PERSONALIZED MEDICINE

Predicting predisposition

> It is unlikely that personalized medicine will be enabled for a wide range of major diseases using genetic knowledge alone.

Much has been made of the potential of pharmacogenetics to identify the genetic variation underlying differences in drug responses between individuals and how this information can be used to improve drug safety and efficacy. However, a particular drug-response phenotype is not determined solely by the genotype — it is also influenced by environmental factors such as nutrition, concurrent medication, underlying disease and age. Jeremy Nicholson and colleagues speculate that it is therefore unlikely that personalized medicine will be enabled for a wide range of major diseases using genetic knowledge alone, and describe in *Nature* a proof-of-principle 'pharmaco-metabonomic' study of paracetamol (acetaminophen) metabolism and toxicity in which they were able to predict an aspect of paracetamol metabolism from the pre-dose metabolic signatures of rats.

Nicholson and colleagues originally came up with the concept of pharmaco-metabonomics — defined as "the prediction of the outcome of a drug or xenobiotic intervention in an individual based on a mathematical model of pre-intervention metabolite signatures" — after finding that rats given galactosamine hydrochloride fell into 'responder' and 'non-responder' groups, which could be distinguished by their pre-dose urinary metabolite profiles.

To investigate this concept further, the authors obtained pre- and post-dose urinary metabolite profiles and post-dose liver mean histology scores from 65 rats given a single toxic-threshold dose (600 mg per kg body weight) of paracetamol. The amounts of the urinary paracetamol metabolites were determined and the variation in these data was modelled in relation to the variation in the pre-dose metabolite profiles. The authors also modelled the variation in the mean histology scores relative to the variation in the pre-dose metabolite profiles in an attempt to predict histological outcome from the pre-dose metabonomic data.

The most convincingly predicted drug metabolite parameter was found to be the mole ratio of paracetamol glucuronide to paracetamol (G/P). The authors therefore built and validated a mathematical model (known as a projection to latent structure) that enabled them to predict expected G/P values from individual pre-dose metabolite profiles.

Although the authors did not produce a fully validated model for predicting post-dose histology, principal components analysis revealed a statistically significant association between the nature of the pre-dose metabolite profile and the extent of the induced liver damage, which was demonstrated by assigning rats into histological classes 1–3, with 3 representing the highest degree of liver damage. In particular, a higher pre-dose level of taurine was associated with a lower severity of liver injury, whereas a higher combined pre-dose level of trimethylamine-*N*-oxide (TMAO) and betaine was associated with a greater degree of liver damage.

More research is needed, but this initial study demonstrates relationships between the nature of the pre-dose metabolite profile and two independent post-dose parameters, and provides validation of the concept of pharmaco-metabonomics. In addition to being used in drug and dose selection in the clinic, where the aim would be to increase drug efficacy and to minimise adverse reactions, metabonomic signatures might be a more reliable method of extrapolating toxicity tests from animals to humans in preclinical development, and could aid the discovery of safety biomarkers for new drugs.

Joanna Owens

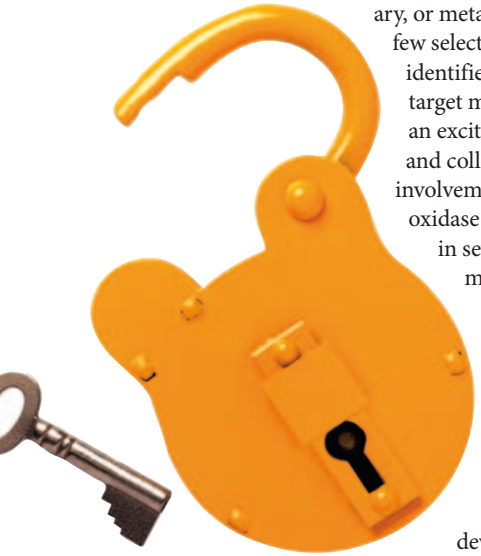
ORIGINAL RESEARCH PAPER Clayton, T. A. *et al.* Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature* **440**, 1073–1077 (2006)

WEB SITE

Jeremy K. Nicholson's lab: http://www3.imperial.ac.uk/people/j.nicholson

CANCER

Blocking LOX prevents cancer spread



The main cause of mortality among cancer patients is generally secondary, or metastatic, tumours, but few selective drugs have been identified that specifically target metastasis itself. Now, an exciting report from Erler and colleagues describes the involvement of the monoamine oxidase lysyl oxidase (LOX) in several stages of the migration of malignant cancerous cells from their primary tumour formation site, establishing a new therapeutic target for preventing and treating metastases.

