Animal Posters

A-2000

Protective Effects of Oat Bioactives on Biomarkers of Gastrointestinal Cell Injury. M. M. Bowen1,2, A. Buigé1,3, Weston W. Bussler1,4, and Slavko Komarnytsky1,4. 1Plants for Human Health Institute, North Carolina State University, 600 Laureate Way, Kannapolis, NC 28081; 2 UNC Gillings School of Global Public Health, 135 Dauer Drive, Chapel Hill, NC 27599; 3 UNC Eshelman School of Pharmacy, 301 Pharmacy Ln, Chapel Hill, NC 27599; and 4 Department of Food, Bioprocessing & Nutrition Sciences, North Carolina State University, 400 Dan Allen Drive, Raleigh, NC 27695. Email: mbowen97@live.unc.edu

Disturbances of the intestinal lining induced by chemical or inflammatory agents may result in increased absorption of toxic substances and amplified immunogenic responses in the gut. Rapid regeneration of the intestinal barrier following injuries is essential to preserving normal homeostasis and can be achieved by cell replication and migration into the wounded area. In this study, we investigated whether bioactives from oat extracts and simulated human in vitro oat digests can promote migration and establishment of tight junctions in the normal human colon CCD 841 CoN cells. Different phenolic components (0.01–10 µM) promoted dose-dependent restitution of wounded monolayers and increased microscopic biomarkers of tight junctions (ZO-1, Tight junction protein-1). Methylated hydroxycinnamic metabolites (ferulic and dihydroferulic acids) showed highest potency to promote gastrointestinal cell migration in vitro, likely due to their increased hydrophobicity and tissue affinity. These findings suggest that oat cultivars with increased levels of bioavailable phenolic metabolites may enhance gastrointestinal cell healing responses and provide a new dietary approach for modulating intestinal wound restitution in subjects consulting for functional gastrointestinal disorders.

A-2001

Absorption and Distribution of the Antimalarial Drug Artemisinin Delivered Orally as Dried Leaves of Artemisia annua. M. Desrosiers and P. J. Weathers. Dept. of Biology and Biotechnology, 100 Institute Road, Worcester Polytechnic Institute, Worcester, MA 01609. Email: mdesrosiers@wpi.edu

The medicinal plant Artemisia annua is a promising option for the treatment of malaria. A. annua produces the antimalarial drug artemisinin (AN) whose derivatives make up the major component of artemisinin combination therapies, the frontline global treatment for malaria. Previously we showed that AN delivered as dried leaves of A. annua (DLA) is >40 fold more bioavailable in mice, 5 fold better at reducing parasitemia, and 3 fold better at slowing the development of AN resistance when compared to pure AN. Recently we showed that AN delivered as DLA is about 4 times more soluble in intestinal fluid and 37% more permeable to the intestinal membrane than pure AN. Here, using the Caco-2 model of the intestinal epithelium we investigate the effects of the essential oil fraction of A. annua on intestinal permeability of AN. We show that digested essential oil at two concentrations equivalent to 0.3 and 4.0% of that known in the plant, and from two sources decreased the intestinal permeability of AN. This result was surprising considering digested DLA increased AN permeability. Interestingly, when essential oil was added undigested, there was no change in the intestinal permeability of AN compared to pure drug. It appeared that the presence of bile changed essential oil permeability. To further characterize differences in bioavailability of AN delivered as DLA vs. pure drug, we performed tissue distribution (ADME) studies in rats. Groups of rats were orally dosed with either pure AN or a slurry of DLA containing an equal amount of AN. One hour after dosing, rats were euthanized, tissues harvested, and analyzed for AN content. AN delivered as DLA was distributed in significantly higher quantities, up to 6 fold greater than from pure AN throughout all the tissues tested. Together these studies begin to explain how bioavailability improves when AN is delivered via the plant than as pure drug.

