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Animal Posters

A-2000

The Role of Various Phospholipase A_2 Enzymes in Key Cell Signaling Events Affiliated with Tumor Promotion. B. L. UPHAM, J. S. Park, P. Babica, O. Adamovsky, and J. E. Trosko. Department of Pediatrics and Human Development, and National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI, 48824. Email: upham@msu.edu

Lower molecular weight polycyclic aromatic hydrocarbons (PAH) with specific structural features are potent inhibitors of gap junctional intercellular communication (GJIC), and activators of mitogen-activated protein kinases (MAPKs) and the release of arachidonic acid (AA), three cellular events linked with tumor promotion and other pathologies. We found that inhibition of GJIC, and activation of MAPKs and AA-release are induced in a rat liver epithelial cell line (WB-F344) within several minutes by 1-methylanthracene (1-MeA), but not 2-methylanthracene (2-MeA). In a second SIVB abstract, Babica et al. presents evidence for the involvement of phosphatidylcholine-specific phospholipase C (PC-PLC) in the dysregulation of GJIC. Here we report that PC-PLC is not involved in the activation of MAPKs and the induction of AA-release. Inhibitors to the various families of phospholipase A_2 (PLA_2) and phospholipase D (PLD) were used to link the activity of PLD and distinct forms of PLA_2 to PAH-induced affects on GJIC, MAPK and AA-release. Secretory ($sPLA_2$), cytosolic ($cPLA_2$), and calcium independent ($iPLA_2$) were not involved in the dysregulation of GJIC and activation of MAPK by PAH. The induction of AA-release by PAH was dependent on $sPLA_2$, but not $cPLA_2$ and $iPLA_2$. PLD was not involved in any of the PAH-induced events on GJIC, MAPK, and AA-release. These results indicate that in response to PAH, GJIC and AA-release in WB-F344 cells are phospholipase specific. Support: NIEHS grant #R01 ES013268–01A2 to Upham.

A-2001

The Established Insect Cell Line, BCIRL-HzAM1, Expresses a Cellular and a Secretory Phospholipase A_2. D. Stanley and C. L. GOODMAN. USDA ARS, Biological Control of Insects Research Laboratory, 1503 S. Providence Rd., Columbia MO 65203. Email: Cindy.Goodman@ars.usda.gov; David.Stanley@ars.usda.gov

Phospholipases A_2 (PLA2s; EC 3.1.1.4) are ester hydrolases that release free fatty acids from the *sn*-2 position of phospholipids (PLs). These are virtually omnipresent enzymes found in all organisms. Secretory ($sPLA_2$s) and cellular ($cPLA_2$s) are known. PLA_2s act in many areas of biology, including digestion, signal transduction and immune defenses. These enzymes act in human medicine and most of our knowledge of PLA_2s comes from work on humans and related model mammals. We have far less information on insect PLA_2s aside from the presence and actions of $sPLA_2$s in insect venoms. Beyond this, PLA_2 actions have been recorded in insect digestion, host defense, reproduction and fat body functions. We posed the hypothesis that established insect cell lines express PLA_2s. Using unfractionated cell homogenates, we detected PLA_2 activity that is sensitive to incubation time, reaction pH, temperature, protein concentration and radioactive substrate concentration. The PLA_2 activity exhibits a strong preference for arachidonyl-containing substrate (indicates a $cPLA_2$) and is not influenced by calcium. There are at least two PLA_2s in the cell line as enzyme activity was sensitive to a $sPLA_2$ inhibitor and to a $cPLA_2$ inhibitor. We detected PLA_2 activity in the cell culture medium, indicating the presence of a $sPLA_2$. The significance of this work lies in the roles of prostaglandins (PGs) in the biology of established insect cell lines. So far we have reported that PGs influence gene expression in BCIRL-HzAM1 cells. In recent unpublished research we found that PGs act in

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baculoviral replication in these cells. Our results presented in this poster indicate that established insect cell lines are competent to release arachidonate from cellular PLs, the first step in PG synthesis.

A-2002

Tumor Promotion-relevant Cell Signaling: Key Roles of Annexins and Phospholipases. P. BABICA[1], J. S. Park[1], I. Sovadinova[1], L. Blaha[1], D. A. Whitten[2], C. G. Wilkerson[2], J. E. Trosko[1], and B. L. Upham[1]. [1]Department of Pediatrics and Human Development, and National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI, 48824; and [2]RTSF - Proteomic Core, Michigan State University, East Lansing, MI, 48824. Email: babica@msu.edu

Lower molecular weight polycyclic aromatic hydrocarbons with specific structural features are potent inhibitors of gap junctional intercellular communication (GJIC) and activators of mitogen-activated protein kinases (MAPKs), two cellular events linked with tumor promotion and other pathologies. We found that inhibition of GJIC and activation of MAPKs are induced in a rat liver epithelial cell line (WB-F344) within several minutes by 1-methylanthracene (1-MeA), and these effects are preceded with the activation of phosphatidylcholine-specific phospholipase C and the release of arachidonic acid suggesting an activation of phospholipase A2. Another signaling enzyme involved in GJIC dysregulation was proteinkinase A (PKA), as indicated by blocking of 1-MeA effects on GJIC by PKA-inhibitors as well as by activation of PKA regulated proteins CREB and ATF-1. Proteomic experiments revealed annexins A1, A3 and A5 responding to 5 min 1-MeA treatment by their disappearance from plasma membrane and reciprocal increase in the fraction of soluble proteins. Western blotting and immunostaining experiments on annexin A3 indicated a translocation from the plasma membrane even within 30 s of exposure followed by reintegration back into the plasma membrane after 60 min. Since annexins A1 and A3 have been previously described as PLA2 inhibiting proteins, we hypothesize that annexins closely interact with phospholipases until removed from the membrane in response to 1-MeA, and the subsequent phospholipase-induced events then regulate PKA, GJIC and MAPKs. Support: NIEHS grant #R01 ES013268–01A2 to Upham.

A-2003

Differential Expression of Full-length and Truncated Cullin-5 is Associated with Granulocytic Differentiation of HL-60 Leukemia Cells. M. J. FAY, G. K. Tan, S. S. Baxter, M. L. Hall, A. M. S. Mayer, and L. A. Carlson. Department of Pharmacology, 555 31st Street, Midwestern University, Downers Grove, IL 60515. Email: mfayxx@midwestern.edu

All-*trans* retinoic acid (ATRA) promotes granulocytic differentiation of HL-60 promyelocytic leukemia cells to neutrophil-like cells. Previously we demonstrated that ATRA-mediated granulocytic differentiation of HL-60 cells is associated with increased mRNA expression for Cullin-5, ROC-2 and ASB-2. The Cullin-5, ROC2 and ASB-2 proteins form an E3 ubiquitin ligase complex that functions to target proteins for ubiquitin-mediated degradation by the 26 S proteasome. The purpose of this research was to examine Cullin-5 protein expression during ATRA-mediated granulocytic differentiation of HL-60 cells. The HL-60 cells were treated for 5 d with 1.0 μM ATRA and Cullin-5 protein expression was evaluated using SDS-PAGE and Western blot analysis. Granulocytic differentiation of the cells was associated with a 6.5-fold increase in the expression of full length Cullin-5 (~84 KDa) versus the vehicle control group. In contrast, truncated immunoreactive Cullin-5 proteins with molecular weights of ~25 and 26 KDa were expressed in the vehicle control cells but not in the ATRA-treated cells. The increase in the expression of full-length Cullin-5 was apparent after 1 d of ATRA treatment and the truncated immunoreactive Cullin-5 proteins were not present at 3 d of ATRA treatment. Since full-length Cullin-5 is reported to be anti-proliferative and truncated Cullin-5 is reported to be proliferative, the differential expression of full-length and truncated Cullin-5 proteins may play an important role during granulocytic differentiation of HL-60 cells. This research was supported in part by NIH CA122003–01 and Midwestern University.

A-2004

Effects of 5-Fluorouracil Treatment on MicroRNA Expression in MCF-7 Breast Cancer Cells. MAITRI SHAH, Mary A. Farwell, and Baohong Zhang. Department of Biology, East Carolina University, Greenville, NC 27858. Email: mys0912@mail.ecu.edu

Breast cancer is one of the leading causes of deaths worldwide. 5-Fluorouracil (5-FU) is one of the chemotherapeutic drugs widely used for breast cancer treatment. Although a study demonstrated that 5-FU induced the aberrant expression of several protein-coding genes in breast cancer cells, the regulatory mechanism of 5-FU-induced gene expression is unknown. In our present study, we found that 5-FU inhibits the growth of MCF-7 cells in vitro with a dose-dependent manner. We are currently performing microRNA microarray and quantitative real-time PCR (qRT-PCR) analysis to test the hypothesis that microRNAs regulate the effect of 5-FU on protein-coding

genes and microRNAs are contributed to the drug sensitivity in breast cancer treatment.

A-2005

The Effect of Green Tea Polyphenon 60 Treatment on miRNA Expression in MCF7 Breast Cancer Cells. LINDSEY N. FIX, Mary A. Farwell, and Baohong Zhang. East Carolina University, Department of Biology, Howell Science Complex S111A Greenville, NC 27834. Email: lnf1122@ecu.edu

The beneficial effect of Green Tea on breast cancer prognosis has been widely documented in Eastern Countries identified as having high tea consumption rates. The biochemical basis of the observed anti-cancer characteristic has been documented as originating from the cellular effects of polyphenols extracted from *Camellia sinensis* leaf. The specific biological mechanism behind these properties has yet to be elucidated but present data implicates that microRNAs (miRNAs) may contribute to this regulatory mechanism. In the present study, MCF7 breast adenocarcinoma cells treated with polyphenon 60 were analyzed for cellular viability under various treatment concentrations. The cytotoxic effects of polyphenon 60 indicate that the biologically active polyphenols alter the cellular environment of breast cancer cells and cause cell death even at low physiological concentrations. We are currently performing miRNA microarray and qRT-PCR analysis to test the hypothesis that polyphenol-induced modification of the miRNA expression pattern contributes to the green tea effect on breast cancer development.

