

## Animal Symposia

### A-1

Japanese Collaboration on Alternative to Animal Toxicology Testing. HAJIME KOJIMA. Japanese Center for the Validation of Alternative Methods, National Center for Biological Safety and Research, National Institute of Health Sciences, 1-18-1 Kamiyouga, Setagaya-ku, 158-8501, Tokyo, JAPAN. Email: h-kojima@nihs.go.jp

In November 2005, the Japanese Center for the Validation of Alternative Methods (JaCVAM) was established as part of the Division of Pharmacology at the National Center for Biological Safety and Research, affiliated with the National Institute of Health Sciences (NIHS) in Japan. One mission of JaCVAM is to promote the practice of the 3Rs (Reduction, Refinement, and Replacement) in the area of animal testing with reduction and replacement prioritized in Japan. The key objectives of JaCVAM are (1) to ensure that new or revised test methods are validated, peer-reviewed, and officially accepted by the regulatory agencies and (2) to expand international cooperation on alternatives to animal testing. The main activities of JaCVAM will focus on the following missions and objectives: (1) coordination of peer review and regulatory acceptance of new and revised test methods; (2) support of validation work for new and revised test methods; (3) promotion of the 3Rs; and (4) working to promote international partnership surrounding the issue of alternative methods. Especially, significant progress has been made in validating alternatives to animal testing in collaboration with international organizations. Particularly important is the JaCVAM cooperation with the European Center for the Validation of Alternative Methods (ECVAM) and NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)/Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). These three centers are closely united, and this may increase international cooperation. Together, our three organizations validated and peer-reviewed a new test method which has been approved by OECD guidelines.

### A-2

Critical Review of the Test Paradigm to Predict Human Hepatotoxicity—the Way Forward. PHILIPPE VANPARYS. Alttoxicon, 2350 Vosselaar, BELGIUM. Email: philippe.vanparys@alttoxicon.com

An ever-increasing part of the investments of pharmaceutical industry is spent on the development of new drugs. Notwithstanding, this increase in R&D spending, the number of new drug approvals (NDAs) decreases over the years. According to analysts, this gap is attributed to less innovation, failure of drug candidates in pre-clinical and clinical testing, and more stringent regulatory requirements. Hepatotoxicity is one of the main causes of attrition of drugs. Existing in vitro and in vivo models are not sensitive enough to detect human hepatotoxins. Therefore, we have to (1) revisit our testing paradigm for toxicity testing and define new test strategies, (2) improve the existing in vivo regulatory test models by assessing more sensitive and predictive endpoints in body fluids despite the absence of conventional hepatotoxicity markers, (3) develop new in vitro models preferentially based on human cells, (4) develop new in vivo models preferentially with lower organisms which are predictive to man, (5) implement high throughput models for compound selection and early decision making before compounds enter into preclinical testing, and (6) integrate new and advanced technical tools such as transcriptomics, proteomics, metabolomics, flow cytometry, and image analysis to better understand the mechanisms of toxicity. This presentation will highlight the problems encountered in hepatotoxicity testing and will elaborate more on improved in vitro cytotoxicity testing and on the evaluation of the zebrafish as a suitable test organism to detect hepatotoxic compounds. The overall outcome will surely reduce mammalian use for hepatotoxicity testing.

### A-3

In Vitro Alternatives in Toxicology: a Current and Future Science Perspective. EUGENE ELMORE. Department of Radiation Oncology, University of California, Irvine, CA 92697. Email: eelmore@uci.edu

The progress in developing, validating, and gaining regulatory acceptance for alternative methods in the US will be reviewed. Efforts to develop and validate in vitro alternatives to animal use in regulatory safety testing have been ongoing since the early 1980s. Much of the effort has been focused on developing assays to detect agents that cause disease endpoints, acute toxicity, or genetic damage, e.g., neurotoxicants, eye irritants, teratogens, mutagens, and carcinogens. With the publication in 2007 of the U.S.

