2014 Meeting of the Society for In Vitro Biology

May 31 - June 4, Savannah, Georgia

The following abstracts will be included in an upcoming issue of *In Vitro Cellular and Developmental Biology*:

A-3000

Incorporation of Biophysical and Biochemical Cues Improve Transfection Rates in DNA Releasing Materials. S. C. VEEN[1], D. Eastlack[2], R. Garcia[2], P. Nealey, and J. Gasiorowski[1]. [1]Midwestern University, Dept of Biomedical Sciences, Biomedical Sciences, 555 31st Street, SH 203-C, Downers Grove, IL 60148 and [2]University of Chicago, Institute for Molecular Engineering, 5801 S. Ellis Ave, Chicago, IL 60637. Email: sveen63@midwestern.edu

Our goal is to develop implantable biomaterials that can support cell growth and release non-viral DNA upon exposure to an electrical trigger. These materials can be valuable tools for gene therapy and tissue engineering. We have functionalized hydrogels with short, positively charged peptides that bind non-viral plasmids and protect the DNA from nucleases. Cells within the hydrogels are transfected only after electrical pulses release the plasmids from the scaffolds. Although these biomaterials show promise in vitro, transfection efficiencies in vivo could be challenging due to a minimal amount of cells that may infiltrate and populate the implantable hydrogel. Therefore, we first created a medium-throughput array method to test a range of cell adhesive peptide concentrations (using the RGD cell adhesion ligand). Mesenchymal and epithelial cells were able to grow on hydrogel surfaces functionalized with as low as 0.4 mM RGD. In addition to cell adhesion peptides improving the adherence of cells to the material, biophysical cues such as topography can be used to potentially enhance gene delivery. Gene array studies have shown that cells on topographically patterned substrates have a significantly different expression profile than cells seeded onto flat control surfaces. Thus, we investigated the potential of topographic information influencing transgene expression. Utilizing luciferase and GFP reporters, we transfected mouse and human mesenchymal cell lines and plated them on surfaces with 200 nm, 700 nm, or 2000 nm grooves or a control flat surface. We compared their relative transfection efficiencies with chemi-luminescence and fluorescence microscopy. In our models, sub-micron grooves increased transgene expression. However, the effect was minimal on features greater than one micron. Through the use of these in vitro models, we hope to incorporate the most efficient biochemical and biophysical cues into our DNA trigger release hydrogels, ultimately improving the effectiveness of these biomaterials in gene therapy and tissue engineering.

A-3001

Collagen Synthesis by a Novel Fibroblastic Cell Line Derived from Caudal Fin of Adult Yellow Perch (*Perca Flavescens*) and Its Use as an Indicator of Chemical Exposure. L. LEE[1], K. Spiteri[2], N. Vo[3], J. Alexander[1], and F. Rojas[1]. [1]University of the Fraser Valley, 33844 King Rd, Abbotsford, BC V2S 7M8, CANADA; [2]Wilfrid Laurier University, Waterloo, ON, CANADA; and [3]University of Waterloo, Waterloo, ON, CANADA. Email: lucy.lee@ufv.ca

Chronic toxicity studies of contaminant effects with fish are expensive and time consuming, and alternative methodologies are being sought. Collagen is a major structural protein, whose synthesis and deposition is regulated by diet, and has been shown to be affected by contaminant exposure. Fish deformities and growth reduction are parameters evaluated in chronic tests, and collagen plays a pivotal role in both structural integrity and growth, thus an in vitro assay evaluating contaminant effects on fish collagen synthesis, rapidly and inexpensively, could reduce the need for whole fish testing. A novel Yellow perch (*Perca flavescens*) cell line, YPF5, was used for toxicological evaluation of chemicals on collagen formation. YPF5 is a fibroblastic cell line derived from the caudal fin of an adult feral perch specimen, and the cells have been maintained for 3 yrs at room temperature. Cells at various passages have been cryopreserved and thawed successfully, and were authenticated as *P. flavescens* by "DNA barcoding" and conventional karyotyping (2n = 48). Immunofluorescence staining with mesodermal cell markers (vimentin and collagen type 1) confirmed the fibroblastic origin of the cells. YPF5 forms distinct collagen bundles in response to Vitamin C (ascorbic acid) treatment in agreement with related literature on in vivo and in vitro collagen production by fibroblastic cells. Exposure to cortisol changed the morphology of the cells to a more epithelioid shape, and co-exposure to ascorbic acid reduced collagen formation. Collagen assembly was also affected by exposure to naphthenic acids and copper sulphate. Within limits, YPF5 collagen synthesis could be used as a sensitive indicator and predictor of fish growth and could be an excellent supplement to chronic toxicity assays.