A-2006

Development of Organ Culture Conditions for Maintenance of Normal and Malignant Human Colon Tissue. N. BHAGAVATHULA, M. K. Dame, M. N. Aslam, and J. Varani. Dept. of Pathology, #4224, Med Sci 1, University of Michigan, Ann Arbor, MI 48109. Email: narasim@umich.edu

We have developed a method to maintain both normal and neoplastic human colon tissue in organ culture. Tissue viability was optimal in serum-free medium supplemented with a number of growth factors and 1.5 mM Ca^{2+}. Tissue was incubated at 90% oxygen and partially submerged with the mucosal layer at the interface. Tissue was cultured for 0, 18, 24 and 48 h in the medium and fixed in 10% buffered formalin. Immunohistochemical analysis of tissue was done for Ki67, E-cadherin and β-catenin. Zero-time fresh tissue was used for comparison. Histologically, tissue structure of the normal and malignant components was maintained. Ki 67 staining was strong in all proliferative regions of the tissue, and was particularly strong in tumor and in the undifferentiated sections of the normal epithelium. E-cadherin was observed in the differentiated regions of the tissue with focal loss of E-cadherin in the tumor sections. β-catenin expression was also evident at the membrane in differentiating cells with diffuse staining in the cytoplasm/nuclear tumor regions. We conclude that human colon tissue can be maintained in organ culture, with preservation of physiological functions unique to normal and malignant tissue.

A-2007

The Canadian Council on Animal Care: Replacement, Reduction, Refinement Alternatives Online. GILLY GRIFFIN, and Nicole Fenwick. Canadian Council on Animal Care 1510–130 Albert St., Ottawa, Ontario, CANADA. Email: ggriffin@ccac.ca

In Canada, continuing improvements to the welfare of animals used in science have occurred over the past few decades partly because of the adoption the Three Rs tenet (Replacement, Reduction, Refinement) to guide the ethical evaluation of animal use. The Three Rs tenet is grounded in the premise that animals should be used only if a scientist's best efforts to find a non-animal alternative have failed, and that when animals are needed, only the most humane methods and care should be used on the smallest number of animals required to obtain valid information. The Canadian Council on Animal Care (CCAC) is Canada's national organization providing peer-review based oversight of animal use in science. To further its mandate, the CCAC has recently established a Three Rs Program and launched a new website dedicated to providing information about the Three Rs to investigators. The "Three Rs Microsite" places a special emphasis on providing researchers at the proposal-development stage with a comprehensive range of Three Rs tools to assist them in designing humane experiments and preparing animal care protocols. The Three Rs Microsite will be continually evolving to include new information and to respond to feedback and requests from researchers. In particular, the CCAC is asking scientists to contribute information to help us further develop the Replacement Alternatives section of the microsite.

A-2008

Efficacy Testing of a Novel Retinoid in Gottingen Mini-pig Skin Organ Culture Yields Comparable Results to That in Intact Animal. M. K. DAME[1], T. Paruchuri[1], M. DaSilva[1], N. Bhagavathula[1], W. Ridder[2], and J. Varani[1]. [1]Department of Pathology, The University of Michigan Medical School, Ann Arbor, MI 48109; and [2]Ricerca Biosciences LCC, Concord, OH 44077. Email: mdame@med.umich.edu

Gottingen minipigs were treated topically with MDI-301, a novel analog to all-trans retinoic acid. MDI-301 has unique potential due to its low skin irritability even at higher doses. The 7-day treatment (0.3 to 30%) of the minipigs did not adversely affect their health (hematological and necroscopy parameters) or produce any changes suggestive of retinoid-induced skin irritation. Although the original project objective was to access the drug in a FDA-approved preclinical safety study, here we focus on the subsequent organ culture of the minipig skin. After sacrificing the animals, skin samples from each treatment site were excised and cultured. As well, untreated skin tissue was dosed in vitro with MDI-301 (0.1–5 μg/ml). After 3 d, the culture supernatants were collected and analyzed for levels of collagen type I. Both skin samples treated in vivo, and skin treated in vitro showed rising collagen production with increasing concentrations of MDI-301 following organ culture. After 6 d, the skin was formalin-fixed and stained for histology. Epidermal hyperplasia was quantified at various MDI-301 concentrations. In vivo and in vitro treatments showed similar results – although the thickness was not substantially changed on average, there were focal areas of hyperplasia at higher retinoid concentrations. Taken together, these data suggest that (1) MDI-301 enhances collagen production in minipig skin, without irritation, and (2) that minipig skin organ culture appears to be equally predictive of this result and can be an alternative model to that of the intact animal for skin drug testing.

A-2009

Healing of Dermal Wounds in the EpiDerm-FT In Vitro Human Skin Model: PATRICK J. HAYDEN, G. Stolper, C. Cooney, and M. Klausner. MatTek Corporation, Ashland, MA. Email: phayden@mattek.com

Dermal wound healing involves interactions between dermal fibroblasts and epidermal keratinocytes, as well as cell and extracellular matrix interactions. The current poster describes wound healing experiments conducted with a full−thickness in vitro human skin model (EpiDerm−FT™). Normal human epidermal keratinocytes (KC) and dermal fibroblasts (FB) were cultured to produce the highly differentiated full−thickness skin model. Small wounds of several mm in diameter were induced in the epithelial model by means of a battery operated cauterizer or a dermal biopsy punch. The wounded EpiDerm−FT™ cultures were fixed at various time points and H&E stained paraffin sections were prepared to evaluate the wound and the wound healing process. Immediately after burn wounding, necrotic epithelium and denatured collagen dermal matrix were evident. Within one day, the denatured collagen matrix began to degrade and epithelial KC were observed migrating inward from the wound edges. Over a time course of seven days, migrating KC repopulated the wounded area to form a fully covered epithelium. Dermal fibroblasts were also observed to be proliferating within the wound area and generating new dermal matrix material. Biopsy punches were used to produce wounds that removed only the epidermis. These wounds also healed within a timeframe of 3−7 d. Increased FB proliferation in dermal areas directly adjacent to migrating KC was observed. These results demonstrate that EpiDerm−FT™ is a useful model for applications designed to elucidate dermal−epidermal interactions during wound healing and to evaluate the role of specific growth factors or new therapeutics in the dermal wound healing process.

A-2010

Use of Cultured Human Skin Cells and a Skin Tissue Construct as Models to Study the Genotoxic Marker γ-H2AX Following Sulfur Mustard Exposure. A. L. MILLER, C. L. Gross, E. W. Nealley, O. E. Clark, N. K. Waraich, K. L. Rodgers, and W. J. Smith. Research Division, United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010–5400. Email: adele.miller@us.army.mil, corresponding author: William.j.smith3@us.army.mil

Sulfur mustard (2–2′-dichlorodiethyl sulfide, SM) is a cytotoxic chemical warfare agent. The skin serves as a principal target site for in vivo toxicity of SM exposure resulting in the formation of blisters and inflammation. To elucidate genotoxic effects of SM, Normal Human Epidermal Keratinocytes (NHEK, Lonza Corp., MD) and a commercially available, multicellular skin tissue construct, EpiDerm™ (MatTek Corp., MA), served as in vitro models to observe the presence of γ-H2AX foci. γ-H2AX is a phosphorylated derivative of the H2AX histone and is tightly bound to double stranded DNA break sites. In its phosphorylated state, H2AX can be is used as a reproducible indicator of genotoxic injury. Cells and constructs were exposed to 0, 50, 100 and 300 μM concentrations of SM for 2 and 24 hrs. Following exposure, tissues underwent fluorescent immunohistochemistry using mouse anti-γ-H2AX antibody. Propidium iodide (PI) was used as a nuclear stain. NHEKs were fixed, permeabilized and incubated with mouse anti-γ-H2AX antibody. Cells were analyzed for γ-H2AX via flow cytometry. Our results show that SM exposure results in the formation of γ-H2AX foci and this change is most prevalent at 300 μM SM then at the lower doses. This indicator of DNA damage will be a useful biomarker for the study of SM toxicity and therapy. This research was supported by the Defense Threat

Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

A-2011

Human Bronchial Epithelial Cells as a Model for Human Lung Airway Injury and Therapeutic Intervention Following Sulfur Mustard Exposure In Vitro. O. E. CLARK[1], E. W. Nealley[1], A. L. Miller[1], Y.-S. Jung[2], and W. J. Smith[1]. [1]Research Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010–5400; and [2]Korea Research Institute of Chemical Technology, P.O. Box 107, Yueseong, Daejeon 305–606 KOREA. Email: offie.clark@us.army.mil, Corresponding author: William.j.smith3@us.army.mil

