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Animal Symposia

A-2

Isolation and Growth of Embryonic and Induced Pluripotent Stem Cells. DEMETRI SPYROPOULOS. Medical University of South Carolina, College of Medicine, Pathology and Laboratory Medicine, 165 Ashley Ave. , RS714A, Charleston, SC 29425. Email: spyropdd@musc.edu

Over the past decade, intensive and high-powered investigations principally involving mouse and human cells have brought the generation and study of induced pluripotent stem cells (iPSCs) to a level that facilitates widespread use in a spectrum of species. A review of key features of these investigations is presented here as a primer for the use of iPSC technology to enhance ongoing animal species studies. iPSC and other cutting edge technologies create the potential to study individuals from "the wild" closer to the level of investigation applied to sophisticated inbred mouse models. A wide variety of surveys and hypothesis-driven investigations can be envisioned using this new capability, including comparisons of organism-specific development and exposure response and the testing of fundamental dogmas established using inbred mice. However, with these new capabilities, also come new criteria for rigorous baseline assessments and testing. Both the methods for inducing pluripotency and the source material can negatively impact iPSC quality and bourgeoning applications. Therefore, more rigorous strategies not required for inbred mouse models will have to be implemented to approach global health issues using individuals from "the wild" for aquatic animal species.

A-3

Stem Cells in Regenerative Medicine and Toxicology. KYLE KOLAJA. Cellular Dynamics, International. Email: kyle@cellulardynamics.com

Induced pluripotent stem (iPS) cell technology offers unprecedented opportunities to move beyond a "one size fits all" approach to pharmacology and toxicology, to a model where individual genetic and molecular profiles are used to guide diagnosis, drug development, and therapeutic decisions. Initially described in 2007, human iPS cells are derived from a patient's somatic cells (e.g. blood, skin) and have the potential to differentiate into any cell type in the human body. In the last 5 years, a rapidly growing body of literature has emerged demonstrating the use of iPS cell-derived differentiated cells to demonstrate human relevant toxicity as well as recapitulate human disease phenotypes in vitro. These novel human cell models are rapidly becoming the standard of choice for disease research and drug discovery as they offer better opportunities for therapeutic decision-making. Several case studies will be presented demonstrating how iPS cell-derived cardiomyocytes, neurons, and hepatocytes are being used today for drug development and discuss their potential future applications.

Disclosure: Author was or is employed by Cellular Dynamics International.

A-4

Overview of 3D Cell Culture Model Systems & Validating Cell-based Assays for Use with 3D Cultures. TERRY RISS. Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711. Email: terry.riss@promega.com

Cells cultured in a 3D arrangement represent a more physiologically relevant environment that has promise for being more predictive of *in vivo* responsiveness compared to cells cultured using standard 2D methods. However, multicellular 3D cultures containing more than one cell type and exhibiting formation of a complex extracellular matrix often represent a challenge for assay chemistries originally designed for measuring events from monolayers of cells on plastic plates. There is an unmet need for guidelines for design and verification of convenient and effective assays useful for larger 3D microtissues. An overview of the advantages and disadvantages of variety of methods used to generate 3D cultures will be presented. We will also describe the critical factors to consider when choosing an assay system to measure markers from 3D cultures, including effective penetration of detection reagents and/or

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complete lysis of microtissue structures using combinations of detergent and physical disruption.

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A-5

Commercial 3D Culture Models: Safety, Product Development, Clinical Applications of Available Products and Future Prospects. PATRICK J. HAYDEN. MatTek Corporation, 200 Homer Avenue, Ashland, MA. Email: phayden@mattek.com

Techniques for in vitro reconstruction of 3D tissues from human cells have become more refined and widely utilized. Epithelial cells are typically cultured on microporous membranes to produce differentiated 3D tissues possessing many physical/biochemical properties of in vivo epithelia. Currently available epithelial models include dermal, ocular, airway, intestinal, gingival, buccal and vaginal epithelium. Second generation models provide additional complexity by incorporating multiple cells types such as fibroblasts, melanocytes or dendritic cells. Non-epithelial cells such as hepatocytes, or tumor cells can be cultured as 3D spheroids using hanging droplets or other techniques. Decellularized organs can also be utilized as scaffolds for re-introduction of cells, recreating functional organs. The trend moving forward is for increased complexity by co-culture of multiple cell types to produce "organ-on-a-chip", and organs connected together to produce "human-on-a-chip" systems. Induced pluripotent stem (iPS) cell technology allows new possibilities for development of models from cardiac, liver, neuronal and other cell types that have been difficult to culture in vitro. Clinical applications of 3D cultures include skin and tracheal grafts, with additional future personalized medical applications of stem cells. The in vivo-like characteristics and barrier properties of 3D tissue models allow application and evaluation of test chemicals, candidate therapeutic compounds and finished formulations in a more realistic manner compared to traditional monolayer cultures. However, the ultimate utility of commercial in vitro culture models for widespread use in research applications and regulatory purposes depends on their performance and validation with respect to interlaboratory transferability, long-term reproducibility, extrapolation to in vivo human results. Examples of successful validation of commercial 3D models for regulatory testing include use of dermal and ocular models for irritation and/or corrosion testing.