A-2002

TRP Channels as Novel Insecticide Target. Ramani Kandasamy1, Damian London1, Lynn Stam1, Wolfgang von Deyn2, Xilong Zhao1, Vincent L Salgado1, and Alexandre Nesterov1. 1BASF Corporation, 26 Davis Drive,
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Two commercial pyridine azomethine derivative insecticides, pymetrozine and pyrifluquinazon, act by perturbing coordination and feeding behavior of sap-sucking insects. These compounds affect chordotonal neurons, which are serially arranged stretch receptors that play an essential role in the senses of proprioception, kinesthesia, hearing, gravity, balance, and acceleration. We found that pymetrozine and pyrifluquinazon, as well as BASF’s new insecticide afidopyropen (trade name: Inscalis™) act by targeting two Transient Receptor Potential (TRP) channels, Nanchung (Nan) and Inactive (Iav), which co-occur exclusively in chordotonal stretch receptor neurons and are essential for mechanosensory function. Simultaneous expression of Nan and Iav was required to form functional TRP channels in a heterologous system. These channels were potently activated by pymetrozine, pyrifluquinazon and afidopyropen. Tritium-labeled afidopyropen bound to membranes expressing Nan and Iav with higher affinity than pymetrozine and pyrifluquinazon. Nan protein formed the main binding interface for afidopyropen, whereas Iav dramatically increased binding affinity. These results define a conceptually novel mode of action whereby insecticides act by modulating TRP channels to perturb insect senses.

A-2003

Identifying the Role of Phosphorylation on Interdomain Communication in Hsp70. LAURA KNIGHTON and Andrew W. Truman. Department of Biological Sciences, University of North Carolina at Charlotte, NC. Email: lknigto@uncc.edu, Corresponding author: atruman1@uncc.edu

Heat shock protein 70 (Hsp70) is a highly conserved ubiquitous molecular chaperone. Hsp70 is essential for cell viability and plays a major role in the folding and activity of a large proportion of cellular proteins. The Hsp70 homologues in yeast are the SSA proteins. Hsp70 is comprised of two functional domains, the nucleotide binding domain (NBD) and the substrate-binding domain (SBD) connected via a flexible linker. Hsp70 binds and hydrolyzes ATP to ADP promoting wide-scale conformational changes communicated from NBD to SBD via the linker. This ‘ATPase cycle’ is critical for Hsp70 function and cell viability. Recent studies have uncovered a role for phosphorylation in the modulation of chaperone function. We exposed cells to a variety of stresses and analyzed the global post-translational modifications on Hsp70. Although the pattern of modification varied substantially for each stress, phosphorylation of T378 (located on the Hsp70 linker) was detected under multiple cellular conditions. Mutation of T378 to a non-phosphorylatable form in yeast (T378A) produced a cellular sensitivity to DNA damaging agents. We are currently optimizing a novel kinase-substrate crosslinking technology in an effort to identify the kinase that regulates T378 phosphorylation.

A-2004

A Histological Comparison of Ear Skin Regeneration in Acomys and Mus. A. SANDOVAL, J. Brant, and M. Maden. University of Florida, Department of Biology, 2033 Mowry Rd, Gainesville, FL 32608. Email: aarsan85@ufl.edu

Regeneration has been studied almost exclusively in lower invertebrates as most mammals are only able to regenerate fetal tissue. The African spiny mouse (Acomys) represents the first time advanced regeneration has been observed in an adult mammal. Acomys has evolved a defense mechanism which involves fragile skin that tears easily when caught by a predator, allowing the mouse to escape. Subsequently, the mouse regenerates extensive parts of its body. The regenerative capabilities of Acomys are being studied by comparing it to a normal mouse (Mus). In order to compare the progression of ear regeneration in Acomys and Mus, ears of both species were wounded using a four-millimeter punch to remove the epidermal and dermal tissue layers, revealing the underlying cartilage. The healing ears were harvested at 2, 4, 7, 14, 21, and 30 day time points. The ears were subsequently embedded in wax, mounted on slides, and trichrome stained to differentiate between erythrocytes, muscle and collagen. Microscopic analysis revealed that although the cartilaginous layer eventually degenerated in both species, extensive degeneration was present much earlier in Mus. Furthermore, Acomys was able to regenerate its cartilage and hair follicles, whereas Mus was only able to regenerate a disorganized, nonfunctioning mass of collagen. Significant scarring was evident in Mus, while no scarring was observed in Acomys. The results of further study of Acomys could prove integral in gaining a comprehensive understanding of the regenerative process. Findings could ultimately improve the entire healthcare field by allowing for the regeneration of human tissue.

