Animal Symposia and Workshops

A-1
Development of In Vitro Toxicity Tests Using Hepatocyte Differentiated from Human iPS Cells. S. ISHIDA. National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, JAPAN. Email: ishida@nihs.go.jp

The evaluation of hepatotoxicity of drug candidates is very important because liver plays major roles in the process of their absorption, distribution, metabolism, and excretion. Many aspects of hepatotoxicity tests depend on animal model. However, species differences often cause the extrapolation limitation of results obtained in animal tests to human. Thus, the establishment of the hepatotoxicity tests using human tissues applicable at the early stage of drug development has been desired. Human primary hepatocytes are widely used for this purpose these days, although they still have problems such as inter-individual differences or securing of their stable supply. Thus, many studies have been done so far to get hepatocytes by differentiating human stem cells. After the establishment of human iPS cells (Yamanaka et al. 2008), the speed of such study accelerated. We have promoted studies to develop in vitro toxicity test using the cells differentiated from human iPS cells for the project “Development of a novel drug toxicity testing system using human iPS cells” led by Cabinet Office, Government of Japan. We have already succeeded to differentiate human iPS cells into hepatocytes. iPS cells are the promising source of an alternative to primary human cells. On the other hand, several difficulties have been clarified during these studies, e.g. it is difficult to go through the complicated differentiation process with reproducibility; it is still insufficient to get the matured cell phenotype, etc. I will introduce recent progress of our project and would like to discuss what is required to establish iPS derived hepatocyte suitable for hepatotoxicity tests.

A-2
iPS-derived Dopaminergic Neurons as an In Vitro Model System for Parkinson's Disease - Promises and Pitfalls. KAH-LEONG LIM1,2,3 and Chou Chai2. 1Department of Physiology, National University of Singapore, SINGAPORE; 2A*STAR Duke-NUS Neuroscience Research Partnership, SINGAPORE; and 3National Neuroscience Institute, SINGAPORE. E-mail: kahleong.lim@duke-nus.edu.sg

Parkinson’s disease (PD) is the most common neurodegenerative movement disorder currently affecting more than four million predominantly elderly individuals worldwide. Despite intensive research, the etiology PD remains elusive. However, a broad range of studies conducted over the past few decades have provided tremendous insights into the molecular events underlying the degeneration of dopaminergic neurons in PD. Further, the recent advent of induced pluripotent stem (iPS) cell technology has opened fresh avenues to better understand the disease pathogenesis. Indeed, the derivation of iPS cells from somatic cells of patients and the subsequent differentiation of these human-derived iPS cells into dopaminergic neurons has provided us an unprecedented opportunity to examine the disease process at the cellular level. For the first time, instead of relying on surrogate animal models, we could now understand directly why dopaminergic neurons in affected individuals are predisposed to degeneration. Such cell-based models would also be useful for drug screening and development. In this talk, I will discuss our progress in using dopaminergic neurons derived via the iPS route from PD models and patients as an in vitro model system to gain insights into the neurobiology of the disease. I will also discuss the strengths and limitations of using such an approach to study PD, and their potential use in the clinic for the...

A-3

Large Animal Induce Pluripotent Stem Cells (iPS) Cells: A Much Needed Bridge to Clinical Applications. JORGE PIEDRAHITA. Center for Comparative Medicine and Translational Research, and Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606. Email: Jorge_piedrahita@ncsu.edu

In the field of regenerative medicine one of the main goals has been the identification of stem cell populations that have high differentiative capacity and are genetically matched to the patient. Patient–derived adult stem cells, such as bone marrow-derived and adipose-derived mesenchymal stem cells have been utilized with limited success. More recently, it has been possible to isolate highly pluripotent stem cells from somatic cells via transcription factor reprogramming. This stem cells, referred to as induced pluripotent stem cells, or iPS, have now been generated from a range of species including mice, rats, and humans. While there are still some issues of stability and homogeneity of different iPS cell lines that need to be resolved it is clear that these cells will have a significant impact in regenerative medicine. However, the bridge between rodent models and humans is a difficult one to cross when developing clinical applications. Fortunately, new advances in somatic cell nuclear transfer coupled with advances in generation of iPS cell from large animal species such as dogs and swine have created a unique opportunity to utilize these large models as a way to speed up translational efforts. This presentation will cover some of the work that we are doing in this area and will describe new advances in iPS generation, and the utilization of large animal models in regenerative medicine.

