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Animal Contributed Papers

A-1000

Assessing CCR1 Antagonists for Chemotaxis Inhibition in a Multiple Myeloma In Vitro Model. S. L. ECHEVERRIA[1] and A. Gilchrist[2]. [1]College of Graduate Studies, Midwestern University, Downers Grove, IL 60515 and [2]Chicago College of Pharmacy, Department of Pharmaceutical Sciences, Midwestern University, Downers Grove, IL 60515. Email: secheverria19@midwestern.edu

Multiple myeloma (MM) is a plasma B cell malignancy characterized by osteolytic bone lesions. MM cells secrete and express CCL3/MIP1α which upregulates osteoclastogenesis. Elevated CCL3 levels display a chemotactic ability on isolated osteoclast precursors. Increased levels of CCL3 in MM patients correlates with a greater disease burden, due to the increase of bone resorption, and is indicative of a worse prognosis when compared to MM patients exhibiting lower CCL3 levels. CCR1, a GPCR chemokine receptor, is endogenously expressed on MM cells, and can bind with CCL3. In a previous study, the RPMI8226 MM cell line, CCL3-mediated CCR1 chemotaxis was inhibited in a dose-dependent manner by six CCR1 antagonists (AZD4818, BX471, CCX354, CP481715, MLN3897, PS031291). In this study, we assessed the MM cell line U266 as well as a transfected cell line, U266_CCR1. While U266 cells express lower levels of CCR1 than RPMI8226, U266_CCR1 express higher levels of CCR1. Cells were treated with a serial dilution of the same six CCR1 antagonists and chemotaxis to either supernatant from osteoclast precursor RAW 264.7 cells or Fetal Bovine Serum (FBS) in which a multitude of growth factors and chemokines are present was examined. We hypothesized the six CCR1 antagonists would result in a dose-dependent inhibition of chemotaxis similar to that seen with RPMI8226. Instead, we found only two of the compounds (AZD4818 and BX471) inhibited chemotaxis of the U266 and U266_CCR1 MM cell lines towards RAW 264.7 supernatant or FBS. For both AZD4818 and BX471 there were differences between the chemotactic response of the U266 and U266_CCR1 cell lines, with the U266_CCR1 cell line having a greater degree of inhibition, suggesting the inhibition is driven by CCR1.

A-1001

Successful Culture of Marine Sponge Cells Using Multiple 3-D Culture Methods. ELIZABETH URBAN-GEDAMKE, Megan Conkling, Peter J. McCarthy, Paul S. Wills, and Shirley A. Pomponi. Florida Atlantic University – Harbor Branch Oceanographic Institute, Ft. Pierce, FL. Email: urbane@fau.edu

Sponges are ecologically, commercially, and biomedically important organisms, but the requirements for sponge tissue for research, restoration, and pharmaceutical production cannot be met by wild harvest alone. A recent study demonstrated the ability to culture marine sponge cells in two-dimensions using an optimized nutrient medium. Here we report the successful implementation of the same optimized nutrient medium to produce three-dimensional cell cultures of the marine sponge *Geodia neptuni* using four different methods - FibraCel© discs, thin hydrogel layers, gel micro droplets, and spheroid formation. Herein we discuss the advantages and disadvantages of each method, as well as make recommendations for future applications. The research furthers our ability to study and culture marine sponge cells, and optimization of these techniques may lead to the *in vitro* production of sponge cultures for the synthesis of marine natural products and for habitat restoration purposes.

