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Protein kinases in drug discovery and development

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The 4th International *Protein Kinases in Drug Discovery & Development* meeting (October 20–22, 2003, Philadelphia, USA) organized by SRI (http://www.srinstitute.com) integrated sessions that aimed to address the key issues for kinase drug discovery. With over 500 kinases in the human kinome [1] and the costs of drug development approaching the billion dollar mark, the meeting focused on strategies to move kinase drug discovery forward in a more rapid and efficient manner, including protein kinase target validation, selectivity and drugability. Topics included kinase assay technology and profiling, novel strategies for kinase inhibitor generation and associated technologies for target identification and validation, including RNAi applications.

Biochemical, cell-based assays and screening methods

Owing to enormous efforts made within the pharmaceutical industry, there are now numerous biochemical and cell-based screening methods for the thorough profiling of kinase inhibitors. Numerous factors such as cost, data quality, throughput and screening-library size need to be taken into consideration and different assays can therefore yield different screening results. Over 13 novel assay formats were outlined during the course of the meeting for tyrosine and serine/threonine kinases, all with the aim of resolving assay development bottlenecks. Gregory Moore (Upstate Discovery; http://www.upstate.com) described assay formats for a range of serine/threonine kinases using monoclonal antibodies based on a small collection of substrates and generic fluorescent polarization assays for tyrosine kinases based on Upstate's anti-phosphotyrosine antibody, 4G10. Upstate currently has the capacity to screen over 120 human protein kinases. Michael Curtin (Promega Corporation; http://www.promega.com) described the development of two novel homogeneous, non-radioactive high-throughput assays to determine the activity of many protein kinases These assays can be readily optimized and data were presented to demonstrate that these formats can produce comparable IC50s to those in the literature for known inhibitors of protein kinase A (PKA) and src family tyrosine kinases.

Phosphorylation of serine, threonine and tyrosine residues is recognised as a key mode of signal transduction and amplification in eukaryotic cells. Pierre Thibault (Caprion Pharmaceuticals; http://www.caprion.com) described how, with protein expression profiling as a principle focus in proteomic research for cancer therapies and biomarkers, Caprion has pioneered a novel mass spectrometry–bioinformatics approach to measure relative protein abundance from purified protein extracts of patient-matched disease and normal specimens.

Steven Pelech (Kinexus Bioinformatics; http://www.kinexus.ca) gave an insightful overview of Kinexus's antibody-based detection method (Kinetworks™), used to track >100 protein kinases simultaneously in diverse cell and tissue types. The multi-immunoblotting services provided by Kinexus allows researchers to accurately measure protein expression, phosphorylation and interactions with many other proteins through the direct capture and quantification of proteins by immobilized peptide probes.

Brian Holaway (Roche Protein Expression Group; http://www.roche.com) described the expression of p38 alpha in inactive or activated forms using a continuous-exchange cell-free (CECF) protein expression system, which potentially overcomes the limitations of current cell-based methods. Rigorous biophysical characterization, including 2D-NMR approaches, also identified a number of conformational differences between the activation states that could potentially be exploited by small-molecule inhibitors.

Structure-based design strategies and applications

Structure-based drug design (SBDD) facilitates rapid compound potency optimization against protein targets. Kinases are readily amenable to SBDD and with >40 novel kinase structures deposited in the Protein Data Bank, there is a growing understanding of how to achieve selectivity. Kumkum Saxena (Vertex Pharmaceuticals; http://www.vpharm.com) discussed how rapid structure determination of kinase target crystal structures is a key tool in the drug discovery process at Vertex. Many hundreds of constructs for each kinase are designed, expressed and purified by high-throughput methods and, following rigorous protein analysis, this approach produces a large increase in crystallization success rates of these

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well-characterized kinases. The protein kinase Pim-1 was given as a worked example, with a staurosporine-bound structure solved to 2.2 Å resolution.

Adrian Gill (Astex Technology; http://www.astex-technology.com) outlined how high-throughput X-ray crystallography can now be used as a screening tool in fragment-based drug discovery. Gill outlined case studies on p38 and CDK-2, showing how millimolar-affinity fragment hits from Astex's Pyramid™ drug discovery platform could be rapidly and efficiently developed into selective, nanomolar-affinity, cell active inhibitors.