National Academy of Sciences Report “Toxicity Testing in the 21st Century: A Vision and a Strategy,” which emphasized the use of modern biology coupled with human cell models, a paradigm shift in toxicology appears to be inevitable. This shift will require that we think of toxic pathways rather than just endpoints. The success of this effort will require the development of novel human cell culture models that express tissue-specific function to facilitate the identification of cellular response pathways that drive toxicity. Perspectives on future directions that are needed to drive acceptance of in vitro alternatives nationally and internationally will be discussed. A global effort to harmonize the development and validation of alternative tests is needed. This may require a paradigm shift on the part of international regulatory agencies to allow the best science for predicting human safety to drive regulatory acceptance. In vitro scientists will play important roles in the development of the appropriate cell models and training future generations of toxicologists in the new science.

#### A-4

Baculovirus Genes Affecting Host Functions. S. M. THIEM. Department of Entomology, 201 CIPS, Michigan State University, East Lansing, MI 48824. Email: smthiem@msu.edu

Baculoviruses are large DNA viruses that infect invertebrates, primarily insects. Those infecting Lepidoptera have been studied extensively owing to their practical uses as biological insecticides and protein expression vectors. An intriguing feature of these viruses is their host specificity in vivo and in vitro. Studies of baculovirus-infected insect cells revealed a number of genes encoding functions that counter host cell defenses and contribute toward host specificity. These include two types of antiapoptotic proteins and a unique protein, host range factor 1 (hrf-1), that relieves translation arrest in cell lines derived from the gypsy moth, *Lymantria dispar*. Translation arrest is postulated to be a host defense, possibly a stress response mediated by an eIF2-alpha kinase. Global translation arrest resulted when some *L. dispar* cell lines were infected with the baculovirus, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), although translatable transcripts were present in cells. Hrf-1, a single gene from the *L. dispar* nucleopolyhedrovirus, restored translation and facilitated AcMNPV replication in the *L. dispar* cells. Moreover, a recombinant AcMNPV bearing hrf-1, but not wt AcMNPV, was infectious for *L. dispar* in vivo by the normal oral infection route. Hrf-1 also rescued translation arrest triggered in *L. dispar* cells

by other baculoviruses, suggesting that the response is specific to *L. dispar*. Hrf-1 has no conserved motifs to suggest function and the only known hrf-1 homolog is a nonfunctional truncated version encoded by another baculovirus. Although mutational analysis suggests that an acidic domain may play a role in relieving the translation block, how hrf-1 functions to restore protein synthesis is still unknown.

#### A-5

Coronavirus Assembly at Intracellular Membranes. B. G. HOGUE. The Biodesign Institute, Center for Infectious Diseases and Vaccinology, Arizona State University, Tempe, AZ 85287-5401. Email: brenda.hogue@asu.edu

With the completion of a finished genome sequence, we must now functionally characterize the rice genome by a variety of methods including comparative genomic analysis between cereal species and within the genus *Oryza*. *Oryza* contains 2 cultivated and 21 wild species that represent 10 distinct genome types. The wild species, in particular, contain an essentially untapped reservoir of agriculturally important genes that must be harnessed if we are to maintain a safe and secure food supply for the twenty-first century. OMAP was established 4 y ago to generate a comprehensive set of genomics resources to investigate genome evolution and enhance positional cloning efforts in the genus *Oryza*. To date, we have generated (1) 14 high-quality BAC libraries that encompass the 10 genome types of *Oryza*; (2) 1,000 Mb of BAC end sequence from these libraries; and (3) SNaPshot fingerprint databases for all 14 libraries. All of these resources are publicly available through the AGI BAC/EST Resource Center, GenBank or at <http://www.omap.org/>. The fingerprints and end sequences (BES) have been combined to develop 14 phase I physical maps. Nine of these physical maps, *O. nivara* [AA], *O. rufipogon* [AA], *O. glaberrima* [AA], *O. barthii* [AA], *O. punctata* [BB], *O. officinalis* [CC], *O. australiensis* [EE], *O. brachyantha* [FF], and *O. minuta* [BCCC] have been heavily manually edited (HME) and aligned to the reference rice genome sequence. These alignments have revealed a large array of genome rearrangements relative to the IRGSP reference sequence and have allowed us to begin draw a more complete picture of *Oryza* genome evolution. In this talk, I will present the current status of OMAP and discuss recent analysis of the HME maps, a global analysis of structural variation across the AA genome species, an analysis of the affect of differential and lineage-specific LTR retrotranspon bursts on genome size variation and comparative sequence analysis of select loci across *Oryza*.