Sulfur mustard (2, 2′-dichlorodiethyl sulfide; SM) is a cytotoxic alkylating agent with vesicating properties. In the airway, SM injury is characterized by acute inflammation of the upper and lower airways, and inflammatory exudate. We have evaluated the normal human bronchial epithelial (NHBE) cells (Lonza Corp. Walkersville, MD) as an in vitro model of human lung airways. Our research goal is to determine the mechanism of toxicity of SM leading to the development of medical countermeasures. The release of cytokines can increase the cytotoxic response to cell injury. In this model we use (1) the release of inflammatory cytokines, (2) cell viability and (3) cellular morphology to characterize the cytotoxic effects of sulfur mustard. Capsaicin and its analogs are known to block neurogenic inflammatory pathways, and have shown the ability to prevent SM damage in the mouse ear vesicant model. We evaluated the ability of the novel compound KR-24095 (capsaicin analog) to prevent SM-induced toxicity. Cultures were grown until they reached 70–80% confluency. Then pretreated for 15 min. with KR-24095 and exposed to SM. Cytokine levels were measured with cytokine multi-plex assay kits (Invitrogen, Carlsbad CA.). Cell viabilities were determined 24 hrs. after SM exposure by flow cytometry. Morphological changes were documented by light microscopy. SM at 100 uM decreased viability, but pretreatment with KR-24095 reduced cell death following SM exposure. The release of Il-10, Il-8, Il-6, Il-5, Il-2, Il-1b, IFN-g, TNF-a, and GM-CSF induced by 300uM SM was reduced in NHBE treated with KR-24095. A capsaicin analog may serve a dual purpose of being anti-inflammatory and protecting against SM-induced cell death. The NHBE model will be an important tool in both defining the mechanisms of SM toxicity and establishing the efficacy of potential medical countermeasures against SM injury. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

A-2012

Lack of Genotoxicity in Cultured Human Small Airway and Microvascular Cells Following Exposure to the Nerve Agent, VX. C. L. GROSS, E. W. Nealley, A. L. Miller, M. T. Nipwoda, O. E. Clark, and W. J. Smith. Research Division, U. S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010–5400. Email: clark.gross@us.army.mil, Corresponding author: William.j.smith3@us.army.mil

Chronic exposure to organophosphate pesticides show a marked genotoxicity in workers involved in their manufacture, as well as workers in the farming community. Nerve agents are much more potent organophosphates than their pesticide cousins, but are not considered to be genotoxic or carcinogenic since their powerful effects on cholinesterase and cholinergic pathways are well known and responsible for their lethality. Chronic low-dose exposure of the potent nerve agent soman in guinea pigs has shown DNA fragmentation in isolated blood leukocytes by using the single cell electrophoresis assay known as the comet assay. In prior experiments, we did not notice any genotoxicity in normal human epidermal keratinocytes (NHEK) acutely exposed to low-dose VX (0.1 nM – 0.1 μM), another very powerful nerve agent. We exposed normal strains of human small airway epithelial cells (SAEC) and microvascular epithelial cells (MVEC) grown in culture to higher concentrations of VX from 0.01 μM to 50 μM for 2 h. The cells were then harvested and processed for genotoxicity analysis by the single cell electrophoresis assay (Comet assay) under alkaline conditions. After staining, a minimum of 50 cells for each VX concentration was analyzed by fluorescence microscopy using Loat's comet analysis software. Results from these studies show no statistical change in Comet moment (a measure of DNA damage) in VX-exposed SAEC and MVEC compared to the unexposed controls. Cytotoxicity of VX-exposed cells was determined by flow cytometry using propidium iodide uptake and also showed no statistical change from controls. It is concluded that VX has no direct effects on genotoxicity or cytotoxicity to SAEC or MVEC under these acute exposure conditions. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

A-2013

Comparison of Fish Cell Line Responses to Chemicals and Process-Affected Waters from the Oil Sands of Alberta, Canada. B. SANSOM[1,2], R. Kavanagh[3], M. MacKinnon[4], D. G. Dixon[1], and L. E. J. Lee[2]. [1]Department of Biology,

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The extraction of bitumen from the Athabasca Oil Sands of Alberta, produces large volumes of tailings and process-affected waters (OSPW) that are stored on the operators' leases before they are reclaimed. Reclamation of the fluid tailings components of the OSPW proposes to utilize end pit lakes (EPLs) where the OSPW is isolated by a water cap in which a lake system will develop. In order to be deemed successful, this lake must be capable of supporting a fish population. However, until the lake is commissioned and allowed to undergo natural bioremediation processes over time, it is difficult to predict how various fish populations will perform with chronic exposure to OSPW. Aspects of toxicity to biota, including fish, will be part of the evaluation of this technique. Through the use of established fish cell-lines, but more importantly new cell-lines developed from native fish to the oil sands region, an economical and non-lethal bioassay is being developed and validated to support reclamation planning. This tool will aid in designing the continuous monitoring programs which will be implemented in the full-scale EPLs. Use of experimental ponds/lakes containing OSPW provides a range of water quality for testing the suitability of this in vitro cell-line approach. Because of the scale and species selectivity, such testing could circumvent the need for transporting and testing using currently available fish bioassays. The fish cell line approach could lead to a prediction of fish survival and success in a range of OSPW impacted aquatic systems without the need to perform fish lethality tests. With the fish in vitro approach, predicting performance capability within aquatic reclamation habitats and impacts of OSPW (runoff, seepage, groundwaters) to fish habitats in receiving aquatic systems (local streams, Athabasca River) could be accomplished without the need to sacrifice of large numbers of test animals. Towards this goal, the present work evaluates the sensitivity of fish cell line bioassays and end-points to detect toxicity of oils sands chemicals such as naphthenic acids (NAs), polycyclic aromatic hydrocarbons (PAHs), surfactants and salts that have been shown to be the main toxic components of OSPW. Additionally, direct testing of OSPW samples on fish cells, by mixing the test waters with media salts and adding directly to the cells in culture without prior extraction procedures, and an innovative triple-cell-line assay for increased sensitivity of chemical exposures will be presented.

A-2014

In Vitro Determination of Protective Antigen Stability in a Novel, Transdermal Anthrax Vaccine. KEVIN E. KNOCKENHAUER[1], Katarzyna M. Sawicka[2], Elizabeth J. Roemer[3], and Sanford R. Simon[1,3]. [1]Department of Biochemistry; [2]Department of Biomedical Engineering; and [3]Department of Pathology, Stony Brook University, Stony Brook, NY 11794–8691. Email: kknockenhauer@gmail.com

The current vaccination paradigm for the prevention of Anthrax is insufficient to deal with a potential, widespread epidemic. The FDA-approved vaccine requires trained medical personnel to administer the intramuscular injection: making the inoculation of large populations difficult. To solve this issue, we propose a transdermal, self-administrable vaccine patch capable of delivering protective antigen (PA), a binding protein secreted by *Bacillus anthracis*, through the stratum corneum. Host immunity from the current vaccine is gained via IgG antibody recognition of PA, which facilitates the entry of the lethal factor (LF) metalloprotease into monocytes: suppressing proinflammatory cytokine production. This patch is comprised of solid polyvinylpyrrolidone nanofibers, containing encapsulated PA, and is produced by the straight forward, cost effective technique of electrospinning. The PA-containing membrane affords an unsurpassed surface area to volume ratio. The current study aims to determine the long-term storage capabilities of the electrospun membrane at differing temperatures and the effect this storage has on nano-encapsulated PA, as compared to that of PA stored in solution (control) and PA dried on silicon wafer substrates. At various time points, the retained functionality of PA has been confirmed and quantified by delivering it along with stock LF onto Mono Mac 6 cells, a human monocytic cell line, in a dose-dependent fashion: where interleukin-6 down regulation was used as proof of function. Sustained immunoreactivity of PA at each time point has been confirmed and quantified using an immunoblotting assay. The current vaccine must be stored between 2–8°C, with formaldehyde and benzethonium chloride as necessary preservatives. The nano-encapsulation process proposed here is an efficient method to confer protein stability, since a PA-containing membrane incubated overnight at room temperature without desiccant retained functionality, while stock PA in solution, according to the certificate of analysis, is stable between 2–8°C for only a few hours.

A-2015

Temperature Induced Morphological Changes and Microsporidia Growth in the EP-1 Cell Line. S. R. MONAGHAN[1,2],

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Microsporidia are obligate intracellular parasites. There are at least 156 species of microsporidia known to infect fish, of which only 4 have been grown in fish cell cultures. Cell lines have been convenient systems to study microsporidia, and these have been widely used for the study of mammalian and/or insect microsporidia. This approach has been infrequently done with fish cell lines. EP-1 is an epithelial cell line derived from Japanese eel (*Anguilla japonica*) that is persistently infected with the microsporidium *Heterosporis anguillarum*. This work evaluates the EP-1 cell line as model to understand the effects of temperature on microsporidial development. It is believed that in wild fish, infections may remain latent until water temperatures increase in late summer and early autumn, when microsporidiosis often becomes symptomatic. EP-1 cells exposed to varying temperatures from 4°C to 32°C have been evaluated. Hemocytometer counts verify that 28° C is the optimal growth temperature for the cell line with an increase in cell number at temperatures from 21 to 32°C but slowly or no growth observed at lower temperatures. As temperatures increase above 21°C, an increase in giant cell formation is observed. These cells when observed under transmission electron microscopy show microsporidial meront accumulation. DAPI dilactate staining of EP-1 also correlates with meront development observed using the transmission electron microscope. Preliminary investigations also indicate a trend toward increased microsporidial growth with increased temperatures, with greatest parasitic growth at 32°C. This was observed with Giemsa staining (indirect microsporidial stain). Thus, temperature appears to have an important regulatory role in microsporidial development in fish cells.

