Human betaine inhibitors

The translation of sequencing data into an understanding of the function of proteins in cells, tissues or whole organisms is the key challenge for functional genomics and proteomics. Small ligands that are able to specifically interact with proteins can be effective tools in the search for proteome function. Classically, new ligands for proteins have been identified by SAR studies, molecular modeling or combinatorial chemistry techniques.

However, these approaches generally use only one protein target. Given the high number of proteins in mammalian organisms, HTS procedures have been developed to handle this task. A potential drawback with such methods is that the full range of proteins that the chosen ligand could interact with are not discovered if the screening is performed with only one or a few proteins. This lack of information regarding how many proteins a given ligand can interact with precludes our ability to completely understand the full spectrum of effects that a ligand might have in a complex medium such as a living cell. Approaches that study the effects of ligands in whole cells are becoming important. Screening libraries using biosensor chips or arrays grafted with proteins enables the real-time recording of ligand–protein interactions. Subsequent elution of these complexes can be used for protein identification by MS.

A method to discover novel protein–ligand interactions has been developed based on affinity capture principles coupled to combinatorial chemistry [6]. Affinity columns were prepared containing 361 different phosphinic peptides, which were used to isolate all interacting proteins from crude rat liver homogenates. By applying a deconvolution process, the most specific ligand was identified within the phosphinate peptide library that had the highest affinity towards one newly discovered protein target, betaine:homocysteine S-methyltransferase (BHMT). The phosphinic pseudopeptides, which served as immobilized ligands for the isolation of rat BHMT, were then tested for their ability to inhibit human recombinant BHMT in solution. The most potent inhibitor also behaved as a selective ligand for the affinity purification of BHMT from a complex media. Further optimization of this active identified compound **ii** as a potent BHMT inhibitor that possessed an IC_{50} value of about 1μM.

Val-Phe-ψ[PO_2^--CH_2]Leu-His-NH_2

(ii)

This work has demonstrated the successful application of a new and simple method for the discovery of new protein targets for artificial ligands of interest. This methodology, in combination with 2D electrophoresis and MALDI-MS holds promise as an additional method for the discovery of new specific protein-ligand interactions.

6 Collinsova, M. *et al.* (2003) Combining combinatorial chemistry and affinity chromatography: highly selective inhibitors of human betaine: homocysteine S-methyltransferase. *Chem. Biol.*10, 113–122

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Biology

Microbiology

Fibronectin-binding in *Streptococcus pyogenes*: proteolytic regulation and virulence fine-tuning

Surface proteins that bind the extracellular matrix and plasma protein fibronectin (Fn) are widespread among bacterial pathogens. In the human pathogen *Streptococcus pyogenes*, Fn-binding is important for cellular invasion and has been suggested to contribute to pathogenesis and virulence.

Nyberg *et al.* now report that the streptococcal cysteine proteinase, SpeB, modulates Fn-mediated cellular internalization of *S. pyogenes* [1]. SpeB degrades the Fn-binding protein F1 (PrtF1) when anchored to the bacterial surface in a plasma environment. In contrast to IgG and fibrinogen, which protect their bacterial surface receptors (M proteins), Fn does not protect protein F1 from SpeB. Instead Fn is degraded by SpeB when bound to the streptococcal surface.

Nyberg *et al.* continued by investigating Fn-binding and virulence, using isogenic PrtF1-expressing or non-expressing strains, cellular adhesion and internalization assays, and transgenic mice not producing plasma Fn (pFn) [2]. They showed that adhesion is dependent on the interaction between PrtF1 and cellular Fn (cFn) and pFn, whereas internalization depends on pFn alone. Furthermore, *S. pyogenes* virulence is attenuated in PrtF1-expressing strains but is partly restored in mice not expressing pFn. Thus, PrtF1-mediated Fn-binding is the first described anti-virulence trait in *S. pyogenes* and could be beneficial in establishing a balanced bacterial interaction with the host. This is particularly interesting because most of the highly virulent *S. pyogenes* strains of the M1 serotype do not express PrtF1. Furthermore, the strains that do express PrtF1 can, under certain circumstances, regulate the surface expression by a bacterial protease, SpeB.

Taken together, these two studies clearly contribute to the understanding of how *S. pyogenes* regulates cellular binding, internalization and virulence on a molecular level. Finally, these studies also emphasizes the importance of taking potential anti-virulence interactions, including PrtF, into account when new therapeutic strategies are developed.