A-6

Bioactives from Natural Products: What Works, What Doesn't, and What's Next. M. LILA. North Carolina State University, 600 Laureate Way, Plants for Human Health Institute, Kannapolis, NC 28081. Email: mlila@ncsu.edu

Effective use of natural products for human health maintenance hinges on success in three arenas: 1) biodiscovery, 2) bioefficacy validation, and 3) practical delivery to consumers. Despite the fact that plants can provide greater molecular diversity and abundant novel molecular chemotypes, random bioprospecting is an inefficient and cost intensive means of sourcing compounds. Local/tribal partnerships and traditional ecological knowledge will identify and prioritize lead candidates, using a strategy of straightforward in-field bioassays. Unlike the high throughput screens used in pharma, the bioassay kits screen for potential hits even when multiple potentiating phytochemicals contribute to the activity. Once primary leads are identified, efficacy for human health interventions must be established. While clinical trials – the gold standard for validation – would be premature and too expensive, standard laboratory in vitro and in vivo bioassays can't assess efficacy or bioavailability in humans. Two alternative strategies were adopted to zero in on these issues. In vitro biolabeling (either with radiolabel or stable isotope) permits metabolic tracking to determine tissue distribution, bioavailability, and clearance of bioactive natural products after ingestion. Human community trials (with volunteer athletes) offer a straightforward means of gauging effects of bioactives on performance and recovery parameters, which are equally critical for metabolic syndrome/diabetes and other chronic diseases marked by inflammation and oxidative stress. Finally, researching biomedicinal activity of natural products is of little value unless consumers ultimately benefit. Novel tactics for concentrating phytoactive compounds for delivery in convenient, stable, and highly bioavailable functional foods formats are underway to counteract the negative trends of Western diets.

A-7

In Vitro Assays of Intercellular Communication as a Biomarker to Determine Chemopreventive Activity of Natural Products. BRAD L. UPHAM. Michigan State University, 243 Food Safety & Toxicology Bldg., 1129 Farm Lane, East Lansing, MI 48823. Email: Brad.Upham@hc.msu.edu

Natural products are chemical compounds produced by living organisms with pharmacological properties that can potentially benefit human health. There are numerous methods to screen for these potential health benefits, and *in vitro* systems are playing a critical role in this process. Selection of an appropriate biological endpoint for in vitro screening is critical in maximizing the identification of a compound(s) that can contribute to beneficial health effects. The primary biological

endpoint used in our lab is gap junctional intercellular communication (GJIC). GJIC plays a central role in maintaining tissue homeostasis through the coordination of intercellular signaling with that of intracellular signal transduction pathways controlling gene expression. The homeostasis of a tissue requires the gap junction channels to be in the open state, and chronic closures of channels result in pathological states. We focus primarily on cancer in which all cancer cells have no or reduced GJIC, compounds that contribute to the cancer process inhibit GJIC, and oncogenes dysregulates GJIC. Restoration of GJIC in cancer cells by the transfection of gap junction genes results in cells reverting back to normal epithelial morphologies, reduced anchorage independent growth in soft agar, and lack of tumor formation in nude mice injected with these re-communicating cancer cells. The screening strategy we use involve experiments that (1) treat cancer cells with a natural product and determine if it increase GJIC, (2) treat cell lines transfected with specific oncogenes with a natural product, and determine if it increase GJIC, (3) pretreat normal cells with a natural product and determine if it reverses the GJIC-inhibitory effects of known tumor promoters. Using this screening method, we have identified natural products that can restore GJIC or prevent the dysregulation of GJIC by cancer causing compounds.

