bind to a target protein. Hence, this approach provides a basis for a new drug discovery technology that uses DCL to identify novel, potent ligands. This technology could be applicable to the discovery of inhibitors of other therapeutically useful proteins.

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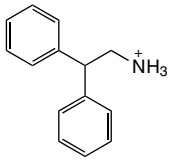
Binders to the angiogenesis marker fibronectin

The formation of new blood vessels from preexisting blood vessels (angiogenesis) is an essential prerequisite for the growth of the majority of aggressive solid tumours but is a rare event in adult physiology, with the exception of the female reproductive cycle. Overexuberant angiogenesis is a characteristic feature of blinding occular disorders (e.g. diabetic retinopathy and age-related macular degeneration) and rheumatoid arthritis. Therefore, markers of angiogenesis represent an ideal target for molecular intervention in, for example, cancer. Fibronectin is a multidomain adhesive glycoprotein that is abundant in plasma and tissues. Alternative splicing of the primary transcript of fibronectin results in the production of several different isoforms of this glycoprotein. One of the extra domains (ED) of fibronectin is ED-B, which comprises a 91 amino acid sequence that is identical in mouse, rat and human fibronectin proteins. ED-B-containing fibronectin (B-FN) has a restricted pattern of expression and is undetectable in normal adult tissues and in mature blood vessels. However, B-FN accumulates in the regenerating tissue around new blood vessels. As a result of the high level of conservation of the ED-B sequence, the generation of monoclonal antibodies by immunisation has been unsuccessful to date [9]. In addition to high-throughput methods of drug discovery, some groups are using screening methodologies that are based on 2D-heteronuclear NMR spectroscopy for protein targets of molecular weights less than 30,000 Da [9]. A marked advantage of using target-based NMR screening methodologies over conventional HTS is the information that is gained about the binding site for the drug under investigation. However, NMR screening methods have low throughput and require substantial amounts of both protein and ligand. Nevertheless, the structural information acquired in the process could be invaluable for, for example, the design of focused affinity-matured libraries of lead compounds. A recent study [10] used NMR screening of a small, rationally designed library of low molecular-weight compounds to identify lead compounds that bind specifically to the ED-B domain of fibronectin. Eighty-five compounds were tested in groups of five compounds for binding to ^{15}N-labelled ED-B. The spectrum of each mixture was recorded and compared with a reference spectrum of ^{15}N-labelled ED-B alone. From this comparison, it was observed that one particular mixture produced a large shift in the resonance of the signal from a single backbone amide group. Deconvolution of this mixture, followed by the re-screening of the five compounds individually, led to the identification of the small molecule **v** as the most potent binder to ED-B, which had a dissociation constant K_d in excess of 5 mM. This work has provided a small molecule starting point for the design of multidentate ED-B binders with improved affinity and this approach warrants further investigation.



(v)

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Biology

Cancer biology

A novel mechanism of anti-estrogen resistance

Anti-estrogens inhibit estrogen receptor-positive breast cancers by arresting cell growth. However, treatment is associated with resistance. In order to improve therapies, it is therefore essential to understand the processes underlying acquired drug resistance.

ACTR is a nuclear hormone receptor co-activator that is overexpressed in breast cancer. While investigating the role of this protein in proliferation, workers in the laboratory of Hong-Wu Chen uncovered a novel mechanism of anti-estrogen resistance [1].

In the presence or absence of estradiol, depletion of ACTR with siRNA inhibited proliferation, while increased expression enhanced cell growth. Remarkably, elevation of ACTR also promoted proliferation in the presence of anti-estrogens. Proliferation correlated with increased cells in S phase and a selective induction of E2F1 target genes critical for the G1/S transition.

Chromatin immunoprecipitation demonstrated that ACTR could bind the promoters of E2F1-regulated genes, suggesting that it may be an E2F1 co-activator. Accordingly, ACTR augmented E2F1 mediated transactivation in promoter-reporter assays. E2F1 bound directly to ACTR via an N-terminal region

1359-6446/04/$ – see front matter PII: S1359-6446(04)03156-3

of the protein distinct from the hormone receptor binding domain. Loss of this interaction abolished the ability of ACTR to enhance E2F1 function.