**A-6**

Viral Gene Transfer Vectors in Studies of Human Smooth Muscle Function. W. T. GERTHOFFER and C. A. Singer. Department of Biochemistry and Molecular Biology, College of Medicine, University of South Alabama, Mobile, AL 36688 and School of Medicine, University of Nevada, Reno, NV 89557. Email: wgerthoffer@usouthal.edu

Smooth muscles are often targets of drugs used to treat cardiovascular and lung diseases. Until recently, studies of signaling mechanisms that were targets for new drug development were hindered by difficulty in genetically manipulating smooth muscle cells in culture. Now a variety of viral gene transfer vectors are used to express proteins of interest which allows direct tests of important hypotheses relevant to chronic diseases including atherosclerosis, hypertension, asthma, inflammatory bowel diseases, and preterm labor. Human adenoviral, Maloney murine leukemia retroviral, and lentiviral vectors have all been applied in culture systems with some success. The strategies used to construct these vectors, the approaches used to verify efficacy, and some examples of novel smooth muscle cell functions discovered will be discussed. Viral vectors have been used to define important signal transduction components of pathways controlling all the major functions of smooth muscles including contraction, cell migration, and proinflammatory gene expression. One particularly useful approach has been to extend the lifespan of cultured human smooth muscle cells by infecting primary cultures with a retrovirus encoding human telomerase reverse transcriptase (hTERT). Having a steady supply of immortalized human smooth muscle cells is highly advantageous for cell signaling and tissue engineering projects.

**A-7**

Mycoplasma Contamination and Cross Contamination in Tissue Culture: a Survey of Major Institutions in Japan. ARIHIRO KOHARA, Tohru Masui, and Hiroshi Mizusawa. Laboratory of Cell Cultures (JCRB Cell Bank), National Institute of Biomedical Innovation, Osaka, JAPAN. Email: kohara@nibio.go.jp

Animal cell culture technique is widely used by scientists in many diverse disciplines such as cell biology to genetics and transcriptome to proteome analyses. Therefore, for biomedical researchers, cell culture is one of the most common research tools. However, this situation has drastically decreased awareness and knowledge on cell culture. For example, there are a lot of scientists who

cannot count cell numbers and cannot do subcultures of cells. In this situation, mycoplasma contamination and cross-culture contamination have been recognized as serious problems, but they would not be paid much attention. Thus, JCRB Cell Bank has recently started a nationwide survey on the mycoplasma contamination by using an inspection kit called "MycoAlert®" in the joint venture with Japan Tissue Culture Association (JTCA). Mycoplasmas are the smallest self-proliferating microorganisms of about 1/10 of a bacterium. Whereas they are contaminating in cell cultures, it is difficult to realize because the medium does not become turbid. But we should not ignore mycoplasma contaminations because they induce bad effects for cells in cultures and, subsequently, spoil experiments. For example, they may induce cell death, abnormal induction of cytokines, or chromosome aberrations. To date, we have analyzed about 3,000 samples of major research institutions in Japan, and the average rate of the contamination was 24.4%. We would also like to introduce the frequency of cross-culture contamination in the next step. In this presentation, I will report on the current situation in Japan and also on the joint activities of JTCA and JCRB Cell Bank to raise consciousness on good cell culture laboratory practices.

**A-8**

Assuring Cell-based Assay Quality by Design and Execution: a Contract Research Laboratory Perspective. HANS RAABE. Laboratory Services, Institute for In Vitro Sciences, Inc., 21 Firstfield Road, Suite 220, Gaithersburg, MD 20878. Email: hraabe@iivs.org

