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Animal Posters

A-2000

Adult Human Fibroblasts Cultured on 3D Silk Fibroin Nonwovens Release Exosomes Strongly Stimulating Neoangiogenesis. UBALDO ARMATO[1,2], Peng Hu[1,3], Jun Wu[1,2], Anna Chiarini[1], and Ilaria Dal Prà[1,2]. [1]Human Histology & Embryology Section, Department of Surgery, Dentistry, Pediatrics & Gynecology, University of Verona, Verona, ITALY; [2]Department of Burns and Plastic Surgery, Second People's Hospital, University of Shenzhen, Shenzhen, CHINA; and [3]Department of Burns & Plastic Surgery, Affiliated Hospital of ZunYi Medical University, ZunYi City, CHINA. Email: uarmato@gmail.com

Deep extended skin wounds heal slowly and often with poor outcomes. Our group is developing novel silk fibroin (from *Bombyx mori*)-based nonwovens (3D-SFnws) that grafted *in situ* as scaffolds should improve wound healing. Vascularization is crucial to the success of any implanted biomaterial scaffold. Our earlier *in vivo* (mice) work had shown that subcutaneously implanted 3D-SF-nws were quickly vascularized via undefined mechanism(s). Here we hypothesized that adult human dermal fibroblasts (HDFs) once stuck to 3D-SFnws keep releasing exosomes transporting angiogenic/growth factors (AGFs). We cultured HDFs on carded/hydroentangled 3D-SF-nws, whose biomechanical features we also studied, and on polystyrene plates in exosome-depleted medium and appraised HDFs growth (DNA) and metabolism (glucose). We isolated the total fractions of CD9-expressing exosomes from pooled HDF-conditioned media and used specific double-antibody arrays to analyze the nature and amounts of AGFs they carried. Finally, we gauged via tube formation assay the exosomes' angiogenic effects on human dermal microvascular endothelial cells (HDMVECs). Our results show that 3D-SFnws biomechanical features met the typical requisites of human soft tissues. HDFs cultured for 2 weeks on 3D-SFnws significantly increased in numbers and actively metabolized *D*-glucose. Exosomes released from 3D-SFnws-stuck HDFs carried remarkably higher amounts of AGFs—i.e. IL-1α, IL-4, and IL-8; Angiopoietin-1 and -2; Tie; GRO-α, GRO-β, and GRO-γ; MMP-1; MMP inhibitor-1; and uPAR—than did those from polystyrene-attached HDFs. At concentrations from 0.62 to 10 μg ml^{-1} the exosomes from 3D-SFnws-stuck HDFs quickly induced the formation of copious HDMVECs tubes *in vitro*. Thus, our results (*i*) further support 3D-SFnws use for skin tissue engineering/regeneration aims; and (*ii*) uphold our hypothesis that SF/HDF interactions cause the exosomal release of multiple AGFs whose joint angiogenic effects can advance 3D-SFnws implants' take *in vivo,* thus improving deep extended skin wounds healing and outcome.

A-2001

Establishment and Transcriptomic Characterization of Cell Lines and Sublines from the Small Hive Beetle, *Aethina tumida* (Coleoptera: Nitidulidae). CYNTHIA L. GOODMAN[1], Jacob Corcoran[1], Stephen Saathoff[1], Joseph A. Ringbauer, Jr.[1], Ya Guo[2], Marilise Stamps[1], Bryony Bonning[2], and David Stanley[1]. [1]Biological Control of Insects Research Laboratory, USDA, ARS, 1503 S. Providence Rd., Columbia, MO 65203 and [2]Entomology and Nematology Department, Institute of Food and Agricultural Sciences, University of Florida, 1881 Natural Area Drive, Gainesville, FL 32611. Email: cindy.goodman@usda.gov

