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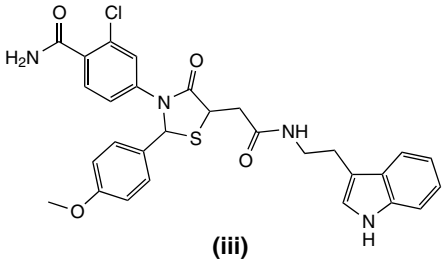
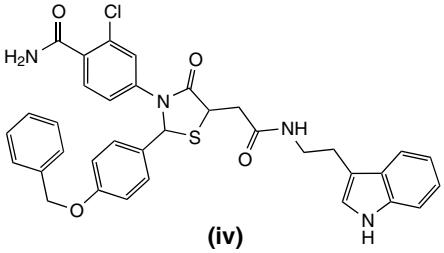
Follicle-stimulating hormone receptor

The split-pool strategy for the preparation of large libraries of compounds enables rapid access to compounds but suffers from the disadvantage of having to deconvolute the resultant mixtures. Encoding of combinatorial libraries affords one solution to this problem for solid-phase split-pool libraries. In this manner, a thiazolidinone library compatible with an encoding strategy had previously been constructed [8].



(iii)



(iv)

From this set, several hits typified by (**iii**) were discovered that possessed agonist activity against follicle-stimulating hormone (FSH), a 31 kDa heterodimeric glycoprotein, by virtue of their ability to stimulate a reported cell line expressing the FSH receptor. The assembly and screening of an encoded thiazolidine library has been undertaken [9]. A library of 42,875 compounds was synthesized on solid phase in mixtures of 1225. These mixtures were tested for agonist activity against FSH.

Two active mixtures were identified which, following deconvolution in two stages via a 'tiered release' allowing deconvolution and identification of all compounds, gave several actives. These were used as the basis for a further round of optimization, which resulted in compound **iv**, which possessed an EC_{50} of 32 nM. This work has provided a novel molecule starting point for the design of improved agonists of FSH, and this approach warrants further investigation.

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Biology

Cancer Biology

Knocking out IKKβ to prevent colorectal cancer

After an introduction which starts in a vaguely familiar fashion [1], the authors of a recent *Cell* paper [2] go on to present results showing that tissue-specific knockout of IκB kinase β (IKKβ) in intestinal epithelial cells or in myeloid cells reduces formation of inflammation-associated colorectal tumours in a mouse model of colitis-associated cancer (CAC). Deletion of the kinase in the colon epithelium does not inhibit experimentally induced inflammation but significantly decreases tumour incidence without affecting tumour size.

Knockout in the myeloid cells, in contrast, results in a decrease in tumour incidence and size. This difference appears to be due to increased p53-independent apoptosis in the enterocytes and to reduced production of tumour growth-promoting factors in the myelocytes lacking IKKβ. Furthermore, the authors report that specific inhibition of cyclooxygenase-2, one of the targets of nonsteroid antiinflammatory drugs (NSAIDs) with chemopreventative activity, did not result in increased enterocyte apoptosis or proliferation in the CAC model.

Based on these results, the authors suggest that specific inhibition of IKKβ could prove effective in the chemoprevention of colorectal cancer as has been found for the less-specific NSAIDs, which inhibit this kinase as just one of multiple activities. There is also evidence indicating that the targeting of the NF-κB pathway might have therapeutic effect, not only in the prevention but also the treatment, of certain types of cancer [1]. However, this appears much less likely to be achievable by specific inhibition of a single drug target alone, be it IKKβ or some other protein.

1 Haefner, B. (2002) NF-κB: arresting a major culprit in cancer. *Drug Discov. Today* 7, 653–663
2 Greten, F.R. *et al.* (2004) IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 118, 285–296

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Deciphering the role of MTA1

Mammary gland development provides an ideal system in which to study mechanisms of tumourigenesis. Maturation occurs post-natally and is characterized by phases of proliferation, differentiation and apoptosis – processes that are deregulated in cancer.

The metastasis-associated protein 1 (MTA1) is an estrogen receptor co-modulator that is overexpressed in breast cancer cells.

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However, the role of MTA1 in mammary development and tumourigenesis is unclear. In order to determine MTA1 function, Kumar and colleagues have therefore generated a mammary gland-specific transgenic mouse [3].

The MTA1 transgene was found to be expressed throughout development; however, profound effects were observed in virgin mice. At this stage, glands morphologically and functionally resembled pregnancy, with increased proliferation, branching and expression of the milk protein, beta-casein. A similar effect was observed in ovariectomized mice and male littermates. Later in development, the transgenic mice displayed delayed onset of involution, with a reduced apoptotic index.

