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Animal Symposia and Workshops

A-1

Single Cell Transcriptomics: Unraveling Heterogeneity in Environmental Health and Cancer. JUSTIN A. COLACINO. University of Michigan School of Public Health. Department of Environmental Health Sciences, Department of Nutritional Sciences, Ann Arbor, MI. Email: colacino@umich.edu

Single cell analytics are poised to revolutionize our understanding of biology. Through unbiased single cell profiling of tissues, we are uncovering previously undiscovered rare cell populations, expanding our knowledge of cellular hierarchies, and redefining our understanding of the plasticity of cellular states. Accompanying this great promise, however, are a number of technical and analytic challenges in high throughput analysis of single cells. In this talk, I will outline the state of the science in single cell transcriptomics. We have recently established the Michigan Center for Single-Cell Genomic Data Analytics. This interdisciplinary group unites researchers from multiple units across the University of Michigan with the unified goal of developed novel analytic approaches for single cell 'omics data in the areas of RNA splice isoforms, reproductive biology, cancer therapeutics, and environmental health sciences. The lecture will highlight ongoing research efforts that apply single cell transcriptomics to understand the mechanisms that drive the influence of environmental exposures during developmental windows of susceptibility on the development of later life disease.

A-2

Treating Cells as Reagents to Achieve Assay Reproducibility. TERRY RISS. Promega Corporation, Cell Health, Madison, WI. Email: terry.riss@promega.com

The reported poor rate of success at being able to reproduce published data has raised many questions in the scientific community and government funding agencies. Improving the reproducibility of cell-based assays is achievable by treating cells as "reagents" and performing routine measurement of quality control parameters such as detecting contamination, confirming cell line authentication, and using standard operating procedures for handling stock cultures. Assay reproducibility also can be improved for many cell-based assays by normalizing to the number of live, dead, or total cell number at the end of the experiment. The presentation will provide an overview of approaches for treating cells as reagents and describe the advantages of multiplex measurement of cell health parameters to normalize data and improve assay reproducibility.

A-3

In Vitro Methods for Measuring Cell Health in Real-Time. TERRY RISS. Promega Corporation, Cell Health, Madison, WI. Email: terry.riss@promega.com

Most methods for measuring live, dead, or apoptotic cells have been developed as endpoint assays that lyse the cell membrane and kill cells to be able to record the desired marker. The harsh reagent ingredients in endpoint assays often leave few options for further sample processing. Recent advances in assay development have provided methods to measure viable cells, dead cells and apoptotic cells using non-lytic assay reagents that do not destroy the cells and provide an opportunity to record data from the same sample for days. This presentation will describe assay chemistries that enable repeated measurement of cell health parameters in real-time using a multimode plate reader and provide examples of multiplexing assays to collect more data or serve as an internal control using an orthogonal assay method.

A-4

Modeling Human Lung Fibroblast Responses in Asthma and Airway Disease. J. L. INGRAM, Department of Medicine, Duke University Medical Center, Durham, NC. Email: jennifer.ingram@duke.edu

Environmental and occupational exposure to allergens and air pollution particulates increases the incidence of

asthma and obstructive airway diseases. Sub-epithelial fibrosis is a component of the airway remodeling that occurs during the pathobiology of these diseases, and airway fibroblasts play an important role in injury-induced airway remodeling. Both airway and lung parenchymal fibroblasts can be cultured from airway biopsy tissue or from parenchymal lung tissue obtained from donor lungs. These primary airway and lung fibroblasts, in submerged and adherent cultures, expand readily with appropriate growth media and incubation conditions. Furthermore, the cells may be manipulated to differentiate from a proliferative state to a synthetic myofibroblast state that is capable of secreting extracellular matrix, expressing alpha-smooth muscle actin, and invading a simulated basement membrane. Functional studies of airway and lung fibroblasts in culture, in response to allergen and other agents that induce injury, include investigations of cell proliferation, differentiation, migration, invasion, extracellular matrix production, growth factor and protease secretion, and gene or protein expression profiling. Airway and lung fibroblasts obtained from healthy or diseased human donors maintain their phenotypes in culture, and cellular processes may be significantly correlated with clinical features and parameters of respiratory function to offer insights into the pathobiology of obstructive airway diseases.