A-4

Automated Cellular Imaging: Acquisition and Analysis Using the ImageXpress® Micro Widefield System. TIMOTHY C. BARANOWSKI. Molecular Devices Corporation, 1311 Orleans Dr., Sunnyvale, CA. Email: tim.baranowski@moldev.com

Capturing images through a stand-alone microscope can be a time-consuming and challenging process, particularly when trying to acquire multiple adjoining fields or the same field at multiple fluorescent wavelengths. High content screening (HCS) systems are designed to alleviate these difficulties by providing an easily configurable process for acquiring and analyzing microscopy images in an automated fashion. This talk will cover general considerations of automated cellular imaging, including aspects of sample preparation, fluorescence versus transmitted light imaging, widefield versus confocal systems, and data storage. Typical examples of HCS assays will be presented. Automated imaging will be discussed in the context of Molecular Devices’ range of hardware and software products for high content screening. These products include the ImageXpress high content imaging systems and the Isocyte laser scanning cytometer for image acquisition. The MetaXpress™ and AcuityXpress™ software tools for image analysis and cellular informatics, respectively, will also be covered.

A-6

Tissue Engineering and In Vitro Models for Disease. DAVID L. KAPLAN. Tufts University, Department of Biomedical Engineering, Medford, MA 02155. Email: david.kaplan@tufts.edu

Tissue engineering emerged a few decades ago as a new approach to regenerate human tissues for clinical repairs. The process was based on the design and implementation of biomaterial scaffold systems to house specific cell types that are usually precultured under tissue-specific conditions prior to implantation. The field has advanced rapidly in recent years due to insights into stem cell biology, cell signaling, improved biomaterial scaffold designs and novel bioreactor systems, all of which have improved both scientific outcomes and translational impact. In the interim, approaches to generate 3D tissue systems have begun to be used as new modes to study cell and developmental biology, disease formation, therapeutic treatments and for toxicological screening. These new targets for tissue engineering research have been prompted by improvement in vitro grown human tissues, such as a versatile approach to study human diseases in ways that bridge 2D cell culture and animal models. We focus on robust protein-based biomaterial scaffolds for long term in vitro culture, stem cells or tissue-specific cell sources from normal or disease sources, and specialized bioreactors, in order to recapitulate in vitro tissue-specific scenarios to generate sustainable tissue systems. The goals are to generate relevant human 3D tissues for a range of diseases, including prostate and breast cancers, obesity, diabetes and kidney disease, among others. Important design issues and noninvasive modes to track cell and tissue outcomes can be integrated...
into these tissue systems in vitro. The structural and functional features of adipose and kidney tissue systems will be described as examples of the approaches utilized.

A-7

Human Skin In Vivo and in Organ Culture: Comparison of Anti-aging Intervention. J. VARANI. Dept. of Pathology, University of Michigan, Ann Arbor, MI 48109. Email: varani@med.umich.edu

Normal human skin can be maintained in organ culture for an extended period of time under conditions that preserve histological structure and biochemical function. Conditions include use of serum-free, growth factor-free basal medium containing a level of Ca\(^{2+}\) optimized for fibroblast function. Growth is at 37°C in an atmosphere of 95% air and 5% CO\(_2\). We have used organ-cultured human skin in parallel with topical treatment studies to assess the capacity of the in vitro culture system to serve as a "platform" for preclinical assessment of anti-aging activity. When skin organ cultures were treated with all-trans retinoic acid (RA) as a prototypic skin-repair agent, the major findings were epidermal hyperplasia, increased type I procollagen production and inhibition of collagenolytic activity / collagen breakdown. Inhibition of collagenolytic activity was largely due to increased elaboration of tissue inhibitor of metalloproteinases-1 (TIMP-1) and suppression of matrix metalloproteinase-1 (MMP-1) activity. RA-treatment also resulted in up-regulation of several pro-inflammatory cytokines including IL-6, IL-8 and MCP-1 and with increased expression of the leukocyte adhesion molecule, intracellular adhesion molecule-1 (ICAM-1). These findings mimic those seen when subjects are topically treated with RA. Additional studies were carried out in which a novel non-irritating synthetic retinoid (referred to as MDI 301) was assessed along with RA. Like RA, there was increased type I procollagen production and decreased MMP activity in organ-cultured skin treated with MDI 301. Of interest, MDI 301 also induced epidermal thickness equivalent to that induced by RA. In contrast, elaboration of pro-inflammatory cytokines and up-regulation of ICAM-1 expression was significantly less with MDI 301 than with RA. Taken together, these findings suggest that human skin in organ culture is capable of detecting cellular changes that underlie retinoid-induced skin repair and are also capable of identifying events that are thought to underlie retinoid skin irritation.