The small hive beetle (SHB), *Aethina tumida*, originated in sub-Saharan Africa and now has a world-wide distribution. Adult SHBs deposit their eggs in honey bee colonies and the larvae feed on honey, pollen and honey bee larvae. Over time this negatively impacts honey bee populations, which translates into a major economic impact on agriculture. A number of measures have been implemented to control SHBs, including the use of biological agents. Current efforts focus on developing novel, SHB-specific insecticides, which could be enhanced with tools to screen candidate compounds against cell lines derived from SHB tissues. Here we report on two continuously replicating SHB cell lines: BCIRL-AtuE-1127-SGS from eggs and BCIRL-AtuNE-1129-SGS from eggs and neonate larvae. Each line consists of a variety of cell morphotypes, including firmly attached asymmetrical cells

and loosely-attached spherical cells. Numerous sublines were produced by limiting dilution to isolate different cell morphotypes; 20 sublines were selected and cryopreserved. Of these, 3 were selected from the AtuNE-1129 parental line and 6 from the AtuE-1127 line for further molecular and genetic characterization. DNA barcoding confirmed that the sublines were derived from SHB tissues. Growth curves indicated their doubling times ranged from 29.2 h to 68.4 h. Gene expression profiles were evaluated in three sublines via RNA-Seq. The transcriptomic libraries and gene expression profiles will aid in the identification of potential insecticidal targets present in the cell lines. Here we present a small subset of genes expressed in these cell lines that could support insecticide development programs, including: various members of ABC transporter families A-G; calcium transporters, such as plasma membrane and endoplasmic reticulum calcium ATPases; and GPCRs with neurophysiological functions, such as octopamine and pyrokinin receptors.

A-2002

Cardioprotective Functions of Prenylated Stilbenoids and Peanut Hairy Root Extracts. ROKIB HASAN[1,2], Sankalpa Chakraborty[1,2], Viswanathan Rajagopalan[3], and Fabricio Medina-Bolivar[1,4]. [1]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401; [2]Molecular Biosciences Graduate Program, Arkansas State University, Jonesboro, AR 72401; [3]Department of Basic Sciences, New York Institute of Technology-College of Osteopathic Medicine, Jonesboro, AR 72401; and [4]Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401. Email: mdrokib.hasan@smail.astate.edu

Despite great therapeutic advances, cardiovascular diseases (CVD) remain a major health problem and the leading cause of mortality worldwide. To improve symptoms and survival of CVD patients, novel therapeutic strategies are needed. Stilbenoids such as resveratrol possess cardioprotective, anti-inflammatory, and antioxidant activities. Resveratrol derivatives, such as the prenylated stilbenoids arachidin-1 and arachidin-3, are produced in peanut and are potentially more bioavailable than resveratrol. However, the molecular mechanisms underlying the bioactivities of prenylated stilbenoids remain unclear. To understand the cardioprotective effects of a peanut hairy root extract enriched in arachidins and purified arachidins, we used H_2O_2-treated H9c2 rat heart cells as a model for oxidative stress. We have also conducted a pilot study with rats to investigate the cardioprotective properties of these extracts. Our preliminary results suggest that the extract, arachidin-1 and arachidin-3 are not toxic to the cells at low concentrations. Both the extract and arachidins pretreatment offer protection against oxidative stress. Furthermore, the in vivo study showed that the peanut hairy root extracts can help to improve cardiac injury of propylthiouracil-treated rats. These studies will increase our understanding of the cardiac function of arachidins and carry important translational implications for the application of prenylated stilbenoids for cardioprotection.

A-2003

The Role of Neuronal Guidance Molecules in the Migration of Melanoma Cells. LAURA LINARES PROANO[1], Gustavo Untiveros[2], and Luigi Strizzi[2]. College of Graduate Studies, [1]Department of Biomedical Sciences and [2]Department of Pathology, Midwestern University, Downers Grove, IL. Email: laura.linaresproano@midwestern.edu

Embryonic neural crest cells give rise to various cell lineages, including melanocytes, which respond to specific guidance signals during tissue specification and positioning. The neural guidance factor Netrin-1 and its receptors DCC, Neogenin, and UNC5 have been shown to regulate migration and positioning of neural crest cells during development. Cells expressing DCC and Neogenin are attracted to Netrin-1, while cells that express UNC5 receptors are repulsed by Netrin-1. Several studies show that Netrin-1 and its receptors may play a role during cancer progression. However, the role of Netrin-1 and Neogenin receptor in melanoma is unclear. Studies of the interaction between Netrin-1 and its receptors in melanoma could explain how melanoma may be attracted to specific Netrin-1 expressing tissues. In fact, postnatal expression of Netrin-1 is detected in brain, colon, liver and kidney – sites of potential melanoma metastasis. Thus, research that defines factors regulating melanoma metastasis is key to developing treatments that halt melanoma progression and prolong patient survival. Here, we investigated whether melanoma cells expressing Neogenin receptor are attracted to Netrin-1. We analyzed expression and function of Netrin-1 and Neogenin receptor in different human melanoma cell lines. Results from Western blot analysis show poor Netrin-1 expression in all melanoma cell lines tested. However, Neogenin receptor expression is higher in aggressive compared to non-aggressive melanoma cells. Migration assays show increased migration of Neogenin receptor positive melanoma cells towards exogenous soluble human rNetrin-1 and towards the Netrin-1 expressing cell line, SY5Y. Finally, migration assays of Neogenin positive melanoma cells treated with Neogenin receptor blocking antibody show a decrease in migration of the anti-Neogenin treated melanoma cells towards SY5Y compared to melanoma cells treated with control IgG. These results suggest that Neogenin receptor may play a role during spread of melanoma towards Netrin-1 positive tissues.