A-8

Pancreatic Cancer and Marine Natural Products. ESTHER A. GUZMAN, Georgios Kallifatidis, Tara P. Pitts, and Amy E. Wright. Harbor Branch Oceanographic Institute at Florida Atlantic University, 5600 US 1 North, Fort Pierce, FL 34946. Email: eguzman9@hboi.fau.edu

Since 1984, the Harbor Branch Oceanographic Institute (HBOI) Marine Biomedical and Biotechnology Research Program has been conducting drug discovery research of novel natural products that can be used to treat or prevent cancer. Our current focus is to find potential treatments for pancreatic cancer, the 4th leading cause of cancer death in the United States. This effort has identified over 100 natural products with cancer fighting properties. Discodermolide, leiodermatolide, aphrocallistin, dictyostatin and neopeltolide cause mitotic arrest. Microsclerodermin A and spongiatriol inhibit the transcription factor NFκB, an important mediator of inflammation which has a strong link with cancer. Some of our compounds prevent mast cells — immune cells that in pancreatic cancer appear to facilitate the initiation and progression of the disease— from degranulating, and thus releasing the growth and blood-vessel-forming factors contained within them. This talk will address the path to the discovery of some of these compounds using various cancer cell lines, as well as highlight manzamine A, an alkaloid first isolated in 1986 from an Okinawan sponge that exhibits exciting activities against pancreatic cancer in vitro. Work done recently at HBOI has shown that although manzamine A is not very cytotoxic on its own, it restores the ability of pancreatic cancer cells to undergo apoptosis induced by other agents. Moreover, manzamine A is a potential anti-metastatic agent in pancreatic cancer as it prevents migration of the highly metastatic pancreatic cancer cell line AsPC-1 through a collagen matrix and reduces the cell dissociation that characterizes these cells. The effects of manzamine A are mediated through its uncoupling of vacuolar ATPases. This mechanism also results in inhibition of autophagy, a process that pancreatic cancer cells use to promote its growth.

A-9

Micellar Nanocarriers for Improved Cancer Therapy. CHALET TAN. Cancer Nanomedicine Laboratory, Department of Pharmaceutical Sciences, Mercer University. Email: tan_c@mercer.edu

Nano-sized drug carriers are emerging drug delivery systems for cancer therapy. Such nanocarriers are advantageous over conventional intravenous formulations because of their capacity to accomplish preferential drug accumulation in the tumor via the enhanced permeability and retention (EPR) effect. Among numerous intravascular nanoparticulate systems that are being investigated in the preclinical and clinical studies, biodegradable and biocompatible polymeric micelles (10-100 nm in diameter) are considered as promising nanocarriers for water-insoluble anticancer drugs. In this presentation, the evaluation of micellar nanocarriers in vitro as well as in tumor-bearing mice will be discussed.

A-10

A Nanobiological Route for the Isolation of Cancer Stem Cells. QIAN WANG. Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter Street, Columbia, SC 29208. Email: wang263@mailbox.sc.edu

Many solid tumor types, including breast and colon cancer, are found to contain small proportions of cells that are capable of proliferation, self-renewal, and differentiation into the various cell types seen in bulky tumors. These cells, termed "cancer stem cells" (CSCs), may be responsible for treatment resistance and tumor relapse. Therefore, isolating, characterizing and culturing CSCs are of critical importance for designing new therapeutic strategies that target this unique tumor initiating cell sub-population. Metabolic labeling of cells

with non-canonical amino acids and glycans combing with fluorogenic probe provides powerful tools to study spatial and temporal variation in protein profile of cells. In this talk, I will introduce our recent research progress in this endeavor. Our work will enable the differentiation between CSCs and non-CSCs in protein level in a spatial and temporal manner, and help to develop new method to characterize and isolate CSCs.

A-11

RNAi as New Target for Pest Control: Challenges with Insect Cell Cultures. GUY SMAGGHE, Kaat Cappelle, Benigna Van Eynde, and Olivier Christiaens. Department of Crop Protection, Ghent University, Ghent, BELGIUM. Email: guy.smagghe@ugent.be

Over the past decade, RNA interference (RNAi), the sequence-specific suppression of gene expression triggered by specific dsRNA molecules, has proven to be a new very promising strategy in crop protection. The main advantages of RNAi are its potential selectivity, even on species level, as well as the lack of persistency in and damage on the environment as a whole. In this paper, we report first on the promising results against some important pest insects such as the coleopteran *Diabrotica virgifera*. In second, a number of challenges will be discussed that need to be addressed to implement RNAi as a widely-used pest control strategy. One of these challenges is variable efficiency that is observed in many insects, especially major pest insect orders such as Lepidoptera, Orthoptera and Hemiptera. Possible causes for this variability in sensitivity are degradation of the dsRNA in the insect body and insufficient cellular uptake. In this paper we report on the challenges to investigate the uptake mechanisms for dsRNA into the target cells of the insect with use of primary and continuous insect cell lines and opportunities to increase uptake and persistence.