A-2006

Over-expression of Recombinant Human Tumor Necrosis Factor Alpha in a Salt Inducible E.coli strain (GJ1158). AMIT DADARYA GUPTA¹, Diamond Jain³, Satya
Tumour necrosis factor-alpha (TNF-α) is a 17-kDa protein consisting of 157 amino acids that is a homotrimer in solution that causes damage to tumour cells but has no effect on normal cells. It is an important inflammatory cytokine and has a central role in the networking to modulate the production and functional activity of several inflammatory cytokines. Currently it is used in cancer treatment in the isolated limb perfusion (ILP) setting for soft tissue sarcoma (STS), irresectable tumors of various histological types, and melanoma intransit metastases confined to the limb. The interaction of TNF-α with TNF receptor 1 and receptor 2 (TNFR-1, TNFR-2) activates several signal transduction pathways, leading to the diverse functions of TNF-α. Although limited amounts of TNF can be obtained from natural sources, relatively large quantities of bioactive, soluble TNF, for structural and functional studies, can be easily obtained by recombinant DNA technology. Here we describe the methods for the expression of human soluble TNF (hTNF) in E. coli, for their extraction, purification, and characterization. Human TNFα cloned in pET20b vector that has a tight regulated T7 promoter upstream of the gene of interest. The recombinant TNFα was over-expressed in a E.coli strain GJ1158, which is a salt inducible host, expressing T7 RNA polymerase that is integrated in its chromosome downstream of PRO-U promoter that gets induced by the osmotic pressure of sodium chloride. The expressed recombinant TNFα was purified using ion exchange chromatography. Purified TNF, obtained according to this method, can be used for in vitro and in vivo studies in animal models.

A-2007

Opportunities for Translating Large-scale Cell Culture Technologies to the Production of Sustainable Clean Meat.

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Clean meat (meat grown using cell culture methods, rather than obtained from animal slaughter) is an emergent biotechnology industry that will ameliorate the serious environmental, sustainability, global public health, and animal welfare concerns of conventional animal agriculture. Critical Technology Elements (CTEs) for clean meat include immortalized cell lines for meat animals (e.g. chicken, pig, cattle, etc.), xeno-free media optimized for proliferation and maturation of these cell lines, edible or biodegradable scaffolding for tissue engineering, and efficient bioreactors for cell proliferation and differentiation. Many biomedical technologies and tools can already be applied to the CTEs of clean meat, but large-scale cell culture for clean meat production presents a number of unique requirements that are not currently met with existing technologies developed for the biomedical industry. Namely, cost constraints and scale requirements for the clean meat industry are notably different than for cellular therapeutics or regenerative medicine, and innovation is needed to develop products that are optimized for the cell types and structures that are relevant for clean meat. Further, developing these tools for clean meat would simultaneously advance the technology and reduce costs for the biomedical and therapeutic applications. We will discuss new applications for cell culture and tissue engineering technologies within the clean meat industry, and discuss potential for symbiotic and synergistic product development through partnerships between biomedical and life science researchers and the emergent clean meat industry.

A-2008

Differential Effects of Oxidative Stress on Cerebral and Cerebellar Neurons Cultured from the Embryonic Chick Brain.