A-2004

Utilization of Enzymes in Seafood Industry. OLUWATOSIN ABIDEMI OGUNKALU and İlknur Ucak. Nigde Omer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Nigde, 51240 TURKEY. Email: ogunkaluoluwatosin1@gmail.com

Enzymes are part of biotechnology instruments and they are applied in seafood and fish industries to achieve particular interest since historical ages. They contain molecules that have potential of transforming biological cells. The quest for enzymes that possess a particular character is on the increased side. Enzymes are crucial part of food because of the following purposes: development, processing, preservation, customer interest. The conventional usage of enzymes in seafood processing are restricted to minimal products like fish sauce and fish protein hydrolytes and their production depend on internal proteases in fish. Seafood enzymes are been utilized now a days in various processes for enhancement of products like selection of skin removal, scale removal and puncture procedure in production of cured roe. Saltwater habitat consists several capacities as origin of enzymes with varieties of genetic matters with characteristics like thermal resistant that are suitable for processing in food industry. This distinct characteristic contained in saltwater organisms could be beneficial for enhancement and formation of new products. Strong application of marine enzymes in seafood processing has a specific influence on the industry because of the development of enzymes as a tools for value addition to discarded parts and monitoring quality of Seafood products.

A-2005

Personalized Cancer Cell Weapons Using CRISPR Genetic Engineering. NINA REDDY. Division of Genetic Medicine, University of Michigan, MI. Email: ninare@umich.edu

Cancer cells have a homing ability and are able to track cells of their own kind. Because of this constant communication and close proximity, cancer cells could be used as targeted weapons that deliver treatments to impede their survival. CRISPR may be used to design a small population of genetically engineered cancer cells that could produce harmful agents to other circulating cancer cells. In designing the engineered cancer cells, IRF7 was activated using CRISPRa. IRF7 is commonly expressed on breast cancer cells and is involved in the production of Interferon 7, a protein observed to inhibit the growth of cancer cells. In order to establish the IRF7 activation, a modified dCas9-VPR cell line was created by transducing the dCas9 protein through lentiviral delivery. After the CRISPR engineered cells were produced, two control groups (MCF-7 and dCas9) and a CRISPR group were made. Neutral red was used to stain CRISPR cells to differentiate and monitor them. An MTT assay was utilized over four consecutive days to determine cell proliferation and viability. The CRISPR cells maintained interaction with the cancer cells in culture when observed under the microscope. Six days after the initial co-culture, the CRISPR group had statistically significant lowered growth rates compared with both control groups ($p < 0.05$). Additionally, the CRISPR group had the lowest total optical density counts for all the MTT days. There could be potential in using cancer cells as a targeted treatment approach in attacking the inner workings of cancer development.

A-2006

Effects of Sodium Butyrate and Nitrite on Rainbow Trout Epithelial Cells. N. C. BOLS[1], E. Braley[2], D. Pritchard[2], J. B. Lee[2], P. G. Pumputis[1], V. R. Dayeh[1], and L. E. J. Lee[2]. [1]University of Waterloo, Department of Biology, Waterloo, ON N2L 3G1, CANADA and [2]Department of Biology, University of the Fraser Valley, Abbotsford, BC, CANADA. Email: ncbols@uwaterloo.ca

