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Animal Contributed Papers

A-1000

Understanding the Mechanism of Action of Anti-microbial Peptides Using a Novel DNA-based Strategy and Enantiomers. SIVA SANKARI, Markus F. Arnold, and Graham C. Walker. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA. Email: sivasank@mit.edu

Antibiotic resistance is a key problem to healthcare worldwide and the rate of development of new classes of antibiotics continues to lag. Anti-Microbial Peptides (AMPs) are a promising class of therapeutic agents, since they are effective against broad spectrum of bacteria and fungi. Moreover, AMPs not only act on bacterial membrane, but they also have various intracellular targets, which makes it harder for bacteria to develop resistance against an AMP. It is important to understand the detailed mechanism of action of AMPs in order to develop them into novel drugs. The classical method of studying their mechanisms of action by treatment of bacteria with chemically synthesized AMPs has a major pitfall. This method cannot differentiate between the distinctive effects of AMPs on bacterial membranes and on intracellular targets in the cytoplasm. In order to study the effect of AMP at a subcellular location, we have developed an innovative, DNA-based regulatable system in *E. coli*. In this system, NCR247 (an antimicrobial peptide produced by plants) is expressed from within the bacteria and is localized to a subcellular location. Our results indicate that NCR247 is bactericidal when expressed as a fusion protein to an outer membrane protein and is presented outside the cell. However, NCR247 is bacteriostatic when it is expressed within the cytoplasm. Our data shows that NCR247 exhibits distinctive physiological effects when expressed in different subcellular, bacterial compartments. We are currently extending this strategy to study the mechanism of action of other AMPs. Since NCR247 is capable of exhibiting pleiotropic effects on bacteria, we wish to understand which of these effects are due to peptide's interaction with bacterial membrane and which are due to stereo-specific peptide-protein interactions. Using D-enantiomer of NCR247 we have shown that peptides effect on membrane permeability and lethality are chirally independent, but other physiological effects are due to peptide-protein interaction. We are currently investigating the protein partners of NCR peptide in bacteria.

A-1001

Dietary Supplementation with Anthocyanin-rich Berries Promotes Healthy Muscle Development Gene Expression Profiles in Diet-induced Obese Mice. JIA XIONG[1,2], John Overall[1,3], Slavko Komarnytsky[1,3], and Debora Esposito[1,2]. [1]Plants for Human Health Institute, North Carolina State University, North Carolina Research Campus, 600 Laureate Way, Kannapolis, NC 28081; [2]Department of Animal Science, NC State University, 120 Broughton Drive, Raleigh, NC 27695; and [3]Department of Food, Bioprocessing & Nutrition Sciences, North Carolina State University, 400 Dan Allen Drive, Raleigh, NC 27695. Email: jxiong5@ncsu.edu; daesposi@ncsu.edu

Anthocyanin pigments are one of flavonoid group of phytochemicals found in colorful fruits like berries, vegetables and other plants. Anthocyanins have demonstrated ability to protect against various human diseases related to increased production of free radicals and oxidative stress. In fact, other mechanisms of action of anthocyanin-rich diets are also responsible for health benefits, like protecting against from DNA cleavage, boosting production of cytokines and regulating immune responses, providing anti-inflammatory and vasoprotective activities, maintaining healthy body weight, and improving glucose and lipid metabolism (Lila MA, 2004, Skates E, et al. 2017). In previous research, diets normalized to 400 μg/g total anthocyanins derived from blackberry, black raspberry, blackcurrant, maqui berry, Concord grape, and blueberry improved body composition and reduced metabolic damage in DIO mouse model. These effects were most pronounced in the blueberry and blackcurrant groups (9.8%–10.2%, $p < 0.05$) (Overall John, et al. 2017). To understand the mechanisms leading to increased lean body mass in

these animals, we analyzed gene expression profiles associated with muscle growth and development in this study. On average, berry diets with structurally diverse anthocyanin profiles were associated with increased abundance of MyoD1 (fold change 1.4-2.8), Myf6 (fold change 1.5-3.4), and Myog (fold change 1.5-3.2) transcriptional factors that coordinate myogenesis and repair of the muscle tissue. Gdf8, Trim63, and Fbxo32 genes associated with the muscle protein turnover showed a weak upregulation (fold change 1.4-1.8) similar to healthy controls. Thus, the return of gene expression in the berry-fed groups back to baseline expression seen in low fat controls demonstrated a dynamic modification of muscle development gene networks that likely contribute to improved metabolic outcomes observed in these animals.