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It has been suggested that oxidative stress-induced damage to proteins and lipids within neurons may underlie the cellular etiology of neurodegenerative diseases. Interestingly, neural disorders differentially affect specific brain regions; for example, motor but not cognitive dysfunction characteristic of Multiple Sclerosis. To understand the cellular basis underlying this phenomenon, our laboratory has therefore employed neurons cultured from different regions of the embryonic chick brain at embryonic day 10 through 14 to study the effects of peroxide-induced oxidative stress at the cellular level. Neurons isolated from the different brain regions were grown in mixed-cell cultures and treated with hydrogen peroxide (from 1 to 100 μM) for up to five days. We focused our assays on overall morphology, cell adhesion protein expression and traffic, and cytosolic protein expression and localization. In vitro, cerebral neurons generally grow longer neurites that can exceed ten times the normal cell body diameter, while neurons from the cerebellum or optic tectum grow shorter processes but with more extensive branching. Oxidative stress induces more extensive process retraction and detachment and decreases in overall cell adhesion protein expression and localization to the cell membrane in the cerebellar and tectal
neurons. In contrast, cerebral neurons subjected to oxidative stress show decreased expression and localization of the cytoskeletal support protein neurofilament-68. These differential effects suggest differential susceptibility of the neuronal subpopulations to oxidative stress, which may offer potential avenues for investigation for the cellular mechanisms affected by different neural disorders using the embryonic chick brain as a model system.

A-2009

The First Wave of Inflammation: Elucidating the Ability of AF and NP Cells to Respond to IVD Herniation Triggered Damage. M. GUERREIRO1, B. Laird2, D. E. Gregory2, and S. J. DeWitte-Orr3. 1Department of Biology, Wilfrid Laurier University 75 University Ave W, Waterloo, ON, CANADA, N2L 3C5 and 2Department of Kinesiology and Physical Education, Wilfrid Laurier University 75 University Ave W, Waterloo, ON, CANADA, N2L 3C5. Email: guer5930@mylaurier.ca

Lower back pain affects up to 80% of the population at some point during their life, with an estimated associated cost of $100 billion per year in North America alone. This results in considerable strains on our healthcare system despite having limited treatment options. Lower back pain can be attributed to mechanical compression of the nerve roots and inflammation caused by the innate immune response triggered by intervertebral disk (IVD) herniation, where a tear in the outer annulus fibrosus (AF) causes the migration of the inner nucleus pulposus (NP) through the AF. Macrophages are known to play a role in initiating inflammation at the site of herniation; however, very little is known of the role of resident IVD cells in initiating the first wave of inflammation and recruiting macrophages to the site of herniation. The present research aims to fill this void by studying resident AF and NP cells derived from rat trail IVDs; first developing primary cell cultures for each cell type, then characterizing their innate immune sensor repertoire and finally studying how these cells respond to inflammatory stimuli, both pathogen and host derived. Understanding the progression of the innate immune response and its role in lower back pain will allow treatments targeting IVD cell sensors in order to stop pain and inflammation before an innate immune response can be mounted.

A-2010

Oxidative Stress-induced Tau Hyperphosphorylation and Redistribution in Cultured Chick Embryonic Brain Neurons. ERIKA BEYRENT and George Gomez. University of Scranton, 800 Linden Street, Scranton, PA 18510. Email: erika.beyrent@scranton.edu

Abnormal phosphorylation of microtubule-associated proteins, such as tau, have been shown to play a role in neurodegenerative disorders, including Alzheimer’s disease and Parkinson’s disease. Aggregates of hyperphosphorylated tau have commonly been shown in post-mortem tissue from individuals afflicted with neurodegenerative disorders such as Alzheimer’s disease. One such condition is oxidative stress, which has been shown to lead to hyperphosphorylation of tau, causing aggregation into non-functional clumps, and the ultimate degradation of the microtubule network. Thus we wish to assess this process in cultured chick neurons isolated from the chick brain, an ideal model for the study of neurons derived from endothermal species. Our goal was to test if tau hyperphosphorylation ultimately leads to neuronal degradation. We treated cells with 10 μM tert-butyl hydroperoxide for 48 hours, then immunostained for phospho-tau and β-tubulin III. In untreated cells, tau protein was tightly colocalized with the microtubule network. Increasing levels of oxidative stress induced a lower correlation between tau and the cytoskeleton, coupled with reductions in neural process length. In addition, oxidative stress induced redistribution of phospho-tau from larger dispersal into larger aggregates. These results suggest that oxidative stress induces hyperphosphorylation of tau protein in live cells, causing them to form aggregates that lead to cytoskeletal degradation and neuronal retraction. Our future studies are focused on determining these effects in neuronal subpopulations derived from the embryonic chick brain.