Cell-based assays are frequently used for preclinical safety assessments by manufacturers of pharmaceuticals, commercial and household products, and personal care products. The in vitro cell-based methods are designed to address specific endpoints of interest, such that the interpretations of the assay results are highly dependent upon appropriate study design for the intended purpose, cell system selection, maintenance and handling, and the proper conduct and execution of the study. Sound experimental design is the foundation for any laboratory activities, as it provides the documentation and communication of study goals and expectations to all parties, and provides the detailed procedures to achieve the goals. Sound study design recognizes that the selection of the cell type and the desired endpoint are relevant to the study goals, and the maintenance and handling of the specific cell cultures assure that the responses of the cells are predictable and reliable under control conditions. Controls and appropriate acceptance criteria included in every trial provide measures of test

system responses and assay execution. Finally, the quality of the study is ultimately dependent upon the execution of the work. Accordingly, training programs assure that the personnel responsible for maintaining the cell cultures, operating and maintaining the equipment, and executing the assays are fully competent to follow the study design and document their work. Training programs are ideally based upon the ability of the trainee to objectively demonstrate functional competency in the various critical steps used in the study.

#### A-10

The Regulation of Cultured Cells and Cellular Products for Transplantation: Current View of the Japanese Regulatory Process. TOHRU MASUI. JCRB Cell Bank, Division of Bioresources, National Institute of Biomedical Innovation, Osaka 567-0085, JAPAN. E-mail: masui@nibio.go.jp

The regulation of cultured cells and cellular products for transplantation in Japan is a complex process involving government guidelines, self-regulation, and public response. Japan initiated the discussion on embryonic stem (ES) research in 1998 after the publication of human ES cell production from leftover embryos of in vitro fertilization patients. The Japanese government understood the necessity of research using human ES cells. In 2001, Guidelines on the Use and Establishment of Human ES Cells for nonclinical application were established and revised in 2006. These guidelines require review by the IRB and a Ministry of Education, Culture, Sports, Science and Technology (MEXT) committee. A drawback to these guidelines was that research using established ES cells was very restrictive. In 2005, the Ministry of Health, Labour and Welfare (MHLW) authorized new guidelines for the use of somatic stem cells in clinical research. At the same time, accompanying guidelines were revised to accommodate updated usage, safety, and efficacy of cellular and tissue products. The Brain Death Transplantation Act was established in 1997, but what constitutes death has not been resolved. The Japanese Society of Tissue Transplantation issued guidelines on transplantation of human cadaver tissue in 2002. The discussions on birth and death and the use of human tissue continue to be considered. Finalized decisions and development to integrate different regulations are slow to accommodate rapid advances in cell culture and cellular product technology.

#### A-11

Navigating Research Strategy, Clinical Integrity, and Current Global Regulatory Compliance. SANDRA L.

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The development of research strategy and experimental design is a key component to meeting regulatory compliance and premarket awards for tissue-engineered cellular products and therapeutic biologics. In September 2007, the Food and Drug Administration (FDA) HR 3580 Act was signed into law. The Act gives FDA far-reaching powers to impose safety restrictions in regulating drugs, biological products, and medical devices. These provisions include requiring manufacturers to conduct additional postmarket studies and disclosure to the National Library of Medicine's Clinical Trials Database. Clinical investigation of tissue-engineered cells and biologics must comply with the FDA informed consent and institutional research board (IRB) regulations. IRB protocols vary by institution, but should be developed with clear scientific and clinical validation of the product to receive FDA premarket biologic approval. The intent of the European Clinical Trial Directive (2001/20/EC), comprised of 25 countries, is to harmonize regulatory clinical study requirements under Good Clinical Practice (GCP) and the pharmacovigilance EudraVigilance database. GCP noncompliance is a criminal rather than a civil penalty, and ethics committees are under statutory control. Spontaneous safety reporting is mandatory from the sponsor regulatory authority, i.e., FDA. The shift in global cooperative collaborations in the development of tissue-engineered and somatic stem cell research for therapeutic biologics requires not only good science and rational research strategy, but understanding global restrictions and regulations.

#### A-12

Mesenchymal Stem Cells and the Development of Therapeutics. MICHELLE GREENE. Millipore Corporation, Bioscience Division, 80 Ashby Road, Bedford, MA 01730. Email: Michelle\_Greene@millipore.com

Mesenchymal stem cells (MSCs) are currently the most easily obtainable and renewable of the common stem cell types. Given their differentiation potential into heart, bone, kidney, tendon, liver, and brain cells among other cell types as well as the lack of political and ethical questions around them, MSCs are the target for much therapeutic development. With the findings that MSCs isolated from adipose tissue function appear to function as those from bone marrow, the idea of treatment with the patient's own stem cells becomes more viable. Perhaps more intriguing is the use of MSCs as screening tools, particularly in the short-term, for therapeutic development.