These findings correlated with increased levels of the cell cycle regulator, cyclin D1, and the pro-survival factor, Bcl-XL. Both genes are downstream targets of the progesterone receptor isoform, PR-A. The transgenic mice were found to have increased levels of PR-A, with a concomitant reduction in the PR-B isoform, suggesting a mechanism for increased expression of cyclin D1 and Bcl-XL. Interestingly, the phenotype of the PR-A transgenic mouse resembles MTA1.

Finally, nulliparous and multiparous MTA1 females, but not wild-type controls, were prone to focal hyperplastic lesions, some of which later progressed to mammary tumours. Consequently, the authors' initial findings indicate that MTA1 is likely to have an important role in mammary development and breast cancer. The transgenic mice generated in this study will doubtless prove a useful tool to test this hypothesis further.

3 Bagheri-Yarmand, R. (2004) Metastasis-associated protein 1 deregulation causes inappropriate mammary gland development and tumourigenesis. *Development* 131, 3469–3479

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Molecular Biology

The DNA repair protein AGT binds DNA by a novel mechanism

Several cancer chemotherapies induce apoptosis by causing DNA alkylation. O6-alkylguanine-DNA alkytransferase (AGT) directly removes alkyl lesions from guanine in DNA by irreversibly transfering the lesion onto a reactive cysteine within the protein. Therefore, inhibition of AGT leads to increased DNA alkylation and apoptosis. Daniels *et al.* have helped our understanding of the mechanism of AGT and the manner in which it is targeted by inhibitors by solving two structures in complex with DNA [6]. The first is bound to a lesion and the second covalently linked to the inhibitor N1,O6-ethanoxanthosine.

AGT binds to DNA by a helix–turn–helix, which is a common DNA-binding motif. However, the recognition helix was inserted into the minor groove and not the major groove, as has been seen in all previous helix–turn–helix structures. An arginine residue inserts into the DNA to flip out the target guanine and a tyrosine sterically forces the phosphate 3′ to the target base to rotate, so that the oxygens are pointing into the centre of the DNA.

The structure is consistent with a previously suggested mechanism, in which an activated water molecule nucleophilically attacks the cysteine to produce a thiolate anion. This forms a much more efficient nucleophile to attack and remove the alkyl group on the guanine.

When AGT binds to DNA in the presence of the inhibitor N1,O6-ethanoxanthosine, it forms a covalent complex with the reactive cysteine residue, thus permanently inhibiting the protein. This therefore causes an increase in alkylation due to endogenous or environmental factors, and eventually apoptosis. Therefore, this structure could help in the development of more efficient inhibitors for use in cancer chemotherapy.

6 Daniels, D.S. *et al.* (2004) DNA binding and nucleotide flipping by the human DNA repair protein AGT. *Nat. Struct. Mol. Biol.* 11, 714–720

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Microbiology

New insights into the chloroquine resistance transporter protein PfCRT

There is now overwhelming evidence that the so-called *Plasmodium falciparum* chloroquine-resistance transporter, PfCRT, plays a major role in conferring chloroquine-resistance in the malaria parasite *P. falciparum*. Chloroquine-resistant parasites from a wide range of geographical areas exhibit a series of different mutations in the gene coding for this protein, but all resistant strains exhibit one common mutation, the K76T mutation.

In the past four years since the discovery of the PfCRT protein, mutations in it have been related to chloroquine-resistance and it has been found to be localized in the food vacuole membrane of the parasite. However, despite these advances, its relationship to other proteins and its normal biochemical function have remained elusive. Now, a new bioinformatic study by Rowena Martin and Kiaran Kirk [4] has revealed that this protein is a member of a large family of integral membrane proteins known as drug/metabolite transporters. Within this family, the CRT proteins of the various *Plasmodium* species appear to be phylogenetically related to the drug/metabolite effluxer superfamily. This discovery has further permitted the authors to gain additional insights into structure–function relationships in PfCRT.

The protein appears to function as a homodimer. Its N- and C-termini are predicted to lie in the parasite cytosol, whereas lysine-76, the residue involved in the crucial mutation resulting in chloroquine-resistance, lies on the food vacuole side of the membrane. Crucially, this residue is predicted to form part of a substrate-selectivity site. The food vacuole is the likely site of action of chloroquine, where it accumulates in its positively charged protonated form, at least in part by pH trapping. Given that the K76T mutation involves removal of a positive charge from the substrate recognition site, it seems likely that the mutant protein acts by effluxing the positively charged chloroquine molecule.

The normal physiological role of CRT remains elusive, but the authors suggest that it could be a transporter of peptides or amino acids. This seems possible, given that a vast quantity of peptides produced as a consequence of prodigious haemoglobin digestion is exported from the food vacuole.