A-2011

Identifying Cytoplasmic DNA Sensors, DHX9 and DDX3, in Rainbow Trout. SHANEE HERRINGTON-KRAUSE. Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA. Email: herr0600@mylaurier.ca

Innate immunity constitutes the first line of defense during viral infections. Viruses produce nucleic acids, both RNA and DNA, during genome replication and transcript synthesis. These nucleic acids are foreign to the cell and are sensed by pattern recognition receptors, based on their type (RNA or DNA), their strandedness (ss or ds) and their location (endosomal, extracellular or cytoplasmic). When a viral nucleic acid is in the wrong compartment (e.g., dsDNA in the cytoplasm), pattern recognition receptors (PRRs) detect it and activate signalling cascades, which culminate in the production of type I interferons (IFNs) and the induction of an antiviral state. Cytoplasmic RNA sensors, such as RIG-I and MDA5, have begun to be characterized in several fish species, but almost nothing is known of cytoplasmic DNA sensors (CDSs) in fish. To this end, two CDSs were cloned from the rainbow trout macrophage-like cell line RTS11. Both CDSs are ATP dependent RNA helicases that
unwind DNA and RNA in the 3’ to 5’ direction as well as functioning as innate immune sensors to initiate an antiviral state via the IFN pathway during a virus infection. In this study the novel rainbow trout DHX9 and DDX3 sequences were compared to known vertebrates sequences to identify conserved protein domains, intron/exon structures and phylogeny. Knowledge of CDSs in rainbow trout will aid in a better understanding of innate antiviral immunity in this commercially and economically important fish species.

**A-2012**

Effects of Resveratrol on Contractions of the Rat Tail Artery: Role of Endothelium. IAN VANANTWERP, Laura Phelps, and Jacob Peuler. Pharmacology Department, Midwestern University, Downers Grove, IL 60515. Email: ivanantwerp42@midwestern.edu

Several published studies have consistently shown resveratrol can directly relax smooth muscle *in vitro*. Our recent preliminary work found that resveratrol’s relaxing action (over 2 hours) on phenylephrine (PE) pre-contracted rat tail arterial tissue rings could not be blocked by any of seven different potassium (K) channel blockers. We also uncovered a novel action of resveratrol to transiently enhance such adrenergic pre-contractions before it’s more delayed and sustained relaxant effect occurred. This same transient enhancement was notably inhibited by some of the same K channel blockers (particularly tetroethylammonium and glibenclamide) that failed to affect it’s relaxant action. We suspected that resveratrol could be acting on K-selective mechano-sensitive ion channels located in the endothelium where they may participate in the release of contracting factors. After initial PE-induced contractions of arterial segments plateaued, acetylcholine (ACh) at 10 μM (a maximally-effective concentration) was administered to produce endothelium-dependent relaxations. Thus, immediate relaxation was indicative of the presence of endothelium while the absence of relaxation was indicative of removal. Chemical removal of the endothelium with saponin (0.1 mg/ml perfusion for 2 minutes) from rat tail arterial tissue demonstrated a significantly less % change in PE-induced contractions prior to relaxation. This same transient enhancement was no-longer present. After chemical removal in regards to compromising the long-term stability of PE-induced contractions necessary to produce fully sustained relaxations. Resveratrol administration generated enhancement of PE-induced contractions prior to relaxation in all endothelium-intact and saponin treated tissues. This suggests the possibility of an endothelium-independent mechanism (perhaps at the level of a smooth muscle receptor or unidentified polyphenol receptor) to be a focus of future studies.