**A-13**

Mesenchymal Progenitors Able to Differentiate into Osteogenic, Chondrogenic, and/or Adipogenic Cells In Vitro are Present in Most Primary Fibroblast-like Cell Populations. KAZUHIRO SUDO and Yukio Nakamura. Cell Engineering Division, RIKEN BioResource Center, Tsukuba, Ibaraki, JAPAN. Email: ksudo@brc.riken.jp

Mesenchymal stem cells are being studied for their potential in regenerative medicine, as various reports have shown that these readily obtainable cells can differentiate into many different cell types. However, the difference between mesenchymal stem/progenitor cells and so-called fibroblasts is unclear. We analyzed 33 distinct populations of fibroblast-like adherent cells derived from various human tissues, including lung, skin, umbilical cord, and amniotic membrane, and found that most of these cells contained cells that were able to differentiate into at least one mesenchymal lineage, including osteoblasts, chondrocytes, and adipocytes. The abilities of different cell populations to differentiate into particular lineages depended on the source tissue. For example, cells derived from lung could differentiate into osteoblasts and chondrocytes but not into adipocytes, whereas cells derived from skin could differentiate into osteoblasts, chondrocytes, and adipocytes. Culture conditions can have a strong effect on the differentiation potential of cells. Inappropriate culture practices, such as infrequent change of media or overgrowth of cultures, often result in the loss of the ability of cells to differentiate. Prolonged passaging of cells can also result in the loss of differentiation potential. The age of the donors might not be so effective for differentiation potentials because a cell population, SF8758, derived from a person of 89 y of age retained the capacity to differentiate into osteoblasts, chondrocytes, and adipocytes. We, therefore, propose that most of the fibroblast-like adherent cells obtained from various human tissues are not actually fibroblasts, but rather that they often comprise instead mesenchymal stem or progenitor cells.

**A-14**

Induction of Hepatocyte-like Cells from Mesenchymal Stem Cells and the Transplantation into Liver-injured Rats. KIYOHITO YAGI<sup>1</sup>, Midori Kojima<sup>1</sup>, Etsuko Ikeda<sup>2</sup>, Katsuhiko Isoda<sup>1</sup>, Masuo Kondoh<sup>1</sup>, and Hajime Ohgushi<sup>2</sup>. <sup>1</sup>Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, JAPAN and <sup>2</sup>Research Institute for Cell Engineering (RICE), National Institute of Advanced Industrial Science and Technology (AIST), Amagasaki Site, 3-11-46 Nakoji, Amagasaki, Hyogo 661-0974, JAPAN. Email: yagi@phs.osaka-u.ac.jp

Stem cell-based therapy has received attention as a possible alternative to organ transplantation owing to the ability of stem cells to repopulate and differentiate at the engrafted site. The isolation of high-performance human stem cells is an important approach for regenerative medicine. Recently, we isolated a stem cell, which we named tooth germ progenitor cells (TGPCs), from discarded third molars, commonly called wisdom teeth. TGPCs had high proliferation activity and differentiation ability in vitro into cells of three germ layers, such as osteoblasts, neural cells, and hepatocytes. The TGPCs were cultured in the presence of growth factors including hepatocyte growth factor and oncostatin M for hepatic induction. After 3 wks of induction, TGPCs were stained using the PKH fluorescent dye. Immunocompromised rats were given an intraperitoneal injection of 1 ml/kg CCl<sub>4</sub>. Two days later, TGPCs were transplanted by injection into the portal vein. The CCl<sub>4</sub> treatment was then performed twice a week for 4 wks to cause persistent inflammation and liver fibrosis. Engraftment of the donor TGPCs was confirmed by PKH26 fluorescence and the presence of the human DNA-specific *alu* gene in the rat liver. The serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increased markedly in the sham-operated rats after CCl<sub>4</sub> treatment. In contrast, the AST and ALT levels were much lower in TGPC-transplanted rats. Azan staining revealed smaller areas of fibrosis in liver sections from the TGPC-transplanted rats than in those of sham-operated rats. These results indicated that the transplanted TGPCs could engraft to suppress liver inflammation and fibrosis. As wisdom teeth are usually discarded, TGPCs are an ethically and functionally promising source for stem cell-based therapy.

