In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-008-9109-x

Animal posters

A-2000

Prostaglandin A_2 Significantly Alters Gene Expression in an Established Insect Cell Line (BCIRL-HzAM1). D. Stanley[1], C. GOODMAN[1], Q. Song[2], S. An[2], and A. McIntosh[1]. [1]USDA, ARS, BCIRL, 1503 S. Providence Rd., Columbia, MO 65203, and [2]Division of Plant Sciences, University of Missouri, 1–87 Agriculture Building, Columbia, MO 65211. Email: David.Stanley@ars.usda.gov

In previous work to determine the biochemical mechanisms of prostaglandin (PG) action in insect cells, we found that PGA_1 and PGE_1 influenced the expression of genes encoding proteins important for a variety of cellular functions. In the present study, we exposed the same cell line, BCIRL-HzAM1, to three PGs of the 2-series to determine if they would have similar effects. Cells were incubated with 15 μM PGA_2 (which interacts with perinuclear receptors), PGE_2, or $PGF_{2\alpha}$ (both of which act via G-protein coupled receptors) for 12 or 24 hr. Differences in protein patterns were determined quantitatively using 2D electrophoresis. Dramatic changes in protein expression were noted in cells exposed to PGA_2 for 12 hr, with significant changes in over 65 proteins. Modifications in protein expression were also noted for the PGE_2 or $PGF_{2\alpha}$ treated cells, but not nearly to the same extent. Identities for some of the proteins of interest had been previously elucidated in our earlier study using MS/MS (MALDI TOF/TOF). Therefore, we can, at this point, clearly state that PGA_2 significantly alters the expression of proteins involved in numerous cellular functions, including cell protection (e.g., catalase and various superoxide dismutases), cell movement/division (e.g., actin-depolymerization factor), protein action (e.g., heat shock cognate), lipid metabolism (e.g., acyl-coenzyme A dehydrogenase), signal transduction (e.g., 14–3–3 protein), and metabolism (e.g., arginine kinase and enolase). We are identifying more proteins via MS/MS to expand our knowledge of those that are up- or down-regulated by PGs, as well as confirming the changes in levels of specific proteins using qPCR. Our results continue to give strong support for our hypothesis that PGs influence gene expression in insect cells.

A-2001

Granulocytic Differentiation of HL-60 Promyelocytic Leukemia Cells is Associated with Increased mRNA Expression for Components of the Cullin-5-Containing E3 Ubiquitin Ligase. SHANEEN S. BAXTER[1,2], Lauren A. Carlson[1], Alejandro M. S. Mayer[1], Mary L. Hall[1], and Michael J. Fay[1,2]. [1]Department of Pharmacology and [2]Department of Biomedical Sciences, Midwestern University, 555 31st Street, Downers Grove, IL 60515. Email: mfayxx@midwestern.edu

The human HL-60 promyelocytic leukemia cell line has been widely used as a model for studying granulocytic differentiation. All-*trans* retinoic acid (ATRA) treatment of HL-60 cells promotes granulocytic differentiation and is effective as differentiation therapy for patients with acute promyelocytic leukemia. The identification of genes that are transcriptionally regulated by ATRA has provided insight into granulocytic differentiation and differentiation therapy. The *Asb*-2 gene has previously been identified as a transcriptional target in ATRA-treated HL-60 cells. The ASB-2 protein forms an E3 ubiquitin ligase complex with the proteins Cullin 5, ROC2, and Elongin B and C. The purpose of this study was to determine if there is increased expression of mRNAs for the other components of this E3 ubiquitin ligase complex. To induce granulocytic differentiation, HL-60 cells were treated for 5 d with ATRA, and differentiation was confirmed by examining superoxide anion production, nuclear morphology, and changes in the expression of CD11b, CD13, and CD15. Quantitative real-time PCR was used to measure ASB-2, Cullin 5, ROC2, and Elongin B and C mRNA expression. Granulocytic differentiation of HL-60 cells was associated with a 23-fold, 1.6-fold, and 1.7-fold statistically significant (*t* test; $p<0.05$) increase in mRNA expression for ASB-2, Cullin 5, and ROC2, respectively. No significant change was found in Elongin B and C mRNA expression. Increased expression of multiple components of the Cullin-5-containing E3 ubiquitin ligase complex with ATRA treatment of HL-60 cells indicates that this complex may play an important role in granulocytic differentiation. This research was supported in part by NIH CA122003-01 and Midwestern University.

