Human tissue in target identification and drug discovery

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The completion of the sequencing of the human genome [1,2] has opened an unprecedented opportunity to develop novel molecular diagnostic and therapeutic approaches, based on a far deeper understanding of the underlying pathophysiology of many complex disorders. Genomics-based research aims to link genotype to phenotype and requires access to high quality human tissue and cell lines, which presents a series of unique challenges. The first IBC *Human Tissue in Target Identification and Drug Discovery* Conference (3–4 December 2001; Boston, MA, USA) specifically addressed this important topic.

Target identification and drug discovery

One of the major challenges facing the drug discovery community is the limitation and poor predictability of animal-based strategies. Many drugs have failed in later stages of development because the animal data were poor predictors of efficacy in the human subject. The case for using human tissue early in the discovery process was elegantly made by Michael Neubauer (Bristol Myers Squibb, Princeton, NJ, USA), who described the multiple points at which microarray technology and human tissue could be integrated to aid in target identification and validation, safety testing, compound selection and crucial decision making on parameters to advance products to clinical testing. Ways of integrating the target discovery phase with the drug discovery phase based on the creative use of human tissues, stem cells and microarray capability were emphasized by the keynote speaker, Klaus Lindpaintner (Hoffmann-La Roche, Basel, Switzerland) and also by Jay Strum (GlaxoSmithKline, Research Triangle, NC, USA).

Sourcing human tissues

Procuring and handling human tissue is an expensive and laborious process that requires considerable technical skills to meet the exacting requirements of modern genomic research. Quality control measures were detailed by Martin Ferguson and Jeffrey Blander (Ardais Corporation, Lexington, MA, USA) and Rebecca Mosher (Millennium Pharmaceuticals, Cambridge, MA, USA). To fill this need for both quality control and well-documented medical information, several biotechnology companies, such as Ardais, Asterand (Ann Arbor, MI, USA), Clinomics (Pittsfield, MA, USA), Genomics Collaborative (Cambridge, MA, USA) and LifeSpan BioSciences (Seattle, WA, USA), have been established [3].

Bioinformatics and databases

Tools are being developed to enable the accurate tracking of tissues and the associated medical information so that all aspects of quality control and ethical standards, such as patient privacy and informed consent, are completed. All biorepository companies are creating such databases and were aptly illustrated by the systems presented by Ardais Corporation (Martin Ferguson and Jeffrey Blander) and Genomics Collaborative (Michael Pellini). Several companies are developing databases of gene expression or proteomics information on large collections of normal and diseased human tissue under carefully controlled conditions. These databases will become a valuable resource for comparative information, identification of novel targets, target validation and as a platform for examining the effects of drugs on the tissues. An overview of one such database from Pharmagene (Royston, UK) was presented by Robert Coleman. Julian Beesley (LifeSpan Biosciences, Suffolk, UK) showed how the company is creating localization databases of major gene families in normal and diseased tissues.

Human cells

Recently, it has become evident that stem cells could be used for target identification and drug discovery. A scientifically interesting, although potentially controversial, presentation was made by Michael West (Advanced Cell Therapeutics, Worcester, MA, USA) on nuclear transfer into human cells and the potential of such cells for discovery and therapeutic use. Unfortunately, these experiments, although failing in their objectives, became the subject of a major debate on human cloning and West was due to appear before a Senate hearing committee on the second day of the conference. Several academic labs and a few companies are now working with stem cells for target identification. For example, Tom Hazel (NeuralStem, Gaithersburg, MD, USA) presented work from the company that has developed several cell lines from different regions of the brain and can efficiently differentiate these pluripotent cells into neurons and glia with phenotypes that differ and

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reflect the brain region from which they were collected. These cells can be used to set up *in vitro* models of CNS diseases and to study drug actions, gene function and lead optimization. Psychiatric Genomics (Gaithersburg, MD, USA) is also using stem cells in this way but has expanded their use to drug discovery.