How dietary and environmental inputs affect the integrity of fish epithelia, such as gastrointestinal and gill, is important to know in order to improve aquaculture and protect fisheries. The short chain fatty acid, butyrate, is being considered as a dietary supplement in aquaculture whereas high nitrite levels are found in aquaculture systems, agricultural watersheds and industrial wastewater. Being a barrier between organism and environment, epithelial cells participate generally in innate immunity and specifically in nutrient absorption and respiration in respectively the gut and gill. Cultures of rainbow trout epithelial cell lines, RTgutGC and RTgill-W1, in L15 were used to study the effect of butyrate and nitrite on these epithelia. The responses to butyrate were influenced by two variables: the presence or absence of fetal bovine serum (FBS) and of closed culture lids. With 10 mM butyrate, cytotoxicity was observed in microwell plate cultures with FBS but not in tightly capped flasks nor in L15 alone, regardless of the culture vessel. In L15, cell migration was stimulated by butyrate at 0.1 mM. At high concentrations (^{3} 2.0 mM), migration was inhibited, F-actin was disassembled, but ZO-1 organization was retained. For nitrite, monolayers remained viable at up to 100 mM, but healing of monolayer wounds was modulated by lower concentrations. The response to combined butyrate and $NO2^-$ exposures will be interesting to investigate in the future.

A-2007

Contextualizing IP3 Receptor-dependent Astrocytic TGF-β Signaling in Blood-brain Barrier Permeability: A Novel

Functional Role. EMILY HASHEM and Donna Leonardi. Bergen County Academies, 200 Hackensack Ave, Hackensack, NJ. Email: hashem.emilyn@gmail.com

Blood brain barrier (BBB) dysfunction is a hallmark of neurodegenerative disease and a known factor in precluding brain recovery following traumatic brain injury (TBI) and stroke. BBB dysfunction is indicated by increased barrier permeability, albumin leakage, and alterations in intracellular signaling pathways in the neurovascular unit, negatively impacting neuronal health and recovery. Astrocytic influence on the BBB via Smad-dependent Transforming Growth Factor β-1 (TGF-β) signaling is increasingly recognized. Still, mechanisms of this action are not fully known and are seldom contextualized within a barrier system exhibiting astrocyte-endothelial cell interaction. One link in the TGF-β signaling process which is not yet clarified is inositol trisphosphate 3 (IP3)-mediated astrocyte-endothelial calcium interactions. Among other factors, TGF-β's effect and dependence on Ca2+ mediated pathways in influencing BBB permeability have not yet been evaluated in the context of a barrier despite mounting evidence for the role of calcium flux in determining outcomes of TGF-β action. The present study is the first to suggest a causal relationship between TGF-β action on BBB permeability and IP3 receptor. In this model, astrocytic TGF-β was determined to significantly reduce BBB permeability, and the IP3R inhibitor Xestospongin-C was utilized to demonstrate a negative modulatory role of IP3R inhibition on BBB-strengthening properties of TGF-β. This study not only directly suggests influence of astrocyte-derived TGF-β at the BBB but provides a vital link in determining TGF-β's role in BBB permeability. Emphasis was placed on physiological relevance through the novel application of a luminal-access *in vitro* BBB model as well as further Gene Set Enrichment Analysis (GSEA) performed using publicly available data from stroke patients. GSEA analysis further supports the relevance to stroke pathology of phenomena observed *in vitro* and lays the groundwork for future investigation into the role of Smad-independent TGF-β signaling and dependence on calcium-mediated processes.

A-2008

Initiation of Cell Cultures from Pandalid Shrimp and Effects of Astaxanthin. L. E. J. LEE, S. E. Cho, D. T. Pritchard, and P. W. C. Lee. Faculty of Science, University of the Fraser Valley, Abbotsford, BC, CANADA. Email: Lucy.Lee@ufv.ca