4 Martin, R.E. and Kirk, K. (2004) The malaria parasite's chloroquine resistance transporter is a member of the drug/metabolite transporter superfamily. *Mol. Biol. Evol.* (2004) DOI: 10.1093/molbev/msh205 (E-publication ahead of print; http://mbe.oupjournals.org)

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Linezolid resistance in *Staphylococcus aureus*

Linezolid, a member of the recently introduced oxazolidinone class of antibacterial agents, is active against multi-resistant Gram-positive pathogens. These antibiotics inhibit protein synthesis by binding to the 50S subunit of the prokaryotic ribosome and preventing assembly of the initiation complex. Although a G2576T mutation in the 23S rRNA gene encoded by the *rrn* operon can confer resistance, linezolid resistance in *Staphylococcus aureus* is rare, presumably because there are 5-6 copies of *rrn* in the genome and at least two copies must carry the mutation in order for the strain to be phenotypically resistant.

Meka *et al.* now report a new mutation and additional genomic changes in linezolid-resistant isolates of *S. aureus* obtained from a patient receiving long-term linezolid therapy [5]. One isolate contained a T2500A mutation in three out of six copies of *rrn* while two isolates carried two copies of the same mutation but had also deleted the sixth copy of *rrn*, thereby increasing the proportion of mutant alleles. Seven months after linezolid therapy had been discontinued, a linezolid-sensitive *S. aureus* was isolated that did not contain any T2500A mutations.

This paper illustrates the adaptability of the *S. aureus* genome and the potential complexity of detecting resistance when there are multiple copies of the target gene. The fact that this resistance mutation appeared to exact a toll on bacterial fitness is an encouraging sign that might be exploited in the future.

5 Meka V.G. *et al.* (2004) Linezolid resistance in sequential *Staphylococcus aureus* isolates associated with a T2500A mutation in the 23S rRNA gene and loss of a single copy of rRNA. *J. Infect. Dis.*190, 311–317

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Business

Collaborations

Large-scale genetic study of diabetes

ParAllele BioScience (http://www.parallelebio.com/), Affymetrix (http://www.affymetrix.com) and Cambridge University (http://www.cam.ac.uk) have announced a collaboration to undertake a large-scale gene-association study to locate new type-1 diabetes genes for use in the development of improved therapies and diagnostics.

Led by John Todd of the University's Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory (DIL; http://www-gene.cimr.cam.ac.uk/todd), the research will use a ParAllele-developed standard panel of 10,000 SNPs to compare genotypes between more than 2000 people. In what is believed to be among the largest studies of its kind to be undertaken, the researchers plan to analyze more than 20,000 DNA samples already collected from diabetes patients and their relatives.

'This research project is the most exciting and important genetics experiment I've ever been involved in,' said Todd. 'We've been collecting samples for quite some time and have been waiting for a technology that would give us the genetic power we needed to commence informative studies. Using this new solution from Affymetrix and ParAllele for a genome-wide gene association study provides us with the best opportunity we've ever had to discover new disease-associated genes and polymorphisms.'

Vernalis and Novartis collaborate

Vernalis (http://www.vernalis.com/) and Novartis (http://www.novartis.com/) have announced a joint R&D programme on Hsp90, a target implicated in several different cancers. Novartis will provide research funding to Vernalis over an initial three-year period.

Simon Sturge, Chief Executive Officer of Vernalis, said: 'I am delighted to be announcing a third major deal for Vernalis in only six weeks. Novartis is a world leader in oncology and an optimal partner to help maximize the opportunity for developing Hsp90 inhibitors as potential cancer treatments. This collaboration further validates Vernalis' research capability and provides additional funding.'

PerkinElmer and Vivascience/Sartorius

PerkinElmer (http://www.perkinelmer.com/) and Vivascience (http://www.vivascience.com), a leading supplier of products and technology solutions for protein purification and analysis and a member of the Sartorius Group, have entered into a collaborative agreement in biomarker screening and discovery.

Vivascience's patented membrane adsorber (MA) chromatography technology will be combined with PerkinElmer's proprietary elution chemistries to create fractionation kits for proteomics-based biomarker analysis. 'By working with a market leader like Vivascience, we are able to accelerate bringing breakthrough innovations to the emerging markets of proteomics-based biomarker screening and discovery', said Peter Coggins, President of PerkinElmer Life and Analytical Sciences.

Kevin Rosenblatt, a leading researcher in biomarker research and clinical proteomics at the University of Texas Southwestern (http://www.utsouthwestern.edu), recently commented: 'The combination of ultra-high mass accuracy and superb resolution over a broad mass range in the prOTOF, coupled with an automated biomarker enrichment platform, make the PerkinElmer approach an ideal biomarker discovery and screening platform.'

Business was written by *Matthew Thorne*

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