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Genetic approach to chemical genetics ▼

Many drugs on the market today were initially identified because they demonstrated activity in patients, animal models or cellular assays that were believed to be physiologically relevant. The advent of molecular biology allowed more detailed dissection of biological processes into their component biochemical processes. Thus, clear links between the physiological effects of compounds and their molecular mechanisms are relatively recent in the history of the pharmaceutical industry. Some of the clearest associations between genes and disease processes come from analyses of naturally arising mutations in the human population. Only in very few cases, however, is there a tight link between a single gene and a particular human disease that identifies a clear target for pharmaceutical intervention.

There are a number of molecular biology approaches that can help to delineate the functions of individual genes at the gene, transcript or protein level. These include genetic tools such as transgenic animals where only one (or a group) of genes are altered. Current techniques enable the expression of the gene to be restricted to specific tissues, and there is some degree of regulation possible by exogenous intervention, but in the vast majority of transgenic animals available today, this type of regulation was not engineered in. Biochemical approaches such as RNA interference (RNAi) can be use to reduce specific protein levels by interfering with the transcript, but it is technically difficult to apply these approaches to a broad range of cells or in whole animals. Alternatively, dominant–negative mutants, either virally encoded or stably transfected, can be used to probe gene function by reducing protein activity.

In their recent paper, Skokat and Velleca [1] describe a hybrid of these approaches where they introduce a mutant copy of a gene of interest that possesses wild-type activity but is engineered to bind to specific unique ligands. Replacement of the wild-type copy of this gene by homologous recombination with this engineered version results in mice where the activities of the encoded mutant proteins can be examined specifically by dosing with exogenous low molecular weight ligands. Because the engineered proteins are wild type in every other respect, these recombinant mice provide excellent models of what might be expected if unique selective inhibitors were developed as therapeutic agents. Not only can the overall physiological response to inhibition be measured, but more detailed analyses can also be performed; for example, assessing changes in transcriptional and/or proteomic profiles or direct quantitation of downstream products.

As with any method designed to be a surrogate for the effects of a novel drug, there are drawbacks. Creating the mutants, although no longer scientifically challenging, is technically demanding. In addition, the high degree of selectivity that can be achieved with compounds interacting with mutants is in many cases extremely difficult if not impossible to achieve using drug-like molecules designed to interact with the endogenous target. Despite these limitations, Shokat and Velleca have demonstrated the potential power that can result when genetics and chemistry are ingeniously combined.

Reference

1 Shokat, K. and Velleca, M. (2002) Chemical genetics: novel approaches to the discovery of signal transduction inhibitors. *Drug Discovery Today* 7, 872–879

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Mining the human ‘kinome’

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Whenever there is a major advance in science, new tools and paradigms change and accelerate the pace of discovery. The sequencing of the human genome has had a major effect on the way we pursue the discovery and development of new drugs.

The now commonly used ‘omics’ terminology has come to refer to the paradigm shift that genomics has had on the way we see biology. At the *2nd International IBC Conference on Protein Kinases* held in Boston (MA, USA; 9–10 September 2002), use of the terms ‘kinomics’ and ‘phosphonomics’ reflect the impact that

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genomics has had on the development of new drugs in this important target area.

The conference covered on-going research in the discovery of therapeutically relevant pathways, technologies for screening kinase targets in HTS, the development of leads and the testing of those leads in the clinic. This provided an exciting glimpse down a path that will hopefully lead to new drugs for treating diseases, such as cancer and inflammation.

Control of complex intra-cellular processes

Protein kinases (PKs) are an important class of intracellular enzymes involved in the regulation of a large variety of biochemical pathways. Because of their regulatory control, PKs have a major role in human disease. More than 500 genes in the human genome code for PKs and ~400 diseases are associated with PK-mediated pathways. A large fraction of these are involved in cancer and inflammation.