**A-2013**

Eicosanoid Inhibitors Impact Protein Expression in an Insect Cell Line, CYNTHIA L. GOODMAN1, Tamra R. Lincoln1, Yaofa Li2, Joseph Ringbauer, Jr1, Kaile Zhou3, and David Stanley1. 1USDA, ARS, BCIRL, 1503 S. Providence Rd., Columbia, MO 65203; 2Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, Ministry of Agriculture, Baoding 07100, CHINA; and 3University of Missouri, Division of Plant Sciences, Columbia, MO 65211. Email: cindy.goodman@ars.usda.gov

Eicosanoids, including prostaglandins (PGs), are oxygenated metabolites of three C20 polyunsaturated fatty acids. They mediate many physiological functions in immunity, reproduction, development, and ion transport in vertebrates and invertebrates. We previously reported that PGs influence the expression of specific proteins in established insect cell lines. Here we extend these observations and show that the inhibition of eicosanoid biosynthesis also impacts the expression of specific proteins. BCIRL-HvAM1 cells, derived from *Heliothis virescens* ovaries, were incubated in eicosanoid biosynthesis inhibitors or, as a comparison, prostaglandin A2 (PGA2) for 12 h and 24 h, harvested and subjected to 2D gel electrophoresis. Proteins that were up- or down-regulated by approximately 2-fold or greater were analyzed via mass spectrometry and identified by bioinformatics. Protein changes were confirmed using qPCR. After 12 h treatments, expression of 36 proteins were altered. Of these, 28 proteins were identified, 8 were up-regulated and 20 down-regulated. After 24 h treatments, expression of 58 proteins were altered. Of these, 38 proteins were identified, 12 were up-regulated and 26 down-regulated. The identified proteins had a variety of functions, including energetics, transcription and protein metabolism. For example, the lipooxygenase synthesis inhibitor, esculetin, and the phospholipase inhibitor, MAFP, led to about 50% reduction in the alpha proteasome subunit at 12 h. The PG synthesis inhibitor, indomethacin, led to about a 5-fold increase in methionine aminopeptidase at 24 h. This work confirms our earlier work and indicates that PGs and other eicosanoids are essential signal elements in the biology of HvAM1 cells, and likely most insect cell lines.

**A-2014**

Real Time Assay for Apoptosis Using Complementation of Annexin V Luciferase Fragments. TERRY RISS1, Kevin Kupcho1, John Shultz1, Jim Hartnett1, Robin Hurst1, Wenhui Zhou1, and Andrew Niles1. 1Promega Corporation, Madison, WI and 2Promega Biosciences, San Luis Obispo, CA. Email: terrry.riss@promega.com

Real Time Assay for Apoptosis Using Complementation of Annexin V Luciferase Fragments. TERRY RISS1, Kevin Kupcho1, John Shultz1, Jim Hartnett1, Robin Hurst1, Wenhui Zhou1, and Andrew Niles1. 1Promega Corporation, Madison, WI and 2Promega Biosciences, San Luis Obispo, CA. Email: terrry.riss@promega.com
We have developed a homogeneous real-time assay for detecting apoptosis that is recorded using a standard plate-reading luminometer. The assay is based on binding of annexin V to phosphatidyl serine (PS) which becomes exposed on the outer leaflet of the cell membrane during the process of apoptosis. We have engineered two genetic fusion proteins composed of annexin V linked to a small or large fragment of luciferase. The purified fusion proteins and a luciferase substrate are added as a reagent to the medium of cultured cells. The individual annexin V-luciferase fragment fusion proteins have very low background luminescence in the presence of non-apoptotic cells. When exposed to apoptotic cells, the annexin V-luciferase fragment fusion proteins bind to PS in close proximity to reconstitute an active luciferase enzyme and generate a luminescent signal in the presence of a time-release luciferase substrate. The reagent can be added to cells for extended periods of incubation enabling detection of the onset of apoptosis in real time. The homogeneous luminescent assay has been multiplexed with a fluorogenic DNA binding dye to demonstrate the onset of annexin V binding precedes loss of membrane integrity and secondary necrosis in vitro. The assay has been validated using a number of anchorage dependent and suspension cell lines and has been shown to correlate with activation of caspase-3/7 activity as an orthogonal marker of apoptosis. Imaging the luminescent signal enables creation of time lapse movies showing individual cells among a population undergoing apoptosis. This new homogeneous apoptosis assay method represents a simplification and improvement over flow cytometry and endpoint assay methods by providing kinetic data from the same sample of live cells in real time using a standard plate reading luminometer.