Pandalid shrimps are commercially important cold-water marine shrimp species found widely distributed throughout the globe with the larger species (also called prawns) abundant in northern Pacific waters. Unlike their aquacultured cousins, the Penaeid shrimps, Pandalids are slow growing and do not fare well in captivity, so mariculture of Pandalid species have seldomly been attempted. Canada exports more cold-water shrimp than any other country in the world, and Pandalid shrimp make up Canada's fourth most valuable seafood export. Pandalid fisheries in Canada is thus economically important with two main species harvested in the northern Atlantic: *Pandalus borealis* and *Pandalus montagui,* while 7 species are harvested in the Pacific coast, with spot shrimp (*P. platyceros*) and humpback shrimp (*P. hypsinotus*) among the most prized in British Columbia, along with *P. borealis, montagui, jordani, danae*, and *goniurus*. Reports of cell culture of pandalid species have been scarce, and there appears that only one attempt of *in vitro* culture has been made with *P. borealis*. In this study, attempts were made to culture *P. platyceros* and *P. hypsinotus* tissues. Cell culture conditions for these species are distinct from the more commonly attempted penaeid shrimp species. Physicochemical requirements, such as temperature, media, adhesion factors, and growth supplements for optimal primary culture will be presented and attempts to develop long term cultures will be discussed. Astaxanthin supplementation will be explored as pandalid shrimp are rich in astaxanthin. Despite many attempts over more than half a century of research, no marine invertebrate cell line has been developed to date, although some *in vitro*-meat start-ups have reported having developed shrimp muscle cell lines without proof. As more *in vitro* meat companies are turning their efforts to other crustaceans' *in vitro* meat such as lobsters and crabs, the race is on to develop a continuously proliferating crustacean cell line. It is hoped that our efforts will add to the growing literature for future crustacean cell line development.

A-2009

NALP1 as a Possible New Player in Cigarette Smoke Induces Cutaneous Inflammation? ROXANE PRIEUX[1], Mascia Benedusi[1], and Giuseppe Valacchi[1,2,3]. [1]Department of Biomedical and Specialty Surgical Sciences, University of Ferrara, Ferrara, ITALY; [2]Plants for Human Health Institute, North Carolina State University, Kannapolis, NC; and [3]Department of Food and Nutrition, Kyung Hee University, Seoul 02447, KOREA. Email: prxrnf@unife.it

Cigarette smoke alters biological processes in the skin such as redox homeostasis and inflammation response that might be involved in promoting skin inflammatory diseases (Smith, 1997; Lee, 2012; Egawa, 1999). Exposure to cigarette smoke has also been linked to a destabilization of the NALP3 inflammasome in the lung epithelium, resulting in a more vulnerable immunological response to several exogenous and endogenous stimuli, such as exposure to ROS (Hans, 2017; Buscetta, 2020; Mortaz, 2010; Ferrara, 2019). Thus,

cigarette smoke has an adverse effect on host defense, increasing the susceptibility to develop infections and pathologies (Ferrara, 2019). In the skin, inflammasome disorders have been linked to an increasing number of diseases such as melanoma, psoriasis, vitiligo, atopic dermatitis and acne (Ye, 2019; De Sá, 2016). The inflammasome protein NALP1 is an important innate immune sensor in human keratinocytes, and, together with ASC and caspase-1, it mediates the activation and secretion of the proinflammatory cytokines IL-1b and IL-18 (Beer, 2014; Jin, 2007; Sand, 2019). However, the role of CS in the NALP1 inflammasome in the cutaneous barrier has still not been investigated. At the University of Ferrara, we aim at understanding how cigarette smoke exposure is involved in the NALP1 inflammasome whether acting as an inhibitor and activator using a 2D and 3D skin models, respectively HaCaT cell lines, primary keratinocytes and reconstructed human epidermis. Oxidative damage will be first evaluated such as formation of 4-HNE protein adducts, ROS and protein carbonyls as well as pro-inflammatory responses in the supernatants IL-1b and IL18. To understand whether CS cutaneous inflammation proceeds via a NALP1-dependent or independent mechanism, we will measure the mRNA levels of NALP1, ASC, CASP1, pro-IL-1β, IL-18 as well as the protein levels of Caspase-1, ASC and NALP1 and finally the release of mature IL-1β and IL-18 in the culture media.

A-2010

Robustness of AFM-based Method to Identify Mechanical Properties of the Cell Body and Pericellular Coat. NADEZDA MAKAROVA[1] and Igor Sokolov[1,2,3]. [1]Department of Mechanical Engineering, Tufts University, Medford, MA; [2]Department of Biomedical Engineering, Tufts University, Medford, MA; and [3]Department of Physics, Tufts University, Medford, MA. Email: nadezda.makarova@tufts.edu