1 Nyberg, P. *et al.* (2004) SpeB modulates fibronectin-dependent internalization of *Streptococcus pyogenes* by efficient proteolysis of cell-wall-anchored protein F. *Microbiology* 150, 1559–1569
2 Nyberg, P. *et al.* (2004) Interactions with fibronectin attenuate the virulence of *Streptococcus pyogenes*. *EMBO J.* DOI: 10.1038/sj.emboj.7600214 (E-pub ahead of print; http://embojournal.npgjournals.com).

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Targets and Mechanisms

ATM directly regulates the transcriptional activity of CREB

ATM is a kinase that is activated in response to DNA damage. Mutations in ATM lead to ataxia-telangiectasia (A-T), the symptoms of which include cancer susceptibility and neurodegeneration. Once activated, ATM phosphorylates several proteins, such as p53, to halt cell growth and initiate DNA repair. However, very little is known about how mutations in ATM can also lead to neurodegeneration. Shi *et al.* [6] now show that ATM also phosphorylates CREB, directly regulating its transcriptional activity.

CREB is a transcription factor that is involved in neuronal development and survival, making it a good candidate for ATM-dependent regulation of neurons. The authors tested whether CREB was phosphorylated at a known activation site in response to ionizing radiation. They found that it was phosphorylated by ATM both *in vivo* and *in vitro*, but at different sites. They showed that phosphorylation at these sites reduced binding to the CREB co-activator, CBP, suggesting that phosphorylation has a genuine inhibitory effect on the transcriptional activity of CREB.

To test gene activation by CREB, the authors fused CREB to the DNA-binding domain of Gal4 and measured expression from a reporter gene. They compared the activity of a fusion containing wild-type CREB and one with the ATM-dependent phosphorylation sites mutated. They found that the mutant increased transcription, indicating that the ATM-dependent phosphorylation sites reduce the activation potential of CREB. Further studies are required to show a direct link between neurodegeneration in A-T and the inhibition of CREB.

6 Shi, Y. *et al.* (2004) Direct regulation of CREB transcriptional activity by ATM in response to genotoxic stress. *Proc. Natl. Acad. Sci. U. S. A.* 101, 5898–5903

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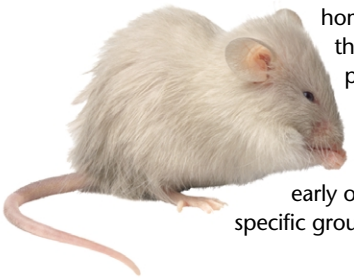
Cancer Biology

Of mice and men: the development of cancer

It has been hypothesized that there is a relationship between the mechanisms of normal development and those of tumourigenesis. A recent study published in *Genes and Development* attempts to address this issue using a novel *in silico* approach [3].

Kho *et al.* used microarray technology to identify genes expressed during mouse postnatal cerebellar development. Genes with known human homologues were then subjected to principal component analysis and partitioned into early or late-stage-specific groups. These groups were compared with a microarray analysis of human medulloblastoma (MB) gene expression. Remarkably, there was a significant association of downregulated genes in MB with the late developmental group; conversely, genes expressed in early development segregated with those that were upregulated in MB. In addition, at the genome level, MB most closely resembled early mouse cerebellar development, whereas normal human cerebellum resembled late mouse development.



To test the general utility of the method, human lung cancers were compared with mouse lung development – similar results were obtained. However, comparing MB with lung development or lung cancers with cerebellar development did not result in significant gene segregations, suggesting that studying tumourigenesis from a developmental standpoint is only valid in an organ-specific context.

There is already a wealth of microarray data publicly available. It is therefore anticipated that the procedure described will enable researchers to exploit this resource to make predictions about cancer biology, prognosis and treatment.

3 Kho, A.T. *et al.* (2004) Conserved mechanisms across development and tumourigenesis revealed by a mouse development perspective of human cancers. *Genes Dev.* 18, 629–640

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Small molecule, great opportunities

Malfunction in apoptotic pathways is one hallmark in molecular carcinogenesis. In this context, the mitochondrial membrane protein Bcl-XL was identified as a potential molecular target for anticancer therapy. Bcl-XL is known to be correlated with resistance of tumours to many anticancer drugs. NSAIDs, such as Sulindac, alter the expression of Bcl-XL, thus leading to mitochondria-mediated cell death.

Wu *et al.* screened a chemical library for their effects on expression of tumour-associated genes [4]. They found that 2[[3-(2,3-dichlorophenoxy)propyl]amino]ethanol (2,3-DCPE) had promising effects on the induction of apoptosis of several human cancer cell lines, but no or minimal influence on the growth of normal human fibroblasts. Compared with 5-fluorouracil and paclitaxel, 2,3-DCPE shows a remarkable selectivity for cancer cells versus normal fibroblasts.