A-1002

Characterization of Polymeric Electrospun Fibers for Tissue Engineering and Biologic Delivery. ALYSSA G. TOGLIATTI and Joshua Z. Gasiorowski. Department of Biomedical Sciences, College of Graduate Studies, Midwestern University, Downers Grove, IL. Email: atogliatti94@midwestern.edu

The degradation of cellular tissue is a significant limiting factor for viable treatment options in structural dysfunction disorders and injuries. Lacking the mechanical and physiological support of the extracellular matrix (ECM), the insulted tissue is functionally devoid of the necessary scaffolding and deprived of its innate biochemical signaling capabilities to support efficient cellular growth. Our research seeks to address this issue by fabricating electrospun fibrous polymeric networks to serve as synthetic scaffolding structures that mimic the biological role of the native ECM. To achieve this, we systematically altered electrospinning parameters to create consistent poly(glycerol-dodecanedioate) fibers with 1.0-2.0 micron diameters and various levels of fiber alignment. Once a reproducible protocol had been established, we expanded our methodology to successfully encapsulate plasmid DNA (pDNA) within core-sheath coaxially spun fibers and examined the passive diffusivity of the pDNA from the core over a 6-day timespan. Preliminary results revealed a binary release profile, suggesting the pDNA exponentially leached out of the polymeric sheath within the first hour and then progressed into a sustained release for the remaining 143 hours. We have found that our electrospun fiber mats support mammalian cell growth and are currently investigating the transfection activity of the functionalized fibers. Upon completion of this study, we aim to use our pDNA-loaded electrospun fibers as a biocompatible scaffold for various tissue engineering purposes.

A-1003

The Revivability and Growth Potential of Primary Chicken Embryo Fibroblast Cells Stored Frozen in Liquid Nitrogen. GIRISH SARMA. Hygieia Biological Laboratories, PO Box 8300, Woodland, CA 95776. Email: girishsarma1@gmail.com

Primary chicken embryo fibroblast (CEF) cells are widely used for isolation, identification, propagation and other research work with avian viruses worldwide. These cells are prepared freshly from embryos at 9 to 10 days of embryonation. Specific pathogen free (SPF) eggs are commonly used for this purpose. In general, preparation of primary CEF cells is time consuming and expensive. Moreover, SPF eggs are not readily available everywhere at any time. Therefore, it would be much more convenient and economical if the primary CEF cells could be stored frozen for long periods for future use. Prior to this report, no published information was available regarding use of uninfected cryopreserved primary CEF cells for virus research. The goal of this study was to find out if these cells can be stored frozen successfully for future use over a long period. The primary CEF cells used in this study were prepared using SPF eggs at 9 days of embryonation. After trypsinization, cells were resuspended in cell freezing medium consisting of medium M-199, fetal bovine serum (FBS), dimethyl sulphoxide (DMSO) and penicillin and streptomycin as preservative. The cells were stored in liquid nitrogen for up to 2 years. At various interval during storage, the cells were revived from liquid nitrogen and subcultured using fresh growth medium containing M-199 supplemented with 4 to 10% FBS. The results indicated that these primary cells could be stored successfully up to 2 years (maximum period tested) in liquid nitrogen and when revived, the cells readily formed confluent monolayers of healthy cells every time. Prolonged storage did not adversely affect its susceptibility to some of the avian viruses tested. The cells could also be subcultured continuously for 11 times (maximum number tested). The results obtained from this study could facilitate maintenance of a standard stock of primary CEF cells for future use as and when required. This would be particularly helpful for laboratories where SPF eggs are not readily available to prepare the cells freshly every time.

A-1004

Fish In Vitro Biology: Another Piece of the Puzzle for the Aquaculture of New Zealand Native Species *Chrysophrys auratus*. GEORGINA C. DOWD[1], Gavril Chong[1], Lucy E. J. Lee[2], Susan N. Marshall[1], and Maren Wellenreuther[1,3]. [1]The New Zealand Institute for Plant and Food Research Limited, Nelson, NEW ZEALAND; [2]University of the Fraser Valley, CANADA; and [3]School of Biological