These results indicate that ACTR plays a key role in proliferation and, consequently, acquisition of drug resistance. Amplification of ACTR in breast cancers presumably bypasses estrogen receptor dependence by utilising the alternative 'E2F1 pathway', rendering cells refractile to the growth inhibitory effects of anti-estrogens. It is therefore conceivable that selective modulation of ACTR will provide a means to overcome this form of resistance.

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Neurobiology

mtDNA instability and mitochondrial disorders

Human mitochondrial DNA (mtDNA) works as a small autonomous cytoplasmic genome featuring a higher level of somatic mutations, compared with nuclear genome (chromosomes). Part of these mutations is pathogenic leading to mitochondrial function abnormalities with numerous clinical syndromes (they can be owing to certain nuclear mutations as well). Mitochondrial disorders are often associated with deletions in mtDNA, which are likely to play also a role in the aging process.

One such disease, which is characterized by sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO), has recently been described [2]. Molecular-genetic testing of four unrelated SANDO patients revealed multiple mtDNA deletions in their muscles and peripheral nerves. The latest case report from Florida Univ. College of Medicine confirmed, by PCR analysis, the association of SANDO with mtDNA deletions and noted that a difficulty in the eyes movement (ophthalmoparesis) hampering normal vision – the most evident manifestation of this rare disease – should lead the physician toward a possible diagnosis of SANDO, if observed together with some other specific conditions (sensory loss, slurred speech and other neurologic signs).

A recent study performed by researchers from Harvard Medical School (Boston) using single-molecule PCR has quantitatively proved the accumulation of mtDNA deletions in brain-related tissue of elderly person [3]. The same analytical approach was used by these researchers with co-workers in an extensive analysis of mtDNA [4] from a muscle tissue of an individual with a mild mitochondrial disorder (exercise intolerance and cellular respiratory chain deficiency). It was found that mtDNA in this case carries a 2-bp deletion leading to the disease. The mutation can also be responsible for the unusual expansion of the paternal genotype discovered in this mitochondrial genome (normally, mtDNA has maternal origin only). This exclusive case with both maternal and paternal mtDNA inheritance allowed to observe rare mtDNA recombinants, to quantitate their frequency (~0.7% of total mtDNA), and to locate three recombination hotspots (most frequent DNA breakpoints) [4]. The identification of break-point hotspots in human mtDNA is important for understanding the origin and propagation of mitochondrial diseases.

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Molecular biology

The structure of a DNA clamp bound to the clamp loader suggests how it is loaded onto DNA

DNA sliding clamps are required for processive DNA replication. In eukaryotes, the clamp loader complex (replication factor C, RFC) loadsthe sliding clamp (proliferating cell nuclear antigen, PCNA) onto primer–template junctions. Clamp loading is driven by ATP hydrolysis, but it is not clear how ATP binding and hydrolysis allows the complex to specifically recognize primed DNA and release PCNA onto the DNA. Bowman *et al.* [5] have solved the structure of an RFC–PCNA complex and suggested a mechanism for primer–template recognition.

RFC is a complex of one large and four small subunits that belong to the AAA+ family of ATPases. In the structure, RFC forms a spiral that sits on the PCNA ring. Between subunits there is a non-hydrolyzable ATP analogue, which bridges the subunits and holds the spiral together, suggesting that this conformation is ATP-dependent. Contacts at the interfaces of the subunits position the catalytic residues, such that they are likely to be optimal for ATP hydrolysis. Interestingly, the large subunit has an extra domain in order to link the two ends of the spiral.

The geometry of the RFC spiral corresponds very well to the twist of DNA, so the authors modelled a segment of primer–template DNA into the structure. The PCNA side of the spiral can accommodate double-stranded DNA, whereas only a single strand, corresponding to a 5′ overhang of primed DNA, can fit out of the other end. This explains the high affinity of the RFC complex for primer–template junctions and why, after ATP hydrolysis, the binding specificity is lost. Therefore, ATP hydrolysis would collapse the RFC spiral and release PCNA onto the DNA.

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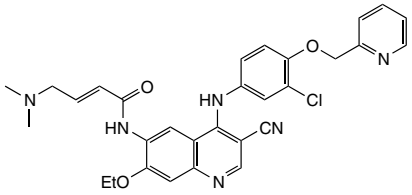
Targets and mechanisms

Competition increases to develop inhibitors of the HER family



There are four members of the human epidermal growth factor receptor (HER) tyrosine kinase family of which at least two, HER-1 (ErbB-1) and HER-2 (ErbB-2), are thought to play a role in certain cancers.