A-5

Expanding the *Xenopus laevis* Invitrome: Establishing and Characterizing Cell Lines For Use in Examining Host-Pathogen-Environment Interactions. B. KATZENBACK. University of Waterloo, Department of Biology, 200 University Ave West, Waterloo, ON, CANADA. Email: barb.katzenback@uwaterloo.ca

More than one third of >7000 amphibian species are threatened globally (AmphibiaWeb. 2017). The etiology is complex and suspected proximal causes include climate change, environmental contaminants and emerging pathogens. Amphibian mortality is often linked to fungal and viral infections and suggests an environment that undermines the ability of the amphibian immune system to defend against infection. Our ability to dissect the impact of singular or cumulative effects on a cellular level in amphibians is limited by the availability of suitable *in vitro* cellular systems to study host-pathogen-environment associations. Recent research in my laboratory has focused on establishing and characterizing cell lines from a variety of tissues from the African clawed frog (*Xenopus laevis*). As the skin epithelial barrier is at the interface between an organism's external and internal environments and represents an important physical and cellular barrier in defense against pathogens, our efforts have concentrated on the development of a *X. laevis* dorsal skin epithelial cell line (Xela DS2) and a ventral skin epithelial cell line (Xela VS2). We have successfully determined growth requirements and cryopreservation conditions for Xela DS2 and Xela VS2, which have achieved >130 passages. In addition, we have begun to characterize Xela DS2 and Xela VS2 susceptibility and transcriptional response to Frog Virus 3 (FV3), an emerging pathogen contributing to amphibian declines. Collectively, these novel cell lines will aid in the expansion of the *X. laevis* invitrome, a newly coined term to describe all cell lines derived from a defined organisms or tissue (Bols *et al*. In Vitro Cell Dev Bio Anim. In Press), and enhance our understanding of frog cellular responses to pathogens and environmental factors.

A-6

Insights from Cell Cultures on the Mode of Action of Insecticidal Proteins from *Bacillus thuringiensis*. JUAN LUIS JURAT-FUENTES[1,2], H. Abdelgaffar[1], J. Jackson[2], A. Castagnola[1], C. Oppert[3], and J. Monserrate[3]. [1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996; [2]Genome Science and Technology Graduate Program, University of Tennessee, Knoxville, TN 37996; and [3]Bayer CropScience, Morrisville, NC 27560. Email: jurat@utk.edu

Midgut cells in lepidopteran larvae are the biological targets of insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt). Consequently, primary midgut cell cultures from lepidopteran larvae have been used as models to determine the host response to intoxication with Bt toxins. However, due to short viability of these primary cultures, continuous insect cell cultures are also used to investigate the Bt toxin mode of action. In this presentation we will present data from using primary midgut cell cultures to identify the cross-talk involved in midgut defense to Bt intoxication and from research aimed at identifying the mechanism of cell killing by Bt toxins. Advantages and disadvantages of each system for the study of insecticidal proteins will be discussed.

A-7

Establishing Cell Lines from Pest Insects: Studies of Prostaglandin Actions. DAVID STANLEY[1], Yao-fa Li[1,2], Hongwei Zhang[3], Cynthia L. Goodman[1], Tamra R. Lincoln[1], Kaile Zhou[3], and Joseph A. Ringbauer Jr.[1]. [1]Biological Control of Insects Research Laboratory, USDA/Agricultural Research Service, 1503 S. Providence Road, Columbia, MO 65203; [2]Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Dongguan Street 437b, Baoding 071000, P. R. CHINA; and

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Insects produce prostaglandins (PGs) that act in specific areas of physiology, including reproduction (e.g., follicle development), ion transport (e.g., primary urine formation in Malpighian tubules) and immunity (e.g., phagocytosis). We used established cell lines to investigate additional PG actions, particularly the influence of PGs on protein expression and cell viability. In the last two years we established new, second generation insect cell lines. By second generation, we mean cell lines from specific tissues that maintain at least some aspects of their in vivo physiological functions. We used the fall armyworm, *Spodoptera frugiperda* and the closely related *S. eridania*, to establish cell lines from testes, nervous system and fat body. Two fat body cell lines have been passaged 24 times and two others ~10 times. Two testes lines have been passaged over 20 times and one nervous system line is in passage 15. We also established a new cell line from squash bugs, *Anasa tristis*, a hemipteran (Goodman *et al.*, In Vitro Cell Dev Biol-Animal, *in press*). We are using this line, and lines from other orders, to investigate the influence of PGs and pharmaceutical inhibitors of PG biosynthesis on cell viability and protein expression. So far we learned that the squash bug cell line is very sensitive to A-series PGs (IC_{50}s ~20 mM) and far less sensitive to D-, E- and F-series PGs, such that IC_{50} values cannot be calculated; there was minimal impact on cell viability after exposure to these PGs at 200 mM. We hypothesize that the differences in sensitivity are due to the structure and modes of action of the A-series PGs. The meaning of this work lies in using established cell lines to gain new knowledge about the biological significance of PGs in insects.

