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MEETING REPORTS

Highlights from the Society for Medicines Research symposium held Thursday March 10, 2005, in London, United Kingdom.

Chemical Genetics and Genomics and Drug Discovery

by Robert Williams and Katherine Brown

Summary

The SMR Symposium *Chemical Genetics and Genomics: What Are They and Are They Helping Drug Discovery* was held on March 10, 2005 at the National Heart and Lung Institute, Imperial College London. The conference program brought together an international line up of speakers representing academia, biotechnology and large pharmaceutical companies to discuss a variety of drug discovery strategies, falling under the umbrella terminology Chemical Genomics and Genetics. Highlights of the meeting are discussed.

The SMR Symposium, held on March 10, 2005, in London, was opened by Dr. Ed Zanders (Cambridge Bioscience Partners, U.K.), who followed the organizer's request for an overview of the field. He cited several definitions of the subject and suggested that chemical genomics could be summarized as the probing of cell function through the use of small organic molecules, and chemogenomics as the systematic investigation of interactions of protein families with small organic molecules. The concepts are only just starting to be explored, despite the wealth of potential information contained within both chemical and biological databases. Dr. Zanders suggested that this is largely because the concepts demand the coalescence of both chemistry and biology, a combination that has not always proved simple to achieve.

Consider the numbers involved. The human genome is estimated to contain from 25,000 to 30,000 genes, which translate to a proteome of about 100,000 protein products of which nearly 10,000 are considered likely drug targets. On the chemistry side, there exists about 10^8 small organic molecules, and theoretically 10^{80} different molecules could be produced! Thus, the chemical potential for interaction far outweighs the total number of potential biological targets in man.

Progress is also currently restricted by the lack of diversity of available compounds, often limited by the constraints of drug production or the methods of their own discovery. Combinatorial libraries, for example, are usually chemically restricted in anticipation of future optimization and development (e.g., the avoidance of chirality). Focused libraries, by definition, are directed at preselected target types and may not be appropriate for diverse target identification. *In silico* design methodologies, however, are not limited in this way; there has been some success, and the sophistication of algorithms appears to be advancing. Natural products have, in recent years, received less attention, owing to fears of poor purity and control over intellectual property rights. However, the audience was reminded that *Gleevec*® is derived from staurosporine, and the statins owe their development to natural product origins.

The classic genomic approach to drug discovery has been to analyze sequence data to identify and classify target families and bring screening to manageable proportions. However, approximately 30,000 proteins have now been structurally determined by X-ray diffraction. These can be used to create arrays for ligand/protein

binding and visualization of the proteome. The biological effects of these chemical probes can be determined by a variety of techniques: high-throughput cell function assays, ligand affinity chromatography, and perturbation of signaling pathways coupled with global analysis of cellular mRNA. These categories signal the arrival of a meeting between chemical genomics and systems biology in which the gaps in our knowledge of signaling pathways can start to be filled. If it is not possible to screen all potential chemicals against all targets, it is possible to assess the statistical likelihood of their association from chemical genomic data.

The impact upon drug discovery is already being felt. Industry molecules and the world's best-selling drugs are being used as models by academics for biological profiling. They are being evaluated both for target generation and off-target effects (for other drug applications and/or the identification of adverse effects). From these data, new sets of drug candidates are being synthesized to provide compounds with improved potency and ranges of selectivity.

Following Dr. Zanders' overview, the next presentation, with the thought provoking title *Chemical Space Meets Biological Space. Now What?* was given by Dr. Jordi Mestres from the Municipal Institute of Medical Research at the University of Pompeu Fabra, Barcelona, Spain. Dr. Mestres described his research as focusing on how to store data and extract knowledge. Knowledge extraction in many areas is hampered by the lack of rigorous classification schemes in many areas, citing protein families such as G-protein–coupled receptors and nuclear hormone receptors. This contrasts with the hierarchical, four-digit classification system that exists for enzymes. Classification systems for small molecules are even worse. Many organizations are familiar with compounds having several numbers assigned to them during their evolution, for example, from hits to leads. Dr. Mestres proposed a numbering system for small molecules relating to the number of rings in the core structure, total number of ring systems, framework identity, scaffold identity and unique molecular identity. This scheme could annotate to biological data (e.g., a binary active/inactive scheme) and match, for example, certain scaffolds with activity. A working example was presented of an annotated chemical library directed to nuclear hormone receptors. One thousand two hundred and sixty ligands had been annotated to 24 receptors and relationships relating compounds to activity against certain receptors and pathways used to identify privileged or promiscuous scaffolds.