A-2016

Establishment of a Monarch Butterfly (*Danaus plexippus*, Lepidoptera: Danaidae) Cell Line and its Susceptibility to Insect Viruses. JAMES J. GRASELA and Arthur H. McIntosh. USDA, Agricultural Research Service, Biological Control of Insects Research Laboratory 1503 S. Providence Road, Research Park, Columbia, MO 65203–3535. Email: graselajj@missouri.edu

A cell line from the monarch butterfly *Danaus plexippus* designated BCIRL-DP-AM/JG was established from adult ovaries. The cell line consisted mainly of round cells and took a prolonged period of time in the growth medium ExCell 401 containing 10% fetal bovine serum and antibiotics before it could be subcultured on a regular basis. The cell line had a population doubling time of 32 h and was susceptible to four baculoviruses (MNPV) but was retractile to a fifth baculovirus, a single nucleopolyhedrovirus (HzSNPV). PxMNPV was the most infectious of the MNPV for BCIRL-DP-AM/JG cell line of *D. plexippus* producing a titer of 5.9×10^7 $TCID_{50}$/ml. The non-occluded Hz-1 virus was also infectious for the *D. plexippus* cell line. The occlusion bodies produced by the BCIRL-DP-AM/JG cell line were infectious for 24 h old *T. ni* larvae and gave LC_{50} values equivalent to or better than the LC_{50} for OB produced in the other susceptible cell lines by the MNPV under study. The identity of the monarch butterfly cell line was established by DAF-PCR.

A-2017

Immunocytochemistry and Image Analysis of Beta-catenin Redistribution in Normal Human Colon Cell Cultures Treated with Disinfection By-products. ERNEST WINKFIELD[1], Mary Moyer[2], and Anthony DeAngelo[1]. [1]U. S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC 27711 and [2]INCELL Corporation, San Antonio, TX. Email: winkfield.ernest@epa.gov, deangelo.anthony@epa.gov

Epidemiological studies have shown an association between the consumption of chlorinated drinking water and increased risk for colon cancer. In vivo studies demonstrated that rodents exposed to chlorination disinfection byproducts developed aberrant crypt foci in the colon, and that some of these aberrant crypt foci developed into tumors. These aberrant crypt foci are characterized by altered Wnt signaling and changes in beta-catenin localization. We are developing an in vitro/in vivo assay to rapidly predict the carcinogenic risks posed by unregulated drinking water contaminants. The assay is based on the phenotypic transformation of NCM460 normal human colonocytes after exposure to test chemicals. Image analysis methods, using Alexa Fluor 488 conjugated antibodies to beta-catenin and DAPI double stranded DNA stain, have been developed to characterize changes in the intracellular localization of beta-catenin after chemical exposure. These methods were used to analyze the intracellular redistribution of beta-catenin in NCM460 colonocytes treated with the disinfection byproducts dibromonitromethane and tribromonitromethane. We were able to demonstrate the relocation of beta-catenin from the cytoplasmic membrane to the nucleus in chemically treated colonocytes by means

of fluorescence immunocytochemistry, object visualization, image capture and processing as well as an analysis of colocalized events using Pearson's correlation coefficient and Manders' overlap coefficients and colocalization patterns. These methods provide a useful approach to identifying and assessing highly colocalized fluorescence activity generated by fluorescent antibody interactions with DNA staining. *This abstract does not necessarily reflect EPA policy.*

A-2018

OLGA: A Case of Mistaken ID. LUCY E. J. LEE[1,2], William J. Martin[1], Andrew Christie[2], Marc Frischer[3], Thomas Soin[4], Guy Smagghe[4], Heather Braid[5], and Robert Hanner[5]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA; [2]Mount Desert Island Biological Laboratory, Salisbury Cove, ME; [3]Skidaway Institute of Oceanography, Savannah, GA; [4]Department of Crop Protection, Ghent University, Ghent, BELGIUM; and [5]Department of Integrative Biology, University of Guelph, Guelph, ON, CANADA. Email: llee@wlu.ca

Development of aquatic invertebrate cell lines have proven elusive and very few continuous cell lines have been reported from molluscan or crustacean species. BGE, a snail cell line derived from *Biomphalaria glabrata* embryos is so far the only molluscan cell line available from the American Type Cell Culture Collection (ATCC CRL 1494). ATCC is also the repository for what is believed to be the only crustacean cell line: OLGA-PH-J/92 (ATCC CRL-2576) reputedly derived from the cerebral ganglia of the crayfish *Orconectes limosus.* Although BGE has been widely used and characterized, the use of OLGA cells have seldom been reported aside from the original publication by Neumann et al. 2000 (In Vivo 14(5): 691–698). In an attempt to develop other crustacean cell lines, we analyzed the growth characteristics of OLGA and attempted to use OLGA's conditioned media to induce cellular proliferation in lobster, crab and shrimp cells. However, the growth characteristics of the cells, including ability to survive in pure water without any media additives and survival at a wide temperature range prompted us to question the origin of the cell line. Molecular and immunological evaluation failed to establish that the cell line was of crustacean origin. DNA amplification and sequencing of both, the cytochrome c oxydase I gene and the 18S RNA gene for several separate batches of OLGA failed to identify the cells as being originated from crayfish. Sequencing of the amplified 18S RNA gene fragment matched most closely to fungal (basidiomycetes) sequences. In contrast, BGE cells were corroborated as originating from *B. glabrata* using multiple genetic markers. We do not believe that OLGA became contaminated with fungal cells as the morphology of the cell line is still identical to the ones reported originally and with micrographs available from ATCC for the said cell line. The evaluated OLGA cells had been obtained separately from the originating source and three separate laboratories were unable to confirm the taxonomic origin of OLGA. Furthermore, immunohistochemical screening for the expression of a suite of invertebrate neuropeptides gave negative results, precluding an invertebrate neural origin. Thus, molecular and other evidence unequivocally indicates that the OLGA-PH-J/92 cell line is not derived from the crayfish *O. limosus*.

A-2019

Development of an *Aiptasia pallida* Cell-culture System to Study Cnidarian-Dinoflagellate Symbiosis. JAN DENOFRIO, and John Pringle. Stanford University, Dept of Genetics, 300 Pasteur Dr., Stanford, CA 94305. Email: denofrio@stanford.edu

Coral reefs have been declining worldwide. The health of reef-building corals depends on the symbiosis between the coral animals and their intracellular dinoflagellate algae, whose photosynthesis supplies 80% or more of the energy used by the coral for growth and calcification. Elucidation of the molecular and cellular mechanisms of this symbiosis and its breakdown is an important step in potentially alleviating the severity of coral-reef decline. Because of the difficulties in propagating and manipulating coral in the laboratory, we use another cnidarian, the small sea anemone *Aiptasia pallida*, as a more tractable model. To enhance the value of this model system, we are attempting to develop an *Aiptasia* cell culture. We have established a method for maintaining short-term cultures starting from pedal lacerates, the products of *Aiptasia* asexual reproduction. Although the cultures are not yet free of contaminants, the *Aiptasia* cells have persisted in culture for over three weeks with periodic genetic identification by PCR. We are now focusing on promoting cell proliferation with the goal of establishing long-term cultures and cell lines. The availability of a cell line would greatly facilitate the study of many aspects of cnidarian molecular and cell biology, including the interactions between cnidarian cells and their symbionts.

A-2020

Development of a Liver Cell Line from Fathead Minnow, *Pimephales promelas*, and their Molecular and Biochemical Characterization. L. E. J. LEE[1], N. Vo[1], J. Werner[2], R. Weil[3], N. D. Denslow[3], and R. D. Law[2]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA;

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Epithelial cells with characteristics of hepatocytes were derived from 3d-post mortem livers of adult fathead minnow (*Pimephales promelas*) that had been shipped in Hank's Buffered Salt Solution (HBSS) from Thunder Bay to Waterloo at room temperature. Viable cells could be consistently obtained in three different occasions from several preparations of fathead minnow liver tissues in HBSS. The livers were cleaned and mechanically dispersed with or without enzymatic treatment and cultured in Leibovitz's L-15 media supplemented with 5 or 10% fetal bovine serum (FBS). Best growth was obtained from non-enzymatic preparations containing outgrowths of cells from small liver tissue chunks. The resulting cellular preparations could be passaged within 3 wk of growth. Liver cells from the second trial (FHML2–6), obtained from an adult female fathead minnow liver were grown in L-15 media supplemented with 5% FBS (v/v). The cells were subcultured using TrypLE, a cell dissociation solution trademarked by Invitrogen and have now been passaged over 15 times in a span of 15 mo. Cryopreserved stock cultures have been successfully thawed from liquid nitrogen storage, with viability greater than 95%. FHML2–6 cells are fast growing with doubling times requiring 2 to 3 d at 28°C. Cell proliferation is temperature dependent and cells can be maintained for several weeks without media changes at room temperature. Molecular and biochemical characterization of the cells in terms of their ability to express liver specific proteins such as albumin, vitellogenin and cytochrome p450 1A1 is currently under way.