**A-15**

Stem Cells: Ancestors in a Somatic Cell Tree. DARRYL SHIBATA. Keck School of Medicine, University of Southern California, Los Angeles, CA 90033. Email: dshibata@usc.edu

Recent studies suggest that cancers contain two types of cells—"cancer stem cells" (CSCs) and nonstem cells. CSCs are postulated to be a minority of cells and have capabilities for limitless numbers of divisions and renewal, whereas non-CSCs can only divide a few times. The xenotransplantation studies used to identify CSCs have a number of potential experimental problems. An alternative approach to characterize CSCs and non-CSCs is by genealogy. An ancestral tree consists of ancestors and dead-ends, which functionally correspond to stem cells and nonstem cells. If CSCs are rare, a cancer tree should have relatively few ancestors. In theory, it should be possible to reconstruct a somatic cell ancestral tree from present-day genomes using molecular phylogeny. The greater the number of divisions

since a common ancestor, on average the greater the number of replication errors (molecular clock hypothesis). Potentially, this approach can be used to study any human tissue because it does not require prior experimental intervention. Preliminary studies using epigenetic patterns (the 5' to 3' order of CpG methylation) illustrate that human cancers are diverse populations maintained by relatively large numbers of ancestors or CSCs.

#### A-16

Mechanism of Self-renewal of Brain Tumor Stem Cells. ICHIRO NAKANO. Department of Neurosurgery, School of Medicine, UCLA, Los Angeles, CA 90095. Email: [inakano@mednet.ucla.edu](mailto:inakano@mednet.ucla.edu)

Despite the dramatic improvements in the outcome of other cancers in the recent decades, brain tumors remain as one of the most devastating diseases. Malignant brain tumors are composed of heterogeneous cell populations. Recent investigations, including our own, have identified a stem cell population called 'brain tumor stem cells' (BTSC). These malignant stem cells self-renew and are capable of recapitulating the entire tumor mass. It is likely that targeted therapeutic strategies, which exploit molecular differences present within a heterogeneous population of brain tumor cells, will lead to the specific eradication of BTSC without causing toxicity. Considering the limited effects of the current therapies on malignant brain tumors, multiple parallel or compensatory oncogenic pathways exist to allow tumor stem cells to escape and survive. Thus, a single agent therapy may not cure malignant brain tumors like the imatinib-like success in the therapy for chronic myeloblastic leukemia, and multiple molecularly targeted therapies are crucially required to terminate malignant brain tumor growth. In this study, I will briefly describe the current knowledge about BTSC.

#### A-17

Prostate Tissue Homeostasis. MONIKA SCHMELZ. Department of Pathology, University of Arizona Health Sciences Center, 1501 North Campbell Avenue, Tucson, AZ 85724. Email: [Schmelz@email.arizona.edu](mailto:Schmelz@email.arizona.edu)

The prostate is the site of two of the most frequent medical problems in elderly men, benign prostatic hyperplasia (BPH) and prostate cancer. BPH is the second most common reason for surgery in men above the age of 65 y. Prostate cancer is the most frequently diagnosed cancer in men in the United States. What causes BPH or cancer to

develop is not known. One hypothesis suggests that an expansion of an epithelial stem cell population is responsible for these disorders. Stem cells have not yet been identified within the prostate utilizing a structural biomarker. We have discovered a new prostatic epithelial cell phenotype-expressing cytokeratin 6a (Ck6a+ cells). The Ck6a+ cells are present within a specialized niche in the basal cell compartment in fetal, juvenile, and adult prostate tissue and within the stem cell-enriched urogenital sinus. In adult normal prostate tissue, the average abundance of Ck6a+ cells was 4.9%. With proliferative stimuli in the prostate organ culture model in which the epithelial-stromal interaction was maintained, a remarkable increase of Ck6a+ expression was noticed up to 64.9%. The difference in cytokeratin 6a expression between the normal adult prostate and the prostate organ culture model was statistically significant ( $p < 0.0001$ ). Within the prostate organ culture model, the increase of cytokeratin 6a-expressing cells significantly correlated with an increased proliferation index ( $r = 0.7616$ ,  $p = 0.0467$ ). The Ck6a+ cells were capable of differentiation as indicated by their expression of luminal cell markers such as ZO-1 and prostate-specific antigen (PSA). Our data indicate that Ck6a+ cells represent a prostatic epithelial stem cell candidate possessing high potential for proliferation and differentiation. As the development of BPH and prostate carcinogenesis are disorders of proliferation and differentiation, the Ck6a+ cells may represent a major element in the development of these diseases.