Whether metastases develop is determined by the interactions between various factors, but it is not always clear how these factors contribute to the mechanisms that drive this multi-component process. Cancer cells that are capable of spreading throughout the body often originate and can thrive in hypoxic (low oxygen) environments, and hypoxic tumours are clinically linked to poor patient outcome. Previous investigations of hypoxic tumour physiology established a link between hypoxia and elevated LOX expression. Building on these findings, Erler and colleagues analysed breast, head and neck cancer studies, and found that hypoxic breast cancer cells had elevated levels of LOX expression, with a lower probability of survival for those patients whose tumour cells expressed higher levels of LOX.

To investigate the therapeutic potential of blocking LOX activity, the authors implanted mice with tumours grown from human breast cancer cells engineered to produce significantly less LOX than normal cells. Metastatic cancer cells were detected in the lungs and liver of control animals that received wild-type tumours. However, mice that received modified tumours expressing lower levels of LOX had fewer metastatic cells in their lungs and none in their liver. Metastasis was completely abolished by giving the control mice β-aminoproprionitrile, an irreversible LOX inhibitor. This response was also achieved using an antibody against LOX.

Cellular invasion, acquisition of motility and cellular adhesion are just three components of the metastatic process. Erler and colleagues identified roles for LOX in all three phases. To investigate the role of LOX in invasion, the authors used collagen gels to recreate the cellular

G-PROTEIN-COUPLED RECEPTORS

Targeting the hotspot of G-protein interactions

The interactions between Gβγ protein subunits and downstream effectors that transmit signals following ligand binding to G-protein-coupled receptors (GPCRs) are potentially attractive drug targets, but are also highly challenging because of the large surface area and flat topology of the interaction surfaces. However, in a recent paper in *Science*, Smrcka and colleagues have identified several compounds that differentially modulate interactions between βγ subunits of G-proteins and their effectors, demonstrating a novel approach for targeting GPCR signalling.

Although protein–protein interactions involve large interfaces, some studies have indicated the presence of 'hotspots' on the protein surfaces — small regions that are responsible for a large proportion of the affinity of interactions. Previous experiments by the Smrcka group involving the screening of phage-display libraries had identified peptides that bound such a hotspot on the surface of the $G\beta_1\gamma_2$ subunit. Intriguingly, these peptides could differentially affect the interaction of this subunit with various effectors.

> “Smrcka and colleagues have identified several compounds that differentially modulate interactions between βγ subunits of G-proteins and their effectors.”

The authors therefore set out to discover more drug-like small molecules that might target the same hotspot using structure-based virtual screening of ~2,000 compounds. The 85 highest-ranking compounds from this screen were then assessed for their capacity to bind to the $G\beta_1\gamma_2$ subunit by using an enzyme-linked immunosorbent assay based on competition with a known peptide binder.

Two compounds, M119 and M201, with affinities of 200–400nM were selected for further studies. Assays measuring their activity on Gβγ-mediated effectors revealed that although both compounds bound to Gβγ subunits and inhibited G-protein receptor kinase-2 binding to Gβγ, they differentially modulated Gβγ interactions with other effectors: M119 attenuated activation of phospholipase-Cβ2, phospholipase-Cβ3 and phosphatidylinositol 3-kinase (PI3K), whereas M201 did not affect phospholipase-Cβ2 activation, but potentiated activation of phospholipase-Cβ3 and PI3K. The two compounds also showed different capacities to modulate second messenger pathways in cellular systems: M119, but not M201, attenuated agonist-induced increases in intracellular calcium, whereas both compounds again inhibited G-protein receptor kinase-2 binding.

Finally, the authors examined the *in vivo* effect of M119 on the analgesic effects of morphine, which acts via μ-opioid GPCRs through a signalling pathway that is known to involve phospholipase-Cβ3. Co-administration of M119 with morphine resulted in an 11-fold increase in the analgesic potency of morphine, which is almost identical to the increase seen in phospholipase-Cβ3 knockout mice, further highlighting the specificity of M119.

Even though such compounds have yet to be tested in disease models, this research highlights that it is possible to identify molecules that selectively target the interaction hotspot between Gβγ subunits and their protein effectors, thereby increasing the possibility that such compounds might one day have potential as drugs for diseases such as heart failure for which Gβγ subunits have been identified as targets.

Charlotte Harrison

ORIGINAL RESEARCH PAPER Bonacci, T. M. *et al.* Differential targeting of Gβγ-subunit signaling with small molecules *Science* **312**, 443–446 (2006)
FURTHER READING Arkins, M. R. & Wells, J. A. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. *Nature Rev. Drug Discov.* **3**, 301–317 (2004)

environment necessary for growth. Control cancer cells incubated on the gel had a branched appearance, showing invasion capabilities, but cells expressing lower levels of LOX were completely immobile, retaining a spherical shape. Next, immunofluorescent imaging of these cells revealed that LOX is concentrated at the leading edge of motile cancer cells, particularly in hypoxic conditions. Linked to motility, the authors also found that LOX, through activation of focal adhesion kinase, is required for formation of cellular adhesion interactions that are essential for cancer cell migration. The involvement of LOX in various steps of the metastatic process underscores its potential as a key therapeutic target for the treatment of cancer.

