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The Screening Asia Conference 2010: an update

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Abstract

The conference was the first of its kind in a series of trend-setting conferences conceived and organized by Select Biosciences. The conference was held in the state-of-the-art Matrix Building, Metropolis. The topics covered were grouped into the following half-day sessions: high-content screening, high-throughput screening, RNAi screening, novel drug-discovery techniques and assay development, and screening natural extracts and small molecules. Petr Bartunek (Group Leader, Institute of Molecular Genetics, Prague, Czech Republic), Rathnam Chaguturu (Director, High-Throughput Screening Laboratories, University of Kansas, USA) and Paul Orange (Business Leader, Bio-Discovery, PerkinElmer) were the keynote speakers. The conference attendees were from academia and the biotechnology and pharmaceutical sectors. The Screening Asia 2010 was designed to address, from a regional and international perspective, how best to approach the one issue that has been at the forefront of the drug-discovery endeavors: who is best suited among academia, biotechnology and pharmaceuticals to discover chemical probes against key therapeutic targets, and how best one could implement the processes.

Drug-discovery research is like searching for a needle in a haystack. Large compound libraries, corporate databases, virtual compound collections and suppliers' databases are routinely screened for lead drug candidates. The situation is, in fact, worse than finding a needle in a haystack, as we do not exactly know the size or shape of the needle. We have very limited knowledge about the 'probe' we desire to find. Often we do not know how it looks or even if it is there. High-throughput screening (HTS) facilitates testing large numbers of compounds against a biochemical target of interest using validated biological or biophysical assays. In recent years, the origins of a significant number of drugs in clinical trials could be traced back to in-house HTS campaigns, validating HTS as a bona fide mechanism for hit finding. New lead discovery against therapeutic targets, especially those involving the rare and neglected diseases, is indeed a monumental task, and requires diligent implementation of appropriate screening-technology platforms for a successful outcome.

This theme was aptly illustrated during the keynote presentation by Rathnam Chaguturu (University of Kansas, USA), with an equally engaging title, 'New therapeutic targets, novel molecular probes and bona fide drug leads: herding the cats to the promised land'. He argued that modern drug discovery is full of promise, yet leaves much to be desired. Despite early promises, HTS has not been the panacea in substantially improving the drug-discovery

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process. The decoding of the human genome has not led to a substantial number of new drug targets. Pharmacogenomics has not yet paved the path to better, safer drugs and increased pharmaceutical R&D spending has not led to a proportionate increase in drug discovery. The pharmaceutical industry is looking backwards to the golden age of phenotypic-based drug discovery and repurposing to ease the drug-discovery bottleneck. In order to accelerate drug discovery, the NIH Roadmap program and the Eu-Openscreen initiative have sought to increase the role of academia and foster collaboration with the pharmaceutical industry. Industry-style probe discovery has now gained unparalleled momentum in academia with the availability of vendor-supplied chemical libraries, ready access to institutional HTS laboratories and pharmaceutical industry-style project-management methodologies. The pharmaceutical industry, in turn, has embraced the concept that a problem shared is a problem solved to engage in open-source drug discovery through a systems-biology approach. Chaguturu's talk outlined the changing landscape in drug discovery and argues that success is ever more promising – perhaps the colloquial 'herding the cats' may not be that impossible.

High-content screening

The morning session on the first day started with Paul Orange (PerkinElmer) discussing the 'Latest techniques using high-content screening (HCS) for the advancement of drug discovery', and illustrating the strategic relevance of HCS in drug discovery. With the desire to better quantify cellular phenotypic changes and get a deeper understanding of complex biological processes, HCS has become a common tool in basic research and drug development. HCS is now commonly used to evaluate:

- Target identification and validation using gene knockdown methods, such as RNAi;
- Target identification and validation in compound screening campaigns;
- Drug safety and efficacy in absorption, distribution, metabolism and excretion/toxicology evaluations.

When performed at high throughput, phenotype and pathway analyses generate a wealth of biologically relevant information, which was not previously achievable. This approach creates its own challenges, but these can be addressed by using image-based readers and their intrinsic capability to perform multiple measurements on cellular systems. Orange discussed the wide range of sample types, data collection methods, image analysis tools and data-storage methods. He emphasized that any laboratory setting up high-content analysis/HCS studies must carefully consider how these choices will impact on the quality of the results generated.