Rabindran *et al.* now report that Wyeth's HKI-272 is an inhibitor of HER-2 (IC_{50} 59nM) that also significantly inhibits HER-1 (IC_{50} 92nM) [8]. However, HKI-272 does demonstrate selectivity over other tyrosine and serine-threonine kinases. In common with it's selectivity profile, the antiproliferative activity of HKI-272 was relatively low in cell lines that lack HER-1 and HER-2 expression (e.g. SW620 IC_{50} 690nM), but more potent in cell lines expressing HER-2 (BT474 IC_{50} 2nM) and HER-1 (A431 IC_{50} 81nM), which correlated with an inhibition of HER protein autophosphorylation. This also resulted in a decrease of cells in S-phase of the cell cycle and some increase in the sub-G1-phase, which is suggestive of apopotosis, although this was not confirmed by any direct measure.

Further experiments confirmed the *in vivo* activity of HKI-272. Dose-dependent inhibition of xenograft tumours (BT474; SK-OV-3; A431) was observed with significant inhibition at 5–40mg/kg following once-a-day dosing. Unfortunately, this *in vivo* activity was difficult to put into context as no detailed pharmacokinetic data was included in the publication. However, near complete inhibition of HER-2 phosphorylation was observed *in vivo* up to six hours following a 40mg/kg single dose of HKI-272, suggesting the compound does have a sustained action. This article suggests that HKI-272 offers an alternative approach in the treatment of HER-positive breast cancer over the currently approved agents Herceptin and Iressa.

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Microbiology

Cytolysin-mediated translocation and microdomain secretion in *Streptococcus pyogenes*

The important human Gram-postitive pathogen *Streptococcus pyogenes* has the ability to deliver effector proteins into the host cell cytoplasm by insertion of the pore-forming protein streptolysin O (SLO), in a manner functionally equivalent to Gram-negative type III secretion.

Meehl *et al.* [6] investigated the specificity of CMT by substituting SLO a related pore-forming cytolysin PFO and by a series of mutations in SLO followed by analysis of translocation of the effector protein NAD-glycohydrolase (SLN) into human keratinocytes. Pore-formation alone by PFO or truncated SLO is not sufficient for translocation of SLN, and the N-terminal part of SLO is required for translocation. This shows that CMT is not a random leakage of secreted proteins into cells, but a specific delivery machinery of effector proteins.

Rosch et al. [7] continued by elucidating if *S. pyogenes* has a localized secretion machinery that could potentially feed proteins into the CMT machinery. The Sec-dependent secretion of the cysteine proteinase SpeB and PhoZ was studied using electron micrographs with gold labelling and fluorescence microscopy. This showed that the general secretion (Sec) machinery is highly concentrated into a microdomain (named ExPortal) specialized in protein export. The targeted secretion through ExPortal could be of importance in several aspects of *S. pyogenes* pathogenesis and might be functionally linked to CMT. These two excellent studies open up the possibility to study how *S. pyogenes* virulence factors are directed from the bacterial cytoplasm all the way into the host cell, where they can exert their activities.

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Stuck in traffic

Severe channelopathies such as long QT syndrome, ataxia and cystic fibrosis can be caused by trafficking deficiencies of potassium, calcium and chloride channels, respectively. The endoplasmic reticulum (ER) has developed a quality-control machinery that involves retention and subsequent degradation of incorrectly folded proteins.

Khanna *et al.* [9] have studied the importance of glycan modifications on Shaker potassium channel as a model in understanding how proteins are subjected to ER-associated degradation (ERAD). Calnexin is a chaperone that interacts with immature Shaker protein in the ER. The authors used co-immunoprecipitation in the absence or the presence of drugs that prevent the interaction between Shaker and calnexin to show that Shaker protein exit rate from the ER is increased when it interacts with calnexin. Properly folded Shaker channels, when artificially retained in the ER, can become a substrate for ERAD only when they are prevented from interacting with calnexin. But more importantly, a transient interaction between Shaker and calnexin confers Shaker protein a long-term protection from ERAD that is independent of the exit rate from the ER.