A-2002

Organ and Monolayer Cell Culture of Gottingen Minipig Skin: A Model for Whole Skin Study and Drug Safety Screening. MICHAEL K. DAME[1], Diana Spahlinger[1], Marissa DaSilva[1], Patricia Perone[1], Robert Dunstan[2], and James Varani[1]. [1]Department of Pathology, The University of Michigan Medical School, Ann Arbor, MI 48109, and [2]Pfizer Global Research and Development, Ann Arbor, MI 48105. Email: mdame@med.umich.edu

Skin from Gottingen minipigs was used as a source of tissue for organ and cell culture, and compared to human skin for growth conditions and sensitivity to irritants. Optimal organ culture conditions were determined, based on the preservation of histological structure. These included serum-free, growth factor-free conditions with a calcium concentration of 1.5 mM. Formulations in which the calcium concentration were low (0.075–0.15 mM) failed to support tissue viability (even in the presence of dialyzed serum). Epidermal keratinocytes were grown from tissue explants and as single cells from enzyme-disrupted tissue. Optimal keratinocyte growth was achieved using a serum-free, growth factor-supplemented culture medium with a calcium concentration of 0.15 mM. Fibroblasts were optimally grown from explant cultures using medium with 1.5 mM calcium and 10% fetal bovine serum. The conditions that were optimal for maintenance of intact pig skin, as well as for the isolated cells, are the same conditions that have been shown previously to be optimal for intact human skin and skin cells. In additional studies, pig skin keratinocytes and fibroblasts were exposed to a panel of contact-irritants and contact-sensitizers. Using growth inhibition as the response, the median effective dose values with each agent were very similar to the values previously determined for human epidermal keratinocytes and human dermal fibroblasts. Taken together, these data suggest that the skin from the Gottingen minipig can be used as a surrogate for human skin in ex vivo skin safety studies.

A-2003

Effects of *Rheum ribes* Ethylacetate Extracts on Cytochromes P450 1B1 Gene Expression and Glutathione-*S*-transferase Activity in HL-60 Cells. P. UYAR. Graduate Program of Biotechnology, Middle East Technical University, Ankara, TURKEY. Email: pembegul@metu.edu.tr

This study was conducted to demonstrate whether *Rheum ribes* L. extracts affect the expression levels of CYP1B1 gene in HL-60 cells and to investigate the susceptibility of the relation between antiproliferation and CYP1B1 expression, and, also, GST activity. Dried and pulverized plant samples were extracted by ethylacetate at a ratio of 1:12 (*w*/*v*). Human Myeloid Leukemia (HL-60) cell line was used as a model system for the studies. HL-60 cells were cultured in the presence of various concentrations of extracts for 72 hr. The percentage of cell viability was determined by metabolism of the tetrazolium salt XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium hydroxide). In the present study, *R. ribes* was found to inhibit the survival of human promyelocytic leukemia HL-60 cells in a concentration- and time-dependent manner. ED50 values of shoot and root extracts were calculated as 114.01 ± 0.65 μg/ml and 98.15 ± 0.54 μg/ml, respectively. HL-60 cells were plated at a density of 1×10^5 cells/ml into T75 flasks. After overnight growth, cells were pretreated for 16 h with *R. ribes* extracts, dissolved in DMSO and diluted with RPMI 1640, to a final concentration 100 μg/ml of growth medium. The final concentrations of dimethyl sulfoxide in the culture medium were <0.1%. At the end of treatment, RNA isolation and protein extraction were performed for all flasks. Isolated RNAs of both treated and non-treated cells were then reversely transcribed to cDNAs using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MuLV-RT). cDNAs were amplified using CYP1B1 and β-actin primer sets. *R. ribes* shoot and root extracts affect HL-60 CYP1B1 gene expression differentially, with respect to non-treated control groups. GST activity of both treated and non-treated cells were measured in the presence of CDNB as substrate in ELISA plate reader at 340 nm. There was a variation in the GST activity of *R. ribes* shoot and root extracts treated HL-60 cells in comparison to non-treated control groups.

