determine standards for this new application of ultrasound.

References

1 Lavon, I. and Kost, J. (2004) Ultrasound and transdermal drug delivery. *Drug Discov. Today* 9, 670–676

2 Fellinger, K. and Schmid, J. (1954) Klinik an therapie des chronischen. *Gelenrhuematismus* 549–552

3 Lee, S. *et al.* (2004) Short ultrasound exposure times for noninvasive delivery in rats using the lightweight cymbal array. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* 51, 176–180

4 Mitragotri, S. *et al.* (1995) Ultrasound-mediated transdermal protein delivery. *Science* 269, 850–853

5 Boucaud, A. *et al.* (2002) Effect of sonication parameters on transdermal delivery of insulin to hairless rats. *J. Control. Release* 81, 113–119

6 Kost, J. *et al.* (2000) Transdermal monitoring of glucose and other analytes using ultrasound. *Nat. Med.* 6, 347–350

7 Chuang, H. *et al.* (2004) Clinical evaluation of a continuous minimally invasive glucose flux sensor placed over ultrasonically permeated skin. *Diabetes Technol. Ther.* 6, 21–30

8 Katz, N. *et al.* (2004) Rapid onset of cutaneous anesthesia with EMLA cream after pretreatment with a new ultrasound-emitting device. *Anesth. Analg.* 98, 371–376

Alain Boucaud
GIP Ultrasons
Faculté de Médecine
2 bis boulevard Tonnellé
37032 Tours Cedex, France
e-mail: boucau_a@med.univ-tours.fr

Cell-based screening

Guido J.R. Zaman, NV Organon, Molecular Pharmacology Unit, PO Box 20, 5340 BH Oss, The Netherlands;
e-mail: guido.zaman@organon.com

The 11th annual *Cell-Based Assays for HTS* meeting, which was organized by the Cambridge Healthtech Institute (http://www.healthtech.com) as part of the *World Pharmaceutical Congress* (17–19 May 2004, Philadelphia, USA), brought together over 100 delegates, including representatives from the pharmaceutical industry and the technology provider sector, to hear experts describe the progress made in cell-based screening, and to discuss the advantages and disadvantages of this technology over cell-free screening. As well as case studies on molecular target screening and 'pathway screening', there were several presentations on emerging technologies. In two lively panel discussions, the major aspects of cell-based screening and assay miniaturization were addressed.

Molecular target screening

Lisa Minor (Johnson & Johnson; http://www.jnj.com) highlighted some of the cell-based methods that have been used for the screening of membrane tyrosine kinases. Growth-factor receptor tyrosine kinases are typically screened with biochemical assays in which only the kinase domain, and not the complete protein, is used. However, agonists or small-molecule mimetics can only be found with cell-based screens. Minor explained how fluorescence methods, such as dissociation-enhanced lanthanide fluoroimmunoassay [DELFIA© (PerkinElmer; http://www.perkinelmer.com)], enable the sensitive measurement of insulin tyrosine kinase phosphorylation in cells.

Cell-based assays are used to screen targets that are refractory to biochemical purification, such as G-protein-coupled receptors (GPCRs) and ion channels. Gareth Waldron (Pfizer; http://www.pfizer.com) described the development of an assay to measure voltage-gated potassium channel activity using membrane-potential-sensitive fluorescent dyes and voltage–ion probe reader (VIPR) technology. Waldron emphasized the necessity of validating ion-channel assays, not only for statistical reproducibility, such as Z-factors [1], but also for the pharmacology of reference compounds (e.g. IC_{50}). In addition, it was reported that comparison of the results generated using VIPR and the patch-clamp technique demonstrates that there is a good correlation between these two methods.

Guido Zaman (NV Organon; http://www.organon.com) described assays for the identification of agonists, antagonists and selective modulators of steroid hormone receptors. Cell-based luciferase reporter assays have been used to identify non-steroidal agonists and antagonists of the classical hormone receptors. The potency and efficacy of these ligands is dependent on the specific cellular environment, and could be influenced by the level of receptor expression. By contrast, radioligand-binding assays enable precise affinity determinations, and are indispensable for the assessment of the selectivity of compounds over closely related receptors.

Functional GPCR assays enable the identification of compounds that have a variety of mechanisms of action. Miguel Garcia-Guzman of Vertex Pharmaceuticals (http://www.vrtx.com) described the use of β-lactamase reporter-gene technology to identify allosteric enhancers of the A_1 adenosine receptor and of selective agonists of the

1359-6446/04/$ – see front matter PII: S1359-6446(04)03241-6

M_1 and M_4 muscarinic acetylcholine receptors. β-Lactamase technology has several advantages over other reporter technologies, such as luciferase, including fluorescence-activated cell-sorting of functional reporter cell lines, the identification of cytotoxic compounds and the possibility of assay miniaturization when assay volumes of <2 μl are used.

Pathway screening

Although cytotoxic library compounds create false-positive hits in antagonistic cell-based screens, these results can be filtered out using the appropriate counterscreen. Another way to circumvent non-specific hits is through the use of phenotypic screens that have a positive read-out, as demonstrated by Hans-Peter Biemann (Genzyme; http://www.genzyme.com). Measurement of the level of rescue of tumor necrosis factor (TNF)-induced apoptosis of mouse L929 cells led to the identification of antagonists of the TNF pathway. In the positive read-out design of this assay, compounds identified as hits give increased cell viability. One example of such a compound is Genz29155, which has demonstrated activity in models of multiple sclerosis.