A-2021

Comparative Gene Expression Analysis of 1- and 2-Methylanthracene in Rat Liver Stem Cells. J. S. PARK, P. Babica, J. E. Trosko, and B. L. Upham. Department of Pediatrics and Human Development, and National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI, 48824. Email: pjoonsuk@msu.edu, upham@msu.edu

Gap junctional intercellular communication (GJIC) can serve as a central-based biomarker of measuring the tumor promotion potential of exogenous chemicals, such as polycyclic aromatic hydrocarbons (PAHs). We previously determined that the 1-methyl, but not the 2-methyl, isomer of anthracene (1-MeA, 2-MeA), which are prominent cigarette smoke components, activated extracellular receptor kinase, inhibited GJIC, and activated phospholipases in a pluripotent rat liver epithelial cell line, WB-F344. Gene expression analysis of WB-F344 cells using Whole-Rat Genome-Oligo-Microarray (Agilent Technologies Inc.) was done after 1, 2 and 12 h following exposure to 1- or 2-MeA. Real time RT-PCR was used to confirm some of the gene responses observed in the microarray. Significant differences were seen in the gene expression profiles between 1- or 2-MeA treated cells. Principal Component Analysis confirmed that most of the temporal responses in the 2-MeA group are downregulated genes, while upregulated genes are unique to the 1-MeA. 37 genes were identified as differentially regulated by 1-MeA, of which just one gene was in common with 2-MeA, with 77 exhibiting comparable expression profiles. A small number of genes were upregulated immediately at 1 h, with little repression at this time point, while many genes were upregulated at 2 h and sustained up to 12 h. Interestingly, some of the genes upregulated by 1-MeA were TCDD-related genes, such as Tiparp, CYP1A1 and CYP1B1. In the responses of aromatic hydrocarbon receptor (AhR) battery genes, 1-MeA was more sensitive than 2-MeA. Support: NIEHS grant #R01 ES013268–01A2 to Upham.

A-2022

Collagen Production by Normal Human Dermal Fibroblast Cells in Serum-free Media. N. NAPOLITANO[1], M. Monaghan[2], E. Roemer[1], and S. Simon[1,3]. [1]Department of Pathology; [2]Center for Biotechnology; and [3]Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11749. Email: nicole.napo@gmail.com

Collagen is a protein found in the skin and bones that is associated with supporting cell structure and strength. Increased collagen production has been shown to advocate healthier, younger skin, and aid wound healing. Assays to help discover collagen inducing compounds would be valuable to academia and biotechnology companies. The purpose of this study was to establish an assay that measured collagen production of normal human dermal fibroblasts (NHDF) grown in standard serum media and serum-free supplemented media. Ascorbic acid has been shown to potently induce collagen production in fibroblasts. NHDF were grown in standard serum media in the absence or presence of ascorbic acid (150 ug/mL) for 7 d and 14 d. Cells were also grown in chemically enhanced serum-free supplemented media under the same testing conditions. The serum free media minimized interactions with the collagen assay while providing several growth factors to help maintain cell function. Collagen was measured using Sirius Red dye, a collagen specific binding dye. Collagen was measured in the growth media

and on the matrix. NHDF grown in the chemically enhanced media and ascorbic acid showed a 2 and 3.5 fold increase of collagen on the matrix at day 7 and 14 respectively, compared to the 1.5 and 2.5 fold increase seen in NHDF grown in standard media. Also at day 14, NHDF grown in the chemically enhanced media with ascorbic acid showed a 2.5 fold increase of collagen in the media. Using this assay, several compounds were tested for the ability to increase collagen production in NHDF grown with chemically enhanced media. Although none of the compounds showed a potent induction of collagen production, there were subtle changes compared to the media control. The Sirius Red Assay successfully measured collagen production in the growth media and on the extracellular matrix. Data suggests that the chemically enhanced media is a good alternative to serum based media and is able to support collagen induction in cells. This model can be used to discover new collagen inducing compounds and expanded to study other skin related proteins.

A-2023

Analysis of Extracellular Matrix Synthesized by Human Bronchial Smooth Muscle Cells In Vitro. S. ZAMURRAD, L. J. Crawford, S. R. Simon, and E. J. Roemer. Departments of Pathology and Biochemistry. SUNY Stony Brook. Stony Brook. NY 11790. Email: szamurra@ic.sunysb.edu

The Extracellular matrix (ECM) consists of an intricate meshwork that provides structure and organization to tissues. The ECM forms a major component of the extracellular environment and helps regulate cell migration, signaling, adhesion, proliferation and differentiation. Cells are constantly remodeling their microenvironment by synthesizing de novo ECM components according to their cell/organ specific functions. There is a need to develop in vitro models that replicate this native extracellular environment so that the tissue degradation seen in inflammatory diseases and metastasis can be studied more thoroughly. Our lab has been long involved in searching for such an optimal and stable ECM equivalent for use in in vitro assays. A human cell based model would provide a more physiologically relevant substrate for the study of protease inhibitors. Here we describe the initial synthesis and analysis of an insoluble ECM synthesized in vitro by normal Human Bronchial Smooth muscle cells (HBSmC). Cells were plated at passages five and seven, grown to confluence, and then cultured for one or two weeks in media supplemented with ascorbic acid. Examination by phase contrast microscopy showed that two weeks of post confluent culture yielded increased ECM deposition for cells plated at either passage. Radiolabeled precursors were added to quantitatively label matrix components. The ECM composition was analyzed by sequential digestion with heparinase I & III, elastin adsorbed trypsin, chondroitinase ABC, collagenase, human neutrophil elastase (HNE) and proteinase K. Preliminary data shows significant amounts of proline to be incorporated into the collagenase sensitive material, and methionine/cysteine in HNE sensitive material. Based upon this pattern of amino acid incorporation and specific enzyme sensitivity, these components are presumed to be collagen and elastin respectively. This ECM has potential for further study of matrix biology and utility as a model for wound healing studies, drug discovery, toxicology, tissue engineering and reparative medicine.

A-2024

The Use of Collagen Matrices for Control of MMP and Serine Proteinase Activity in Chronic Wounds. K. D. FENWICK[1], S. R. Simon[1,2], F. Daccueil[1], and E. J. Roemer[1]. [1]Department of Pathology; and [2]Department of Biochemistry, SUNY Stony Brook, Stony Brook 11794. Email: kfenwick21@gmail.com

Inflammation triggers activation of neutrophils, resulting in secretion of serine proteinases and matrix metalloproteinases (MMPs). These two classes of proteinases synergize to degrade most components of connective tissue in nonhealing chronic wounds. This study employed native fibrillar collagen matrices, manufactured by Suwelack Health Care and distributed by Medline Industries, for controlling pathologically excessive activity of neutrophil proteinases in chronic wounds. Our laboratory has previously shown that these matrices can bind MMPs and neutrophil-derived serine proteinases from solution, so that their proteolytic activity is effectively depleted from the fluid phase. We now show here that these matrices also prevent the activation of MMP precursors by two mechanisms. First we confirmed that MMP-8 can be activated by cathepsin G and that MMP-9 can be activated by neutrophil elastase, using a FRET-quenched substrate for quantitating MMP activity. We then demonstrated that in the presence of the native fibrillar collagen matrices activation of MMPs by serine proteases was diminished. Most recently, we have shown that the non-enzymatic activation of pro-MMP-8 by aminophenyl mercuric acetate (APMA) is reduced in the presence of fibrillar collagen matrices. A possible cause of this diminished activation is sequestration of pro-MMP-8 so that it is no longer accessible for activation. If pro-MMP-8 is activated with APMA before addition of the collagen matrix, the fluid phase retains significantly greater MMP activity. These data support that native fibrillar collagen matrix dressings may reduce the proteolytic activity in chronic wound bed fluid by not only binding and sequestering active proteinases, but by also reducing pro-

MMP activation at the point of secretion from activated neutrophils.

A-2025

Validating MTS as an Alternative Viability Assay to MTT on the Human 3-D Tissue Models, EpiAirway™ and EpiDerm™. C. R. KAVANAGH[1], L. J. Crawford[1], S. R. Simon[1,2], and E. J. Roemer[1,2]. [1]Dept. of Pathology; and [2]Biochemistry and Cell Biology, SUNY Stony Brook, Stony Brook, NY, 11794. Email: ckavanag@ic.sunysb.edu

MTT is currently the standard viability assay recommended for use with MatTek's three-dimensional organotypic models. MTT is reduced by metabolic activity to a formazan product that requires solubilization through lysis of the cells. In certain cases, maintenance of the tissue may be of importance and a non-lytic assay would be a better alternative. Prior evaluation of the non-lytic assays: Alamar Blue, CalceinAM, and MTS proved MTS to be the most consistent and reliable method of viability measurement. Of the three alternatives, MTS is the most chemically similar to the standard MTT. Like MTT, it is a tetrazolium salt-based assay; however, it is reduced to a soluble formazan product, which can be measured by absorbance without cell lysis. MatTek's organotypic models are frequently used in our lab, mostly the EpiAirway™ model for nasal drug delivery testing and EpiDerm™ to evaluate transdermal vaccine delivery. We have determined that MTS is an acceptable alternative to MTT for use on EpiAirway™. We currently are adapting the EpiAirway™ protocol to EpiDerm™ to determine if MTS will be a dependable assay for this model as well. Preliminary EpiDerm™ data suggests an appropriate endpoint of 2.5 h for MTS incubation. We have seen robust MTS signals in time course Triton X 100 (Tx100) assays comparable to those obtained by MTT. Assays are ongoing to establish a set protocol for EpiDerm™, like that which we have for EpiAirway™. Future studies will examine the usefulness of MTS on MatTek's other models.