A-2004

In Vitro Investigation of Individual and Combined Cytotoxicity of Ochratoxin A and Arsenic in Chinese Hamster Lung Fibroblasts and Human Urothelial Cells. M. AGGARWAL[1,2], C. Behm[1], W. Foellmann[1], J. K. Malik[2], and G. H. Degen[1]. [1]Institut für Arbeitsphysiologie an der Universität Dortmund, Leibniz Research Centre for Working Environment and Human Factors, Ardeystraße 67, D-44139 Dortmund, GERMANY and [2]Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar-243 122 (UP), INDIA. Email: drmanoj_2k@yahoo.com

Ochratoxin A (OTA), a mycotoxin that frequently occurs as a contaminant in various foods and feeds, is a potent rodent carcinogen, with kidney and urinary bladder as major target organs. OTA is known to cause porcine nephropathy and is suspected to be involved in Balkan endemic nephropathy in humans. Arsenic, a metalloid, found in ground drinking water in many parts of the world, is also a potent

carcinogen with multiple target organs including urinary bladder, kidney, and skin. As both substances appeared simultaneously as contaminants of food or water, it is indicated to study their interaction. The present study was designed to determine the individual and combined cytotoxic effects of OTA and arsenic in Chinese hamster lung fibroblast (V79) and human bladder carcinoma (5637) cells by using the cell titer blue (based on mitochondrial activity) and the neutral red (based on lysosomal activity) assays. Concentration–effect curves for OTA alone, arsenic alone, and their combinations for various treatment times (up to 72 h) were generated. Cytotoxicity was observed at low micromolar concentrations of OTA or arsenic in both cell lines, with 5637 cells appearing more sensitive than V79 cells. When tested as combinations, the observed cytotoxicity was higher than for the individual substances alone but some of the concentration pairs tested produced lower combined cytotoxicity than the sum of the cytotoxicity induced by OTA and arsenic. These results are evident for future risk assessment of genotoxic effects of this mixture.

A-2005

In Vitro Evaluation of Nano-encapsulated Protective Antigen Functionality Through the Use of a Monomac 6 Assay. KEVIN E. KNOCKENHAUER[1], Katarzyna M. Sawicka[2], Elizabeth J. Roemer[1,3] and Sanford R. Simon[1,2,3]. [1]Department of Biochemistry, [2]Department of Biomedical Engineering, and [3]Department of Pathology, Stony Brook University, Stony Brook, NY 11794. Email: eroemer@notes.cc.sunysb.edu

Anthrax, a disease caused by the gram positive bacteria *Bacillus anthracis*, has become an increasing threat to public health in the last several years, due to its use as an agent of biological warfare. The currently utilized human anthrax vaccine, which confers immunity through the host antibody recognition of protective antigen (PA), requires a three dose regimen and annual booster shots after the initial vaccination to maintain its efficacy. The long term goal of this project is to produce an anthrax vaccine that is capable of delivering protective antigen through human skin. The novel method for transdermal vaccine delivery that we propose utilizes the high surface area to volume ratio offered by protein-containing nanofiber membranes, prepared by the electrospinning technique. Research has already been undertaken to study the effect the main virulent agent of anthrax, lethal toxin (LT), has on Monomac 6 cells (MM6), a human monocytic cell line. Lethal toxin is said to comprise of a Zn^{2+}-dependent metalloprotease known as lethal factor (LF), and a binding protein known as protective antigen. The in vitro MM6 assay was designed to test the bioavailability of electrospun PA. The assay required stimulating MM6 cells with equal amounts of PA and LF, together and independently, as well as in the presence and absence of lipolysaccharide (LPS), a component of a bacterial cell wall. Using a Ray Bio multiplex assay kit, it was qualitatively determined that the interactions have a direct effect on the production of two cytokines: IL-6 and IL-8. Quantitative analysis of cytokine concentrations was performed through the use of IL-6 and IL-8 enzyme linked immunosorbent assay (ELISA) kits. Control PA was observed to interact with LF and resulted in a down regulation of cytokine production. The MM6 cells dosed with electrospun PA, along with LF, were compared and their IL-6 and IL-8 production was subsequently down regulated, proving that PA retained its functionality through the process of electrospinning. This finding provides an innovative platform for the development of a transdermal anthrax vaccine.