The atomic force microscopy (AFM) indentation method in combination with the brush model is a unique way to determine the mechanical response of the cell body and the physical properties of the pericellular layer at the subcellular resolution. It was shown that this method allows for quantitative segregation between various cancer and normal cells. Furthermore, it was possible to differentiate between dormant and active cancer-initiating melanoma cells in a zebrafish model (all cells had BRAF/p53 onco-mutation). Application of the described method to cardiovascular and blood-related diseases, cancer, and aging could provide further quantitative clues to the previously unknown physical properties of cells. Therefore, it is paramount to prove that the described method is sufficiently quantitative and repeatable. Here we report on such proof. We demonstrated that the method is only weakly dependent on the uncertainties within the model and experimental data, with an error of less than 3% when measuring the elastic modulus of elasticity of the cell body. The errors in the derivation of values of parameters of the pericellular coat (equilibrium length and the effective grafting density) can be as high as 25%, though. However, these errors of the brush parameters are mostly due to the limitation of the model used to extract the brush parameters, which assumes the pericellular cell layer to be an ideal steric brush. The deviation from this model may be considered as useful biophysical information by itself. In addition, the pericellular layer parameters are independent of the calculation of the elastic modulus of the cell body.

A-2011

Combined Effect of Prenylated Stilbenoids from Peanut and Paclitaxel on Triple Negative Breast Cancer Cell Lines. SEPIDEH MOHAMMADHOSSEINPOUR[1,2], Linh-Chi Ho[1], and Fabricio Medina-Bolivar[1,3]. [1]Arkansas Biosciences Institute, [2]Molecular Biosciences Graduate Program, and [3]Department of Biological Sciences, Arkansas State University, Jonesboro, AR. Email: sepideh.mohammad@smail.astate.edu

Breast cancer is the most prevalent type of cancer in women worldwide. Triple-negative breast cancer (TNBC) is known to be one of the deadliest types, owing to its unresponsiveness to typical hormonal treatments. Therefore, it is essential to investigate alternative treatments to increase survival rates for this disease. The goal of this study was to examine prenylated stilbenoids from peanuts as an adjuvant for paclitaxel, a chemotherapeutic drug with severe side effects. To induce the production of stilbenoids, peanut hairy root cultures were co-treated with elicitors and further purified via semi-preparative high-performance liquid chromatography. The cytotoxicity of the prenylated stilbenoids arachidin-1 and arachidin-3 and the non-prenylated stilbenoid resveratrol was studied in TNBC cell lines MDA-MB-231 and MDA-MB-436. The cytotoxicity of combining prenylated stilbenoids and paclitaxel were studied in MDA MB 231 cell lines by checkerboard drug assays. Cytotoxicity and apoptosis were measured by the RealTime-Glo™ MT Cell Viability Assay and Apo-ONE Homogeneous Caspase-3/7 assays, respectively. To further investigate the apoptosis and cell cycle stages, cells were studied using flow cytometry after treatment with prenylated stilbenoids or resveratrol. Cytotoxicity, checkboard and flow cytometry assays showed a decrease in paclitaxel dosage when combined with prenylated stilbenoids. This highlights the significance to continue research with prenylated stilbenoids as an adjuvant in TNBC treatment. Current studies focus on elucidating the signaling pathways affected by these compounds in TNBC cells to increase our understanding of the anticancer mechanisms of these natural products.

A-2012

CRISPR Genetic Engineering of Double Agent STAT3 in MCF7 Human Breast Cancer. NEIL REDDY. Satellite High School, Biological Sciences, 300 Scorpion Ct, Satellite Beach, FL 32937. Email: neilreddy04@gmail.com

CRISPR genetic engineering is an editing technique to delete genes. Via a guided Cas9 nuclease, cellular pathways can be permanently altered to impede cancer proliferation. Certain proteins and transcription factors may possess double-sided roles. STAT3 is an example as it has a strong presence in both healthy individuals and cancer patients. Many times, STAT3 is recruited for typical functions such as inflammatory responses in the immune system. Additionally, STAT3 regulates apoptosis, the regulated programmed cell death cycle. Due to cytokine and external stimuli, STAT3 transactivates pro-survival genes that negatively regulate apoptosis, allowing for cancer cell escalation in the body. To control STAT3 activity, the pathway was modified in the MCF7 breast cancer cell line. CRISPR genetic engineering was used to delete the STAT3 recruiter JAK1. JAK1 is known to recruit and phosphorylate STAT3 and may be a cause of the transactivation of pro-survival genes. STAT3 should still be able to phosphorylate for normal bodily functions through its specific protein kinase. Three groups were established: Cas9 group (Cas9 nuclease only), CRISPR group (Cas9 with Guide RNA), and Untreated group (MCF7 only). Jak1 was deleted in the CRISPR group. Proliferation was compared through three time intervals (24 h, 48 h 72 h). The CRISPR group had significantly less cell growth than the Untreated group at 48 h with a p value of 1.6E-12 using an alpha level of 0.05. STAT3 could be a potential target in creating individualized treatments via vaccinations for patients using the genetic markers expressed. Furthermore, escalations from stage 1 breast cancer to stage 4 breast cancer may be prevented due to the altered cellular pathway, reducing the possibility of activating anti apoptotic genes. The cellular pathway will then be altered at all surrounding tumor sites, not just the original injection site as an effect of the JAK1 recruiter's presence and cellular pathway throughout the entire body. This will allow for a potentially versatile and effective approach to breast cancer treatment and prevention.

