the inhibition of either EIF2 α dephosphorylation with salubrinol or JNK activity with a peptide inhibitor rendered cardiomyocytes resistant to the imatinib-induced collapse of mitochondrial membrane potential and cell death. Therefore, the imatinib-mediated induction of the ER stress-response pathway leads to cell death through the activation of JNKs.

These findings not only suggest that patients who are on imatinib should be monitored closely for signs of left ventricular dysfunction, but also that clinical trials of new agents that target ABL should prospectively assess left ventricular function so that rates of cardiotoxicity can be determined.

Ezzie Hutchinson,
Nature Reviews Cancer

ORIGINAL RESEARCH PAPER Kerkelä, R. et al. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nature Med.* **12**, 908–916 (2006)

were used to demonstrate recovery of motor function in the treated animals. These effects lasted several months after treatment ended, providing further evidence of changes in the underlying neuronal pathways.

These findings show that endogenous progenitor cells can be stimulated to contribute to the functional repair of damaged neuronal tracts, providing hope for neurodegenerative disease therapy and giving further support for targeting the D_3 receptor as a therapeutic strategy for Parkinson's disease. Several D_3 receptor agonists are already in use for Parkinson's disease, which could encourage the development of similar therapeutics and lead to a better understanding of the underlying molecular pathways, revealing further therapeutic targets.

Katherine Whalley

ORIGINAL RESEARCH PAPER Van Kampen, J. M. & Eckman, C. B. Dopamine D_3 receptor agonist delivery to a model of Parkinson's disease restores the nigrostriatal pathway and improves locomotor behaviour. *J. Neurosci.* **26**, 7272–7280 (2006)

CANCER

Stress can fuel tumour angiogenesis

The possible effects of stress on cancer are a matter of hot debate. While most studies investigating this connection focus on the dampening effects of stress on the immune response to arising tumours, a more direct link has now been uncovered. Reporting in *Nature Medicine*, Thaker *et al.* demonstrate that stress hormones can accelerate cancer progression by affecting tumour angiogenesis, thereby enhancing tumour growth and metastasis, in mouse models of ovarian cancer.

To investigate the stress–cancer link, immune-deficient ‘nude’ mice were inoculated into the peritoneal cavity with human ovarian cancer cell lines after one week of experimental stress. This was induced using a physical restraint system, in which periodic immobility induces high levels of hypothalamic–pituitary–adrenal and sympathetic nervous system activity that is characteristic of chronic stress. Two weeks after tumour inoculation, there was a marked difference in tumour aggressiveness: stressed mice had two- to threefold more tumour nodules, with similar increases in tumour weight gain, compared with ‘unstressed’ controls. Furthermore, whereas in control mice tumours were confined to the peritoneal cavity, tumours had spread to the liver and spleen in 50% of the stressed mice. Similar experiments with a different ovarian cancer cell line and in an orthotopic breast cancer model showed that these stress effects were evident in a wide range of tumour cell lines.

At the molecular level, this effect was found to be conveyed by stress-induced tissue catecholamines activating β_2 -adrenoreceptors on tumour cells, a receptor overexpressed in most ovarian cancer cell lines. The effect of stress could be mimicked with β_2 -adrenoceptor agonists, and blocked with the beta-blocker propranolol. Natural

β_2 -adrenoceptor-deficient ovarian cancer cell line variants, and tumour cells in which the β_2 -adrenoceptor was knocked down by small interfering RNA, were ‘immune’ to the stress effect, further confirming the central importance of this receptor. Downstream of the β_2 -adrenoceptor, it was found that catecholaminergic stimulation induces expression of vascular endothelial growth factor (VEGF) via a cyclic AMP–protein kinase A-dependent pathway. The resulting elevated levels of VEGF led to increased tumour vascularization — and the ovarian cancer cells seem to exploit this pathway to boost their own blood supply.

Although the effects of stress on immunological, neurochemical and endocrinological functions are well known, this is the first report to demonstrate that the neuroendocrine stress response can also directly affect the growth and activity of malignant tissue via hormone receptors on tumour cells. If this mechanism is confirmed in the human setting, it could have exciting therapeutic implications for the management of ovarian cancer and possibly other types of cancer — especially as beta-blockers are safe and readily available. The authors also speculate that other neuroendocrine signalling pathways might modulate tumour cell biology under other circumstances, implying that interventions targeting neuroendocrine function at the level of the CNS could represent a new strategy to protect patients from the detrimental effect of stress on the progression of malignant disease.

Alexandra Flemming

ORIGINAL RESEARCH PAPER Thaker, P. H. *et al.* Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nature Med.* **12**, 939–944 (2006).

IN BRIEF

OBESITY

Neuronal PTP1B regulates body weight, adiposity and leptin action.

Bence, K. K. *et al. Nature Med.* 16 July 2006 (doi:10.1038/mm1435)

Protein tyrosine phosphatase (PTP) 1B is a potential therapeutic target for obesity and type 2 diabetes. To investigate how PTP1B affects adiposity, Bence and colleagues generated mice with tissue-specific deletions of *Ptp1b* in brain, muscle, liver or fat. Only mice lacking neuronal PTP1B were resistant to high-fat diet-induced obesity and were protected from developing leptin resistance. Neuronal PTP1B also regulated adipocyte leptin production and insulin sensitivity independent of changes in body weight. So, it seems for effective obesity and type 2 diabetes therapy, PTP1B inhibitors should be directed to the brain.