A-8


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In vitro models that accurately predict human response and sustain long-term physiologic function to enable chronic and disease progression evaluation have been limited. Investigators have made efforts to improve culture function by more closely mimicking in vivo three-dimensional, multi-cellular, tissue structure through alteration of cell culture medium composition, use of extracellular matrices, and application of co-cultures. These technologies enable creation of a more natural cellular structural organization and thereby minimizes probability for artifactual responses resulting from binding sites or cellular reactivity of monolayer or unicellular cultures that are not normally functional in vivo. Understanding how to combine these factors is critical to achieving full 3D tissue formation with sustained physiologic function that can overcome the short-term, poor predictive function of unicellular, 2D culture systems. Examples of 3D liver, skin and bone-marrow cultures in long-term drug discovery and regenerative medicine applications will be presented. These are among the first culture systems to display physiologic tissue architecture and function both in vitro (e.g. simultaneous expression of liver Phase I, II and III metabolism) and upon implantation (including integration into host tissue and stimulation of tissue healing and remodeling).

A-9

Bioengineered Human Skin Tissues: Clinically Relevant Models for Identification and Validation of New Therapeutic Targets to Inhibit Squamous Cell Carcinoma Development. Yonit Szwee-Levin1, Harold Hatch2, Adam Sowalsky2, Larry A. Feig2, Jonathan A. Garlick3,4, and ADDY ALT-HOLLAND1,4. 1Department of Endodontics, School of Dental Medicine; 2Department of Biochemistry School of Medicine & Sackler School of Graduate Biomedical Sciences; 3Department of Oral and Maxillofacial Pathology School of Dental Medicine; and 4Division of Cancer Biology & Tissue Engineering, School of Dental Medicine, Tufts University, Boston, MA 02111. Email: Addy.Alt_Holland@tufts.edu

Bioengineered, three-dimensional (3D) human skin tissues that can model the structure and function of normal and cancerous skin epithelium provide a powerful tool to advance our understanding of the molecular mechanisms that direct squamous cell carcinoma (SCC) development. We have developed a spectrum of skin tissue models that incorporate in their epidermal compartment human Ras transformed HaCaT keratinocytes in which E-cadherin-
mediated intracellular adhesion was maintained or suppressed, as well as complex microenvironments that consists of a viable supporting dermal compartment, defined media, and distinct growth conditions. These physiologically relevant 3D skin tissues closely manifest the premalignant nature of SCC in vivo, and allow us to bridge between studies performed in rudimentary monolayer cell cultures and those performed in animal models in vivo. Furthermore, they enable us to reveal new signaling molecules that regulate tumor cell motility and invasion, validate them, and predict their therapeutic potential as novel targets for inhibition of SCC progression. This presentation will demonstrate how these clinically relevant in vivo-like human skin tissues can widen our insights of the mechanisms that regulate the progression of precancer to malignancy. By elucidating these mechanisms, novel effective and applicable therapeutic intervention strategies can be designed to block tumor cell motility and impair early cancer invasion thus preventing SCC development or reoccurrence.