A-2013

Effects of Dietary Supplementation with Cabbage Extract on Brain Mineral Compositions and Inflammatory Marker Expressions in SAMP8 Mice. JIA XIONG[1,2], Sierra Bonney[1,2], Fernanda V. Matta[1,3], Nick Thompson[1,2], 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]ICP-MS Facility, Department of Chemistry, University of Surrey, Guildford, Surrey GU2 7XH, UK. Email: jxiong5@ncsu.edu; daesposi@ncsu.edu

Changes in mineral status and increased risk of stroke with age have been reported (Morita A, et al., 1994; Hughes CM. et al., 2015). Inflammatory mechanisms play an important role in the risk of stroke and brain ischemia, and some inflammatory cytokines including interleukin-6 and tumor necrosis factor-α are useful in the diagnosis of ischemic stroke (Rodríguez-Yáñez M and Castillo J., 2008). Our previous research showed a diet supplemented with cabbage seed extract had protective effects in an accelerated aging model. The aim of this research was to determine whether diets with low fat, high fat and supplementation with cabbage seed have effects on 18 mineral contents and 5 inflammatory cytokines in male and female mice brain using an accelerated aging mouse model. The results showed obvious differences of brain mineral compositions between male and female mice. More mineral contents found in male mice, especially for Fe, Mn, Al, Ca, V, Cr, Co, and Ni, and there is a good correction between them. However, different diets didn't show significant effects on the brain mineral compositions. The proinflammatory gene expressions in male brains had no significant differences. High fat diet up-regulated IL1β expression in female brains at 50 weeks. Cabbage seed diet down-regulated gene expression of COX 2 and IL6 at 32 weeks. In conclusion, although cabbage seed and high fat diets show slightly effects on some proinflammatory markers, gender had more effects on brain mineral compositions and inflammatory marker expressions in SAMP8 mice than dietary supplementations.

A-2014

Evaluation of Cornulin and DJ-1 as Novel Prognostic Biomarkers for Cutaneous Squamous Cell Carcinoma. RACHNA KARUMURI[1] and Hilal Arnouk[1,2]. [1]Midwestern University Chicago College of Osteopathic Medicine and [2]Midwestern University Chicago College of Graduate Studies, Downers Grove, IL 60515. Email: rachna.karumuri@midwestern.edu

Cutaneous Squamous Cell Carcinoma (cSCC) is the second most common skin cancer. With the current methods of detection, 14% of cSCC's are found to have metastasized by the time of detection. Moreover, cSCC is responsible for the majority of deaths caused by non-melanoma skin cancers. This study looks into the role of Cornulin and DJ-1 as novel prognostic biomarkers in order to have earlier detection and more objective prognosis for cSCC patients. Cornulin is a biomarker of epidermal differentiation; it has been found to be expressed in the upper layers of stratified squamous

epithelium. DJ-1, an oncogene, is known to show expression in the basal epithelium layer; DJ-1 has been shown to have a protective role in UV exposed keratinocytes. This experiment looks into the differential expressions of Cornulin and DJ-1 in the different tumor progression steps of cSCC. We hypothesized that there is an inverse relationship between the level of Cornulin expression and the degree of differentiation of cSCC and a linear relationship between the level of DJ-1 expression and the degree of differentiation of cSCC. Immunohistochemistry staining was performed using anti-Cornulin and anti-DJ-1 antibodies on Tissue Microarrays of cSCC samples representing the different grades and TNM clinical stages. Qualitative analysis shows that there is an inverse relationship between Cornulin expression and the level of differentiation of cSCC and a linear relationship between DJ-1 expression and the level of differentiation of cSCC. Further data collection and quantitative analysis are ongoing to establish the utility of Cornulin and DJ-1 as diagnostic and prognostic biomarkers of cSCC.