A-2006

Update on the COLIPA Eye Irritation Programme for Development of In Vitro Methods. PENNY JONES[1], Sandrine Bessou-Touya[2], Lieve Declercq[3], Ann De Smedt[4], Bart De Wever[5], Claudine Faller[6], John Harbell[7], Béatrice Le Varlet[8], Pauline McNamee[9], Monique Marrec-Fairley[10], Wolfgang Pape[11], Uwe Pfannenbecker[11], Klaus Schroeder[12], Magalie Tailhardat[13], Christine Van den Berghe[14], and Freddy Van Goethem[4]. [1]Safety and Environmental Assurance Centre, Unilever, Sharnbrook, UK; [2]Laboratoire Pierre Fabre, Castres, FRANCE; [3]Estee-Lauder Companies, Oevel, BELGIUM; [4]Johnson & Johnson Pharmaceutical Research & Development, Beerse, BELGIUM; [5]Phenion, Frankfurt, GERMANY; [6]Procter & Gamble, Cosmital SA, Marly, SWITZERLAND; [7]Mary Kay Inc, Dallas, TX; [8]Links Ingénierie, Paris, FRANCE; [9]The Procter & Gamble Company, Egham, Surrey, UK; [10]COLIPA, Brussels, BELGIUM; [11]Beiersdorf, Hamburg, GERMANY; [12]Henkel, Dusseldorf, GERMANY; [13]LVMH, St. Jean De Braye Cedex, FRANCE; and [14]L'Oreal, Aulnay Sous Bois Cedex, FRANCE. Email: penny.jones@unilever.com

Success in fully replacing the Draize eye irritation test with in vitro methods has not yet occurred in part due to lack of understanding of underlying physiological mechanisms of eye irritation. The COLIPA eye irritation programme for development of in vitro methods incorporates integrated research projects and collaborative activities with external partners. The integrated research projects focus on understanding mechanisms of eye injury and identification of new in vitro endpoints more predictive of the in vivo human response to chemical injury resulting in new or improved in vitro methods that would proceed to formal validation. There are three projects: (1) investigation of

whether kinetics/patterns of change in physiological function/signals of injury released from the cornea in vitro can predict a chemical's potential to damage the eye with a focus on recovery; (2) identification of endpoints related to magnitude of injury and repair in three-dimensional human corneal constructs and (3) a genomics project using a pattern recognition approach to identify new endpoints for injury and repair that builds on corneal models including that being evaluated in project 2 for potential use in current/future in vitro assays. Equally important to achieve validated in vitro methods is industry collaboration with producers of in vitro models, academia, external scientific organisations, and regulators. COLIPA is working with: (1) producers of Human Reconstructed Tissue (HRT) models on method development/optimisation of current in vitro assays and (2) with ECVAM by actively participating in its Eye Irritation Task Force and providing support for post-hoc statistical analysis of current (in vitro) methods. This poster provides a detailed overview and update on the integrated elements of the COLIPA eye irritation programme.

A-2007

Evaluation of Collagen-containing Materials for Management of Chronic Wounds: Preservation of Native Structural Integrity and Reduction in Fluid-phase Inflammatory Enzyme Activities. F. DACCUEIL, E. J. Roemer, and S. R. Simon. Department of Pathology, Stony Brook University, Stony Brook, NY, 11794. Email: dacfarah03@yahoo.fr

Overproduction of Elastase and Matrix Metalloproteinases (MMPs) at the site of chronic wounds is detrimental to the normal wound-healing process. Many biochemical companies have focused their studies on engineering wound dressings using some type of collagen with the goal of diminishing or sequestering the activities of these enzymes. While collagen has the capacity to sequester the activities of destructive proteinases, the type and structural integrity of the collagen appears to be a major determinant of efficacy. In these studies, we set out to investigate the importance of type I native collagen in providing superior ability to diminish activities of Elastase and MMPs in a surrounding fluid phase. Several dressings were evaluated, including products containing collagen alone or in combination with oxidized regenerated cellulose (ORC) to assess their efficacy in depleting the activities of both MMPs and Elastase. In order to assay for retention of the triple helical structure of the collagens in the different wound dressings, a procedure for detecting collagen damage was employed in which the dressings were treated with α-chymotrypsin, a proteinase which degrades damaged, but not native, collagen. The levels of triple helical native collagen before and after chymotrypsin treatment were then quantitated by binding of the dye Sirius Red. My data shows that dressing material from Johnson and Johnson containing a mixture of collagen and ORC (Promogran™) has relatively lower levels of the triple helical structure of native collagen than a product containing pure collagen from Suwelack Health Care (Puracol Plus™), and that the collagen in the collagen/ORC product appears to be damaged. Additionally, my measurements show that the Suwelack pure collagen dressing has a higher capacity than the collagen/ORC dressing to deplete Human Neutrophil Elastase from the fluid phase, while its capacity to deplete gelatinolytic MMPs from the fluid phase is comparable to that of competing products.