A-3002

Characterization A-549 Lung Adenocarcinoma Using *In Vitro* Metastatic Models. M. J. ROSSI and S. Kolli. University of New Haven, 300 Boston Post Rd, West Haven, CT 06516. Email: mrossi@newhaven.edu

Background: Previous work from our lab has shown that using 3D gelatin hydrogels as a substitute extracellular matrix induces an epithelial to mesenchymal-like transition by A-549 cells. It is important to fully characterize the cells from this model system as well as develop models for other stages of the metastatic process. Events to model include dissociation of cells from the primary epithelium, migration into the surrounding tissue, transit through the circulatory system and colonization of a secondary

site. The aim of this study was to further characterize the phenotype of cells cultured in a 3D hydrogel as well as develop additional models for metastasis. Materials and methods: A-549 cells were grown as monolayers for 2D cultures on tissue culture plastic surfaces, 2D cultures on fibronectin-coated plates, in suspension and in gelatin hydrogel for 3D cultures. Morphological changes were assessed by bright field microscopy and staining with FITC-labeled Phalloidin. Gene transcription data was determined for E- and N-cadherin, integrin subunits α_v, β_3 and β_6, fibronectin, ILK, FoxC2, and Twist-1 by qPCR. Scratch wound assays were performed in 24-well plates. Expression data was determined by Western blot. Results: Cells grown as monolayers with or without fibronectin were predominantly epithelial in morphology. There wasn't a significant difference in their wound healing capacity. Viable cells grown either in hydrogels or in suspension had significantly reduced mRNA per cell and the relative amounts of most of the genes examined changed significantly. The comparison of the suspension qPCR data to the hydrogel data also revealed significant differences Expression of proteins also varied between the various systems. Conclusions: We have developed three distinct models of steps in the progress of metastasis, each with unique phenotypic profiles. Studying these models may allow for a better understanding of the susceptibility of chemotherapeutic treatments over the course of cancer progression.

A-3003

A Novel Method for Drug Screening Using Human Mesenchymal Stem Cell (hMSC) Spheroids. B. XU[1], S. A. Samy[2], J. Alley[2], L. Fitzgerald[2], M. L. Shuler[1], and M. Ariza-Nieto[1]. [1]Cornell University, 223 Thuston Ave. 305, Ithaca, NY 14850 and [2]Guthrie Clinic, Ltd, Guthrie Weight Loss Center, One Guthrie Square, Sayre, PA 18840. Email: bx53@ cornell.edu

Here, we introduce a novel drug screening method using human mesenchymal stem cell (hMSC) spheroids (Ylöstalo et al, 2012) for personalized medicine and/or to predict long term secondary effects due to genetic predispositions. Isolation, expansion, and differentiation of hMSCs have been well documented; however *in-vitro* cell signaling and target metabolic interactions are still to be elucidated. The 3D characteristics of spheroids allow the formation of microenvironments required for advanced and complex cell functions that are not present in 2D cultures. MicroRNAs are small molecules known for their signaling regulatory roles in eukaryotic gene expression. This group previously presented evidence of miR-22-3p deregulated in patients with aortic stenosis (Samy SA., et al 2013). The goal is to develop cells capable of expressing miR22 and its secretion into their surrounding media. This exploratory research will use statins (Atorvastatin, Simvastatin, Rosuvastatin) and ACE inhibitors (Lisinopril, Ramipril, and Trandolapril). The specific aim is to observe whether these drugs are capable of changing the expression profiles of miR22. Cells previously isolated and cryopreserved were placed in MEMα (Gibco cat lot 1376676) supplemented with 20% calf bovine serum (ATCC 30-2030), and 100 IU penicillin with 100 µg/mL spreptomycin (ATCC 30-2300). Post-detachment with Trypsin/EDTA 0.05% (Gibco cat lot 25300-054), Passage 1 was seeded to a density of 0.05 $x10^6$ cells/200 mm^2. Spheroids were allowed to grow for 20 days prior to drug assay at standards growing conditions 37°C and 5%CO_2. Higher levels of miR22 transcripts were found in hMSC spheroids compared to cells growing in monolayer in cell line C001-EDBC. These *in-vivo* and *in-vitro* studies comply with IRB approved clinical trial GHS # 1207-27. This research is funded by NIH/NCI supplemental grant and PS-OC (CMM) at Cornell University and a physician's grant from the Guthrie Foundation Investigator-Initiated Research Grant. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

