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High content screening – from cells to data to knowledge

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The second annual *IBC High Content Screening Symposium* (11–13 June 2001, Miami Beach, FL, USA) was sponsored by Cellomics (Pittsburgh, PA, USA) and Beckman Coulter (Fullerton, CA, USA) and brought together scientists from academia and industry with interests in cell-based screening. Presentations covered the capabilities of high content screening (HCS), its existing and potential applications, and how it can be improved in the future. In particular, there was significant input from Cellomics application scientists regarding ArrayScan II™ (AS; Cellomics) who discussed the different applications of this technology platform, in addition to product demonstrations and interactive discussions.

HCS versus HTS

Several companies are now providing technology that enables scientists to make multiple measurements of the cellular phenotype on a cell-by-cell basis. Compared with single-measurement well-based assays, multiparameter cell-based HCS assays yield data with much higher biological information content. Whereas HTS is used as a fast primary screen to identify hits for further testing, HCS can be used to identify leads from hits. The ability to obtain quantitative data from multiple endpoints, from both individual cells and cell populations, greatly enhances the information obtained from whole-cell screens in drug discovery. This could provide new insights into cell function and mode-of-action studies that were previously labour intensive and difficult to interpret.

ArrayScan™ technology

Keith Olson (Cellomics) reviewed the new software of the AS system, which enhances user interactions and improves assay throughput. The addition of Image-Pro™ software places greater flexibility for image-analysis algorithm development in the hands of the end user. The sub-population analysis feature enables users to select or reject cells based on nuclear size, shape, and intensity, or to gate on (that is, select a subpopulation of) cells that are above, below or between thresholds for a particular marker. Responders or non-responders to a stimulus (e.g. a cytokine) can be analyzed separately and compared with the entire cell population.

The advantages of the AS technology highlighted were: (1) images are acquired by a cooled CCD camera via standard inverted microscope optics (for most HCS applications the resolution is sufficient so three-dimensional detail obtained by laser-confocal approaches is unnecessary); (2) spatial information on individual cells (e.g. changes in cell shape or size), organelles (e.g. nuclear morphology) or cellular targets (e.g. transcription factor translocation to nucleus) can be obtained; (3) experiments are performed in standard clear-bottom 96- or 384-well microtitre plates; (4) cells can be live or fixed; (5) AS uses a UV arc lamp source, therefore, it can simultaneously quantify molecules that fluoresce over a wide spectral range.

Bright future for fluorescence technology

Fluorescent markers that are currently used by cell biologists could be adapted for use on the AS platform, according to Richard Haugland (Molecular Probes, Eugene, OR, USA). These include markers for organelles, nitric oxide and reactive oxygen species, intracellular Ca^{2+} (for G-protein-coupled receptor activation), enzyme substrates, fluorescent resonance energy-transfer (FRET) and enzyme-coupled assays. Haugland also described a

1359-6446/01/$ – see front matter PII: S1359-6446(01)01927-4

range of markers for cell viability, proliferation and apoptosis, some of which have already been successfully used with AS, such as Calcein AM for viability and Annekin V for apoptosis.

In a talk entitled *Watching Proteins in the Wild* Klaus Hahn (Scripps Research Institute, La Jolla, CA, USA) discussed developments in the field of fluorescent biosensors for live cell-imaging. FRET is particularly useful for examining protein–protein interactions, or protein and peptide cleavage. New protein 'transducers' give a change in FRET signal only when in a particular conformation. Similarly, 'domain' biosensors, which contain a fluorescent moiety bound to a specific protein motif, will only bind to a target protein when it is in a particular conformation. These approaches are useful for investigating proteins such as the Rho family, which are involved in both motility and cell-death pathways depending on their position in the cell. By using biosensors and image analysis, the spatio-temporal dynamics that enable the same proteins to induce different outcomes can be elucidated.

Automation

Automation of assays was an important issue, and Joseph M. Zock (Cellomics) discussed the HCS-specific issues of automation, for example, cell density is crucial for image-analysis based assays. A KineticScan™ (KS) platform (Cellomics) has been specifically developed for the continuous analysis of live cells, and features on-board fluidics, plate handling and incubation of up to 300 plates. Meina Liang (Abgenix, Fremont, CA, USA) described the use of KS for calcium-flux and receptor-internalization assays and suggested that it might also be used for apoptosis assays such as Annexin V. Although much slower than fluorometric imaging plate reader (FLIPR; 4 h per plate compared with 2 min), KS can analyze data from individual cells and identify heterogenous responses.

Repurposing existing technology

Signal transduction

Several users demonstrated the validation of AS in target validation and primary screening stages. Ann Hoffman (Hoffmann-LaRoche, Nutley, NJ, USA) described the use of AS in high-throughput cellular screening assays for nuclear factor-κB (NF-κB), extracellular-regulated kinase (ERK) and c-jun.

The effects of interleukin-20 (IL-20) on signal transduction pathways in HaCaT cells and IL-20-receptor-transfected baby-hamster kidney (BHK) cell lines can be examined using the AS platform, as described by Donna Prunkard (ZymoGenetics, Seattle, WA, USA). Using Cellomics HitKits™ for STAT1 and STAT3 (signal transducers and activators of transcription 1 and 3, respectively) they demonstrated that IL-20 elicited a dose-dependent STAT3 induction but not a STAT1 induction.