A-2008

Retaining Cell Integrity during Organotypic Model Viability Assays: Alternatives to MTT. C. R. KAVANAGH[1] L. J. Crawford[1], K. M. Sawicka[3], S. R. Simon[1,2] and E. J. Roemer[1,2]. Departments of [1]Pathology, [2]Biochemistry and Cell Biology and [3]BioMedical Engineering, SUNY Stony Brook, Stony Brook, NY, 11794. Email: ckavanag@ic.sunysb.edu

Our lab frequently uses organotypic models. Among these are several from MatTek, including EpiAirway™: to evaluate compounds for nasal drug delivery; EpiDerm™: to study transdermal vaccine delivery systems; and MelanoDerm™: to study skin color mediators. In each case MTT is the recommended end-point assay for cell viability. However, we often need to retain tissue integrity and MTT requires tissue lysis. Our other research routinely uses viability assays that do not require cell lysis and we have endeavored to adapt these to the organotypic models. The three assays examined to date are MTS, Alamar Blue, and CalceinAM. MTS, like MTT, is based on tetrazolium salt cleavage in the mitochondria of metabolically active cells to form a formazan product that can be measured by absorbance. But with MTS, this colored product is aqueous and soluble, and does not require cell lysis. Alamar Blue uses resazurin, a blue nonfluorescent dye, which is reduced by cell metabolic activity to pink fluorescent resorufin. The resulting signal is proportional to the number of living cells in the assay. CalceinAM is membrane-permeant and can be introduced into cells via incubation. Once inside the cells, it is hydrolyzed by endogenous esterase to green fluorescent calcein, which is retained in the cytoplasm. Thus, CalceinAM provides a more direct estimate of viable cell number than do either tetrazolium or resazurin, which rely on metabolic activity. To supplement the viability assays and provide a concurrent measure of cell death, lactate dehydrogenase (LDH) released by cytolysis in the models is measured in the incubation medium. After initial success

using all three of these non-lytic assays we are now working to establish final protocols for their routine application to organotypic models.

A-2009

In Vitro Investigation of Antioxidant and Antiproteolytic Properties of the Clove Extracts: Tellimagrandin I and Casuarictin. S. ZAMURRAD, S. Parrino, F. Daccueil, E. J. Roemer, and S. R. Simon. Department of Biochemistry and Cell Biology, Department of Pathology, SUNY Stony Brook, Stony Brook, NY 11794. Email: szamurra@ic.sunysb.edu

Neutrophils secrete a variety of substances including reactive oxygen species (ROS), matrix metalloproteinases (MMP) and human neutrophil elastase (HNE) during inflammation. MMPs and HNE are proteases which degrade elastin, cartilage proteoglycans, several collagens, and fibronectin, as well as foreign materials ingested during phagocytosis. Proteinase inhibitors normally inhibit damage to connective tissue caused by leakage of these enzymes. Insufficient levels of these inhibitors can lead to prolonged inflammation, which is disastrous to human health. Prolonged inflammation can be a major contributor to age-related damage, heart disease, diabetes, dental caries, eye disease, arthritis, and dementia to name a few. Our lab frequently tests compounds for their antioxidant and antiproteolytic activity so that they can be used as anti-inflammatory agents in topical creams and drugs. Antioxidants and proteinase inhibitors are important to overcome the actions of ROS in vivo. Among the most important dietary antioxidants are plant phenols such as those which can be isolated from tea, rosemary, and spices such as sage, turmeric, and clove. We tested the clove extracts: Tellimagrandin I and Casuarictin (Nacalai Tesque Inc, Japan) and both showed significant antioxidant and antiproteolytic activity. Tellimagrandin and Casuarictin are gallic acid glycosides which are esters of gallic acid bound to hydroxyl of sugars. Gallic acid is a subclass of polyphenol responsible for the antioxidant activity in many plants. Both extracts were diluted to varying concentrations as low as 0.01 μg/mL and assayed separately for antioxidant, anti MMP-8, and anti HNE activity in 96-well plates. Both extracts showed antioxidant and antiproteolytic activity and are readily available, which makes them promising compounds for potential applications in chronic wound therapy, skin care, and oral hygiene.