A-2026

Limited Success in the Long-term Culture and Maintenance of Function of Non-plateable Lots of Cryopreserved Primary Human Hepatocytes on a 2-dimensional Surface. T. J. FLYNN and M. C. Garcia. Division of Toxicology, US FDA, MOD-1 Laboratories, 8301 Muirkirk Rd., Laurel, MD 20708. Email: thomas.flynn@fda.hhs.gov

Primary human hepatocytes are considered the "gold standard" by the pharmaceutical industry for rapid screening of lead compounds for both liver metabolism and liver toxicity. However, freshly isolated human hepatocytes are not available on a regular basis. Cryopreserved hepatocytes, while readily available, have a limited survival in culture unless they are plated in complex, 3-dimensional matrices. We have cultured cryopreserved human hepatocytes on uncoated, 96-well Primaria® plates using Williams E medium containing 10% FBS and supplemented with growth factors including nicotinamide, lipoic acid, ethanolamine, dexamethasone, HGF and EGF. After an initial die off of cells, proliferation began in many wells on day 5–6. Proliferating cells had either an epithelial (Ep) or fibroblastic (Fi) morphology. Cells reached confluence by day 20–21. The Ep cells went on to form secondary structures comparable in appearance to hepatic cords. The Ep cells immunostained primarily for cytokeratin-18 (a hepatocyte marker), but a small number of cells immunostained for cytokeratin-19 (a cholangiocyte marker). Over the period evaluated, days 14–42, the Ep cells made and secreted physiological levels of albumin. At days 25 and 39, the Ep cells expressed comparable basal and inducible EROD (CYP1A1/2) activities. Although all cells were plated and treated identically, we would typically observe on each 96-well plate Ep cells in about 1/3 of the wells, Fi cells in about 1/3 of the wells, and no proliferation or growth of cells in the remaining 1/3 of the wells. The Ep cells were morphologically and functionally consistent with hepatocytes and remained so for at least 42 d after plating. We do not yet know why we are achieving apparent success in only 1/3 of the plated wells.

A-2027

Evaluation of Apoptosis During Static Storage of Pancreatic Islet Cells. L. H. CAMPBELL, A. Vazquez, Z. Chen, and K. G. M. Brockbank. Cell & Tissue Systems, Inc. North Charleston, SC 29406. Email: lcampbell@celltissuesystems.com

Organs for transplantation are commonly harvested and placed in storage solution (Viaspan) on ice for transport to the donor or until they are ready for transplant. Strict guidelines are in place for each organ to provide maximum viability and function upon transplantation. For example, the guidelines for pancreas include no warm ischemic time and <6 h of cold ischemia. In an effort to improve the quality of whole pancreas and/or the islets isolated from the pancreas, our lab has been engaged in improving storage conditions and the solutions used for storage. One aspect of this research involves using islet cell lines to evaluate solution formulations. As a first step, the pancreatic islet cell line, βt3, was used to evaluate the induction of apoptosis when cells were stored for ~16–24 h at 4°C in various storage solutions. The cells were then followed for 4 d post storage. Metabolic activity and live/dead analysis

were evaluated each day. Apoptosis was also evaluated via nexin staining, caspase activity and measurement of DNA laddering that is a hallmark of apoptosis. Preliminary experiments evaluated cell viability for several days post storage after 16–24 h of storage at 4°C using various solutions. Observations of cell viability during this post storage period demonstrated that viability did not increase at a steady rate over time but rather there was a lag in viability followed by an increased rate of cell recovery by day 4 post storage. For example, cells stored in Belzer's Machine Perfusion solution (BPS) demonstrated an initial viability 35% on days 0–2 with a large increase in viability (84%) observed by day 5 post storage. Experiments evaluating apoptosis suggest that while there may be a peak of apoptotic activity that occurs within 1 to 2 d after rewarming there is still significant apoptotic activity that continues for several more days. Apoptosis measured by nexin staining on cells stored in BPS demonstrated ~10% staining at day 0 that increased up to ~25% by day 2–3 with a gradual decline on day 4 to ~15%. This apoptotic activity is not readily observed when only metabolic activity is measured due to the proliferation of recovered cells. These studies support the idea that the storage solution can have an impact on organ health and provides avenues for improvement of storage solution formulations that may ultimately improve the quality of organs used in transplantation.

A-2028

Pertussis Composite Nanofibrous Membranes as an Acellular Transdermal Whooping Cough Vaccine. T. A. GAWADE[1], K. M. Sawicka[2], E. J. Roemer[3,4], and S. R. Simon[2,3,4]. [1]Department of Biology; [2]Department of Biomedical Engineering; [3]Department of Biochemistry; and [4]Department of Pathology, State University of New York-Stony Brook, NY 11794. Email: tgawade@ic.sunysb.edu

Whooping Cough has globally resurfaced due to the suboptimal quality of the traditional vaccine, cyclic variations in its pattern and discovery of new strains of the causative agent, *Bordetella pertussis*, making the need for an improved vaccine immediate. We propose to develop a novel, solid state transdermal vaccine by immobilizing Pertussis Toxin (PT) in nanofibrous membranes of the polymer, Polyvinylpyrrolidone (PVP) utilizing electrospinning. The retention of the protein's biological functionality was verified using an in vitro assay in which Chinese Hamster Ovary (CHO) cells clumped in the presence of biologically active PT. This assay was utilized to confirm transdermal delivery of the protein through an uncompromised Epiderm-200 human skin model (EFT-200, MatTek). Retention of PT functionality was confirmed in the supernatant around the tissue 24 h after dosing, as well as in the homogenized tissue samples. To establish the proposed vaccine for long-term immunity, a longevity study compared the retention of PT bioavailability in standard solution, dried, and electrospun form maintained at different conditions: at room temperature, dessicated and refrigerated. The preliminary results show that solvated PT retained functionality until day 14 at a comparable PT standard range of 0.025–0.0375 ng/ul, whereas electrospun and dried solution of PT demonstrated a clumping comparable to a PT standard range of 0.05–0.075 ng/ul. The biologically active retention of PT and its delivery through the epidermal layer of the human skin model can be applied to the creation of a novel transdermal vaccine that will address the evolving nature of Whooping Cough, address the issue of weakened immunity, and set a precedent for revolutionizing traditional methods of vaccination.

A-2029

Warning: The Serious Problem of Mistaken Identities of Cultured Human Cell Lines. ARIHIRO KOHARA[1], Wilhelm G. Dirks[2], Hans G. Drexler[2], Yukio Nakamura[3], Miho K. Furue[1], Tohru Masui[1], and Hiroshi Mizusawa[1]. [1]7–6–8 Saito-Asagi, Ibaraki, Osaka, Natl. Inst. of Biomedical Innovation, JCRB, JAPAN; [2]Inhoffen St. 7b, 38124 Braunschweig, Human and Animal Cell Lines, German Biological Resource Centre, DSMZ, GERMANY; and [3]3–1–1 Koyadai, Tsukuba, Ibaraki, Cell Engineering Division, BioResource Center, RIKEN, JAPAN. Email: kohara@nibio.go.jp

Cell culture is a technique that is widely used by scientists in diverse disciplines such as cell biology to genetics and transcriptome to proteome analyses. Therefore, cell culture is one of the most common research tools for biomedical research. However, lack of observance of basic rules for good laboratory practice can drastically decrease the quality of cell cultures. In these situations, the risk for infection by mycoplasmas and the risk for cross-contamination among human cell lines increases and has been recognized as a serious problem, with little attention paid to it. The major non-profit cell banks are aware of the problem of cross contamination and have developed PCR-based STR-typing analysis of their deposited cell lines using the Promega PowerPlex 1.2 system. This technical approach is applied at the cell banks of DSMZ (Germany), RIKEN (Japan), ATCC (USA), and JCRB (Japan). Information regarding STR reference profiles and cross-contaminated cell lines is accessible at the homepage of JCRB (URL: http://cellbank.nibio.go.jp/cellbank_e.html). Cross-contaminations were found as follows: at DSMZ: 27/650 cell lines (4.2%), at RIKEN: 53/565 cell lines (9.4%), at JCRB: 38/638 cell lines (6.0%). Since even scientific institutes with large cell

culture facilities have no DNA reference profiles for detecting cross-contaminations, JCRB has generated an online search engine for human STR profiles. Cell culturists can generate the composition of relevant alleles in their own lab and use the online search engine for STR profiles for authentication. This system is free, on the web and examines authenticities of cell lines by comparison with DNA reference profiles of a STR database shared by DSMZ, JCRB and RIKEN.

A-2030

Interindividual Variation Affects Development of Primary Explant Culture. N. OGATA, and K. Iwabuchi. Laboratory of Applied Entomology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchu, Tokyo 183–8509, JAPAN. Email: kikkuo@cc.tuat.ac.jp

Primary explant culture and dissociated cell culture are used for the purpose of establishing insect cell lines. However, with explant culture, each experiment requires fresh explantations, which implies poor reproducibility of the sample. This limits studying of cell line development. Interindividual variation of experimental animals is thought to be one of the critical factors restricting the reproducibility of explant culture. One way to overcome this limitation is to use many cultures from a single insect. In this study, using many explant cultures of *Allomyrina dichotoma* larval fat body, we have assessed how interindividual variation affects development of primary explant culture. 10–50 fat body explants from an insect were isolated using "burning sterilization" technique where 70% ethanol is swabbed on the surface of insect and then caught on fire. Explants were plated in 35-mm tissue culture dishes in Shields and Snag M3 insect medium with 10% fetal bovine serum. Migrated cells from explants were observed under a phase contrast microscope. All observed cells were categorized by morphological characteristics: size and polarity (Size: larger/smaller than 50 μm, intermediate type was scarcely found throughout the observation; Polarity: high polar cells/low polar spherical cells). Additionally, spindle shaped cells were especially defined, as these cells are fast-growing. Characteristics of observed cells were recorded with time passage based on definitions mentioned above. Then, developmental stages of cultures were defined in 5 stages according to the category of cells observed in the culture. Statistical analysis using the generalized linear mixed model was carried out to evaluate correlations between culture development and time passage from donors examined in this study. The intraclass correlation was strong at the individual level, i.e. interindividual variation of experimental animals is the primary factor of poor reproducibility in primary explant culture.