Samantha Barton

ORIGINAL RESEARCH PAPER Erler, J. T. *et al.* Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* **440**, 1222–1226 (2006)

ANTI-INFLAMMATORY DRUGS

New clues in the COX2 mystery

Why do cyclooxygenase-2 (COX2) inhibitors increase the risk of heart attack and stroke? And can such adverse effects be avoided while retaining anti-inflammatory efficacy? Potential answers to both questions are suggested by a recent study from Garret FitzGerald and colleagues published in the *Journal of Clinical Investigation*.

The development of selective COX2 inhibitors was initially stimulated by the hypothesis that COX1 was a constitutively expressed isoform whose inhibition could cause the gastrointestinal side effects characteristic of traditional non-steroidal anti-inflammatory drugs (NSAIDs), whereas COX2 was an inducible isoform whose inhibition results in the anti-inflammatory effects of these drugs. However, the hypothesis seems over-simplistic in the light of the problems with COX2 inhibitors, and considerable attention is now being focused on gaining a deeper understanding of the mechanisms underlying the beneficial and adverse effects of COX inhibition.

A key to understanding these effects seems to be the relative influence of COX1 and COX2 inhibition on the levels of the various prostanoids that result from further enzymatic modification of the product of both COX enzymes, prostaglandin H_2. For example, suppression of the production of the prostanoid prostaglandin E_2 is believed to underlie the anti-inflammatory efficacy of COX2 inhibitors. However, it has been proposed that concomitant alterations of the levels of other prostanoids — thromboxane A_2, which has prothrombotic effects, and prostacyclin, which has antithrombotic effects — might cause cardiovascular side effects.

To investigate the importance of the levels of various prostanoids, and the effects of COX inhibition on them, FitzGerald *et al.* used several mouse models with knockout, knockdown or mutation of one or both of the COX enzymes, and also selective COX2 inhibitors. These experiments provided evidence that COX2 is the dominant source of prostacyclin *in vivo*, as is COX1 for thromboxane A_2, supporting the proposal that disruption of the balance between prostacyclin and thromboxane A_2 could underlie the cardiovascular problems associated with selective COX2 inhibition. Indeed, inhibition, deletion or inactivation of COX2 augmented the response to thrombogenic stimuli and also elevated blood pressure, and these responses were attenuated by knockdown of COX1, which mimics the known antithrombotic effects of low-dose aspirin.

So, is there any way to obtain the beneficial anti-inflammatory effects of COX2 inhibition without increasing the risk of serious cardiovascular events? Intriguingly, a previous study had shown that deletion of microsomal PGE synthase 1 (mPGES1), which synthesizes prostaglandin E_2 from prostaglandin H_2, is as effective as traditional NSAIDS in models of pain and inflammation, and so the authors investigated the effects of mPGES1 deletion further. Prostaglandin E_2 was depressed, prostacyclin was augmented, there was no effect on thromboxane A_2 and, most importantly, mPGES1 deletion did not affect either thrombogenesis or blood pressure. Taken together, these observations suggest that inhibitors of mPGES1 could retain the anti-inflammatory effects of COX2 inhibitors while being less prone to their adverse cardiovascular consequences, and so might represent a promising focus for future drug development efforts.

Peter Kirkpatrick

ORIGINAL RESEARCH PAPER Cheng, Y. *et al.* Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function. *J. Clin. Invest.* **116**, 1391–1399 (2006)
FURTHER READING Mitchell, J. A. & Warner, T. D. COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. *Nature Rev. Drug Discov.* **5**, 75–86 (2006) | Grosser T., Fries, S. & FitzGerald, G. A. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J. Clin. Invest.* **116**, 4–15 (2006)

IN BRIEF

CARDIOVASCULAR DISEASE

Targeting C-reactive protein for the treatment of cardiovascular disease.

Pepys, M. B. *et al. Nature* **440**, 1217–1221 (2006)

Studies show that human C-reactive protein (CRP), which binds to ligands in damaged tissue and activates complement, increases myocardial and cerebral infarct size in rats. Targeting CRP is therefore a potential therapeutic strategy in humans against heart attack and stroke. This paper reports the design and synthesis of a small-molecule inhibitor of CRP, 1,6-bis(phosphocholine)-hexane, and shows that administration of the compound to rats undergoing myocardial infarction completely abolished the pathogenic effects caused by human CRP.