A-1002

Multidimensional Tomography of Cells by a Combination of Atomic Force Microscopy and Dynamic Mechanical Spectroscopy. RENATO AGUILERA[1], Maxim Dokukin[1,2], Nadja Makarova[1], and Igor Sokolov[1,3,4]. [1]Tufts University, Medford, MA; [2]NanoScience Solutions, Inc. Arlington, VA;

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Cell phenotyping by their mechanical properties is gaining increasing interest as a type of label-free biomarker. Alterations to the cytoskeleton and nucleus due to cell instability, passage, or differentiation lead to significant changes in cell mechanics. Additionally, precise mechanical measurements enable viscoelastic modeling of cell deformation for high-speed rheological studies. Here, we present the application of our new technique, FT-NanoDMA, to create a multidimensional tomography of the dynamical mechanical properties of cells. FT-NanoDMA stands for Fourier transformed nano dynamical mechanical analysis, which is implemented by means of atomic force microscopy (AFM). It allows for simultaneous recording at different frequencies of multiple dynamical mechanical properties of cells, such as the storage and loss moduli, loss tangent, etc. The FT-nanoDMA is a modality that allows for mechanical analysis of nanointerfaces and single cells with a nanoscale spatial resolution (up to 10–70 nm when recorded on fixed cells) and spans the entire biological spectroscopic range (up to 300 Hz). The images of the distribution of these parameters can be recorded at each particular depth of the cell, thereby creating a stag of images suitable to create a multidimensional tomography of single cells. We demonstrate examples, which show that the tomographic images show unique features not present in the complementary fluorescent/optical images of cells. The biological significance of this novel information has yet to be understood.

A-1003

Use of Rainbow Trout Fish Cell Lines to Study Cellular Uptake of Fluorescently DiI-labeled Lipid Nanoparticles Encapsulating siRNA In Vitro. HEATHER M. KELLY[1], Sam Chen[2], Yuen Yi C. Tam[2], Lucy E. J. Lee[1], and Justin B. Lee[1]. [1]Department of Biology, University of the Fraser Valley, Abbotsford, BC, CANADA and [2]Integrated Nanotherapeutics, Burnaby BC, CANADA. Email: Heather.Kelly1@student.ufv.ca

Lipid nanoparticles (LNPs) can be used for the delivery of bioactive molecules such as nucleic acids for various applications, including the treatment of multiple diseases. siRNAs, in particular, have been used in eukaryotic cells to induce RNAi and cause subsequent degradation of target mRNA. In contrast to humans, studies on the therapeutic use of LNP systems in aquaculture have been poorly investigated. One of the major problems threatening the aquaculture industry is the detrimental effect of infectious disease on fish mortality. Although antibiotics are currently used to treat rainbow trout and other fish diseases, some of the antibiotics are the same ones used to treat humans. Therefore, the overuse of antibiotics may lead to antibiotic resistance in both humans and aquaculture. The future direction of this research is to treat rainbow trout using LNP encapsulating siRNA (LNP-siRNAs) in order to offer an alternative therapeutic. The present research aims to investigate cellular uptake of LNP-siRNA formulations on three different established rainbow trout (RT) cell lines, which include a gill epithelial cell line (RTGill-W1), a gut epithelial cell line (RTGutGC), and a macrophage cell line that stably expresses green fluorescent protein (RTS-11-GFP). Fluorescently Dil-labeled LNP-siRNAs with varying lipid concentrations of 0, 5, 10, and 100 μg/mL were tested for cellular uptake *in vitro* using all three cell lines. The effects of Dil-LNP-siRNAs on epithelial cell migration was also evaluated through wound healing assays using the RTGill-W1 and RTGutGC cell lines. Preliminary results showed no negative effects of Dil-LNP-siRNAs on epithelial cell migration and significant fluorescent LNP uptake was observed at both 24 and 48 hour timepoints.

A-1004

High-resolution Nanothermometery of Biological Cells Using Ultrabright Polysaccharide Near-IR Fluorescent Nanoparticles. B. PENG and I. Sokolov. Tufts University, Dept of Biomedical Engineering, 200 Boston Ave, Suite 2600, Medford, MA 02155. Email: bpeng01@g.ucla.edu

In order to better understand metabolic dysfunction and disease, it is vital to create tools that can interrogate nanoscale thermodynamic processes in biological systems. Fluorescent nanosensors provide a noninvasive approach to perform thermometry with high sensitivity and response speed. Here, we synthesized a biocompatible, ultrabright polysaccharide NIR nanosensor to spatially image thermal distributions at single nanoparticle resolution in real-time. Utilizing NIR fluorescence reduces error from scattering and endogenous sources while enabling *in vivo* application. Also, the ultrabright nature of the particles mitigates error from low sensor concentration and high excitation power densities. We present 2D distributions of single nanoparticle temperatures as well as thermal distributions within MCF10A and HeLa cells to assess metabolic activity. The direct observation of heterogeneous intracellular temperature distributions at individual nanoparticle resolution raises new possibilities in nanoscale sensor innovation.