A-12

RNAi Technology to Understand the Virus-host Interface. RALPH TRIPP. University of Georgia, College of Veterinary Medicine, Department of Infectious Diseases, Athens, GA, 30602. Email: ratripp@uga.edu

Advances in RNA interference (RNAi) have facilitated the application of a systemic cell-based loss- or gain-of-function and cell response screening platform that enables genome-wide analysis of the host genes/factors required for virus replication or resistance to infection. Application of both experimetal and computational biology approaches have uncovered critical events that occur at the virus-host interface and the host genes that affect virus infection, replication and disease pathogenesis. A better understanding of the spatial and temporal host gene intereactions during viral infection has enabled insights into the mechanisms by which viruses co-opt host cell function and host regulatory mechanisms that influence disease treatment and outcome.

A-13

Engineered Microvesicles as Targeted Delivery Agents for Cancer. THOMAS D. SCHMITTGEN[1], Dhruvitkumar S. Sutaria[1], Jinmai Jiang[1], Ola A. Elgamal[1], Ana Clara Azevedo-Pouly[1], Ryan Pavlovicz[2],Chenglong Li[2], and Mitch A. Phelps[1]. [1]Division of Pharmaceutics & Pharmaceutical Chemistry and [2]Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus OH 43210. Email: Schmittgen.2@osu.edu

Oligonucleotide technology has emerged as an exciting and promising strategy for cancer therapy, however successful delivery is a major bottleneck in their clinical development. Microvesicles are a small subtype of membrane vesicles that have been reported to naturally contain nucleic acid contents including microRNA, protein and mRNA. We have developed a unique microvesicle drug delivery system to target the therapeutic miR-199a-3p in its modified precursor form to hepatocellular cancer cells and tumors. The most novel feature of this system is that the nucleic acid cargo is synthesized by the same cells that produce the microvesicles, thus abrogating the need for synthetic oligonucleotides. Targeted microvesicles were produced from HEK293T cells by overexpressing a lysosomal associated membrane protein Lamp-2A which was engineered to contain an HIV TAT peptide sequence on the C-terminal. The pre-miR-199a loop region was modified such that it resembled the HIV-1 transactivation response (TAR) RNA and was inserted within the intron of the Lamp2A gene. Correct splicing of the intron portion and processing of the mature miRNA was evaluated after transfection into HEK293T cells. The pre-miR loop was modified so that it binds to the TAT peptide which is expressed within the luminal side of the microvesicles. An in silico study was performed using computational modeling to ensure a stable interaction between the two. EMSA gel shift assay, in-vitro DICER assay and luciferase assay were performed to study the correct binding, processing and functionality of the modified pre-miR-199a-2 sequence. Western blotting confirmed correct expression of the fusion protein on ultracentrifuged purified microvesicles. Future work will be focused on in vitro and in vivo studies in cell lines and a mouse model to determine the efficacy of this novel delivery system.

A-14

The Language, Molecular Players, and Regulation of Epigenetics. JOSEPH M. WU, Tze-Chen Hsieh, and Barbara Doonan. Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, New York 10595. Email: Joseph_Wu@nymc.edu

Epigenetics investigates heritable changes in gene expression occurring without changes in DNA sequences. Several epigenetic mechanisms, notably, DNA methylation, and stable histone modification can change genome function. Epigenetics-relevant DNA methylation is a post-replicative covalent event, heritable by somatic cells after cell division, that involves the addition of a methyl group to the C5 position of cytosine in DNA, primarily CpG dinucleotide sequences, using DNA methyltransferases and S-adenosylmethionine (SAM) as the methyl donor. In general, DNA methylation modifications act in concert with covalent changes in the histones, primarily in the unstructured N-termini containing a high concentration of lysine and arginine residues that are often extensively modified by post-translational reactions, such as, methylation, acetylation, and ADP-ribosylation. Epigenetic mechanisms have been linked to regulation of chromatin remodeling, gene transcription, development, aging and to the development of various pathological conditions such as cancer, obesity and type 2 diabetes. Here, we summarize advances in our knowledge of the epigenetic phenomenon, focusing on the heritable maintenance of gene expression-restrictive states through regulated heterochromatization. We will present evidence on modification of epigenetic phenomena and the accompanying alteration in gene expression by bioactive food components such as genistein, tea catechins and resveratrol. We will review role of actin and actin-related proteins and silent information regulators, and control of their expression by aforementioned food components. We also propose implications of these findings for future research in disease prevention and management.