Nathalie Aulner (Institut Pasteur) shared the experiences at Leishdrug (a European FP7-funded interdisciplinary project) and the challenges of applying the HCS platform as a strategy to identify leads that selectively target amastigotes (hosting primary macrophages) in *Leishmania*. Leishdrug employs a highly multi-disciplinary strategy for leishmanicidal drug development. An established bench-top visual phenotype-based assay, utilizing mouse bone marrow-derived macrophages and DsRed2 transgenic *Leishmania amazonensis* amastigotes, has been adapted to a robust HCS platform. The high-content strategy relies on the detection and counting of fluorescent amastigotes, parasitophorous vacuoles and viable macrophages as readouts and allows the simultaneous assessment of selective leishmanicidal activity and host cell toxicity under physiological conditions. Aulner described the challenge of working with physiologically relevant primary macrophages as *Leishmania*-hosting cells and the clinically relevant amastigote stage for assay miniaturization, the statistics and sampling approaches as well as preliminary results from pilot screens.

The talk by Jonathan Cechetto (Institut Pasteur Korea) dealt with the applicability of HCS for small-molecule lead discovery against infectious and neglected-disease targets. A critical feature of a number of infectious and neglected-disease agents is their ability to survive and multiply within human cells. The killing of these intracellular microbes is a key requirement for efficient treatment. For organisms such as *Mycobacterium tuberculosis*, *Leishmania donovani* and *Trypanosoma cruzi*, identification of potent inhibitors has been hampered by labor-intensive techniques and a lack of validated targets. At the Institut Pasteur Korea, Cechetto's team has developed phenotypic, cell-based assays that use automated confocal fluorescence microscopy and automated image analysis for the HTS of small molecules that interfere with the survival of these organisms within host cells. Application of these assays enabled the novel small molecules active against either the infectious organism directly or host cell targets required for infection, survival or replication of the infectious agent to be identified.

Hakim Djaballah (Memorial Sloan Kettering Cancer Center, USA) discussed his laboratory's adaptation of a biosensor-based technology to monitor EGF receptor activation, internalization and recycling in live cells, using high-content assay methodologies. In addition, he highlighted this strategy as a novel biosensor-screening approach with applications to other signaling receptors for discovering novel modulators with potential therapeutic use.

High-throughput screening

The afternoon session started with a presentation by Horst Flotow (Experimental Therapeutics Center, Singapore) titled, 'Translational research driven by high-throughput screening approaches'. The focus of his research was gastric cancer, the second leading cause of global cancer mortality. One reason for this is the large heterogeneity in oncogenic profiles between gastric adenocarcinomas, which leads to a poor response to standard chemotherapy. To identify drugs that can reduce tumor growth in a broad spectrum of gastric cancers, his team used a panel of six molecularly diverse gastric cancer cell lines to screen a library of over 3000 pharmacologically active compounds. A high-throughput ATP-dependent luminescence-based cell-viability assay served as the primary screen. An imaging-based method was used as a secondary screen to directly identify compounds that can reduce cell proliferation and induce apoptotic cell death. The results showed that each cell line displayed a unique drug-response profile. Compounds that impair the growth of five out of six cell lines were deemed potential drug candidates for standard gastric cancer chemotherapy. On the other hand, compounds that kill selective cell lines were considered to form the basis of targeted therapy for patients with gastric tumors showing similar molecular profiles.

A natural product toolbox for the HTS paradigm was the theme of the talk delivered by David Camp (Eskitis Institute, Griffith University, Australia). The wealth of chemical diversity that has evolved with biological diversity has been used as a source of medicines by indigenous people for thousands of years. Research into natural products reached a peak between the 1970s and 1980s. However, by the 1990s research on natural products by the pharmaceutical industry was declining. This decline was the result of various factors, including the low throughput of screening/isolation of pure compounds from natural product extracts. Drug discovery turned to HTS of large libraries of pure compounds synthesized via combinatorial chemistry. Since the chemical diversity of these libraries was not always relevant to biological function, this approach was not as successful as hoped. The potential rewards from natural product drug discovery are massive despite being regarded by some as a risky, speculative 'fishing expedition' with no guarantee of finding compounds of interest. Timelines, governed by the separation of compounds from extracts using bioassay-guided

fractionation, are slower than alternative lead-generation strategies. Camp outlined various process improvements implemented at the Eskitis Institute that have streamlined the conventional biodiscovery paradigm. The efficiency gains facilitated delivery of novel chemical leads from natural product screening within timelines expected by big pharmaceutical companies, and complement HTS as a lead-generation strategy.