In drug discovery, complex and interdependent regulatory pathways often serve as therapeutic targets. In his keynote address, Lewis Cantly of Harvard Medical School (http://www.hms.harvard.edu) described the signaling pathways controlled by phosphoinositide 3-kinase (PI3K). He showed how gene knockouts and bioinformatics could be used to map these pathways and their relation to disease. Stressing the importance of bioinformatics, he described how ScanSite (http://scansite.mit.edu), a data mining tool co-developed by researchers at the Massachusetts Institute of Technology (http://med.mit.edu/) and Harvard Medical School, can help identify and rank structural motifs that confer selectivity for many PKs. Using ScanSite, tuberin was identified as an AKT substrate that biochemically linked PI3K to tuberous sclerosis.

The need for new technology

The demand to increase the magnitude and comprehensiveness of 'kinomic' drug discovery requires new technology. Topics covered in the conference ranged from cellular and biochemical tools to computational approaches.

The architecture of the kinome is key to understanding the cellular pathways involved in disease. We need new cell-based technology to map these pathways. The Food and Drug Administartion (http://www.fda.gov) and the National Cancer Institute (http://www.nci.nih.gov) have formed a new jont research program called the Clinical Proteomics Program, directed by Emmanuel Petricoin and Lance Liotta. Petricoin and co-workers have taken an ambitious approach to profiling pathways at the bedside. They have developed laser capture micro-dissection to excise healthy and diseased cells from patient biopsies. Protein expression can be probed directly and disease profiles can be derived. More than 130 proteins that are associated with numerous cancers, including PKs, have been identified. Changes in protein expression can be followed over the course of treatment. This work will impact the development of individualized therapies for the treatment of cancer.

Kinexis (http://www.kinexis.ca) uses immunoblotting to enable comprehensive analysis of the expression patterns of >250 known signaling proteins. The company has evaluated >1400 antibodies in >4000 immunoblots. Steven Pelech, President and CEO of Kinexis, described how this information is used to develop analytical tools to provide a 'bar-code readout' to quantify phosphorylation and decipher kinase pathways.

A dominant theme of the conference was the importance of structural knowledge in the development of potent and specific inhibitors. BioFocus (http://www.biofocus.co.uk) combines ligand SAR and structural knowledge of the enzyme to develop 'family–activity relationships', where SAR is understood in the context of specific families of targets. Brad Sherborne, Head of Computational Chemistry at BioFocus, reviewed the development of several focused combinatorial libraries based on amidine functionalized heterocyclic core-structures. Using CDK2 as an example, he showed how the approach could be used to identify potent and selective chemotypes in support of 'hit-to-lead' development programs.

Cellular and computational approaches are not the only ways to move kinase targets through the discovery and development pipeline. New biochemical approaches to screening kinase targets are being developed. Miniaturization in the HTS laboratory is becoming crucial to efficiently screen the growing number of targets. Development of miniaturized HTS assays must be simplified. Homogeneous assays like fluorescence polarization (FP) and time resolved fluorescence resonance energy transfer (TR-FRET) are popular. Companies such as Promega (http://www.promega.com), Molecular Devices (http://www.moleculardevices.com) and Panvera (http://www.panvera.com) offer kits that are suitable for screening kinases using FP.

New technology is being developed to address specific problem areas. Bill Ricketts from Ribapharm (http://www.ribapharm.com) described his evaluation of a new approach to screening serine and threonine kinases using fluorogenic substrates developed by Promega that does not require substrate-specific antibodies. Luis Stancato of Eli Lilly (http://www.lilly.co.uk) described the Caliper mobility-shift assay technology.

Steven Anderson of Abbott Laboratories (http://www.abbott.com) reported on the development of μARCS (Microarrayed Compound Screening) for screening kinases. The technology uses compounds arrayed at high density on plastic sheets that are transferred to gels containing assay reagents. Anderson also described ASMS (Affinity Selection Mass Spectrometry). This approach is used to identify compounds that bind directly to tyrosine, serine and/or threonine kinases.

Detailed knowledge of enzyme structure can be indispensable to a development

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program. Syrrx (http://www.syrrx.com) uses nano-volume crystallization to simplify and speed the production of protein crystals that can be used to solve the 3D structures of proteins in important target classes such as kinases. Twelve novel structures have been solved this year and the company is targeting another 13 by the end of 2002. Dominique Perrin of Serono (http://www.serono.com) described the use of discrete sub-structural analysis (DSA) to identify chemical determinants related to compound activity. DSA helped Serono complete a hit–lead program for JNK within 90 days.