The final talk of the morning session was delivered by Dr. Edgar Jacoby from Novartis Institute for Biomedical Research in Basel, Switzerland, entitled *Chemogenomics Knowledge-Based Strategies for Drug Discovery*. In a similar vein to the previous presentation, Dr. Jacoby stressed the importance of annotation of compound activity and relationships to protein families. Novartis combines a molecular information system (based on an MDL drug database) with annotated high-throughput screening and profiling data to design screening collections for target families such as G-protein–coupled receptors. Following screening of focused libraries and identification of hits, further compounds are identified for screening based on two-dimensional fingerprints and "similog keys." A challenge highlighted by Dr. Jacoby was identifying and building in desired selectivity profiles for compounds active against proteins from target families.

The second conference session was comprised of three presentations falling into the area of forward chemical genomics (phenotype first approach). The first presentation entitled *VASTox Chemical Genomics Approach: Waging War on Attrition* was given by Dr. Andy Mulvaney (Vastox, U.K.). Vastox's strategy is based on phenotype first screening to validate targets and identify leads in a single step. The approach is focused on the use of zebrafish and Drosophila and the identification of chemotypes (chemically induced phenotypes).

Zebrafish are vertebrates of high fecundity for which there are 8,000 recognizable phenotypes associated with single gene mutations. Dr. Mulvaney cited an example of how targets could be identified from analysis of genetics following identification of a chemotype in this model. An inhibitor of PKC was found to induce edema and bent body shape, matching the phenotype induced by genetic mutation of the gene. Similar correlations between chemotypes and genetic mutations have been observed for GSK-3β. Dr. Mulvaney suggested that off-target effects might be identified by screening compounds in zebrafish and cited atorvastatin-induced chemotypes being indicative of modulation of the hedgehog pathway. Reference was also made to a paper by Peterson et al. (Nat Biotechnol 2004, 22: 595–9), where a novel compound had been identified that suppressed a mutation-induced cardiovascular defect. Dr. Mulvaney closed by proposing the wider utility of zebrafish in toxicology screening including rapid analysis of specific organ toxicity.

Dr. Curtis Keith (CombinatoRX, U.S.A.) highlighted that many disease conditions were treated by combinations of drugs and thus required inhibition of multiple target sites. He cited the example of *Augmentin* (amoxicillin trihydrate/clavulanate potassium; a β-lactam and β-lactamase inhibitor) and the many combination regimens deployed in cancer chemotherapy. Dr. Keith did not believe that we possessed sufficient knowledge to identify relevant points of intervention from the study of anatomical network maps. He proposed that a relevant, pragmatic strategy to the identification of new combination drugs was the use of cell-based forward chemical genetic screens with "disease-rele-

vant" readouts such as inflammatory mediator production. Dr. Keith described experiences at CombinatoRX that have revealed a number of instances where drugs displayed little or no activity alone but clear synergistic activity in combination. In the oncology area, **chlorpromazine** (which possesses activity against mitotic kinesin) and **pentamidine** (which possesses activity against PRL phosphate) display potent antiproliferative activity in combination. Similar findings had been made with compounds in combination when tested for inhibition of MRSA replication.

Dr. Nick Westwood (St. Andrews University, U.K.) opened his presentation, *Forward Chemical Genetics—Successes and Challenges*, by citing the discovery of **monastrol** as one of many examples where a drug had been identified by phenotype-first, chemical genetic screening, followed later by identification of the compound target. Dr. Westwood described a number of forward genetic screening campaigns undertaken in his laboratory but highlighted also the value of the "interdisciplinary training" of researchers in this setting. Dr. Westwood claimed that young researchers gained broad technological awareness, skills in compound synthesis and handling, and the ability to frame relevant biological questions. Recently, equipment for drug screening has become affordable to academia for investigation of the activities of novel compounds. A specific screening experience was described where 12,160 compounds were tested at 10 μM for activity in a *Toxoplasma gondii* motility assay modeling host cell invasion. Forty-four active compounds were identified and 24 further confirmed as pure compounds. Dr. Westwood's lab had pursued some early chemical optimization work aimed, for example, at increasing potency for affinity labels.