A-3004

Gene Expression Profiles of Adiponectin and CD14 In Vivo and In Vitro. Y. LI[1], J. Alley[2], S. Samy[2], L. Fitzgerald[2], M. Shuler[1], and M. Ariza-Nieto[1]. [1]Cornell University, Ithaca, NY 14853 and [2]Guthrie Clinic, Ltd, One Guthrie Square, Sayre, PA 18840. Email: yl2373@cornell.edu

Rising rates of obesity in the US have many implications, among them a risk of certain cancers (Eheman C et al 2012). Widely published evidence has shown that individuals with excess weight (overweight and obese) are at higher risk for cancer and metabolic disorders. Individuals with above normal body mass index (BMI) demonstrate low levels of adiponectin (hypoadiponectinemia). Similarly, these individuals generally have other comorbid metabolic disorders including insulin resistance, metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D) and cardiovascular disease (CVD). CD14 is a cell surface antigen that was recently presented as an important biomarker associated with dysregulated adipose pads and lipid panel parameters in microvesicles/exosomes, which are known for their importance in cell signaling with carriage of proteins and microRNAs (Kranendonk MEG et al 2014). The aim of this study is to observe the relationship between adiponectin and CD14 both *in vivo* and *in vitro*. This study is compliant with ethics review, as an IRB approved clinical trial (GHS # 1207-27). Subjects enrolled were patients who underwent gastric bypass surgery for weight loss. All donors (n=10) signed informed consent. Liver, omental, adipose tissue and mononuclear cells were collected as part of the surgery and assayed for adiponectin, CD14 and GAPDH (reference) transcript abundance. Strict MIQE guidelines for specific and non-specific amplification were followed. Mesenchymal stem cells isolated from the same donors are being used in an attempt to study cell signaling

interactions using both adiponectin and CD14 as biosensors. The discovery of biomarkers that target genetic, epigenetic and phenotypic profiles are in great demand for personalized and regenerative medicine. One potential goal could be the prevention of obesity, especially for children. The content is solely the responsibility of the authors and does n ot necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

A-3005

Preclinical Evaluation of Aurora Kinase Inhibitors in SW-872 Human Liposarcoma Cells. O. ZAROU, L. Alt, N. Chandar, S. Noronha, and M. Fay. Midwestern University, Biomedical Sciences/SH203, 555 31st Street, Downers Grove, IL 60515. Email: ozarou22@midwestern.edu

Aurora kinase inhibitors are being evaluated as potential chemotherapeutic agents; however, their effectiveness against human liposarcoma has not been investigated. Previously we demonstrated differential expression of aurora kinases A and B in SW-872 human liposarcoma cells versus differentiated adipocytes. The purpose of this research was to compare the effects of an aurora kinase A inhibitor (MK-5108), an aurora kinase B inhibitor (AZD-1152), and a pan-aurora kinase inhibitor (AMG-900) on cellular growth and the ability to induce an increase in the percentage of multinucleated cells in SW-872 human liposarcoma cells. The cells were treated with the inhibitors (0 – 1,000 nM) for 24, 48, and 72 hours and the total number of viable cells was determined using trypan blue and automated cell counting. At 72 hours of treatment, the IC50 values were 365.7 nM for MK-5108, 54.0 nM for AZD-1152, and 4.9 nM for AMG-900. To evaluate the drug-induced increase in the percentage of multinucleated cells, we treated the SW-872 cells with the inhibitors (0 - 1,000 nM) for 72 hours, and the percentage of cells with DNA content >4N was determined with propidium iodide staining and flow cytometry. AMG-900 caused a statistically significant increase in the percentage of multinucleated cells starting at 25 nM, while AZD-1152 significantly increased the percentage of multinucleated cells starting at 100nm, and MK-5108 was only effective at the 1,000 nM dose. These results indicate that aurora kinases may be a viable chemotherapeutic target for human liposarcoma, and that a pan-inhibitor may be the most effective therapeutic strategy.