Prabha Ibrahim (Plexxikon; http://www.plexxikon.com) described the company's Scaffold-Based Drug Discovery™ platform for kinase lead generation, which uses a combination of low-affinity biochemical screening of >20 000 selected scaffold-like compounds and high-throughput co-crystallography. Three kinase targets are currently being investigated, namely c-abl (and a number of key clinical mutations), Pyk-2 and c-met. To date, >53 novel scaffolds have been discovered that have a range of activities against these targets.

Michael Vieth (Eli Lilly; http://www.lilly.com) outlined how knowledge of the kinome and abundant kinase selectivity data allows for significant advances in the understanding of the relationship between kinase targets and inhibitors. Coupled with computational docking and X-ray crystallography, chemogenomic approaches can be used to advance compounds through the drug discovery pipeline. Novel classes of kinase inhibitors based on a pyrazole–quinoline scaffold targeted against TGFBR1 kinase were highlighted.

New targets, early discovery and development

Given the complexity of kinase signaling, target identification and validation have long been key issues in kinase drug discovery. Recent advances in the use of RNA interference (RNAi) methods provide a powerful new approach to study the genetics of human disease. Luk van Parijs (Center for Cancer Research – MIT; http://web.mit.edu/ccr/index.html) described how lentivirus-based RNAi systems can silence gene expression in primary cells, tissues and animals. This technology has been used to perform reverse and forward screens for genes that control the development of autoimmunity and immune cell cancers, allowing the identification and validation of novel targets for drug discovery.

Mark Valleca (Cellular Genomics; http://www.cellulargenomics.com) described an integrated chemical-genetic approach to kinase drug discovery by coupling Analog Sensitive Kinase Allele (ASKA) technology to capabilities in medicinal chemistry and HTS. Case studies centred on the protein kinases Btk and EphB4, with potent, selective and cell active compounds being identified in a rapid and efficient manner from their high-throughput accelerated lead optimization (HALO) platform.

David Dudley (Pfizer; http://www.pfizer.com) described how the discovery of the non-ATP competitive mitogen activated protein kinase kinase 1 (MEK) inhibitors PD 98059 and PD 184352 (CI-1040) facilitated significant insight into the biological role of the MAP kinase signaling pathway. Recent structural, enzyme kinetic and mutation experiments have identified an inhibitor-binding site adjacent to the ATP binding site of the MEK isoforms.

p38 MAP kinase has a central role in the mediation of chronic and acute inflammatory diseases. Andrew Protter (Scios Inc; http://www.sciosinc.com) described new insights into the pharmacology of the potent and selective p38 MAPK inhibitors SCIO-323 and SCIO-469 (currently in phase I and phase II trials, respectively). Eric Springman (Locus Pharmaceuticals; http://www.locuspharma.com) described a biochemical approach to understand the unique signatures of two alternate inhibition mechanisms in p38, namely allosteric inhibition resulting from conformational changes in the DFG (Asp-Phe-Gly) activation loop and docking inhibition resulting from binding directly in a protein docking site.

New therapeutic areas

The involvement of protein kinases in a wide range of human diseases has generated interest in inhibitors to target metabolic and viral diseases. Jennifer Moffat (Suny Upstate Medical University; http://www.upstate.edu) described how Roscovitine, a cdk inhibitor, prevents replication of varicella zoster virus at the level of viral gene transcription and genomic DNA replication. Elaine Kilgour (AstraZeneca; http://www.astrazeneca.co.uk) described how targeting the pyruvate dehydrogenase (PDH) multienzyme complex with a tool compound such as AZD7545 could be used to develop future treatments for type 2 diabetes.

Concluding remarks

Overall, the meeting attendees considered this to have been an informative gathering of pharma, biotech and academic researchers in the protein kinase field, with a range of crucial questions concerning the drugability of protein kinases as a target class addressed. Despite the recent approval of Gleevec® (c-abl inhibitor), Iressa® (EGFr inhibitor) and the clinical success of BIRB-796 (p38 inhibitor), together with their involvement in many crucial cellular roles, kinase signaling pathways remain poorly understood. A number of significant challenges still lie ahead for the drug discovery industry with regards to safety and tolerance as a consequence of kinase inhibition and off-target effects.

Reference

1 Hunter, T. *et al.* (2002) The protein kinase compliment of the human genome. Science 298, 1912–1916