A-2010

Optimization of a New Method of Characterizing Live Basal Keratinocytes Using Chariot Transfection Reagent. Y. FUKUDA, E. J. Roemer, S. R. Simon, and M. Matic. Departments of Biochemistry and Pathology, SUNY Stony Brook, Stony Brook, NY 11794. Email: yfukuda@ic.sunysb.edu

An intracellular epitope, keratin 14 (K14), is a marker for basal keratinocytes that is used for quantitative analysis by flow cytometer. Traditionally, keratinocytes have to be isolated, fixed, permeabilized and labeled before flow cytometric analysis. To date, using these conventional methodologies, live cells could not be isolated by use of intracellular epitopes. In addition, no intracellular marker has ever been used for obtaining live cells employing a fluorescence-activated cell sorter (FACS). The transfection reagent called Chariot, developed by Active Motif, is a peptide based reagent that allows proteins, peptides, and antibodies to be delivered inside live cells. In our study, we evaluated whether Chariot was toxic to ex vivo keratinocytes and optimized intracellular protein delivery via Chariot by varying incubation time and temperature. Obtaining optimal delivery time is significant for adherent cells such as keratinocytes, because they do not tolerate non-adherent conditions very well. Although Chariot delivery works with adherent cells, we chose to work with cells in suspension because we ultimately need to sort the cells by FACS. Samples were analyzed quantitatively for expression of intracellular markers by flow cytometer. The data indicated no significant difference between any of the times or temperatures tested, demonstrating that the final incubation can be as short as 10 min and still provide efficient intracellular antibody delivery. In addition, there was no significant difference in viability between Chariot-treated cells and untreated control cells. We have shown that Chariot is an efficient reagent for successful intracellular delivery of antibodies into living keratinocytes. We hope to further optimize the incubation time to maximize the efficient delivery of antibodies within the shortest time frame for other cell types.

A-2011

Comparison of Growth Characteristics, Neurochemical Parameters, and Response to Toxicants for Neural Tissue Derived Cell Lines from Goldfish and from Crayfish. L. E. J. LEE, M. R. Bufalino, and M. P. Wilkie. Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA. Email: llee@wlu.ca

Neural cell lines derived from lower vertebrates and invertebrates could prove crucial for understanding the mechanisms of neural cell regeneration, as many aquatic vertebrates and invertebrates have been shown to retain a high capacity for regeneration and functional recovery. Whereas several neural cell lines have been developed

from mammalian species, very few neural tissue-derived cell lines have been reported for aquatic vertebrates or aquatic invertebrates. In this study we report on the development of a goldfish brain derived cell line GFB3C-W1 and compare growth and physiological characteristics with the only aquatic crustacean cell line OLGA-PH-J/92 (Neumann et al., In Vivo 14:691, 2000) derived from crayfish brain ganglia. Optimal conditions for growth, ability to form undifferentiated masses of cells (neurospheres), response to a common differentiation agent (retinoic acid), and sensitivity to metals were evaluated and compared using the two cell lines. The growth conditions assessed were optimal medium type, osmolality, temperature, glucose concentration, and growth factor supplementation. The results from this study can be used to optimize the initial cell culture conditions in future attempts to develop new aquatic vertebrate or invertebrate cell lines or for aquatic toxicity monitoring as well as in many aspects of aquaculture and biotechnology.