A-2031

Regulatory Requirements for In Vitro Systems to Meet Performance Standards During Validation and Over Time. M. KLAUSNER, J. Kubilus, S. Ayehunie, Y. Kaluzhny, H. Kandarova, P. Kearney, P. J. Hayden, and J. Sheasgreen. MatTek Corporation, Ashland, MA 01721. Email: mklausner@mattek.com, phayden@mattek.com

A recent US National Research Council report (June, 2007) envisions humane alternative toxicological tests that are faster, less expensive, and more accurate than animal tests. In vitro tissue models have been or are in the process of being validated as alternatives to animal testing for safety evaluation of cosmetics, pharmaceuticals, and consumer products. Currently available organotypic human models include dermal (EpiDerm, EpiDerm-FT), ocular (EpiOcular), ectocervical (EpiVaginal) and airway (EpiAirway). As they become formally validated, regulatory agencies and other users need to be assured that the models will provide consistent, high quality data over time, not just during the validation process (K. Gupta et al., *Regul Toxicol Pharmacol*. 2005 Dec;43(3):219–24). Recommended guidelines include "full characterization of cells or tissues, sampling of each lot … for performance, and regular use of controls and benchmark chemicals to provide assurance of consistency of assay performance" (A. Rispin et al. *Regul Toxicol Pharmacol.* 2006 Jul;45(2):97–103). The current poster summarizes long-term reproducibility and performance of in vitro epidermal (EpiDerm) and ocular (EpiOcular) models against benchmark chemical treatment. Quality control testing of weekly batches of EpiDerm and EpiOcular was performed using the MTT assay. The exposure time needed to reduce viability to 50% (ET-50) for Triton X-100 was determined. For EpiOcular, yearly average ET-50 values have ranged from 22.0 min to 27.3 min. Coefficients of variation (CV) for negative control tissues (exposed to ultrapure H2O) have averaged under 6% for every year since 1997. In addition, the yearly average CV for all tissues has never exceeded 6.7%. For EpiDerm, yearly average ET-50 values have ranged from 5.9 hrs to 7.5 hrs. The coefficients of variation (CV) for negative control tissue (exposed to ultrapure H2O) have averaged under 7.5% for every year since 1996. These results over the past 14 yrs of commercial production address regulatory concerns regarding performance standards over time.

A-2032

Use of the EpiOcular Tissue Model for Testing of Ultra-mild Eye Care Cosmetics. MITCHELL KLAUSNER[1], Patrick J. Hayden[1], Joseph Kubilus[1], George R. Jackson[1], and Jessica McDonnell[2]. [1]MatTek Corporation, Ashland, MA and

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Eye care cosmetics (ECC) need to be non-irritating in order to be successful in the marketplace. In addition, in order to avoid complaints by customers with sensitive eyes, many ECCs are formulated to be ultra-mild. However, testing of, and discrimination between ultra-mild formulations is difficult since traditional Draize rabbit eye testing is insensitive to the low levels of irritation caused by ECCs. Furthermore, animal testing is not possible due to animal rights concerns and due to current European legislation banning cosmetics that have been tested using animals. Human clinical testing can be performed but because only a low level response is expected, large numbers of subjects would be necessary and hence testing costs would be high if not prohibitive. Cells in monolayer culture could be used for testing however the test materials would need to dissolved in aqueous media which for many non-water soluble cosmetics is not possible. The current study investigated use of the organotypic EpiOcular tissue model as a means of discriminating between ultra-mild formulations. Ten (10) commercially available mascara products were purchased and tested using EpiOcular with an extended time exposure protocol. Because the tissue model is cultured at the air-liquid interface (apical tissue surface left dry), both water soluble and water insoluble test materials could be applied neat to the apical tissue surface. Exposure times between 8 and 24 h were used after which the tissue viability was determined using the MTT assay. Dose response curves were constructed and the exposure time that reduces tissue viability to 50% (ET-50) was determined by mathematical interpolation. For the 10 mascaras tested, a broad range of ET-50s was obtained from 8.7 h to > 24 h. Other studies with low levels of surfactants known to be irritating at higher concentrations could also be discriminated by ET-50s. As such, the extended time exposure protocol appears to be a facile, cost effective means to screen ultra-mild ECCs and other materials.

A-2033

ECVAM Validation of the EpiDerm Skin Irritation Test. H. KANDAORVA, P. J. Hayden, and M. Klausner. MatTek Corporation, Ashland, MA. Email: hkandarova@mattek.com, phayden@mattek.com

In April 2007, ECVAM endorsed 2 alternative methods (EPISKIN and EpiDerm Skin Irritation Tests (SIT)) as replacements of the in vivo rabbit skin irritation test. While EPISKIN assay was recognized as a stand alone method, EpiDerm SIT was endorsed for use in a tiered testing strategy (OECD TG 404), where irritating results are accepted and non-irritating results may require further testing by another method. Based on results published by Faller and Bracher (2002), and analysis of results of the ECVAM validation study, there was evidence that differences in the barrier properties between the 2 models were responsible for the lower sensitivity of EpiDerm SIT when using an identical protocol as used for EPISKIN. Therefore, modifications of the exposure conditions were introduced to the EpiDerm protocol: a) exposure time was increased from 15 min to 60 min; b) the temperature during the exposure was increased to 37°C. With these modifications, when testing chemicals from the pre-validation and validation studies, a significant increase in sensitivity (84%) was obtained, while maintaining an acceptable specificity of the method. In autumn 2007, an international validation study between 4 laboratories was performed to evaluate reproducibility and confirm the predictive ability of the modified EpiDerm SIT. Results of the study are presented here. Overall, sensitivity and specificity of 80% were obtained, which is comparable to results for the EPISKIN SIT for the same set of chemicals (sensitivity of 70%, specificity 80%). The inter-laboratory reproducibility of the modified EpiDerm SIT and its concordance with the in vivo rabbit data was also very good. The method was formally accepted by ECVAM as a validated method in November 2008. These results together with 14 yrs of consistent Quality control data for the EpiDerm tissue model make it the ideal choice for skin irritation testing without the use of animals.

A-2034

Drug Metabolizing Enzyme Activity in Human In Vitro Dermal (EpiDerm) and Airway (EpiAirway) Epithelial Models. P. J. HAYDEN, G. R. Jackson, J. Bolmarcich, H. Cohen, and M. Klausner. MatTek Corporation, Ashland, MA. Email: phayden@mattek.com

Human dermal and airway epithelia contain xenobiotic metabolizing enzymes (XME) that can cause biotransformation of drugs and environmental/occupational chemicals, resulting in altered drug activity or formation of toxic/mutagenic metabolites. The present work evaluated expression of XMEs in highly differentiated in vitro models of human dermal (EpiDerm) and airway (EpiAirway) epithelia. RT-PCR experiments were conducted to evaluate baseline and inducible expression of cytochrome P450 (CYP) isoforms in the epithelial cultures. EpiAirway cultures constitutively expressed CYP1A1 (weak), CYP1B1, CYP2A6, CYP2B6 (weak), CYP2C8 (weak), CYP2C19, CYP2D6, CYP2E1 and CYP3A5, while CYP3A4 and 3A7 were not detected. 3-Methylcholanthrene (3MC) strongly increased expression of CYP1A1 and slightly increased CYP2B6 and

CYP2C8 expression in EpiAirway. In EpiDerm, CYP1B1, CYP2C19, CYP2D6, CYP3A4 (weak) and CYP3A5 were constitutively expressed. 3-Methylcholanthrene (3MC) strongly increased expression of CYP1A1 and CYP1B1 in EpiDerm. Enhanced metabolism of the CYP1A1 and CYP1B1 substrate ethoxyresorufin confirmed increased activity following treatment with 3MC. Thus CYP expression in EpiAirway and EpiDerm showed a high concordance with CYP expression reported for in vivo human airway and dermal epithelia. Total Glutathione S-transferase (GST) activity in the epithelial models was also evaluated by measuring conjugation of glutathione with 1-chloro-2,4-dinitrobenzene and UDP-Glucuronyltransferase activity was determined by 4-methylumbellipherone conjugation. High baseline GST and UDP-glucuronyltransferase activity in both models was not further enhanced by 3MC treatment. The results demonstrate that the EpiDerm and EpiAirway in vitro human epithelial models possess in vivo-like XME activities and may thus be useful for evaluating epithelial metabolism of drugs and environmental/occupational chemicals.