Salmaan Inayat-Hussain (Universiti Kebangsaan, Malaysia) continued with the theme of pharmacognosy, with specific reference to styryllactones, which can induce apoptosis in a variety of cell lines. The mechanisms of styryllactones-induced apoptosis was discussed, focusing on the roles of the caspases, mitochondria and effects of oncogenes.

Martin Everett (MerLion Pharmaceuticals) discussed the use of extract fingerprinting to identify new sources of natural products (pharmaceutical leads and novel scaffolds). Chemical characterization of botanical and microbial extracts has traditionally relied on coupled HPLC methods, which are laborious and present limited opportunities to compare data from many different samples. MerLion Pharmaceuticals has developed a proprietary system for the rapid, high-throughput chemical characterization of natural extracts. The system employs UPLC coupled to MS, evaporative light scattering and photodiode array detectors, and the resulting data are processed to provide a unique profile or 'fingerprint' for each extract. Individual fingerprints are stored within a comparative database and can be analyzed to determine relative productivity, similarity and chemical diversity. Furthermore, fingerprinting data can be queried for matches to reference standards to identify new sources for compounds of interest (e.g., pharmaceutical leads and natural ingredients), or to identify promising sources for the isolation of new chemical scaffolds.

RNAi screening

Although the Inaugural Screening Asia Conference was colocated and cohosted along with the 4th Annual RNAi and miRNA World Congress by Select BioSciences in the adjacent conference hall, the Screening Asia Conference also held a session with three eminent talks on RNAi screening. RNAi is a basic cellular mechanism for silencing gene expression and has become a powerful and widely used means for developing therapeutics against viral infections, cancer, vascular diseases and other diseases. RNAi is induced by dsRNAs, also known as siRNAs. In living cells, internal siRNAs originate from longer double-stranded precursors via the activity of Dicer, a protein of the RNase III family of endonucleases (York Zhu, Biomics). RNAi holds promise as a platform technology for the rapid identification of novel targets and generation of key validation data prior to investing significant chemistry resources (Xiaoyu Lin, Abbott).

Jianzhong Xi (Peking University, China) described a novel, self-assembled cell microchip for HTS of functional miRNAs capable of regulating cell migration and metastasis. miRNAs constitute a recently discovered group of endogenous, small noncoding RNA with the length of 18–25 nucleotides in plants and metazoans. These miRNAs were demonstrated to play important roles in a wide range of biological processes, including cell migration and cancer metastasis, and a large number of miRNAs are deregulated in cancer samples. While miR-10b, miR-373, miR-520c, miR-520e and miR-31 have been implicated to be involved in metastasis, the magnitude of miRNA involvement in cancerogenesis, especially metastasis, has not been fully ascertained. Xi presented a novel self-assembled cell microarray, which is amenable for the HTS of functional miRNA genes regulating cell migration, and how the primary screen results have guided the unraveling of the molecular mechanism of a couple of miRNAs in the process of colon metastasis.

Xiaoyu Lin discussed Abbott's efforts related to cancer target identification and validation by siRNA library screening. Abbott has been conducting large-scale siRNA library screens

for cancer target identification over the last 8 years. However, the outcome of Abbott's screening exercises using earlier generation siRNA libraries was suboptimal due to severe siRNA-mediated off-target effects. To overcome the off-target issue, Lin's group had investigated siRNA off-target mechanisms and identified 'seed match' as the main source of siRNA-mediated off-target effect. Based on the understanding of the off-target mechanism, Lin has identified an siRNA library (On-Target Plus siRNA library) with much reduced off-target effect, and a retest strategy enabling efficient elimination of 'off-target hits' from the screen. After completing the On-Target Plus siRNA library and the optimized retest strategy, Lin carried out siRNA library screens to identify genes that are essential for cancer cell growth and survival. These optimized screens revealed attractive biological pathways and novel targets that may represent unique opportunities for cancer therapy. For further validation of the targets from siRNA library screens, Abbott has developed RNAi-based methods to address key issues such as target druggability and *in vivo* efficacy that enables target validation without the time and labor-intensive step of generating '*in vivo* grade' tool compounds. It is hoped that obtaining a better understanding of the target biology by applying these RNAi-based tools and other methods will allow the triaging of novel targets before embarking on a significant chemistry effort.