In keeping with the STAT theme, Sheri-Lynn Bradshaw (Schering-Plough, Kenilworth, NJ, USA) showed how STAT translocation could be used to measure interferon (IFN) activity. She has been developing an IFN-α 2b bioassay for use in the clinical and preclinical research and demonstrated the feasibility of quantifying activation of STAT1, STAT2 and STAT3 in several cell-lines using AS. This results in significant time-saving: 2 days rather than a 5-day 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. However, for application to clinical stages, the need for GLP acceptance criteria was discussed; and although the possibility of multiplexing the three STAT assays appears practical, it was doubted that the FDA would accept results from multiparameter assays.

Robert Blake (SUGEN, Redwood City, CA, USA) talked about challenging the 'old-style' screening cascade and argued that a phenotype-based assay using automated image-analysis can leap-frog the need for a biochemical screen. He demonstrated *src*-transformed NIH-3T3 cells that contain actin-ring structures. Immunostaining revealed the colocalization of src to the actin-ring structures, the formation of which was decreased by small-molecule src inhibitors, thus causing the cells to revert to the non-transformed phenotype. Blake used AS to image and quantify the actin rings and thereby developed a screen to identify src-selective compounds.

Measuring multicolour immuno-fluorescence on the AS was used as a high-throughput alternative to western blotting. Quantifying p53 and p21 up-regulation in cells transfected with MDM2 (mouse double minute 2 protein) antisense oligonucleotides was demonstrated by Cathy Liptrot (AstraZeneca, Macclesfield, UK). Simultaneous measurement of fluorescein isothiocyanate (FITC)-tagged-oligonucleotide uptake was directly correlated with cytoplasmic–nuclear translocation of p53 and a decrease in cell number in a single multiparameter assay. Liptrot also described the comparison of conventional methods for measuring apoptosis, cytotoxicity and proliferation with methods adapted for quantification on the AS.

Cytotoxicity, apoptosis and differentiation

In the cell-cycle field, Susan Catelano (Rigel, South San Francisco, CA, USA) and O. Joseph Trask (Sphinx, Durham, NC, USA) both showed that measuring the mitotic index using AS compared well with fluorescence-activated cell sorting (FACS) analysis, and had easier sample preparation for adherent cells and a 96-well format. Susan also showed that comparable DNA-content histograms could be produced but need some refining, such as the discrimination of dead cells

In another project by Prunkard, AS was used to quantify adipocyte differentiation by adapting the 'receptor internalization' algorithm of AS to identify and count cells that contained Nile Red-stained lipid droplets. This also enabled

quantification of growth-factor inhibition of differentiation. Prunkard also uses AS to quantify markers of differentiation in dispersed cells from embryoid bodies using immunostaining techniques. Finally, she discussed using the 'cytoplasm to nucleus translocation' algorithm to measure cytokine induction of a retrovirally transfected NF-κB–green-fluorescent-protein fusion protein in live cells and the identification and analysis of small sub-populations of responding cells.

Multiparameter apoptosis assays were also discussed by Mark J. Lynch (Bayer, West Haven, CT, USA). He emphasized the complexity of cellular responses and the difficulties of applying curve-fitting algorithms when compounds show pleiotropic effects. Although biologists are inspired by the amount of information these assays produce, it can cause problems generating IC_{50} values to feedback to chemists!

Similarly, Jeffrey Haskins (Pfizer, Ann Arbor, MI, USA) explained multiparameter cytotoxicity assays and illustrated these with some beautiful time-lapse videos. The challenges facing toxicologists are similar to those faced by other disciplines in drug discovery – more data and the need for earlier decision-points based on more information. In his opinion, the power of 'multicolour' analysis lies in looking at the coexpression of markers.

Disease targets

For CNS targets, Linda Rowse (Pfizer, Ann Arbor, MI, USA) and Peter Simpson (Merck Sharp and Dohme, Harlow, UK) showed how using the 'neurite outgrowth' algorithm to replace time-consuming microscopical analysis had greatly increased throughput and reduced bias.

The future

So, what is the future for HCS assays? John Emery (GlaxoSmithKline, Philadelphia, PA, USA) asked how industry can increase the rate of product approvals, because R&D spending is increasing 15-fold and yet product approvals only 0.7-fold. Using cell-based assays earlier in the screening cascade should lead to higher quality leads and less attrition in later stages. However, the genome and proteome have unleashed a vast amount of new targets and the challenge is for cell biology to keep pace. Although this might be met, in part, through assay miniaturization, kinetic assays and the use of multiple biosensors, there is a corresponding need to manage the vast amounts of data coming from what has been termed the 'cellome'.

William Busa (Cellomics) explained how data could be turned into knowledge using 'computer-aided thinking tools'! Converting data into knowledge-representation systems enables the biologist to query data in new ways. Data inputs (which can be anything from compounds, proteins, lipids and ions, to cell types, organisms or disease states) are converted into 'nodes' in a database. Text-mining tools automate the extraction of data from flat text such as journals and add this to the database. Then, by making millions of logical associations between nodes, pattern-recognition software generates pathways and networks. The probabilities that the relationships are biologically significant are estimated from associations with known pathways and published data that can be incorporated into the 'cellome'.

Finally, Franklyn Prendergast (Mayo Clinic, Rochester, MN, USA), in an entertaining and thought-provoking presentation, urged us to consider the complexity of the 'tissueome', 'humanome' and even the 'diseaseome'. He challenged the traditional 'target-centred' drug-discovery process and suggested the need to reverse the reductionism that the genomics era has started. He argued that biocomplexity was not only the pharmaceutical industry's challenge, but also its future. Harnessing the power of the cell could lead, ultimately, to individualized patient analysis and therapy.