A-3006

The Biological Role of NPM1 in Neuroblastoma Cell Lines. N. RE[1], J. Kwak[1], S. Volchenboum[2], and K. Kristjansdottir[2]. [1]Midwestern University, 555 31st Street, SH 403, Downers Grove, IL 60515 and [2]The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL. Email: nre85@ midwestern.edu

Neuroblastoma remains a challenging pediatric cancer to treat due to the heterogeneous nature of the disease. The MYCN transcription factor is the most utilized biomarker for prognosis of neuroblastoma, with high-levels of MYCN correlating with high risk disease. Patients with high risk disease have poor prognosis, with the 5-year survival rate of only 40-50%. Although it is used as a prognostic indicator, MYCN has not been successfully used as a therapeutic target. Thus targeted therapies for high risk neuroblastoma are needed. Increased NPM1 (nucleophosmin) protein levels have recently been linked to high levels of MYCN in neuroblastoma cell lines. These high levels of NPM1 may play a role in increasing aggressiveness of the cancer cells. NPM1 has been shown to be important in a number of cancers including ovarian and gastric cancers and inhibitors of NPM1 have been shown to modulate the susceptibility of these cancer cells to apoptosis. However, the role of NPM1 in neuroblastoma has not been studied. Here we examine the role of NPM1 in neuroblastoma by manipulating NPM1 protein levels using siRNA and/or overexpression in cell lines modeling low-risk and high-risk disease. Proliferation was examined using WST-1 assays and cell counts. NPM1 levels were manipulated using overexpression of exogenous protein and siRNA of endogenous protein. The decrease in NPM1 levels may result in a less aggressive phenotype. We are also examining the effect of NPM1 protein levels on neuroblastoma cell migration and invasion. Together these results may provide insight into the relationship between NPM1, MYCN, and high risk disease and may identify NPM1 as a novel therapeutic target for neuroblastoma patients.

Disclosure: This work was supported by Start-up funds from College of Health Sciences at Midwestern University.

A-3007

Investigating the Consequences of Pharmacological Inhibition of CCR5 on Monocytes and Plasma Cells. J. BAGINSKI[1] and A. Gilchrist[2]. [1]Midwestern University, College of Health Sciences, 900 South 5th Street, St. Charles, IL 60174 and [2]Department of Pharmaceutical Sciences, Chicago College of Pharmacy, Midwestern University, 555 31st St, Downers Grove, IL 60515. Email: johnjbaginski@ gmail.com

Maraviroc (Selzentry®) is a CCR5 antagonist approved by the FDA for treatment of individuals with human immunodeficiency virus (HIV). While maraviroc is quite effective at blocking HIV entry into T-cells, it is currently undergoing clinical trials for autoimmune diseases such as rheumatoid arthritis and graft versus host disease. However, very little research has been done looking at additional effects this drug may have on other components of the immune system. This is surprising given that the chemokine receptor CCR5 is found on a wide range of immune cells including macrophages, monocytes, lymphocytes and dendritic cells. CCR5 and its activating ligands (e.g. CCL3, CCL4, CCL5) play a role in the chemotactic response of immune cells such as lymphocytes and monocytes. Thus, we explored the effects of maraviroc on chemotaxis to CCL3, CCL4 and CCL5 using a plasma cell line and a monocytic line. Additionally, flow cytometry was used to examine the surface

expression of CCR5 and CCR1 on immune cells before and after exposure to maraviroc in combination with CCL3, CCL4, or CCL5. Our studies indicate that maraviroc, at physiologically relevant concentrations, appears to affect both plasma cells and monocytes. Exposure to maraviroc in combination with CCL3, CCL4 or CCL5 appears to influence surface expression of CCR1 and CCR5 as well as alter the chemotactic response in both cell lines.

A-3008

Characterization of a Recombinant Phospholipase A_2 from *Heliothis Virescens* (Lepidoptera: Noctuidae). C. GOODMAN, J. Ringbauer Jr, and D. Stanley. USDA, ARS, BCIRL, 1503 S. Providence Rd, Columbia, MO 65203. Email: cindy.goodman@ars.usda.gov