A-2035

Mechanisms of Innate Immunity Involvement in Asthma Exacerbations: Experiments with In Vitro Models of Human Airway Epithelial Cells (EpiAirway™) and Epithelial Cell/Fibroblast Co-cultures (EpiAirway-FT™). P. J. HAYDEN, G. R. Jackson, A. Armento, J. Bolmarcich, H. Cohen, and M. Klausner. MatTek Corporation, Ashland, MA. Email: phayden@mattek.com

Respiratory viral and bacterial infections are a major cause of asthma exacerbations. The airway epithelium is known to express innate immune responses to these agents via toll-like receptors (TLRs). The current studies investigated the effect of TLR stimulation in well differentiated in vitro models of human airway epithelium consisting of normal airway epithelial cells (AEC) (EpiAirway™) and AECs co-cultured with normal airway fibroblasts (EpiAirway-FT™). Both models display a well-differentiated mucociliary phenotype similar to in vivo airway epithelium and are cultured at the air-liquid interface. RT-PCR experiments confirmed expression of TLR 1, 2, 3, 5, 6, and TOLLIP by the models. Apical stimulation with TLR agonists resulted in decreased barrier function as determined by measurement of transepithelial electrical resistance (TEER), concomitant with secretion of numerous cytokines and chemokines. Forty-four cytokines, chemokines and growth factors were evaluated by bead based multiplex assays and/or ELISA assays. High levels of IL-8, fractalkine, G-CSF, IL-1α, IL-1ra, IL-6, IL-8, IP-10, MIP-1α, MIP-1β, MIP-3α, RANTES, TNFα and VEGF were observed after TLR stimulation. Moderate amounts of eotaxin-3 but only slight amounts of eotaxin-1 were detected in the absence of fibroblasts. However epithelial cell/fibroblast co-cultures produced high levels of eotaxin -1 after apical TLR stimulation. The most potent inducer of chemokine secretion was poly (I:C) (TLR3 ligand). Weaker induction was observed with PAM (TLR 1/2), Flagellin (TLR5) and FLS-1 (TLR6/2). TH2 cytokines that are characteristically associated with asthmatic disease (e.g. IL-13) synergized with Poly (I:C) in production of IL-8 and eotaxin-1. These data provide additional evidence of mechanisms by which epithelial/fibroblast TLR activation synergizes with TH2 conditions to produce chemokines that promote influx of neutrophils and eosinophils into the airway, hallmark features of asthmatic disease.

A-2036

Trans-epithelial Electrical Resistance (TEER) Measurement on In Vitro Cutaneous and Ocular 3-D Models: a Sensitive Parameter Needing Standardized Conditions. T. DELANNE, Pascale Justine, and José Cotovio. L'OREAL Research, Aulnay-Sous-Bois, FRANCE. Email: tdelanne@rd.loreal.com

Two major routes are involved in tissues barrier function (BF), thus driving small molecules flux. While transcellular route of chemicals depends on their lipophilicity, paracellular route takes place into the intercellular spaces and is under epidermis tights junctions (TJs) control. Together with the proteo-lipidic complex of the cornified layers, TJs are important effectors of tissues BF. When TJs are not mature or damaged, hydrophilic compounds (electrically charged) are able to diffuse from the upper layers to the basal one (for both skin epidermis and eye corneal epithelium). Consequently, modifications of BF linked structures can influence the tissue trans-epithelial electrical resistance (TEER). Steady state and quality of the BF of in vitro models could be detected by using TEER measurements. This parameter could give some information about the tissue integrity modifications after chemical treatments. We studied three commercially available in vitro human epithelial engineered models: two epidermis models (EpiSkin large model, RHE small) and a corneal epithelial model (RHCE) supplied by SkinEthic Laboratories. A comparison between models on TEER measurements was made before and after surfactant treatment. TEER was measured by using a specific epithelial tissue Volt-Ohmmeter. TEER was monitored during a time period in order to define acceptable and standardized steps where TEER could be regarded as stable prior to measurements. TEER assays were carried out after topical surfactants treatments: SLS treated EpiSkin/L or RHE small and Triton X-100 treated RHCE. Results showed a sharp TEER

decrease since the lowest concentrations tested reaching low resistance values at the highest surfactant concentrations. Complementary cellular viability assays showed clear effects only at the highest surfactant doses. TEER standardized method could be a useful endpoint for quality control assessments and comparisons between models. In addition it could be used as a suitable easy to use tool, to describe BF, complementary to TEWL and other permeation studies.

A-2037

Identifying Respiratory Toxicity Using the EpiAirway™ Human 3-D Model Combined with Multiple Endpoint Analysis. J. A. WILLOUGHBY, Sr. and J. M. McKim, Jr. CeeTox, Inc., 4717 Campus Drive, Kalamazoo, MI 49008. Email: jwilloughby@ceetox.com

There is a growing need for new in vitro alternatives to animal toxicity testing in both the chemical and personal care industries. The registration of new chemicals under REACH as well as Amendment VII to the Cosmetics Directive in Europe requires the development, validation, and utilization of new in vitro methodologies. In the past, analysis of respiratory toxicity was performed using animal models, however these studies are costly and time consuming. An in vitro human cell model combined with multiple endpoint analysis may provide a robust model for evaluating many types of respiratory toxicity. The objective of this study was to assess the toxicity of several known respiratory toxicants in the three-dimensional in vitro human EpiAirway™ system. This model contains highly differentiated human tracheal/bronchial epithelial cells cultured at the liquid-air interface, very closely resembling the epithelial tissue of the upper respiratory tract. The EpiAirway™ system was purchased from MatTek (Ashland, MA) in 96-well format. The cells were apically treated with bleomycin and doxorubicin over a broad range of exposure concentrations. Cells and media were collected after incubation periods of 24 and 72 h. Toxicity was assessed by measuring cell viability (MTT assay), cellular glutathione levels, TNF-α, IL-1α and IL-6 cytokine expression levels, and histology. Doxorubicin reduced cell viability in a dose dependant manner after 72 h. Total cellular GSH levels were reduced in a dose dependant manner to 10–20% of control levels by 72 h. At 24 h, qRT-PCR results showed doxorubicin significantly induced expression (>5-fold) of TNF-α and IL-6 while histology showed significant sloughing of cells and structural breakdown. Bleomycin reduced cell viability to about 90% of control levels by 72 h, however total cellular GSH levels were reduced in a dose dependant manner to 75–80% of control after only 24 h. qRT-PCR results showed bleomycin significantly induced expression (>5-fold) of IL-1α and IL-6 at 24 h while histology showed little sloughing of cells but significant structural breakdown. In conclusion, the 3-D EpiAirway™ system combined with multiple endpoint analysis provided toxicity data consistent with those observed in vivo.

A-2038

Protein Kinase-C Reduces Recovery of TBP and Causes Changes in the pI of TBP-associated Proteins from In Vitro Transcription Reactions. CALVIN B. L. JAMES, and Albert Saez. Department of Biomedical Sciences, Ohio University, Athens, Ohio, 45701. Email: jamesca@ohio.edu

Signaling through protein kinase C (PKC) plays a role in regulating polymerase III (pol III) transcription. In in vitro transcription reactions, PKC appears to act via changes to the transcription initiation complex, specifically affecting TATA-Box Binding Protein (TBP) and possibly TBP-associated proteins. Here we report the use of exogenously added recombinant 6x-histadine tagged TATA-box Associated Protein (r-His-TBP) in pull-down assays to isolate pol III associated transcription factors from HeLa cell extracts (WCE). Western blot analysis of the recovered r-His-TBP from pol III in vitro transcription reactions show that reduced quantities of TBP is recovered from WCE following PKC activation. Further, 2-D PAGE analysis of proteins from these pull-down assays show that following PKC activation, a number of high molecular weight proteins become more basic. How changes to the pI of transcription complex-associated proteins correlate with a reduction in TBP recovered from these complexes remains unclear at this time. However, the recovery of r-His-TBP will allow for the isolation TBP and associated proteins, and provides an opportunity to further probe the role of TBP in PKC-induced repression of pol III transcription.

A-2039

Molecular Cloning of p38 MAP Kinase cDNA from Killifish (*Fundulus heteroclitus*). J. DENRY SATO[1], Erin E. Flynn[1], M. Christine Chapline[1], Jordan A. Francke[1], Cecily J. Swinburne[1], Joseph R. Shaw[1,2], and Bruce A. Stanton[1,3]. [1]MDI Biological Laboratory, Salsbury Cove, ME 04634; [2]School of Public and Environmental Affairs, Indiana University, Bloomington, IN 47405; and [3]Dept. of Physiology, Dartmouth Medical School, Hanover, NH 03755. Email: dsato@mdibl.org

Serum- and glucocorticoid-inducible kinase (SGK) regulates the cystic fibrosis transmembrane regulator (CFTR) chloride ion channel in the adaptation of killifish, a euryhaline teleost, to increased environmental salinity. SGK function is regulated at the levels of transcription, kinase activation, and protein

degradation. The stress-responsive kinase p38 MAP kinase (p38 MAPK/MAPK 14) regulates mammalian SGK gene transcription. Our working hypothesis is that in response to increased salinity p38 MAPK increases CFTR activity in killifish gill tissue through its stimulation of SGK activity. We are cloning killifish p38 MAPK cDNA to design anti-sense morpholino oligonucleotides to test our hypothesis by knocking down p38 MAPK expression in killifish transferred from fresh water to sea water and measuring survival and SGK levels in the gills of treated fish. First strand cDNA was synthesized from killifish liver total RNA, and putative p38 MAPK cDNA fragments were amplified by PCR with paired primers based on areas of homology between carp, salmon, and zebrafish p38 MAPK sequences. Several cDNA fragments were cloned, purified and sequenced. A 1 kb p38 MAPK cDNA fragment was generated from two independent cDNA preparations. The DNA sequence of this fragment corresponded to approximately 90% of the coding region of p38 MAPK in human, carp and zebrafish. Over a span of 330 amino acid residues p38 MAPKs from these four species were more than 92% similar. By sequencing multiple cDNA fragments, we have detected putative allelic polymorphisms in killifish p38 MAPK. 5'-RACE experiments have been done to obtain the nucleotide sequence spanning the p38 MAPK cDNA translation start site, which will be targeted with anti-sense morpholino oligonucleotides. This research was supported by INBRE grant P20-RR016463 from the NCRR, grant RO1-DK45881 from NIDDK to BAS, and a Cystic Fibrosis Foundation grant to BAS. EEF and JAF were supported by STEER grant 1-R25-ES016254.