A-1005

Protective Effects of Blackcurrant Extract in Senescence Accelerated Mouse (SAMP8) Models. NICK THOMPSON[1,2], Jia Xiong[1,2], Sierra Bonney[1,2], Fernanda V. Matta[1,3], and Debora Esposito[1,2]. [1]Department of Animal

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Anthocyanin pigments are flavonoid phytochemicals that exhibit a wide range of pharmacological activity, including but not limited to antioxidation, cardiovascular and neurological protection, and prevention of muscle atrophy (Chen et al. 2019). Delphinidin is one of the anthocyanins of dietary importance, reported to effectively reduce muscle weight loss and improve cardiovascular and metabolic risk factors associated with obesity (Overall et al. 2017). Blackcurrant berries contain a high amount of the anthocyanin delphinidin, and previous research by Xiong et al. (2019) concluded that supplementation of anthocyanin rich berries may improve muscle regeneration. This study aimed to investigate the effects of a diet supplemented with blackcurrant extract on the physical fitness in male and female accelerated aging mouse models. Throughout the procedure physical endurance, feed intake, body weight, body composition, and reactivity and passivity related to senescence were monitored. Male and Female mice receiving the blackcurrant extract treatment showed fewer age related changes in reactivity and passivity. The results showed obvious differences in body composition between the male and female SAMP8 models, and suggest that diet and gender contribute to fitness and muscle regeneration in SAMP8 mice.

A-1006

A Novel *In Vitro* High Throughput Bioassay Screening System to Assess the Effects of Environmental Contaminants on Gap Junctional Intercellular Communication. BRAD L. UPHAM[1], Lizbeth Lockwood[1], Alison Bauer[2], Richard Neubig[3], and Jinu Lee[4]. [1]Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI 48824; [2]Department of Environmental and Occupational Health, University of Colorado-Denver, Aurora, CO 80045; [3]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824; and [4]College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon 21983, SOUTH KOREA. Email: upham@msu.edu

The selection of GJIC as an endpoint is a significant step in developing a systems-based *in vitro* model, as this biological phenomenon is crucial for integrating signaling mechanisms within cells with that of neighboring cells in a tissue, and is an important early stage event in abnormal cell proliferation within tissues. Most *in vitro* assessments of GJIC rely on fluorescent dye transfer techniques that require introduction of the dye through scrape loading, microinjection, or electroporation techniques, and detection with microscopes that all tend to be problematic in developing HTS assays. Thus, there is a need to develop and validate a bioassay system to assess GJIC in response to environmental toxicants and drug candidates that is conducive to high throughput screening (HTS) using *in vitro* cell model systems. The parent cell line used is the F344 WB cell line, which is an excellent *in vitro* cell model of liver oval cells, a bipotent stem/progenitor cell that give rise to hepatocytes and hepatic biliary duct cells, and self-renew. We present an HTS assay that depends on a subsets of donor and subset of receptor cells where the donor cells are stably transfected with the iodide transporter gene and the acceptor cells with the yellow fluorescent protein (YFP) gene. The addition of iodide initiates the bioassay by entering the donor cells via the iodide transporter, and then transfers through gap junctions to the receptor cells, in which iodide quenches the YFP-fluorescence. Closed or partially closed gap junction channels prevents or partially prevents quenching in the receptor cells from iodide. Multiple well plates are used and fluorescent plate readers measure the fluorescence, which makes this bioassay quite amendable to HTS. Research supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number R21ES031345. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health."