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Chrysophrys auratus (Australasian snapper) is one of the most abundant teleost species in the in-shore waters of New Zealand. Commercially, the in-shore snapper fishery is one of the largest and most valuable in New Zealand contributing NZD$86 million per annum to GDP, and employing over 700 people. Snapper are also one of the most in-demand recreationally caught fish in New Zealand. To ensure sustainability of this sought-after species, snapper stocks are managed within the Quota Management System (QMS). The New Zealand Institute for Plant and Food Research Limited started a genomics-assisted selective breeding programme in 2016 to investigate the suitability of snapper as an aquaculture species. The establishment of a strong broodstock, combined with high-throughput genotyping and phenotyping of selected individuals in a three generation pedigree, has led to a greater understanding of the teleosts life cycle, from hatching larvae through to optimising nutrition for adults. As part of the breeding programme a high-quality snapper genome has been assembled. Regions in the genome associated with commercially important traits have been identified, leading on to the selective breeding of high-quality fast-growing individuals. We are currently working on the development of cell lines from snapper to support research into the captive breeding of the species. In this presentation we will discuss the growth of cells extracted from various tissue types, with a specific focus on gill cells. We will present sequence data distinguishing snapper of New Zealand and Australian origin, and show where the snapper gill cells fall in this differentiation. Finally, we will discuss applications of cell lines as a research tool for the breeding and further development of new species for aquaculture.

A-1005

Cisplatin-induced Apoptosis Requires Functional p53 in a Neuroblastoma Cell Line. ALEXANDER CAIRO, Kelly Keeler, and Kolla Kristjansdottir. Department of Biomedical Sciences, College of Graduate Studies, Midwestern University, Downers Grove, IL. Email: acairo37@midwestern.edu

Neuroblastoma is the most common extracranial solid tumor in children. Cisplatin, a chemotherapeutic agent used to treat neuroblastoma, induces DNA damage and in response cells initiate DNA repair and apoptosis through activation of the p53 pathway. Activation of p53 pathway involves stabilization of the protein by several mechanisms including phosphorylation of p53 and an interaction with NPM1, a molecular chaperone important for DNA double strand break repair. Furthermore, 100 M cisplatin treatment for 6 hours induced a 4-fold increase in apoptosis in WS neuroblastoma cells as compared to untreated cells as measured by the Apo-One Caspase 3/7 assay. Apoptosis was also detected by the appearance of cleaved PARP after 20 M cisplatin treatment for 24 hours in WS cells as measured by immunofluorescence. p53 pathway activation was confirmed by detection of phospho-p53 in cisplatin treated cells using immunofluorescence. To confirm that the apoptosis was p53 dependent, we treated neuroblastoma cells containing mutated or non-functional p53 protein, SK-N-BE(2) and SK-N-AS, with 100 M cisplatin treatment for 6 hours and found that apoptosis was no longer detectable using the Apo-One Caspase 3/7 assay. In addition, cleaved PARP was no longer detectable using immunofluorescence after treatment of SK-N-AS and SK-N-BE(2) cells. Thus, apoptosis induction by cisplatin requires functional p53 in neuroblastoma cells. Given the importance of p53 and the interaction between NPM1 and p53, we hypothesize that NPM1 localization is important for cisplatin-induced apoptosis in neuroblastoma cells. We have generated a cytoplasmic mutant of NPM1 similar to the one found in acute myeloid leukemia patients. We are currently transfecting full-length NPM1 and the cytoplasmic NPM1 mutant into both p53 wild-type and non-functional neuroblastoma cells. We will examine the localization of p53, the activation of p53 pathway and induction of apoptosis in these cells. Taken together these data will help elucidate the relationship between NPM1 and the p53 pathway in neuroblastoma.

A-1006

Translating Large-scale Commercial Cell Culture Technologies to the Production of Cell-based Meat. LIZ SPECHT. The Good Food Institute, Washington, DC. Email: lizs@gfi.org

Global demand for meat is expected to rise by nearly 70% in the next 30 years, yet current meat production methods will be unable to meet this demand due to their intense resource and land requirements. Furthermore, the most efficient meat production methods— those that involve intensive, industrialized systems—are plagued by a host of externalized harms such as severe environmental pollution and public health risks. One solution is to develop technologies for making delicious and price-competitive meat that satisfies consumer demand with a fraction of the resource burden of conventional meat production. Cell-based meat (meat grown using cell culture methods, rather than obtained from animal slaughter) is one such emergent biotechnology-based solution. In the past three years, more than three dozen startup companies have emerged to commercialize cell-based meat, but this sector is still nascent from a technology development standpoint. This talk will explore the state of the cell-based meat industry with a particular focus on specific

areas of need within the technological landscape, where an influx of creative approaches and concerted resource allocation seems best poised to advance the field. Many technologies and tools that have been developed for other animal cell culture applications like cell therapy and biopharma can be applied to cell-based meat, but scale-up and economically viable commercialization of this technology presents a number of unique challenges. We will discuss new applications for existing cell culture and tissue engineering technologies within the cell-based meat industry as well as opportunities for completely novel technology development.