BIOMARKERS

Taking out the trash

Despite years of intensive analysis, only a small number of plasma proteins have been validated as cancer biomarkers, such as prostate-specific antigen and cancer antigen 125. Now, Josep Villanueva and colleagues show that peptides in the serum of cancer patients that are generated as a result of tumour protease activity can be used for the detection and classification of cancer.

Many researchers have considered the thousands of proteolytically derived peptides — products that are the result of the high levels of active proteases that tumours produce — to be 'biological trash'. However, Villanueva *et al.* have developed a mass-spectrometry approach to identify tumour-specific peptidome patterns in serum samples. Although researchers have previously attempted similar approaches, their studies were not adequately validated. These authors set out with the goal of developing a mass-spectrometry-based system of serum analysis that could be reproduced in independent samples.

Villanueva *et al.* used an automated procedure for the simultaneous measurement of peptides in serum that used magnetic reverse-phase beads for analyte capture and matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) mass-spectrometry read-out — a more sensitive type of analysis than other mass-spectrometry-based approaches. To fully interpret their results, they developed a minimal-entropy-based algorithm that simplifies and improves statistical analysis of the data.

Using this system, the authors profiled 106 serum samples from patients with advanced prostate cancer, bladder cancer or breast cancer. On the basis of an analysis of 61 signature peptides, all of which were breakdown products, the authors were able to identify specific proteolytic patterns that were not only cancer-specific, but also cancer-type-specific. They then demonstrated that this signature could be used to discriminate between patients with advanced prostate cancer and control

KINASES

New route to kinase inhibition

> “GNF2 inhibited cellular BCR–ABL kinase activity through an allosteric non-ATP-competitive mechanism.”

Most kinase inhibitors that have been tested in clinical trials, such as the successful anticancer drug imatinib (Gleevec), act by directly competing with ATP at the ATP-binding site of the kinase. Reporting in *Nature Chemical Biology*, Adrian and colleagues now describe a new class of compounds that potently inhibit the same kinase as imatinib — BCR–ABL — but through a novel allosteric, non-ATP-competitive mechanism. Furthermore, these compounds maintain potency against some clinically relevant imatinib-resistant BCR–ABL mutants.

Typically, to discover new kinase inhibitors, screens are performed against recombinant catalytic kinase domains. However, this approach tends to identify ATP-competitive inhibitors that are often members of well-explored compound classes. So, to address this issue, and search for compounds with new mechanisms of inhibition, the authors developed an assay in which the cytotoxicity of compounds against cells specifically transformed with BCR–ABL was compared with their isogenetic parental cell line.

From an initial screen of 50,000 compounds, those that showed good differential cytotoxicity were investigated further, and compounds that were well characterized as targeting the ATP-binding site were discarded. Of the remaining compounds, one class in particular showed excellent differential cytotoxicity and became the focus of further optimization efforts. These led to compounds such as GNF2, which showed comparable potency to imatinib in inhibiting BCR–ABL-expressing cells but, remarkably and in contrast to imatinib, did not inhibit BCR–ABL kinase activity *in vitro*.

This high cellular selectivity — coupled with the observations that GNF2 did not inhibit any of 63 kinases including BCR–ABL in biochemical assays and binding experiments that demonstrated that GNF2 specifically bound to ABL — suggested that GNF2 inhibited cellular BCR–ABL kinase activity through an allosteric non-ATP-competitive mechanism. On the basis of knowledge about the mechanisms regulating BCR–ABL and their experimental data, the authors proposed that GNF2 stabilizes the inactive conformation of BCR–ABL by mimicking the binding of the myristoylated amino terminus of normal ABL to a binding pocket located near the carboxyl terminus of the kinase. BCR–ABL is not myristoylated and so lacks this auto-inhibitory mechanism. In support of this proposal, mutations to the myristate binding pocket were shown to render BCR–ABL resistant to GNF2, but not to imatinib.

Binding to this pocket distant from the active site of the kinase is consistent with the excellent cellular and enzymatic selectivity of compounds such as GNF2. In addition, GNF2 and imatinib showed synergistic antiproliferative effects when combined, further supporting the idea that they bind to different sites on BCR–ABL.

Compounds such as GNF2 showed good pharmacokinetic behaviour, and so could represent a promising starting point for the development of new drugs with diminished off-target activity. The demonstration that the cellular activity of BCR–ABL can be pharmacologically modulated by non-ATP-competitive inhibitors in a highly selective fashion also suggests that such inhibitors could be found for other kinases of therapeutic interest for which selectivity is a key concern.

Peter Kirkpatrick

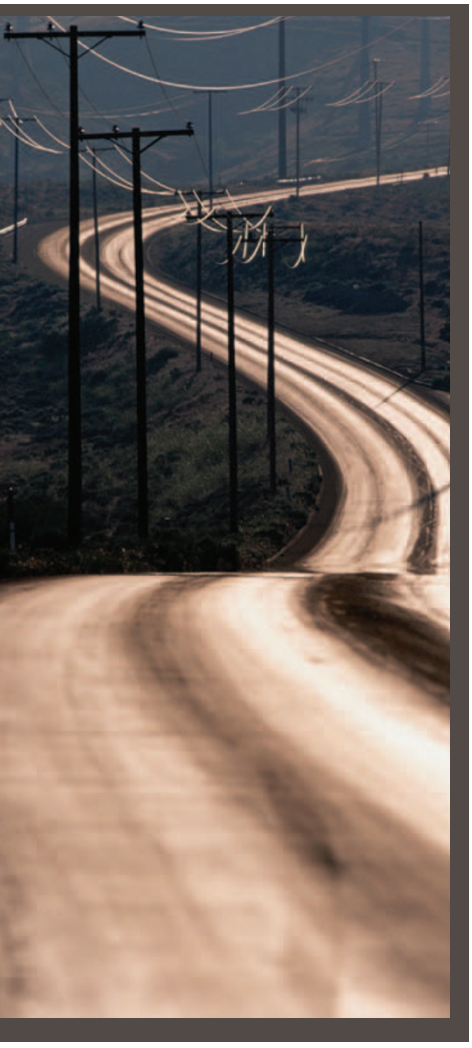
ORIGINAL RESEARCH PAPER Adrian, F. J. *et al.* Allosteric inhibitors of BCR–ABL-dependent cell proliferation. *Nature Chem. Biol.* **2**, 95–102 (2006)