Phospholipases A_2 (PLA_2s) hydrolyze *sn*-2 fatty acid moieties from phospholipids (PLs). A crucial PLA_2 action is the release of arachidonic acid from PLs for the production of prostaglandins (PGs). PGs are signal molecules operating in animals, including insects. We have uncovered several PG actions that mediate insect immune reactions to infection and invasion. Our long-term vision is developing novel insect pest management tools by exploiting the insect PG system. We have characterized PLA_2s from a variety of insect species and reported that bacterial infections stimulate PLA_2 activity. Here we report for the first time the *in vitro* expression of a PLA_2 sequence from a pest insect, the tobacco budworm, *Heliothis virescens*. We cloned the sequence into an expression vector and transformed *E. coli* for recombinant PLA_2(rPLA_2) production. The rPLA_2 consists of 609 base pairs, 203 amino acids and has a theoretical molecular weight of 23.4 kDa. We partially purified the rPLA_2 on a nickel column and used radioactive substrates to show that the rPLA_2 activity is time- and protein concentration-dependent. The rPLA_2 was optimally active at 28 to 37^{o}C and pH 7 to 8. Most important for our work, it was specific for arachidonyl-associated PL substrate and inhibited by a classic cellular PLA_2 inhibitor, MAFP. We compared the activities of our rPLA_2 with PLA_2s in insect cell lines, BCIRL-HvAM1 from *H. virescens* and the closely related species *Helicoverpa zea* (BCIRL-HzAM1). We found many similarities among all three PLA_2 activities, especially in substrate specificity and response to inhibitors. This work sets the stage for developing gene silencing constructs, dsRNAs, to test hypotheses on specific gene function(s) in immunity and whether silencing a specific PLA_2 gene lethally cripples insect immunity.

A-3009

Identifying the Interactome of NPM1 in Neuroblastoma Cell Lines. A. ALVAREZ[1], J. Kwak[1], S. Volchenboum[2], A. Truman[3], and K. Kristjansdottir[1]. [1]Midwestern University, 555 31st Street, Downers Grove, IL 60515 and The University of Chicago, [2]5841 S. Maryland Avenue, Chicago, IL and [3]929 E. 57th St., Chicago, IL. Email: aalvarez95@midwestern.edu

Neuroblastoma is a cancer arising from neural crest cells and is among the more common childhood cancers. Patients with the high-risk form of neuroblastoma have poor survival rates despite intensive medical interventions. Therefore, there is great need for new therapeutic targets. The MYCN gene is associated with the high-risk form of neuroblastoma. The NPM1 protein, which is correlated with MYCN levels in neuroblastoma, has a role in a number of cancers including leukemias, lymphomas, ovarian and gastric cancers. The N-terminal domain of NPM1 modulates the susceptibility of cancer cells to apoptosis. This research investigates the role of NPM1 in neuroblastoma by identifying the interactome of NPM1. Protein interactors have been identified using both a full length and a mutant form of NPM1 without the N-terminal domain. The interactomes of NPM1 were compared in cell line models of both high and low-risk neuroblastoma. Validation of the interactomes were done by probing them with antisera to known interactors of NPM1. To increase the coverage of NPM1 interactors, we combined the data from a high-throughput yeast two-hybrid screen (using NPM1 as a bait) and a combination of NPM1 immuno-precipitation and mass spectrometry. Identification of the interactome of NPM1 may identify novel therapeutic targets and result in new treatments for neuroblastoma. This work was supported by Start-up funds from College of Health Sciences at Midwestern University and with a CHS Research Facilitation Grant from Midwestern University.

A-3010

Effects of the Emergent Pesticides, Diflubenzuron and Spinetoram, on Gap Junctional Intercellular Communication, a Biomarker of Homeostasis in a Rat Liver Epithelial Cell Line. K. VI[1], M. Polanco[1], A. Wheeler[1], J. Wise[2], and B. Upham[1]. Michigan State University, [1]Department of Pediatrics and Human Development and [2]Department of Entomology, East Lansing, MI 48824. Email: vikim@msu.edu

Gap junctional intercellular communication (GJIC) is a critical cell process needed in maintaining the homeostasis of tissues, and the disruption of this cell signaling mechanism alters gene expression that has been linked to adverse health effects, including tumor promotion. Thus, determining the effects of environmental toxicants and toxins on the function of GJIC is a potential biomarker of toxicity. Using a simple dye transfer assay in a F344 rat liver oval cell line, we commpar3ed the effects of two emergent insecticides used in fruit orchard pest control, diflubenzuron and spinetoram, on GJIC with that of the legacy pesticides, DDT and lindane. Preliminary results indicate that both diflubenzuron and Spinetoram dysregulate GJIC that was dose and time dependent. However, these compounds were 2-3 times less potent than the legacy pesticides, DDT and lindane, thus potentially less toxic to mammalian systems. Further experiments are being conducted to determine if all four of these pesticides dysregulate GJIC through similar or different mechanisms.