A-2012

Assessing the In Vitro Respiratory Toxicity of Fine Particles of Al_2O_3 and SiO_2: A Precursor Study for Lunar Dust Toxicity. J. A. JORDAN, A. M. Verhoff, and D. G. Fischer. Dept. of Natural Sciences, Clayton State University, Morrow, GA 30260, Biosciences and Technology Branch, NASA Glenn Research Center, Cleveland, OH 44135. Email: dgfisher@nasa.gov

Chemical and physical analysis of lunar soil suggests a particulate composition that may contribute to the development of acute and chronic respiratory disorders. In this study, fine Al_2O_3 (0.7 μm) and fine SiO_2 (1.6 μm) particles were used as simulant materials to assess the cellular uptake and cellular toxicity of lunar dust particles. Respiratory cells, murine alveolar macrophages (RAW 264.7) and human Type II epithelial (A549) were cultured as the in vitro model system. We first determined the phagocytic activity of both macrophage and epithelial cells using ultrafine (0.1 μm) and fine (0.5 μm) fluorescent polystyrene beads. Following a 6-h exposure, RAW 264.7 macrophage cells had extended pseudopods, with polystyrene beads localized in the cytoplasmic region of these cells. After 24 h, the macrophage cells were rounded, clumped, and lacked pseudopods, which indicated the impairment of phagocytosis. Interestingly, A549 epithelial cells did not contain polystyrene beads and, after 24 h, the majority of the polystyrene beads appeared to primarily coat the surface of the cells. Next, we investigated the cellular response to fine SiO_2 and Al_2O_3 (up to 5 mg/ml) particles. RAW 264.7 cells exposed to 1.0 mg/ml of fine SiO_2 for 6 h exhibited pseudopod development and cellular damage, along with apoptotic and necrotic states. On the other hand, A549 cells showed only a slight toxicity when exposed to fine SiO_2 of the same time and dose. A549 cells had particles clustered on the surface of the cells. Only a higher dose (5.0 mg/ml), or a 24 h treatment, of A549 cells to fine SiO_2 resulted in a significant cytotoxicity. Most importantly, both cell types showed minimal cytotoxicity following exposure to fine Al_2O_3. Overall, this study suggests differential cellular toxicity associated with exposure to fine mineral dust particles.

A-2013

D-Glucose Protection Against MPP+ Induced Cell Death in Human Lung Carcinoma A549 Cell Line. DAVID ELMASHAT, Ramesh B. Badisa, and Karam F. Soliman. Florida A&M University, College of Pharmacy and Pharmaceutical Sciences, Neuroscience Section, Tallahassee, FL 32307. Email: sayem2007@yahoo.com

1-methyl-4-phenylpyridinum (MPP+) is shown to cause selective death of nigrostriatal dopaminergic neurons in the brain, and is widely employed to induce Parkinson's disease symptoms in animal models. Previous studies from our laboratory showed that 10 mM D-glucose protected MPP+-induced toxicity to central nervous system derived malignant neurons and glial cells under in vitro conditions. It would be interesting to investigate if glucose protection against MPP+ toxicity is also observed in other type of malignant cultures. For this purpose, the present study was designed to investigate the effect of MPP+ on human lung adenocarcinoma epithelial cell line (A549) by testing at 2.5, 5, 10, 25, 50, 75, 100, 200, 300, 500, 700 and 1,000 μM for 48 h in the presence of 2 and 10 mM D-glucose. The cell viability was evaluated by dye uptake assay using crystal violet. The data on cell viability study indicated clearly that in the present experimental conditions, the cytotoxic effect of MPP + in 2 mM glucose was dose dependent and that significant cell death with respect to control was observed at concentrations above 10 μM of MPP+. As anticipated, the 10 mM glucose protected the cells against MPP+ induced toxicity at all concentrations. The extent of protection was observed in the range of 5 to 50%. The average LC50 value in 2 mM glucose was 29.5 μM, while in 10 mM glucose this value was 265 μM, a nine-fold increase. In conclusion, 10 mM glucose was shown to protect MPP+ induced toxicity in lung carcinoma cells. (Supported by NIH grant 03020).

A-2014

Migration of Human Mesenchymal Stem Cells is Inhibited by Norepinephrine Through the Beta1-Adrenergic Pathway. SHIVAM VERMA, Kenneth Clark, Vivek Verma, Xueyin N. Huang, Wen Yan, and Jorge L. Sepulveda. Department

of Pathology, University of Pittsburgh, Pittsburgh, PA 15213. Email: Shivam222@gmail.com