Once the underlying pathophysiology of a disorder is known, it could be possible to manipulate 'normal' human cells to exhibit the appropriate disease-specific abnormalities. One such potential technique involving regulated gene expression was presented by Mohan Phillips (RheoGene, Charlotsville, VA, USA). RHeoPlex™ is a ligand-inducible gene regulation technology for the precise regulation of one or more target genes in the same cell, enabling the study of gene expression, cell signaling and protein interactions *in situ*.

Adipocytes are more readily available and a presentation from Anindita Sen (Zen-Bio, Research Triangle, NC, USA) was the subject of considerable interest. Major antiobesity research in the area of adipocyte metabolism has been conducted on rodent adipocyte-like cell lines. There are key differences in the genetic and metabolic make-up of humans and rodents that can only be evaluated after careful investigation using human adipocytes, which can form the basis of a useful target and drug discovery tool.

Target identification and validation

Applying the latest technologies to target identification and validation directly in human tissues is the first and crucial step in the holy grail of drug discovery and was the central theme of this conference. Many of the speakers illustrated how the use of microarray technology and/or proteomics could enable a massively parallel approach to target identification. Excellent examples of using microarray technologies to obtain unique insights into the role of myelination in the prefrontal cortex of schizophrenics were presented by Kenneth L. Davis (Mount Sinai School of Medicine, New York, NY, USA). Several new targets identified in post-mortem tissue from bipolar patients were presented by Michael G. Palfreyman (Psychiatric Genomics) and novel cancer targets from Emmanual F. Petricoin (CBER/FDA, Bethesda, MD, USA) and Krishnarao Appasani (Perkin Elmer™ Life Sciences, Boston, MA, USA).

Drug targets are for the most part proteins. To date, high-throughput proteomics technologies are not as advanced as microarray technologies in terms of numbers of items per array, nor in sensitivity. However, through the use of high-resolution 2D gel electrophoresis and MS technology, comparisons between diseased and healthy tissue, and drug treated and untreated human cell lines, protein targets can be validated, drug efficacy determined and drug toxicity profiles examined. This was presented by Andreas Kopke (Wita Proteomics AG, Berlin, Germany) with further examples, from histopathological cancer samples, given by Robert J. Penny (Ameripath Pharmaceuticals, Indianapolis, IN, USA).

Although neither proteomics nor gene expression analysis are ideal technologies they both have the powerful potential to accelerate the discovery of new targets, particularly when used in combination with other tools, such as RT-PCR, *in situ* hybridization, antibody localization and functional studies. These technologies, when applied to human tissue produce the sort of breakthrough synergies that significantly accelerate and improve the efficiency of the drug discovery process.

Drug discovery

Predictions of tens of thousands of new targets, compared with the 500 or so we currently have drugs for, could be an overestimation. Typically, the gene expression information has been used to pick a favorite gene for expression in a suitable cell system and subsequent HTS using the familiar substrate or ligand displacement assays. A newer and potentially more powerful approach is to use Multi-Parameter HTS℠ (MPHTS℠). This new technology, described by Palfreyman, uses cell-based screening and quantitative changes in gene expression as the read-out and has the advantage that multiple genes can be read in parallel and the screening is information rich. Typical HTS measures a single target and is binary in its output (i.e. it shows if the unknown compound produces an effect or not). MPHTS℠ screens compounds against multiple genes in parallel and, even if the effect on the 'gene signature' is not the desired one, it could be of interest for another objective. Most importantly, MPHTS℠ does not require an understanding of the gene product function, only that the change in gene expression is a reliable indicator of disease or drug action. This enables assays to be set up quickly as it is not necessary to express and purify the protein or create a recombinant cell line expressing the receptor. One significant advantage of using differentiated human stem cells is the similarity of gene expression to the native tissue.

The technology has been developed to enable up to 16 genes to be measured at any one time but it is certainly likely that miniarrays of 100+ genes could be devised. This approach, although universal in its application to any therapeutic area, has particular attraction for complex polygenic disorders, such as cancer and psychiatric disorders. Microarray analysis of tissue from patients with these disorders aids the analysis of gene interactions in a pathway and identifies multiple genes that contribute to the 'disease signature', which can then form the basis of a drug screening approach.