subjects in an independent validation set of serum samples.

The authors propose that the proteolytic degradation patterns in the serum peptidome might not only be used in cancer detection, but also to distinguish indolent from aggressive tumours. Such tests are urgently needed to identify men with prostate tumours who might safely avoid surgery or radiation therapy. The findings also indicate that proteomic analysis should not involve inhibition of proteolysis in *ex vivo* samples, which could limit biomarker discovery.

Kristine Novak, Nature Reviews Cancer

ORIGINAL RESEARCH PAPER Villanueva, J. *et al.* Differential exoprotease activities confer tumour-specific serum peptidome patterns. *J. Clin. Invest.* **116**, 271–283 (2006)
FURTHER READING Coombes, K. R. *et al.* Serum proteomics profiling — a young technology begins to mature. *Nature Biotechnol.* **23**, 291–292 (2005)



G-PROTEIN-COUPLED RECEPTORS

Bridging the GPCR gap

Evidence is accumulating for the existence and functional significance of GPCR heterodimers. Following the recent report that GPCRs form tissue-specific heterodimers *in vivo*, research just published in *Proceedings of the National Academy of Sciences* shows that bivalent ligands that target both μ- and δ-opioid receptors have comparable analgesic benefit to morphine without the side effects chronic exposure causes, and could lead to the development of a new generation of improved analgesic drugs.

Research suggests that δ-opioid receptors are involved in the development of tolerance and dependence to μ-opioid receptor agonists, and studies with the δ-antagonist naltrindole (NTI) have shown that the chronic side effects of morphine treatment can be blocked without reducing analgesia. Philip Portoghese and colleagues therefore proposed that a series of bivalent ligands that could modulate both receptors might make improved analgesics with fewer side effects and would also provide a useful tool for studying receptor synergy.

The authors designed and synthesized a series of bivalent ligands, the MDANs (μ–δ agonist–antagonists), consisting of a μ-agonist pharmacophore (oxymorphone) and a δ-antagonist pharmacophore (NTI) linked by variable-length diamine spacers (consisting of 16, 19 or 21 methylenes). They administered the compounds to mice by intracerebroventricular (i.c.v) injection and found that the length of the spacer had profound effects on receptor function, providing evidence for bridging activity between the μ- and δ-receptors. The antinociceptive activity of the bivalent ligands was enhanced by increasing the distance between the pharmacophores, but no such increase in potency was seen for monovalent μ-opioid ligands or when μ-agonist and δ-antagonist were co-administered. This suggests that the increased μ-agonist potency results from receptor association rather than the presence of both ligands, and that a certain length of spacer is required to achieve optimal bridging.

It is also possible that the δ-receptor exerts negative allosteric cooperativity on the μ-receptor, and so the authors studied this by pretreating mice with the δ-antagonist, NTI, which they speculated would improve potency. As expected, NTI increased the potency of the bivalent ligands compared with control ligands possibly by displacing the bound MDAN δ-antagonist and enabling the μ-opioid receptor to be liberated from the negative allosteric effect of the δ-opioid receptor.

Measuring the ED_{50} value of a ligand can be indicative of the development of tolerance to a drug. It was therefore interesting to see that after pretreatment with NTI, the ED_{50} value for MDAN-19 and MDAN-21 was the same as the monovalent μ-opioid ligand, suggesting that δ-antagonism reduces the development of tolerance to μ-opioid agonists. To further investigate this, the authors studied the development of tolerance and withdrawal after chronic i.c.v administration. Although none of the bivalent ligands caused tolerance or dependence to the same extent as morphine, MDANs with shorter spacers produced tolerance without physical dependence and those with longer spacers produced neither tolerance nor dependence. Moreover, the MDAN-21 ligand was 50-fold more potent than morphine by i.c.v or intravenous administration yet does not cause tolerance or dependence, suggesting that there is promise for the development of bivalent ligands as a new generation of analgesic drugs that do not cause physical dependence or tolerance with chronic use. These findings suggest that as we learn more about how cooperativity and other mechanisms of GPCR behaviour mediate distinct pharmacological effects it will become feasible to design targeted GPCR drugs with fewer and less severe side effects for a variety of indications.

Joanna Owens

ORIGINAL RESEARCH PAPER Daniels, D. J. *et al.* Opioid-induced tolerance and dependence in mice is modulated by the distance between pharmacophores in a bivalent ligand series. *Proc. Natl Acad. Sci. USA* **102**, 19208–19213 (2005)
FURTHER READING Owens, J. It takes two… *Nature Rev. Drug Discov.* **4**, 627 (2005)
WEB SITE
Philip Portoghese's laboratory: http://www.pharmacy.umn.edu/faculty/portoghese_philip/home.html