A-10

3D Co-culture Systems in Drug Discovery and Regenerative Medicine. D. R. APPLEGATE and B. A. Naughton. RegeneMed, Inc., 9855 Towne Centre Drive, Suite 200, San Diego, CA 92121. Email: dapplegate@regenemed.com

An overview of RegeneMed’s tissue engineering technology supporting products and services to address medical needs regardless of disease progression state; from miniaturized tissues for high throughput compound safety and efficacy assessment, to chemical/biological warfare biosensors, molecular diagnostics, personalized medicines, medical devices and tissue implants will be presented. Liver3, Human and animal 3D tissue cultures in multiwell plates and specialized bioreactors, integrate critical technologies to provide physiologically relevant replacements to current industry-standard animal and cell-based tests that are often not predictive of the human response to toxic compounds. These 3D liver co-cultures, sustain tissue function for months, enabling pre-characterized tissues from fresh and cryopreserved hepatocytes for previously unattainable in vitro endpoints such as bioavailability, drug-drug interactions, chronic toxicity, disease progression, tissue regeneration and tumor formation. Skin3, 3D full- and partial-thickness skin models, to include immune competent models for sensitization and inflammation, address the growing animal alternative market and regulations. Stem cell-derived 3D tissues, including liver, cardiac and neuronal, for patient-specific and genomic diversity research are in development. Product translation clinical application will be presented skin (FDA approved/marketed) cardiovascular (clinical trials), liver, cartilage (large animal studies), other tissues (small animal studies).

A-11

Thermo Scientific HyClone Hystem Hydrogels. PAUL PRICE. ThermoFisher, Cellomics Division, Logan, UT. Email: paul.price@thermofisher.com

Hystem Hydrogel kits consist of the natural extracellular matrix component hyaluronic acid, a chemically modified gelatin for cell attachment and a cross-linker to attach one to the other. The Hystem systems can be tailored for hydrogel stiffness and controlled release of user supplied growth factors and ECM components. This workshop presentation will introduce and demonstrate how this rapid, very-user friendly system can be used to form scaffolds for 3-D cell growth, coat plates or encapsulate cells.

A-12

Authentication of Cell Lines. YVONNE A. REID. Cell Biology Program, ATCC, Manassas, VA. Email: yreid@atcc.org

Propagation of animal cell lines is widely used by scientist in many diverse disciplines such as basic cell biology, genetic mapping, gene expression, and gene therapy. Cell line authentication is a crucial activity and yet it is one of the most under-appreciated tasks performed by most research scientists. The validity of conclusions drawn by researchers demand that consistent and unequivocal identification of cell lines are made. Yet over the years many cell lines have been shown to be misidentified, due in part, to lack of adequate authentication protocols available in previous years. Advances in new technologies have lead to more accurate authentication of cell lines. The financial loss due to these errors is estimated in the millions of dollars. An overview of the current technologies used to authenticate animal cell lines will be presented.

A-13

Screening Cultures for Adventitious Agents. JOHN W. HARBERLL. Mary Kay Inc, Dallas. TX. Email: john.harbell@mkcorp.com

Occult infection in cell cultures by adventitious agents such a mycoplasma has been an issue discussed by this society since its founding. Improvements in the purity of reagents and protective equipment have reduced some sources of infection
but not all. The problem persists because the primary source of infection is contaminated cultures maintained in the same laboratory. This workshop will focus on mycoplasma as one of the most common adventitious agents in cell culture and one that has a clear negative impact on the scientific validity of the most common adventitious agents in cell culture and laboratory. This workshop will focus on mycoplasma as one infection is contaminated cultures maintained in the same but not all. The problem persists because the primary source of infection is contaminated cultures maintained in the same laboratory. This workshop will discuss the “how to” of detection methods, prevention of cross contamination and possible cleansing of infected cultures.

A-14

Real Time Phenotypic Characterization of Cultured Cells. THOMAS J. FLYNN. Division of Toxicology, FDA Center for Food Safety and Applied Nutrition, 8301 Muirkirk Road, Laurel, MD 20708. Email: thomas.flynn@fda.hhs.gov

The importance of the genotypic characterization of cell lines has been made eminently clear in recent years. However, just because a cell has been shown to have the correct genotype, that does not prove that it is still behaving phenotypically in the expected ways. Culture medium composition, day in culture, growth phase (e.g., log vs. stationary), passage number, etc. can all impact the phenotypic expression of cells in culture. While techniques such as immunohistochemical expression of phenotypic markers and gene expression using microarrays are effective means of characterizing the phenotypic expression of cells, neither of these procedures are useful for real-time monitoring of cell phenotype. A number of technologies are now available to monitor cell function in real time. Some of these are: 1) the xCELLigence system from Roche which is a microphysiometer that monitors cell growth in real time; 2) the Bionas analyzing system which monitors several cell metabolic parameters in real time; 3) Biolog phenotypic arrays which can provide a phenotypic fingerprint of cultured cells; 4) metabolomics assessment of cell culture medium using NMR or LC/MS/MS. While this list is not meant to be all inclusive, it will provide a background for discussion of some newer technologies that are available to monitor cell function in real time.