ANTI-INFECTIVES

The protozoan inositol phosphorylceramide synthase; a novel drug target which defines a new class of sphingolipid synthase.

Denny, P. W. *et al. J. Biol. Chem.* 22 July 2006 (doi:10.1047/jbc.M600796200)

Infections caused by kinetoplastid protozoan parasites are prevalent in developing countries, but treatments are often toxic. Denny and colleagues identified and characterized a protozoan inositol phosphorylceramide (IPC) synthase, an enzyme essential for the synthesis of cell-membrane sphingolipids. Because this enzyme has no mammalian equivalent, it raises the possibility of developing antiprotozoal drugs with reduced side effects.

BIOTECHNOLOGY

Stem cell-derived erythroid cells mediate long-term systemic protein delivery.

Chang, A. H. *et al. Nature Biotechnol.* 16 July 2006 (doi:10.1038/nbt1227)

Chang and colleagues have developed a novel method of long-term, systemic therapeutic protein delivery by exploiting the globin-synthesis system in erythroid cells. By targeting human clotting factor IX expression to late-stage erythropoiesis, high-level secretion of clotting factor IX was achieved in a murine model of haemophilia B, resulting in phenotypic correction of the coagulation disorder. Advantages of this method include resistance to transcriptional silencing, induction of immune tolerance and a reduction of the risk of insertional oncogenesis.

TARGET VALIDATION

Expression of mammalian GPCRs in *C. elegans* generates novel responses to human ligands.

Teng, M. S. *et al. BMC Biol.* 20 July 2006 (doi:10.1186/1741-7007-4-22)

Current *in vitro* methods used to study mammalian G-protein-coupled receptor (GPCR)-ligand interactions might not accurately reflect *in vivo* interactions. Teng and colleagues have developed a simple *in vivo* method to study and screen GPCR ligands using the nematode *Caenorhabditis elegans*. In an avoidance assay, *C. elegans* expressing either mammalian somatostatin-2 receptor (Sstr2) or chemokine-5 receptor in gustatory neurons were able to detect and respond to appropriate agonists. Pre-exposure to ligand led to receptor desensitization and behavioural adaptation, and structure-function relationships could be established for Sstr2 ligands.

CARDIOVASCULAR DISEASE

SNP to the rescue?

Can a common single nucleotide polymorphism (SNP) have sufficient impact to affect therapeutic targeting in heart disease? This possibility has recently been raised in a paper by Liggett and colleagues, who showed that a polymorphism in the β_1 -adrenergic receptor (β_1 AR) altered cardiac function and response to the adrenergic receptor antagonist bucindolol in human heart failure.

Clinical trials of β_1 AR antagonists in heart failure have often revealed substantial interindividual variability in outcomes, which the authors considered could be due to differences in the β_1 AR gene. Indeed, the Liggett group had previously found a common non-synonymous SNP that results in either Arg or Gly being encoded at amino-acid position 389 of the β_1 AR. Compared with Gly- β_1 AR, Arg- β_1 AR displayed increased stimulation of adenylyl cyclase in transfected fibroblasts, enhanced contractility in transgenic mouse hearts and caused a larger increase in left ventricular ejection fraction after β_1 AR antagonist administration in human studies.

The authors therefore set out to investigate the relevance of this SNP for β_1 AR antagonist therapy in heart failure by using bucindolol, a drug that has previously failed to show efficacy in clinical trials. Ventricular tissue from failing and non-failing human hearts was used to investigate the impact of β_1 AR genotype on contractile responses. In both heart types, Arg homozygotes displayed fourfold larger agonist-promoted contractility than Gly carriers. In transfected fibroblasts expressing β_1 ARs of either genotype, those expressing the Arg- β_1 AR displayed a substantially greater degree of noradrenaline-stimulated cAMP accumulation, and bucindolol showed a larger absolute cAMP-lowering effect in these cells compared with those expressing the Gly- β_1 AR.

To understand the clinical implications of the β_1 AR SNP, the authors undertook genotyping of the β_1 AR allele in patients who had taken part in a large placebo-controlled clinical trial of bucindolol for the treatment of moderate and severe heart failure. Each patient cohort consisted of >200 subjects, grouped by treatment and genotype. No outcome was associated with genotype in the placebo group, indicating little impact on the natural course of heart failure. However, Arg homozygotes treated with bucindolol had an age-, sex- and race-adjusted 38% reduction in mortality and 34% reduction in mortality or hospitalization compared with placebo. In contrast, Gly carriers had no change in clinical response to bucindolol compared with placebo. The therapeutic advantage of Arg homozygotes was due to the degree of adrenergic activity that could be antagonized by bucindolol.

Although it has yet to be shown whether these results can be extrapolated to other β_1 AR antagonists with differing pharmacological properties to bucindolol, this study has shown that a drug that was not efficacious in a mixed patient population displayed greater efficacy in patients of a specific genotype, and so could set the stage for genetic-based treatments in heart failure.

Charlotte Harrison

ORIGINAL RESEARCH PAPER Liggett, S. B. *et al.* A polymorphism within a conserved β_1 -adrenergic receptor motif alters cardiac function and β -blocker response in human heart failure. *Proc. Natl Acad. Sci. USA* 103, 11288–11293 (2006)

FURTHER READING Weinshilboum, R. & Wang, L. Pharmacogenomics: bench to bedside. *Nature Rev. Drug Discov.* 3, 739–748 (2004)