By investigating the role of glycan and their modifications, the authors demonstrated that ERAD can also be prevented by mannosidase I inhibitors but not by mannosidase II inhibitors, thereby illustrating the importance of mannosidase I in the fate of glycosylated Shaker protein. This study firmly highlights the importance glycan modifications the quality-control machinery associated with the ER.

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Pentamidine congeners show activity against malaria parasites

Aromatic diamidines are currently used in the treatment of fungal and protozoal infections, but their potential as antimalarials has hardly been explored. Now, however a collaborative study between the laboratories of Tien Huang and Donald Krogstad and others has explored the activities of a series of analogues of pentamidine [10]. In this study, the linker between the two aromatic rings is varied and various groups are attached to the amidine nitrogen atoms. Some of these compounds are found to exhibit striking activity against malaria parasites in culture, show no cross-resistance with chloroquine against chloroquine resistant parasites and have selectivity indices in excess of 1000, relative to cultured human lung epithelial cells.

The authors report that the linker between the two aromatic rings appears to have a drastic effect on biological activity, with a piperazine linker exhibiting the strongest activity by far. By contrast, attachment of groups to the amidine moiety has little effect on activity as long as the amidine group remains intact. Intriguingingly, although these compounds are found to bind to DNA, no correlation between the strength of interaction with AT-rich DNA (a characteristic of the malaria parasite) and biological activity is seen. However, activity does appear to correlate with the ability of the compounds to block haem detoxification in the parasite. Compounds incapable of inhibiting synthetic malaria pigment (haemozoin or β-haematin) formation are essentially inactive. Thus, this class of compound appears to exhibit a mechanism of action similar to that of chloroquine, but avoids the chloroquine resistance mechanism of the parasite.

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Business

Announcements

National Institutes of Health launches a center for chemical genomics

The National Institutes of Health (NIH; http://www.nih.gov/) has recently launched the NIH Chemical Genomics Center – the first of its kind – which is based at the National Human Genome Research Institute's (NHGRI) Division of Intramural Research in Betheseda, MD, USA.

Many academic and government scientists find it difficult to access large libraries of organic chemical compounds. However, the new center should change that, by forming the first part of a nationwide network that aims to provide innovative chemical tools for drug development and biological research. Elias A. Zerhouni, Director of NIH, said: 'Providing public-sector researchers with this unprecedented opportunity will greatly broaden the scope of biological exploration'. He continued by explaining that: 'The NIH-supported chemical genomics network will have a transformative effect on medical research by expanding our understanding of how the human genome and proteome function, which in turn will speed the development of new ways to fight disease and improve human health.'

In addition to this announcement, Jim Inglese, who was at Merck Research Laboratories (North Wales, PA, USA; http://www.merck.com/mrl), has now been appointed as head of biomolecular screening at the new NIH Chemical Genomics Center. Christopher P. Austin, who will direct the Center, commented that: 'We are very excited that a researcher of Dr. Inglese's stature in the pharmaceutical and chemical genomics communities is joining our team. His expertise in high-throughput screening technologies and assay development will be a tremendous asset to our center'.

Aventis and Astex collaboration is extended

Astex Technology (http://www.astex-technology.com) is pleased to announce that its collaboration with Aventis (http://www.aventis.com) in the area of cytochrome P450s research has been extended. Astex, a fragment-based drug discovery company, will be able to continue supplying Aventis with their proprietary crystal structures of the human drug-metabolising cytochrome P450s.

Tim Haines, CEO of Astex, commented on the extension agreement: 'We are very pleased to be extending our existing collaboration with Aventis. The considerable successes we have achieved in the last four years, including solving the first crystal structures of human cytochrome P450s, reflects Astex's pioneering expertise in the use of high throughput X-ray crystallography'. Indeed, Astex has solved and recently published the crystal structures of two important members of the human cytochrome P450 family – 2C9 and 3A4. In addition, the structural data on P450 provides Astex and its collaborators a promising lead in developing compounds with optimal DMPK properties, thereby decreasing attrition rates for drug development.

Merger between Strakan and ProSkelia

The two companies ProSkelia (http://www.proskelia.com), based in

1359-6446/04/$ – see front matter PII: S1359-6446(04)03150-2