A-15

Microelectrode Arrays for High Content Neurotoxicity Screening. J. ROSS and M. McClain. Axion Biosystems, ATDC Bioscience Cntr., 311 Ferst Drive, Atlanta GA 30332. Email: jross@axionbio.com

Over the course of a lifetime, humans are continually exposed to thousands of household, industrial, and agricultural chemicals. Although many of these chemicals are relatively benign to human health, a subset may have harmful neurotoxic effects. Ironically, the most severe neurotoxicants are not the greatest threat to human health, as they present readily apparent symptoms of toxicity. Rather, the most dangerous substances are those that have unrecognized but subtle influences on neurological function that, under chronic exposure, can lead to diseases such as Parkinsonism or autism. Recently, the need to develop in-vitro platforms for rapidly screening such compounds was highlighted by the National Research Council’s report, “Toxicity Testing in the 21st Century,” and the European Union’s REACH program for chemical testing and regulation. Microelectrode arrays (MEAs), culture wells with integrated stimulation and recording electrodes, have emerged as a technology with the capacity to screen neuroactive compounds. Although current MEA technologies measure complex changes in cellular dynamics, significant advances in both the throughput and analysis are still required. To address MEA throughput limitations, we combined simultaneous stimulation/recording electronics and microsensor fabrication techniques to develop a high-content multiwell MEA system. Using this system, we present preliminary results and data from the literature, demonstrating network-level neuroactivity changes at nanomolar concentrations for a variety of single-mechanism neuroactive compounds. Further development holds promise for a standardized in-vitro neurotoxic screening platform.

A-16

Differentiation and High-throughput Screening Techniques for a Library of Mutant Mouse Embryonic Stem Cells. DEEANN WALLIS, Stacy Galaviz, Lauren Schilling, and Jim Sacchettini. Texas A&M University, Department of Biochemistry and Biophysics, College Station, TX. Email: dwallis@tamu.edu

We have developed an entirely novel discovery platform utilizing a library of murine knockout ES cell clones to define gene function and identify genes involved in specific cellular functions/responses. This can be utilized to identify mammalian, host-based targets to various types of genetic disease, infections, or toxins including cancer, bacteria, virus, neurotoxins or environmental pollutants. There are many advantages of using ES cells as a discovery platform over traditional tissue culture models or other gene silencing technologies. ES cells have unlimited capacity for expansion without malignant transformation and maintain physiological signaling pathways. ES cells are uniquely capable of induced differentiation into single or multiple tissue types. Equally important, each of the ES cell clones from our library has a stable heterozygous inactivation at an individual gene, thereby reducing, but not completely eliminating gene function. To date, we have been able to
successfully grow and differentiate ES cells as embryoid bodies, gut structures, neurons, and lung tissues. We are in the process of developing protocols for differentiation into dendritic cells. We are already utilizing ES cells in HTS assays for cell death/viability and for gene expression. Other assays currently in development include: agent uptake and subcellular localization, endocytosis/exocytosis, neurotransmitter release, and quantitation of immunofluorescent cell-type specific markers. Many of these assays utilize high content analysis. This work was supported by the Transformational Medical Technologies program contract HDTRA1-10-C-0063 from the Department of Defense Chemical and Biological Defense program through the Defense Threat Reduction Agency (DTRA).