York Zhu presented a new technical platform from an entire siRNA target library construction to successfully screen for RNAi therapeutics (virus and cancer) in a high-throughput manner. An ideal siRNA library should contain every site represented by multiple overlapping sequences, and individual sequences should have the widely accepted rational length and should easily and simply meet a high-throughput format to accelerate screening for RNAi therapeutics. An EcoP15I (a type III restriction enzyme)-based entire siRNA target library technology has been developed by Biomics, with the resulting pool of cloned siRNA constructs not only completely mimicking the length (19–23 bp) of siRNA naturally generated by the Dicer enzyme in living cells, but also demonstrating the representative sites of a target mRNA.

Novel drug-discovery techniques & assay development

Angela Dunne (GlaxoSmithKline [GSK]) presented an intriguing talk on GSK's efforts in the utilization of primary cells for phenotypic screening in hit identification and compound profiling. She described how primary cells have been used successfully at GSK to support HTS campaigns of more than one million compounds with subsequent compound profiling in excess of 500 compounds per week. The challenges of large-scale production of purified primary cell samples originating from multiple donors were discussed, and key assay-development parameters that have been optimized to deliver high-throughput, reliable and disease-relevant primary cell assays in miniaturized screening formats were highlighted. The logistics of scale-up and monitoring of assay performance in a production environment resulting in the identification of reproducibly active compounds supported the utilization of primary cell assays in early-phase drug discovery.

The final afternoon session started with Petr Bartunek (Institute of Molecular Genetics, Prague, Czech Republic) discussing the usefulness of small molecules as tools in stem cell biology. Several signaling pathways have been implicated in the maintenance of the stem cell fate or in promotion of cell differentiation. He illustrated the systematic and combinatorial approach to identify small molecules as modulators of Wnt and other signaling pathways and discussed the wide applicability of these approaches in stem cell and cancer biology.

Haian Fu (Emory Chemical Biology Discovery Center, Emory University, USA) discussed the strategies to probe protein–protein interaction (PPI) space for small molecule modulator

discovery. As advances in genomics reveal critical genetic and epigenetic alterations in various human diseases, it has become clear that these genomic changes are often accompanied by a dysregulated network of PPI. Thus, these altered PPIs interfaces have emerged as a new class of challenging but highly promising molecular targets for novel drug discovery. Case studies on multiplexed assay technologies and ultra HTS were presented to illustrate the discovery of small-molecule PPI inhibitors and challenges for therapeutic development.

Takashi Funatsu (University of Tokyo, Japan) presented the most elegant talk of the conference, titled, 'Single-molecule analyses of functions and interactions of biomolecules by nano- and micro-devices'. To analyze weak interactions of protein molecules, he used a single-molecule imaging technique with zero-mode waveguides. As an application of zero-mode waveguides, he performed single-molecule imaging of the chaperonin reaction cycle. Formation and decay process of a symmetric GroEL-(GroES)₂ complex (the football-shaped complex) were analyzed to find that the first GroES to interact with GroEL did not always dissociate from the football-shaped complex prior to the dissociation of a second GroES. As another approach to study PPIs, his team is developing a microfabricated fluorescence-activated biomolecule sorter that uses a thermoreversible gelation polymer as a microvalve, with the opening and closing of the sorter controlled by laser-induced heating under epifluorescence microscopy, and a throughput of 100 particles per second.

While the number of poster presentations were few (a total of eight), the quality of the posters was high. In conjunction with the conference, three training courses (label-free assays, fluorescence assays, and enzyme and binding assays in drug discovery) were presented by Jack Owicki (Owicki Consulting).

The conference organizers are to be commended for addressing a timely topic, and bringing together the experts in the field to share their views on the current status of target and probe discovery in finding new, novel and safer drugs.