A-8

Databases and Analysis Tools Supporting Toxicology in the 21st Century. N. S. SIPES. National Toxicology Program Division, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. Email: nisha.sipes@nih.gov

Publicly available data and in vitro and computational methods/models are required to move toxicity testing from individual in vivo mammalian screens to be able to predict the ability of a chemical to affect biological pathways or lead to a toxic outcome. The Toxicology in the 21st Century (Tox21) federal collaboration aims to develop these databases and models through involvement of the National Institutes of Health (including the National Toxicology Program at the National Institute of Environmental Health Sciences and the National Center for Advancing Translational Sciences), the U.S. Environmental Protection Agency, and the Food and Drug Administration. Within the collaboration, thousands of chemicals (including pharmaceuticals, environmental, consumer- and industrial-use) have been screened in tens to hundreds of in vitro high-throughput screening assays (e.g., cell stress, cytotoxicity, mitochondrial, nuclear receptors) and are publicly available. Further, computational models have been developed using these data to predict potential toxicity. This talk will describe the data, tools to access and analyze the data, and provide a modeling example on how to interpret in vitro assay readouts toward estimating the likelihood of an in vivo interaction for prioritizing potential human health risk.

A-9

The Application of Global Metabolomics to Plant Sciences. DANNY ALEXANDER. Metabolon, Inc. 617 Davis Dr., Morrisville, NC 27560. Email: dalexander@metabolon.com

Metabolomic analysis provides a means to simultaneously evaluate changes in all the major biochemical pathways in plant tissues, yielding knowledge about the metabolic effects of genetic differences (natural variation, mutations), plant development, plant nutrition, biotic and abiotic stresses, agricultural chemicals (*e.g.* mode of action), and genetic engineering, among others. In recent decades the great expansion of information brought about by genome sequencing and expression analysis provide important, but incomplete representations of the molecular events underlying plant growth and development. Metabolomics can serve to fill a critical void in the long range goal of developing a comprehensive systems approach to plant biology.

A-11

Development of a Tumor-on-a-Chip for Hypoxia Studies. G. WALKER, S. Beach, and M. Gamcsik. UNC/NCSU Joint Department of Biomedical Engineering, North Carolina State University, 4206C Engineering Building III, 911 Oval Drive, Raleigh, NC 27695–7115. Email: gmwalker@ncsu.edu

Hypoxia is a hallmark of most tumors, yet its role in the metastatic process remains unclear. *In vivo*, tumors possess a dynamic spatiotemporal distribution of oxygen that stands in stark contrast to the static, constant-hypoxia chambers that are commonly used *in vitro*. Furthermore, metastatic potential is commonly assayed by invasion assays (e.g., Boyden chambers), but adding a dynamic hypoxia environment to these assays is impractical. Microfluidic devices provide a way to combine the functionality of a Boyden chamber with precise

oxygen control that mimics *in vivo* conditions. We have developed devices in our lab to demonstrate one possible approach. Finite element models of oxygen transport were used to guide the design of multi-layer microfluidic devices. Initial results within polydimethylsiloxane (PDMS) devices showed the effects of hypoxia on cell culture. Because of the difficulty of working with PDMS, a new approach to fabricating microdevices using xurography and thin sheets of polystyrene has been adopted. Initial results with these devices show a promising path forward for creating a tumor-on-a-chip.

A-12

Liquid Metals for Microfluidics. MICHAEL D. DICKEY. North Carolina State University, Department of Chemical and Biomolecular Engineering, Raleigh, NC 27695. Email: mddickey@ncsu.edu

This talk will describe efforts in our research group to use liquid metals as components in microfluidic systems. Liquid metals based on gallium offer low toxicity alternatives to mercury. These liquids have low viscosity and can therefore be injected into microfluidic channels. Importantly, these metals form thin oxides on their surfaces that stabilize their shape inside microchannels. Injecting the metal into microchannels can be used to make components such as electrodes, valves, and pumps in a facile manner. It also allows liquid metals to be patterned into useful structures such as ultra-stretchable wires, sensors, antennas, and microelectrodes. The metal can also be patterned by direct-write 3D printing. It can be embedded in elastomer and used as a sacrificial material to create microfluidic channels. Within the context of biology, it is possible to use liquid metal to make direct electrical contact to cells and neurons within microchannels.