The Redistribution® technology of BioImage A/S (http://www.bioimage.com) relies on measuring the intracellular translocation of proteins that are tagged with green-fluorescent proteins. Translocation is visualized in real-time with high-throughput image-based techniques. Len Pagliaro presented the progress made by BioImage in the identification of inhibitors of the PI3K pathway for cancer. Redistribution® assays have been developed for several targets in the PI3K pathway [e.g. forkhead (FKHR) and Akt1], enabling rapid identification of the mechanism of action of active compounds. Promising xenograft data were presented for a compound (SCR0044001) that had been identified using a translocation assay for FKHR.

Drug screening with primary cells

Cell lines used for HTS are usually derived from immortalized human cell lines (e.g. HEK293) or rodent cell lines [e.g. Chinese hamster ovary (CHO)], that have been manipulated by recombinant DNA techniques to express the target of interest. Cultured primary cells, blood platelets or neurons all possess characteristics that often represent *in vivo* physiology better than immortalized cell lines. Assays in these cell types could give a more accurate prediction of the activity of compounds in *in vivo* models and in the clinic, and thus could contribute to a decrease in attrition in the whole process of small-molecule drug discovery. However, primary cell types are in short supply, and are therefore rarely used for drug screening. Two presentations highlighted developments in technologies that facilitate cell-based screening with lower numbers of cells than are normally needed. William Janzen (Amphora Discovery: http://www.amphoracorp.com) described the screening of calcium-flux GPCR assays on the HTS250 LabChip™ microfluidics system manufactured by Caliper (http://www.caliperls.com). The adaptations to the system developed by Amphora enable the use of suspended (e.g. CHO) and adherent cell-lines (e.g. HEK293). One key advantage that microfluidics have over microtiter plate-based screening is that there is a reduction (tenfold) in the volume of cells and reagents required, which makes the procedure considerably more labor- and cost-efficient. Microfluidics and fluorometric-imaging plate reader performed equally well with respect to assay performance and the pharmacology of reference compounds.

A second new technology that enables the use of relatively low numbers of cells per datapoint is the CellCard™ system developed by Vitra Bioscience (http://www.vitrabio.com). Simon Goldbard described the CellCard™ as a coded microcarrier on which cells are grown and assayed. The CellCard™ technology enables a 100-fold reduction in primary cell usage. Whereas 50 cells attached to the bottom of a well of a conventional 96-well microtiter-plate correspond to a culture that is <1% confluent, 50 cells on a CellCard™ can equal 50% confluency. Because the CellCard™ microcarriers are coded, incubation reactions for different cell types (e.g. cells from different patients) can be simultaneously mixed in a single microtiter-plate well, thus the reactions proceed under identical environmental conditions and all the cells are exposed to the same concentration of the compound under investigation; the platform is compatible with a wide variety of imaging based assays.

Nuclear translocation of G-protein-coupled receptors

Brian O'Dowd (University of Toronto; http://www.utoronto.ca) presented a novel approach for measuring the interaction of compounds with GPCRs or transporters. The technology is based on the nuclear localization signal, which is present in the cytoplasmic tail of some GPCRs such as the angiotensin (AT) II AT_1 receptor and the bradykinin B_2 receptor [2]. The binding of a compound to the receptor prevents receptor translocation to the nucleus, which was visualized using green-fluorescent protein-tagged receptors and confocal microscopy imaging. O'Dowd reported that the tagging of other GPCRs with the nuclear localization signal directed the translocation of these receptors to the nucleus. The assay works irrespective of the

G-protein type that is coupling to the receptor.

Complementation of β-galactosidase applied to drug screening

The DiscoveRx technology (http://www.discoverx.com) is based on the complementation of *Escherichia coli* β-galactosidase fragments with ligands of interest, a principle that has been applied in recombinant DNA technology for more than 20 years [3]. Degradation of β-galactosidase produces two fragments, a large protein fragment and a small (~4 kDa) peptide enzyme donor fragment. The two fragments are individually inactive, but rapidly recombine in solution to form an active enzyme. Various chemicals (e.g. cAMP, steroid hormones and kinase inhibitors) have been attached to the small peptide fragment without affecting its ability to re-associate with the large protein fragment and thus reform the active enzyme. Based on this technology, DiscoveRx has developed several ligand-competition assays for a number of targets. Richard Eglen (DiscoveRx) presented several novel assays designed to measure intracellular inositol-1,4,5-triphosphate levels and to detect protein degradation and receptor internalization by tagging the protein of interest with the 4 kDa enzyme donor fragment. The DiscoveRx technology uses fluorescent and chemoluminescent substrates of β-galactosidase, and this technique can be used on conventional HTS readers; this property affords this technology a particular advantage over other methods that require specialized equipment.

Concluding remarks

The choice of the right assay format strongly impacts on the probability of success of a screening campaign, and sets the direction of the subsequent hit optimization program. The *Cell-based Assays for HTS* conference addressed several aspects of cell-based screening. Many speakers emphasized that cell-based assays provide an important means of evaluating the activity of compounds in a more physiologically relevant environment, but disadvantages of these assays were also addressed. Pathway screening enables the identification of compounds that target novel intervention points. However, it could prove more difficult to drive SAR on these compounds using cell-based assays. Finally, interesting novel developments in technologies that could enable the incorporation of primary cells of patients as an early screening tool in the drug discovery process were reported.

References

1 Zhang, J-H. *et al.* (1999) A simple statistical parameter for use in evaluation and validation of high-throughput screening assays. *J. Biomol. Screen.* 4, 67–73
2 Lee, D.K. *et al.* (2004) Agonist-independent nuclear localization of the apelin, angiotensin AT_1, and bradykinin B_2 receptors. *J. Biol. Chem.* 279, 7901–7908
3 Messing, J. *et al.* (1981) A system for shotgun DNA sequencing. *Nucleic Acids Res.* 9, 309–321