Into the clinic

Nearly every major pharmaceutical and biotechnology company has compounds in their development pipelines that are focused on kinase-related diseases. According to Jeffrey Hanke of AstraZeneca (http://www.astrazeneca.com), there are ~50 programs in clinical testing. Many of these programs are targeted toward tyrosine kinases, which are often implicated in cancer. Major tyrosine kinase targets include EGFR and VEGFR (cancer) and P38 MAP kinase (inflammation) and PDGFR.

For PKs, selectivity is an important determinant for a viable drug candidate. There can be as many as 200–300 protein kinases mediating a large number of pathways, for example in a single cell. Action at a specific pathway requires target selectively; this can depend on the mechanism-of-action of the drug. Drugs that inhibit the enzyme by competing with substrate binding are often selective, although selective ATP-inhibitors have also been identified. Chemistry based on substituted purines and pyrimidines are common, as would be expected for inhibitors of ATP binding. Compounds described by Pfizer (http://www.pfizer.com), Scios (http://www.sciosinc.com), Vertex (http://www.vpharm.com), Boehringer Ingelheim (http://www.boehringer-ingelheim.com), and Johnson & Johnson (http://www.jnjpharmarnd.com), use different classes of core structures.

P38 MAP kinase is associated with several inflammatory diseases, including rheumatoid arthritis. Jeffery Madwed of Boehringer Ingleheim showed that BIRB796 inhibits the enzyme with a potency of 24 nM and is 10,000-fold selective over a panel of kinases, including ERK, another member of the MAP kinase family. BIRB796 inhibits P38 via a novel mechanism involving two distinct sites that interferes with the binding of ATP. Preclinical results show that the compound inhibits production of tumour necrosis factor-α (TNFα), and prevented the progression of collagen induced arthritis in a mouse model. Phase II studies are currently under way.

Scott Wadsworth of Johnson & Johnson showed that small-molecule inhibitors of P38 MAP could be used for novel therapeutic uses, including allergy and HIV infection. Robert Kaufman of Vertex described how genetic and structural information has been used to take a 'chemogenomics' approach to the discovery of potent and highly selective inhibitors, such as VX745. David Liu of Scios described how they have used human and rat cDNA microarrays to identify novel pathways associated with disease. This approach was used to identify 'cross-talk' between P38 and TGF-β signaling pathways.

MEK is a member of the MAP kinase family and is involved in tumour proliferation. Haile Tecle (Pfizer) showed results of the development of a series of compounds with activity against MEK. CI1040 is extremely selective (>10,000-fold) against a large panel of kinases including P38. Inhibition is not dependent on ATP concentration, implying a substrate competitive binding mechanism. CI1040 is currently in Phase II clinical trials.

EGFR is a receptor tyrosine kinase (TK) that mediates several downstream signaling pathways that control cellular proliferation and survival. EGFR-TK is associated with a large number of cancers, including prostate, lung colon and pancreatic cancer. There are several programs actively pursuing the development of EGFR-TK inhibitors. Recently, the FDA has put Iressa (AstraZeneca) on an accelerated approval track for the treatment of non-small cell lung carcinoma. Jeffery Hanke described the use of a structure-based approach to find selective inhibitors of ATP binding. Tarceva™ is another EGFR inhibitor being developed by Hoffman-La Roche (http://www.roche/com) in collaboration with OSI Pharmaceuticals and Genentech (http://www.gene.com). Tarceva™, like Iressa™, acts to block ATP binding to EGFR. Novartis has an orally bioavailable drug PKI166 in Phase IB trials; this is a dual inhibitor of EGFR –Her2 tyrosine kinases.

Conclusion

This is the second conference on protein kinases sponsored by the IBC this year. Both conferences were extremely well attended, each with >250 delegates. The conference presented a broad and comprehensive view of the current status of drug discovery in the area of protein kinases. This is, and will continue to be, an active area in pharmaceutical research. Based on the broad role that PKs have in disease, we should expect more activity in this area in the near future.