A-13

Programming Bacteria in Time and Space. LINGCHONG YOU. Duke University, Dept of Biomedical Engineering, 2355 CIEMAS, 101 Science Drive, Durham, NC 27708. Email: you@duke.edu

Microbes are by far the most dominant forms of life on earth. In every imaginable habitat, they form complex communities that carry out diverse functions. Microbial communities drive the geochemical cycling of diverse chemicals and through these activities shape the earth's climate and environment. They are also intimately tied to human physiology and health. Members of each microbial community may compete for resources, collaborate to process the resources or to cope with stress. They communicate with each other by producing and responding to signaling molecules. And they innovate by exchanging genetic materials. These interactions raise fundamental questions regarding the evolutionary and ecological forces that shape microbial consortia. Our lab has adopted a combination of quantitative biology and synthetic biology to explore these questions. We engineer gene circuits to program dynamics of one or more *Escherichia coli* bacterial populations and use them to examine questions in cellular signal processing, evolution, ecology, and development. Analysis of these systems has provided insights into bacterial tolerance to antibiotics, developmental pattern formation and scaling, as well as strategies to use bacteria to fabricate functional materials.

A-15

Fluor-tethered Inhibitors of Hsp90 Reveal Secretion and Reinternalization of Hsp90 Is Associated with Metastatic Progression. TIMOTHY A. J. HAYSTEAD. Department of Pharmacology and Cancer Biology, Duke University, Durham NC 27701. Email: timothy.haystead@dm.duke.edu

Heat shock protein 90 (Hsp90) is an essential molecular chaperone that has been in clinical development as a therapeutic target for almost 3 decades. Cancer cells induce Hsp90 and malignant tumors promote expression of a unique form of the protein on their cell surface, eHsp90. The ectopically expressed form is thought to facilitate migration of malignant tumor cells from the primary tumor site. Because of these oncogenic associations, 17 different Hsp90 inhibitors have been tested in Phase 1–3 clinical trials for multiple indications in cancer. Although classical Hsp90 inhibitors have proven to have therapeutic efficacy in the majority of cancers, especially in combination with other cutting edge therapies (e.g. Herceptin), they are not curative. In a typical Hsp90 inhibitor clinical trial, the majority of responsive patients go into stable disease i.e. their tumors stop growing as long as drug surveillance is maintained. The reason for this is mostly related to two factors, the abundance of cellular Hsp90 (1–2% of expressed protein in all cells) and its ubiquitous chaperone functions (protein folding, stabilization and transport) in all cells. Many of the proteins chaperoned by Hsp90 are involved in cell growth and proliferation, such as protein kinases and transcription factors, and are generally highly redundant i.e. inhibit one and another can take its place. Tethered inhibitors of Hsp90 are specifically designed to target the ectopically expressed form of Hsp90, eHsp90. This unique form of Hsp90 has been largely ignored by the oncology community and no one other than our group has developed a strategy that can selectively target this protein *in vivo*. What is exciting about eHsp90 is that it is only expressed on the surface of malignant tumor cells and not normal cells. Moreover, the Haystead lab at Duke University has shown that when

eHsp90 is bound to a tethered Hsp90 inhibitor, the protein aggregates (forming e90 bodies) and becomes rapidly reinternalized with the bound drug. This phenomenon can be observed readily in intact tumor cells in culture using fluorophor-tethered Hsp90 inhibitors using high resolution confocal microscopy. More importantly, when injected into animals bearing human tumors, the tethered inhibitors can be visualized by fluorescence imaging or by PET. These studies conclusively demonstrated that tethered Hsp90 inhibitors are only recognized by aggressive tumor cells and not any other cell within the body.

A-16

Development of Exosome-encapsulated Paclitaxel to Treat Cancer Metastases. E. V. BATRAKOVA[1,2], M. S. Kim[1,2], M. J. Haney[1,2], Y. Zhao[1,2], I. Deygen[3], N. L. Klyachko[1,2,3], and A. V. Kabanov[1,2]. [1]Center for Nanotechnology in Drug Delivery; [2]Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, 125 Mason Farm Rd., Chapel Hill, NC 27599; and [3]Deparment of Chemical Enzymology, Faculty of Chemistry, M.V. Lomonosov Moscow State University, Leninskie Gory, 119992, Moscow, RUSSIA. Email: batrakov@email.unc.edu