Apart from downregulation of Bcl-XL 8–16 hours after treatment, western blot analysis revealed that application of 2,3-DCPE results in the apparent activation of caspase-8, caspase-3 and caspase-9 and cleavage of poly(ADPRibose) poymerase in various cancer cell lines but not normal fibroblasts. In addition, Wu *et al.* detected the release of cytochrome *c* into the cytosol in these cells. These effects can be negotiated by overexpression of Bcl-XL.

Real-time PCR analysis showed that 2,3-DCPE does not decrease Bcl-XL mRNA, suggesting a translational mechanism of effect. It is possible that other cellular molecules are involved in 2,3-DCPE-induced apoptosis. The exact mechanism of action of 2,3-DCPE still remains to be characterized, but this

molecule seems to be a potential new anticancer agent.

4 Shuhong, W. *et al.* (2004) Induction of apoptosis and down-regulation of Bcl-XL in cancer cells by a novel small molecule, 2[[3-(2,3-Dichlorophenoxy)propyl] amino]ethanol. *Cancer Res.* 64, 1110–1113

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Neuroscience

Allevation of Alzheimer's disease pathology in mice by a small heparin

The accumulation of β-amyloid plaques and the immune response and antibodies directed against them is thought to exacerbate the pathology of Alzheimer's disease (AD) through a variety of distinct mechanisms, including activation of the contact and complement systems. Using a transgenic mouse model overexpressing human amyloid precursor protein$_{751}$ (typically present with numerous extensive plaques in the cortex and hippocampus from five months in age onwards), Bergamaschini and colleagues have shown that chronic peripheral injection of the small heparin enoxaparin (ENO) alleviates AD-related pathology [5].

Specifically, immunocytochemical analysis revealed that the drug reduced the number, size and concentration of plaques observed in the neocortex, and diminished the degree of activation of astrocytes immediately surrounding the plaques. Preliminary analyses suggested that these effects were specific, and were without appreciable side-effects. Parallel *in vitro* work showed that ENO had no effect on Aβ fibrillarity, but reduced the cytotoxic effects of Aβ(1-40) and Aβ(1-42) (as indexed by cell viability) and, in contrast to other glycosaminoglycans, attenuated the activation of the complement and contact systems (as indexed by the percentage cleavage of C4 and HK, respectively).

The authors suggest two possible sites of action for ENO, the CNS (where it might protect against soluble Aβ neurotoxicity or elute cell-bound Aβ) or the blood (where it could affect the brain-plasma dynamics of circulating Aβ). Whatever the mechanism of action, the study suggests that ENO represents a potentially valuable therapeutic for AD through limiting brain Aβ accumulation and deposition, and through decreasing the associated immune response.

5 Bergamaschini, L. *et al.* (2004) Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and β-amyloid accumulation in a mouse model of Alzheimer's disease. *J. Neurosci.* 24, 4181–4186

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Business

Merger

Agreement between Sanofi-Synthelabo and Aventis to create Sanofi-Aventis

Sanofi-Synthelabo (http://www.sanofi-synthelabo) have announced an improved offer for Aventis (http://www.aventis.com), which has been unanimously approved by the Board of Directors and principal shareholders (Total and L'Oreal) of Sanofi-Synthelabo and by the Management and Supervisory Boards of Aventis, who have recommended that Aventis shareholders tender their shares into Sanofi-Synthelabo's offer.

The combination of the two companies will create Sanofi-Aventis, the third largest pharmaceutical group in the world, and the number one in Europe. The management team will be drawn equally from both groups and will be chaired by Jean-Francois Dehecq, Chairman and CEO of Sanofi-Synthelabo.

Collaboration

MedImmune and Cerus to co-develop therapeutic vaccine

The biotechnology company MedImmune (http://www.medimmune.com) have reached an agreement with Cerus Corporation (http://www.cerus.com) to develop and commercialize a novel therapeutic vaccine against breast, colon, prostate and metastatic melanomas.

Cerus will participate in the development of the vaccine whereas MedImmune will be responsible for clinical testing and commercialization of any product resulting from the collaboration. Commenting on the collaboration, Peter Kiener, Vice President of Research at MedImmune, said: 'Cerus Corporation's therapeutic vaccine technology greatly complements MedImmune's existing program targeting the EphA2 protein in cancer…Because EphA2 is overexpressed by many types of human cancers, we believe Cerus' technology may be employed to develop a vaccine that can stimulate the immune system to attack cancerous cells expressing EphA2.' Stephen Isaacs, President and CEO of Cerus Corporation, said 'We are excited to enter into a collaboration with MedImmune, a leading biotechnology company with a track record of developing successful products and an important new cancer target in EphA2.'

Business was written by *Christopher Watson*

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