**A-17**

Functional Expression of Odorant Receptor In Vitro. H. MATSUNAMI. Duke University Medical Center, Department of Molecular Genetics and Microbiology, Durham, NC 27710. Email: hiroaki.matsunami@duke.edu

Humans and other mammals use hundreds of odorant receptors (ORs) to detect and discriminate thousands of volatile flavor chemicals (i.e. odorants). High-throughput in vitro systems have been a boon to studies of non-olfactory G protein-coupled receptors, allowing both academic and industrial labs to identify agonists and antagonists for target receptors. Though the establishment of an in vitro system in cell lines is essential for conducting a large scale analysis of OR ligand specificities, exogenously-expressed ORs in cell lines pose critical problems, namely, poor protein expression, poor plasma membrane trafficking, and/or poor coupling to signal transduction components in these cells. The bane of these systems lies in the inability to functionally express transfected ORs on the plasma membrane, due to endoplasmic reticulum (ER) retention. Receptor-transporting protein (RTP) family members were among the first of accessory proteins that were shown to improve in vitro systems by promoting cell-surface expression of various ORs. RTP1 and RTP2 are specifically expressed in the olfactory neurons. With the development of a high-throughput in vitro system for ORs we are positioned to identify a large set of agonists and antagonists. This information is useful because we not only understand olfactory coding, but we will be able to manipulate the olfactory system.

**A-18**

Micro/Small RNA Detection on a Microfluidics Microchip. CHRISTOPH EICKEN¹, Xiaochuan Zhou², Xiaolian Gao¹, and Chris Hebel¹. ¹LC Sciences LLC Houston, TX and ²Atactic Technologies Inc. Houston, TX. Email: ceicken@lcsciences.com

Detection of micro and small RNAs using a microarray offers the opportunity to examine all known and/or predicted micro/small RNA transcripts in a single experiment. A successful micro/small RNA microarray detection system consists of a high quality microarray platform, a reliable sample preparation and labeling process, and a comprehensive data analysis capability. Additionally, micro/small RNA detection is a rapidly expanding field. A detection system must be highly flexible to be able to serve the changing needs. Here we present an advanced µParaflo™ microfluidics microchip technology that was developed to enable a comprehensive micro/small RNA microarray service. Modified nucleotides are incorporated into the detection probes to enhance their binding to short micro/small RNAs without sacrificing specificity. The detection probes are in situ synthesized using PGR (Photo-Generated Reagent) chemistry to afford the highest synthesis yield and complete flexibility in the sequences synthesized. This capability has proven to be highly valuable to scientists in their discovery studies of new micro/small RNAs and their biogenesis and functional mechanisms. The high quality of the µParaflo™ array microchips will be demonstrated by several key parameters, including detectivity, specificity, and feature uniformity. Diverse application examples will be provided to illustrate the usefulness of this highly sensitive, specific, flexible, high dynamic range, and low noise microarray technology in the evolving fields of micro/small RNA research.

**A-19**

Comprehensive Suite of MicroRNA Research Tools for and Their Applications. SUN LU¹,², Shuwei Yang¹, Qi-Heng Yang¹, Huaping Li², Li Lin², Dan Lin², Guiliang Zhou², Jinkuo Zhang², and Luming Niu¹. ¹GeneCopoeia, Inc., 9620 Rockville, MD 20850 and ²FulenGen Co. Ltd., #3 Juquan Road, Science Park, Guangzhou, P. R. CHINA. Email: slu@geneCopoeia.com

MicroRNAs (miRNAs) are small, non-coding single stranded RNA molecules found in eukaryotic organisms. They are highly conserved and usually 21–23 nucleotides in length. MiRNAs are involved in almost all cellular functions and regulate gene expression by binding to the 3’ untranslated regions (3’ UTRs) of targeted mRNAs. Irregularities in miRNA expression level and abnormalities in their regulation of gene expressions have been found to be associated with cancers, cardiovascular disorders and
many other diseases. There are practical implications and applications of miRNAs as biomarkers and therapeutic targets for diagnosis, prognosis, and treatments of many diseases. Discussion points will include the introduction of a comprehensive suite of research tools that are now available to study 1) the expression profiles of individual and genome-wide whole set of miRNAs among different tissues, diseases and treatment conditions; 2) overexpression of mature miRNAs and their regulatory effects on targeted genes and proteins; 3) validations of miRNAs and their targeted genes and proteins; 4) transient and stable inhibition of miRNA mediated regulation of gene expression and protein functions; 5) restoration of gene expression and protein functions that are negatively regulated by miRNAs. Also discussed will be examples and case studies of these tools in cancer and other fields of research.