A-15

Epigenetic Predictors of Child Behavior: From Prenatal Exposures to Psychopathology. ALICIA K SMITH. Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA. Email: alicia.smith@emory.edu

Epigenetic patterns established during early development may guide the developmental trajectory of an individual across the lifespan. Our recent population-scale studies provide insight into how epigenetic modifications connect prenatal exposures to development of behavior problems during childhood. In a prospectively characterized cohort, prenatal exposure to antiepileptic drugs associates with lower average DNA methylation levels in neonates at birth and with more internalizing problems, anxiety, depression and sleep problems at 3-5 years of age. Similarly, DNA methylation of specific genes associates with both prenatal AED exposure and the development of childhood behavior problems. For example, methylation of *PDGFRB* (platelet-derived growth factor receptor-β) associates with AED exposure through pregnancy and with increased emotional and sleep problems in exposed children; mice that lack *PDGFRB* have deficits in cognitive functions, memory formation and socioemotional behaviors. Interestingly, this gene exhibits genotype-dependent DNA methylation patterns, which are more likely to be consistent across multiple tissues including blood and brain. These data suggest that AED-associated differences in DNA methylation may influence long-term development.

A-16

Genome-wide Alteration of 5-hydroxymethylcytosine in a Mouse Model of Fragile X-associated Tremor/Ataxia Syndrome. BING YAO[1], Li Lin[1], R. Craig Street[1], Zachary A. Zalewski[2], Jocelyn N. Galloway[2], Hao Wu[3], David L. Nelson[2], and Peng Jin[1]. [1]Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, 30322, [2]Department of Human and Molecular Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, and [3]Department of Biostatistics and Bioinformatics, Emory University Rollins School of Public Health, Atlanta, GA 30322. Email: bing.yao@emory.edu, peng.jin@emory.edu

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder in which patients carry premutation alleles of 55-200 CGG repeats in the FMR1 gene. To date, whether alterations in epigenetic regulation modulate FXTAS has gone unexplored. 5-hydroxymethylcytosine (5hmC) converted from 5-methylcytosine (5mC) by the ten-eleven translocation (TET) family of proteins, has been found recently to play key roles in neuronal functions. Here we undertook genome-wide profiling of cerebellar 5hmC in a FXTAS mouse model (rCGG mice) and found that rCGG mice at 16 weeks showed overall reduced 5hmC levels genome-wide compared to age-matched wild-type littermates. However, we also observed gain-of-5hmC regions in repetitive elements, as well as in cerebellum-specific enhancers, but not in general enhancers. Genomic annotation and motif prediction of wild-type- and rCGG-specific differential 5-hydroxymethylated regions (DhMRs) revealed their high correlation with genes and transcription factors that are important in neuronal developmental and functional pathways. DhMR-associated genes partially overlapped with genes that were differentially associated with ribosomes in CGG mice identified by bacTRAP ribosomal profiling. Taken together, our

data strongly indicate a functional role for 5hmC-mediated epigenetic modulation in the etiology of FXTAS, possibly through the regulation of transcription.

A-17

Conquering Chaos in the Age of Networked Science: The Importance of Data Management. KATHRYN M. HOUK. Tufts University Hirsh Health Sciences Library, 145 Harrison Ave. Boston, MA 02111. Email: katie.houk@tufts.edu

Do you know where all of your data is stored and if it's up-to-date? Would your neighbor across the hall be able to find and interpret your latest experimental results on your computer? Would you pass safely through an NIH audit of your research? If any of these questions made you nervous, learning more about proper data management would be a good use of your time! Attend this highly interactive, 2-hour session to network and engage with your colleagues over the struggle to maintain your research data. Using a real lab's research project as an example we'll cover the importance of good data management practices, seven common issues in data management, and resources to assist you along the way. By the end of this fun session you will have tools to help your lab better manage your data and feel more comfortable creating the 2-page data management plans required by NSF and NIH grants.