In recent years, a number of stem cells have been proposed as potential candidates for cellular cardio-myoplasty. One such cell type is the human bone marrow mesenchymal stem cell (MSC). These cells have been shown to migrate to and seed the heart after myocardial infarction, augment cardiomyocyte function, and to a minor degree, differentiate into a cardiomyocyte phenotype. What is not known, however, is how the post-infarction environment affects MSC migration. We investigated, in cell culture, how elevated levels of norepinephrine affect the migration of these cells. Human mesenchymal stem cells were cultured in standard 12-well dishes with a variety of alpha and beta-adrenergic agonists and antagonists in the presence of fetal bovine serum or hepatocyte growth factor. Motility was assessed by a wound assay or by migration through an 8 μm pore membrane. At 3–4 d after wounding, cells subjected to physiologic and supra-physiologic concentrations of norepinephrine exhibited a statistically significant reduction in migration in a dose-dependent manner. This effect is mediated primarily through the beta1 adrenergic receptors, which are shown to be expressed in undifferentiated human MSC's by RT-PCR. Migration of human mesenchymal stem cells is inhibited by norepinephrine primarily through the beta1 receptor mediated pathway. Although this effect is subtle at normal physiologic catecholamine concentrations, it is amplified at elevated levels often seen in post-infarcted myocardium. These results have potential implications for myocardial infarction treatments involving beta-blockers and cell therapy.

A-2015

Spots-On-Dots™: A Frameless Microarray Platform for High-throughput Protein Analysis. NICHOLAS CARUCCIO, William Patterson, Tobias Zutz, and Thomas Burke. Primorigen Biosciences, 510 Charmany Drive, Madison, WI 53719. Email: nick.caruccio@primorigen.com

Protein microarrays are a powerful tool for multiplexed biomarker profiling; however, current platforms require cumbersome frames and costly equipment, restricting assay layout and throughput. Spots-On-Dots™ is a versatile platform for multiplexed protein detection that features a grid of nitrocellulose dots on a glass or plastic substrate. Individual dots are arrayed (printed) with protein targets (>100 spots per dot). Test samples applied to the printed nitrocellulose dots are isolated within the hydrophobic surface, eliminating the need for frames. Colorimetric assays can be read with a standard flatbed scanner, expanding assay throughput and layout options and also eliminating the need for dedicated readers. Fluorescent assays compatible with existing equipment can also be developed. Frameless microarrays support a broad variety of immunoassays including; multiplexed bio-marker expression analysis, rapid selection and characterization of matched-pair antibodies, and determining kinetic and equilibrium binding data. Primorigen Biosciences is using Spots-On-Dots™ to develop content-based, printed antibody microarray kits including: (1) Determ-A-Derm™, a multiplexed array to determine early stem cell lineages, (2) an array for rapid, low-cost, isotyping of antibody samples, (3) an array with proprietary content to monitor early pancreatic β-cell-lineage markers, (4) a multiplexed array to characterize mature pancreatic β-cell function. Primorigen Biosciences is interested in identifying collaborators to co-develop additional assays.

A-2016

An In Vitro Cellular Model for Neurodegeneration with Proteasomal Mutation. ZONGMIN LI[1] and Maria Figueiredo-Pereira[2]. [1]Department of Biological Sciences, New York City College of Technology of City University of New York, Brooklyn, NY 11201, and [2]Department of Biological Sciences, Hunter College of City University of New York, New York, NY 10021. Email: zli@citytech.cuny.edu

Accumulation of ubiquitinated proteins in inclusions is common to various neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, although it occurs in selective neurons in each disease. The mechanisms generating such abnormal aggregates and their role in neurodegeneration remain unclear. Proteasome impairment triggered by aging or conditions such as oxidative stress may contribute to protein accumulation and aggregation in neurodegeneration. To test this hypothesis in mouse neuronal cells, we overexpressed a 20S proteasome beta-5 subunit with a mutation on its active site. The N-terminal threonine to alanine substitution resulted in impairment of the chymotrypsin-like activity, which is a rate-limiting step in protein degradation by the proteasome. The Thr1Ala mutation was not lethal under homeostatic conditions. However, this single amino acid substitution significantly hypersensitized the cells to oxidative stress, triggering not only the accumulation and aggregation of ubiquitinated proteins, including synuclein, but also cell death. Our results demonstrate that this genetic manipulation of proteasome activity involving a single amino acid substitution causes the formation of protein aggregates in stressed neuronal cells independently of the occurrence of mutations in other cellular proteins. This cellular model could be a useful tool to further address the molecular mechanism of neurodegeneration associated with proteasomal impairment.