Complementary to this approach is the analysis of gene expression patterns that result from the action of therapeutic agents. These 'drug signatures' can also be used to find new drug classes with

improved properties. A crucial part of this 'gene signature' discovery paradigm is access to human nerve cells that can be grown in culture under controlled conditions. One such system uses human neuronal stem cells that can be differentiated into neuronal systems that reflect the complexity of the CNS and include the multiple interactions between neurons, astrocytes and oligodendrocytes that most closely approximate the function of both the normal and diseased brain. Finally, discovery of drugs that act on the human CNS are best studied in human cell-based systems and differentiated neural stem cells present such an opportunity for MPHTS℠, providing drug candidates for families of new targets that will enable the discovery of therapeutics for complex psychiatric diseases.

Pharmacokinetics, pharmacogenomics and diagnostics

Examples of human systems that are suitable for *in vitro* studies of ADME properties include Caco2 cell lines for determining the relative permeability of different analogs of a given chemotype, human hepatic microsomal or S9 fractions of human or animal hepatocytes for determining metabolic stability and human blood cells for protein binding and metabolism studies. Norman Huebert (3-D Pharmaceuticals, Exton, PA, USA) gave a comprehensive overview of the way these human systems can aid the study of new drug candidates. Pharmacogenetics/genomics and diagnostics require blood samples for DNA analysis; however, assessment of gene expression levels in human cells is being considered as a potential pharmacogenomic or diagnostic predictor of drug response and as such will become a valuable tool.

Bioethics and legal issues

No discussion on the use of human tissues would be possible without a consideration of bioethical issues. Throughout this conference all speakers referred to, and took significant steps to respect and adhere to, all bioethical guidelines on the use of human tissues. In particular, George Annas (Boston University, Boston, MA, USA) provided reasoned arguments about informed consent being 'informed', the appropriate restrictions on the use of information, the right of refusal and the potential impact on discrimination of employment and insurance when using genetic information or human materials. Many of the issues, such as confidentiality of information, are similar to those that are faced with all types of medical information, but there is a strong perception that genetic information is regarded as 'life's future diary'. Such a 'diary' of an individual reveals their potential for illness and could easily, if not properly protected, be the basis for discrimination. Privacy issues, state laws versus federal laws, legal issues, intellectual property rights, pass-through of benefit both medical and financial to the donor of tissue, ownership of material and, most importantly, the confidentiality issues were all addressed by Annas.

What to look for in stem cell and biorepository investing also raises several challenges. In addition to the normal requirements, stem cell and human tissue investments raise several legal and bioethical issues and many of these points were elegantly elaborated as 'A Venture Capital Perspective on Investing in Stem Cell and Tissue Companies' by Michael Lytton (Oxford Bioscience Partners, Boston, MA, USA).

All participants at this first conference on the use of human tissue in target identification and drug discovery reached a strong consensus on the significant value for understanding these complex disorders and the potential to substantially increase the efficiency of the discovery and development of new therapeutics. Further symposia on this subject are being planned.

References

1 International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. *Nature* 409, 860–921

2 Venter, J. *et al.* (2001) The sequence of the human genome. *Science* 291, 1304–1351

3 Editorial (2001) Biorepositories. *Start-up*, July/August, 19–27

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Erratum

Please note a correction to the *Private Prescription* article entitled *Formulating fortunes – the tale of a medicated lozenge*, published in *Drug Discovery Today*, 1st March 2002, Volume 7, No. 5, pp. 286–287. This article should have contained the following Table to go with its citation in column 3 on page 286.

The Editorial team of *Drug Discovery Today* would like to apologize for this inaccuracy and for any confusion that we might have caused.

5 Reynolds, J.E.F. (ed.) (1982) *Martindale, The Extra Pharmacopoeia*. (28th edn), p.1779, The Pharmaceutical Press

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Table 1. Active components of Fisherman's Friends Lozenges [5]

Ingredient	Extra strong original (% w/w)	Aniseed flavour (% w/w)
Menthol	0.9	0.5
Eucalyptus oil	0.153	
Cubeb oil	0.305	
Anise oil		0.17
Tincture of capsicum	0.02	
Extract of liquorice	7.317	
Liquorice powder		7.6