#### A-18

Using Cell Sensor Impedance Technology for Label-free and Real-time Cell-based Assays. YAMA ABASSI. Acea Biosciences Inc., 6779 Mesa Ridge Road, #100, San Diego, CA 92121. Email: [yabassi@aceabio.com](mailto:yabassi@aceabio.com)

The RT-CEST<sup>TM</sup> and RT-CIM<sup>TM</sup> systems allow for label-free and real-time monitoring of cellular processes such as cell proliferation, adhesion, viability, and migration using electronic cell sensor array technology. Real-time monitoring of cellular processes by the RT-CEST<sup>TM</sup> and RT-CIM<sup>TM</sup> systems offers distinct and important advantages over traditional endpoint assays. First, the avoidance of label allows for more physiologically relevant assays and saves on time, labor, and resources. Second, a comprehensive representation of the entire length of the assay is possible, allowing the user to make informed decisions regarding the timing of certain manipulations or treatments. Finally, the actual kinetic response of the cells within an assay before or subsequent to certain manipulations provides important information regarding the biological status of the cell such as cell growth, arrest, morphological changes, and apoptosis.

**A-19**

Extracellular Flux Measurements Provide a New Window on Cellular Bioenergetics. GEORGE ROGERS. Seahorse Bioscience, 16 Esquire Road, N. Billerica, MA 01862. Email: GRogers@seahorsebio.com

Current research has increasingly implicated metabolic and mitochondrial dysfunction in a variety of disease states, including cancer, diabetes, obesity, neurodegenerative disorders, and aging. The ability to obtain time-resolved measurements of mitochondrial and cellular bioenergetics under many different conditions is enabling investigators to link various forms of mitochondrial dysfunction with these disorders and degenerative processes. In this study, we present a novel method based on the extracellular flux (XF) of oxygen and protons to study mitochondrial function and overall cellular bioenergetics. The Seahorse XF24 Analyzer noninvasively measures cellular oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), indicators of aerobic respiration and glycolysis, respectively. Discussed are examples of using established cell lines and primary tissue cultures to measure mitochondrial uncoupling, OXPHOS capacity, in vitro cytotoxicity, and fatty acid oxidation (FAO). Also presented are methods to measure the effects of various compounds on the glycolytic pathway, mitochondrial complexes, and electron transport chain function. In conclusion, we show how this XF technology may be applied to

investigate mitochondrial function and cellular bioenergetics in the context of cell health and quality, bioprocessing, and disease caused by metabolic dysfunction.

**A-20**

A Hybrid CMOS/PDMS Microsystem for Autonomous Cell Culture and Incubation. JENNIFER BLAIN CHRISTEN. Department of Electrical Engineering, Arizona State University, GWC 350, Tempe AZ 85287-5706. Email: Jennifer.blainchristen@asu.edu

The design, fabrication, and testing of a hybrid, closed-loop microsystem for stand-alone cell culture and incubation will be presented. The device allows for the observation and testing of cells while maintaining an incubation environment. The microincubator is engineered through the integration of silicon CMOS electronics with multilayer silicone (PDMS) microfluidics. The implementation of such a system, cells grown atop a CMOS die, allows for the integration of any discrete electronic device (for the detection of voltage, current, conductivity, pH, etc.) at the substrate, permitting more accurate measurements with finer spatial resolution. The complete structure has a size of  $2.5 \times 2.5 \times 0.6 \text{ cm}^3$ . The device has been used to successfully culture BHK-21 cells autonomously over a 3 day period in ambient environment.