In the final session, there were two presentations from U.S. speakers who had braved the East Coast snowstorms to attend the symposium. Dr. Andrew

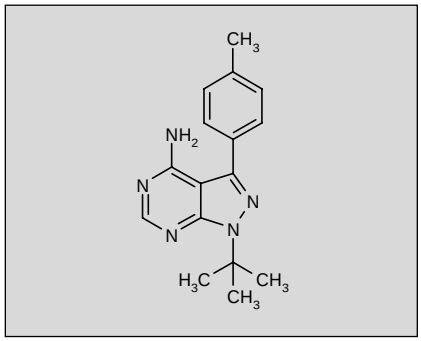

Fig. 1. PP1: Synthetic inhibitor used by Cellular Genomics that does not inhibit wild-type kinases.

Whitney (Cellular Genomics Inc.) described the use of *Analogue Sensitive Kinase Allelles* (ASKA) in drug discovery efforts at Cellular Genomics. The ASKA technology makes use of genetically modified kinases in which a binding pocket is introduced that confers susceptibility to potent inhibition by a synthetic inhibitor, **PP1** (Fig. 1), which does not inhibit wild-type kinases.

PP1 possesses good pharmacokinetic properties and a good safety profile, which makes it suitable for use in *in vivo* target validation studies. A similar, labeled probe (rather than a pharmacological inhibitor) has been developed for use in pathway mapping and substrate identification studies.

Dr. Whitney described some specific examples of the utility of the ASKA technology. EphB4 is a kinase overexpressed in tumor tissue, and knockout of this enzyme is embryonic lethal in mice. Eph4 ASKA mice, however, are viable and show a tissue distribution of enzymes comparable to that of wild-type animals. Studies using EphB4 ASKA mice have confirmed a role for this enzyme in supporting tumor growth and provided useful information relating to biomarker development and target safety.

A Btk–ASKA model was described that had been used predominantly in *in vitro* studies. This enzyme plays a key role in signaling in multiple inflammatory cells. Studies conducted at Cellular Genomics have utilized HEK293 cells, which do not normally express Btk. These cells have been used to define "transcriptional fingerprints" arising from Btk–ASKA transduction and specific inhibition by **PP1**. Such studies have proved useful in determining specificity of test compounds.

Dr. Steve Hall (Serenex, U.S.A.) gave the final talk of the day. The focus of Dr. Hall's talk was *Chemoproteomics-Driven Drug Discovery*. At the heart of the Serenex approach is use of a technology to bind compounds specifically to the purine-binding proteome, which includes kinases and a range of other protein families. Compounds are screened for their ability to displace binding of specific proteins and have the advantage that no compound modification is required prior to test. To date, Serenex has tested 12,000 purified compounds and eluted 650 unique proteins. Dr. Hall described two examples of the validation of this technology. Compounds have been identified that bind and inhibit HSP-90 but not ADE6, the latter of which is involved in purine biosynthesis and a cause of toxicity associated with the prototypical HSP-90 inhibitor **geldanamycin**. These compounds have been licensed and are currently undergoing phase II clinical evaluation. A further example of the value of profiling of compounds against the purine subproteome was given for two inhibitors of the epidermal growth factor receptor (EGFR). One compound was found to bind five other targets including a liver enzyme and was known to have been dropped because of toxicity issues. A second compound was shown to be a specific inhibitor of EGFR and HER2 and is known to still be progressing in development.

Dr. Hall went on to describe areas of current interest for Serenex. Inhibition of quinone reductase 2 is believed to underpin the antiinflammatory effects of **chloroquine**, while side effects are linked to inhibition of

aldehyde dehydrogenase. The Serenex technology is being deployed to identify quinone reductase-specific compounds. Similarly, the toxicity of **methotrexate** is associated with activity against a number of liver proteins, and a chemistry program is underway to find compounds devoid of liver-binding activity.

Dr. Hall finished by highlighting that this technology had the power to identify potent inhibitors of specific targets even when starting from the point of screening with no particular target in mind. Eight thousand adenosine derivatives were screened against two proteomes and 300 compounds were identified that displace protein binding with an IC_{50} of 10 μM or less, 95 compounds with an IC_{50} of 1 μM or less, 250 selective for a single target and 100 dual inhibitors. HSP-90 has been a particular company interest and in 9 months, single digit nanomolar inhibitors have been identified. This discussion was a fitting end to a day of thought-provoking presentations from scientists at the leading edge of drug discovery in the postgenomic era.

This symposium can be seen as a webcast with audio and synchronized slide presentation at webcast.prous.com/SMR_Mar_2005.

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