PHARMACOGENETICS

Thousands of samples are needed to generate a robust gene list for predicting outcome in cancer.

Ein-Dor, L. *et al. Proc. Natl Acad. Sci. USA* **103**, 5923–5928 (2006)

Researchers need to exercise caution when correlating gene expression signatures to cancer treatment outcomes because of significant ambiguities between different published gene lists. Variation between datasets is thought to stem from the small number of tumour samples used. Ein-Dor and coauthors report a mathematical model called 'probably approximately correct' (PAC) sorting that they used to evaluate the robustness of several published gene lists. Their calculations show that to achieve 50% overlap between two predictive gene sets for breast cancer, the genetic profiles of several thousand patients would be required.

DRUG DELIVERY

Transdermal protein delivery by a coadministered peptide identified via phage display.

Chen, Y. *et al. Nature Biotechnol.* **24**, 455–460 (2006)

Recent studies that used phage-display to identify peptide sequences that facilitate phage transport across the gastrointestinal mucosa prompted Chen *et al.* to look for peptide sequences that could aid transdermal delivery. Application of a phage library to the skin of mice and subsequent *in vivo* selection of phage from the systemic circulation identified eight phage clones expressing a common sequence. Co-administration of a cyclic peptide expressing multiple copies of this sequence was able to enhance the transdermal delivery of both insulin and growth hormone in a sequence- and dose-dependent manner.

STROKE

Role of matrix metalloproteinases in delayed cortical responses after stroke.

Zhao, B.-Q. *et al. Nature Med.* **12**, 441–445 (2006)

Matrix metalloproteinases (MMPs) are thought to exacerbate the effects of stroke because of their role in degradation of the neurovascular matrix, and there has been much interest in the development of inhibitors of MMPs for acute stroke therapy. However, this study suggests that MMPs might have a beneficial role in plasticity and remodelling of neurovascular tissue during stroke recovery. MMP9 was shown to be upregulated 7–14 days after stroke, and treatment with MMP inhibitors inhibited neurovascular remodelling and increased brain injury. The results suggest that rather than aiming to inhibit MMPs, compounds that can modulate MMPs to promote stroke recovery should also be explored.

ION CHANNELS

Fast track to ion-channel modulators

Although ion channels are well-established therapeutic targets for diseases including neuropathic pain and cardiac arrhythmia, screening ion-channel modulators is relatively low-throughput and creates a bottleneck in drug discovery in this area. However, a new screening technology developed by González and colleagues could provide a much-needed boost to drug discovery efforts.

Alterations in membrane potential govern many cellular functions and are strictly controlled by regulating ion flow through cell membrane ion channels. Conversely, opening of voltage-gated ion channels is regulated by membrane potential changes. Compounds that preferentially influence active ion channels are termed 'use-dependent' and have enhanced safety profiles; by contrast, toxins often block all channels, irrespective of activity.

Given the therapeutic potential for ion-channel modulators, demand for high-throughput screening (HTS) methods is high. The 'gold-standard' technique, whole-cell patch clamping, allows detailed characterization of channel activity but is only amenable to low-throughput screening, whereas existing HTS methods based on voltage-sensitive probes lack the temporal resolution necessary to determine a compound's mechanism of action. The authors therefore sought to combine key features of patch clamping — such as membrane potential control, repetitive stimulation and high temporal resolution — with the high-throughput capabilities of optical dyes.

In this technique, termed E-VIPR, cells in multi-well plates are subjected to electrical field stimulation using an electrode array. Fast-acting voltage-sensitive fluorescent dyes indicate changes in membrane potential, which reveals the effects of drugs on channel activity. E-VIPR was compared with patch clamping in cells expressing a human voltage-gated sodium channel, $hNa_v1.3$. Several known sodium-channel blockers were successfully characterized according to potency, and use-dependent and non-use-dependent compounds were distinguished. Finally, 400 commonly used drugs were screened. In several cases, unexpected sodium-channel-blocking activity was demonstrated, suggesting that E-VIPR could identify compounds otherwise overlooked.

The technique is applicable to many different ion channels and could significantly advance HTS of ion-channel modulators. Further experimentation is essential, including extending its application to different cell types and channel subtypes. E-VIPR lacks the fine control of membrane potential achieved by patch clamping but has several advantages, including cost, efficiency and the capacity to identify compounds that elude patch clamping, such as slow-developing channel blockers. E-VIPR would probably be used to complement, rather than replace, existing methodology, facilitating the initial selection of promising compounds for more detailed investigation.

Katherine Whalley

ORIGINAL RESEARCH PAPER Huang, C. J. *et al.* Characterization of voltage-gated sodium-channel blockers by electrical stimulation and fluorescence detection of membrane potential. *Nature Biotechnol.* **24**, 439–446 (2006)