A-2015

First Record of High Mortality of the Cottony Cushion Scale After Application of the Contact DNA Insecticide. N. V. GAL'CHINSKY[1], R. Z. Useinov[1], I. A. Novikov[1], E. V. Yatskova[2], A. K. Sharmagiy[2], Yu. V. Plugatar[2], and V. V. Oberemok[1,2]. [1]V. I. Vernadsky Crimean Federal University, Simferopol, CRIMEA and [2]Nikita Botanical Gardens – National Scientific Centre Russian Academy of Sciences, Yalta, CRIMEA. Email: pcr.product@gmail.com

Dense *Icerya purchasi* Maskell population damage plant health is mostly caused by extracts significant quantities of sap from the host plant, which results the shoots dry up, defoliation occurs and branches or whole trees may die. All life stages of *I. purchasi* are covered with wax, which reduces the effectiveness of most chemical insecticides. In addition, the use of insecticides prevents regulation by natural enemies, it could be reason damage to the populations of biocontrol agent can contribute an outbreak of *I. purchasi*. In this brief report describing experiments carried out on the larvae of *I. purchasi* (feeding on *Pittosporum tobira* Thunb.), we emphasize the extremely promising potential of DNA insecticides for the control of sap-sucking insects. We designed 11 nt long antisense oligonucleotide (5′-ACACCGACGAC-3′ – IP-11) from the *I. purchasi* 28S ribosomal RNA gene, respectively, and applied them to target plant (1 mg of DNA per m^2 of plant leaves). The experiment was performed in triplicate between November and December 2020 within the grounds of the Nikita Botanical Garden (Republic of Crimea, Yalta). In the groups treated with water, CTRL-11 (control group – 5′-AGGAAACGATG-3′), and IP-11, we observed larval deaths of 8.06, 29.27, 41.64%; 12.25, 34.47, 67.08%; 18.44, 37.41, 83.02% respectively, on the 4th, 7th and 10th day after treatment (IP-11 vs. control: $\chi2 = 29.04$, $p < 0.001$, $N = 200$, df = 1; IP-11 vs. control: $\chi2 = 61.01$, $p < 0.001$, $N = 200$, df = 1; IP-11 vs. control: $\chi2 = 81.93$, $p < 0.001$, $N = 200$, df = 1). Compared to controls the mortality in the IP-11 group was accompanied by a significant 6.4-fold decrease in the expression of target gene on the 7th day.

A-2016

Impact of Klotho Gene Variability and Methylation on Traumatic Brain Injury Outcomes. DANIELA NAUMOV[1,2], Sandra Deslouches[2], and Yvette Conley[2]. [1]Yale University, New Haven, CT and [2]University of Pittsburgh School of Nursing, Pittsburgh, PA. Email: daniela.naumov@yale.edu

Traumatic brain injury (TBI) affects about 1.7 million individuals annually. It is one of the leading causes of death and disability in the U.S. The Klotho gene, expressed in the brain choroid plexus which produces cerebrospinal fluid (CSF), may influence patient outcomes after TBI. Genetic variations in Klotho have shown to dramatically shorten lifespan, while its overexpression is associated with improved survival and cognitive function. It was hypothesized that DNA variability and DNA methylation within the Klotho gene would significantly impact TBI outcomes. Participants were recruited if they suffered a severe TBI (Glasgow Coma Scale score ≤ 8) and had CSF passively drained as standard of care. The study population ($n = 369$) consisted of majority white males, with an average age of 38. A genome-wide association study was conducted to identify potentially influential single nucleotide polymorphisms (SNPs). The discovery data collection led to a follow-up allelic discrimination data collection that assessed DNA variability. Group-based trajectory analyses were used to characterize patterns of DNA methylation for DNA extracted from CSF days 1–5 after injury. Regression analyses were run to relate DNA variability and methylation to TBI outcomes. The discovery analysis identified one SNP, rs508394, that showed significance to death at 3 and 12 months post-TBI ($p = 0.048$, 0.016) and disability ($p = 0.046$) at 3 months after TBI. While there was no significance observed between methylation and TBI outcomes, the Klotho methylation trajectory plots provided insight into the genetic basis of recovery after TBI. Together, these findings may be important when evaluating Klotho as a potential biomarker for TBI outcomes, thereby furthering our knowledge on genetics-based therapies.