Exosomes have recently come into focus as "natural nanoparticles" for use as drug delivery vehicles because they lack many drawbacks inherent to other nanoformulations. Many potentially useful chemotherapeutics possess undesirable attributes such as low solubility in aqueous solutions, immunogenicity, or inefficient accumulation in target cancer cells due to multidrug resistance (MDR) mechanisms. Our objective was to assess the feasibility of an exosome-based drug delivery platform for a potent chemotherapeutic agent, paclitaxel (PTX), to treat MDR cancer. Herein, we developed and compared different methods of loading exosomes released by macrophages with PTX (exoPTX) and characterized their size, stability, drug release, and *in vitro* antitumor efficacy. A reformation of exosomes upon sonication resulted in high loading efficiency, and sustained drug release. Importantly, incorporation of PTX into exosomes increased cytotoxicity more than 50 times in drug resistant $MDCK_{MDR1}$ (Pgp+) cells. Furthermore, exoPTX demonstrated significantly greater cytotoxicity against all cell lines tested, as compared to Taxol and PTX. The biodistribution and antitumor effects of exoPTX were further evaluated in a model of murine Lewis Lung Carcinoma pulmonary metastases. Our studies demonstrated nearly complete co-localization of airway-delivered exosomes with cancer cells, and a potent anticancer effect in this mouse model. Overall, exoPTX holds a significant potential for the delivery of various chemotherapeutics to treat drug resistant cancers.

A-17

The Potential of Ocular Exosomal Biomarkers as Therapeutic Targets, and as Diagnostic and Prognostic Indicators. M. KLINGEBORN[1], C. Bowes Rickman[1,2], and W. D. Stamer[1,3]. Departments of [1]Ophthalmology, [2]Cell Biology, and [3]Biomedical Engineering, Duke University, Durham, NC 27710. Email: mikael.klingeborn@duke.edu

Interest in utilizing 30–150 nanometer sized exosomes and other extracellular vesicles (EVs) as biomarkers of disease has increased exponentially in recent years. EVs (including exosomes) have several unique features that define ideal biomarkers: (i) a lipid bilayer provides protection for their RNA, DNA, and proteins cargo; (ii) they contain tissue-, cell-, or disease-specific proteins and nucleic acids; and (iii) their hardiness enables a wide range of methods for isolation and enrichment from a range of body fluids (e.g. plasma, serum, urine, aqueous humor, tears and vitreous). To identify biomarkers for retinal disease, we defined the proteome of exosomes from the retinal pigmented epithelium (RPE), which forms the outer blood-retinal barrier in the eye. The RPE is a highly polarized barrier, leading to the directional secretion of proteins, lipoprotein particles and EVs. Such a division dictates directed interactions between RPE and the systemic circulation (basolateral side) and the retina (apical side). As a model, we used primary cultures of differentiated porcine RPE monolayers on permeable supports. EVs were isolated from conditioned medium bathing either apical or basolateral RPE surfaces, from which exosomes were purified and processed for proteomic profiling. In parallel, EV size distribution and concentration were determined. Using protein correlation profiling mass spectrometry, a total of 634 proteins were identified in exosome preparations, 302 of which were uniquely released apically, and 94 uniquely released from the basolateral side. Basolaterally released exosomes and EVs from RPE cells theoretically enter the systemic circulation and thus basolateral-RPE specific exosomal proteins that we identified, such as Bestrophin-1, represent targets for immunoisolation of RPE-derived exosomes from blood. These data serve as a foundation for comparative studies aimed at elucidating the molecular pathophysiology of retinal diseases and to help identify potential therapeutic targets and systemic biomarkers for such diseases.

A-18

Integrative Approaches to Defining the State and Architecture of the Breast Cancer Kinome. SHAWN M. GOMEZ[1,2,3]. [1]Joint Department of Biomedical Engineering, UNC Chapel Hill and NC State University, Chapel Hill, NC 27599;

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Understanding complex cellular diseases such as cancer requires a multifaceted picture of cellular function and dysfunction. While continued technological developments have enhanced this understanding through the detailed characterization of multiple "omes", integrating and interpreting these data still poses significant challenges. Here, we describe recent work focused on characterizing the global behavior of the network of kinases, the kinome, within breast cancer. Utilizing proteomic, expression, interaction and drug perturbation data, we provide a broader view of the architecture of the kinome as well as its behavior in response to targeted perturbations. We further identify regions of the kinome that are poorly characterized and/or poorly targeted by existing therapies. This systems view of the kinome presents potential opportunities for disease classification, identification of potential drug targets and the broader design of combination therapies.