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Animal Contributed Papers

A-1000

Scavenging for Bacteria: Identification and Characterization of Rainbow Trout MARCO. SARAH J. POYNTER[1], Andrea Monjo[2], Gabi Micheli[2], and Stephanie J. DeWitte-Orr[2,3]. [1]Department of Biology, University of Waterloo, Waterloo CANADA; [2]Department of Biology, Wilfrid Laurier University, Waterloo CANADA; and [3]Department of Health Sciences, Wilfrid Laurier University, Waterloo CANADA. Email: sdewitteorr@wlu.ca

Class A scavenger receptors (SR-As) are a family of key innate immune receptors, which bind to a wide range of polyanionic ligands including bacterial components and nucleic acids. Macrophage receptor with collagenous structure (MARCO) is a SR-A that has been studied in mammals largely for its role in binding bacteria. To date there is little information about SRAs in fish, and what ligands specific SR-A family members bind remains largely unknown. In this present study a novel rainbow trout MARCO transcript has been identified and its sequence and putative protein domains have been analyzed. While there is only moderate sequence similarity to mammalian MARCO sequences there are notable protein domain similarities and MARCO clusters with MARCO sequences from other species more closely than other fish scavenger receptors. MARCO transcript was found in rainbow trout gonadal cell line RTG-2 and the macrophage/monocyte splenic cell line RTS-11; presence was not detected in the gut or gill cell lines RTgutGC or RTgill-W1. When overexpressed in CHSE-214, a cell line that lacks functional scavenger receptors, rainbow trout MARCO is able to bind the class SR-A ligand, acLDL as well as gram-positive, and gram-negative bacteria (of both mammalian and aquatic sources). MARCO did not show any binding to the yeast cell wall component zymosan. When the MARCO sequence was truncated to remove a domain necessary for bacterial binding in mammals, the scavenger receptor cysteine-rich domain, MARCO no longer bound bacteria or acLDL. This is the first time rainbow trout MARCO has been identified, and the first in-depth study exploring a fish class A scavenger receptor ligand binding profile. This study provides novel insight into the role of rainbow trout MARCO in bacterial innate immunity.

A-1001

Characterizing Functional Differences in Sea Anemone Hsp70 Isoforms Using Budding Yeast. SHAWN J. WALLER, Adam M. Reitzel, and Andrew W. Truman. Department of Biological Sciences, University of North Carolina, 9201 University City Blvd, Charlotte, NC 28223. Email: swalle11@uncc.edu, Corresponding author: atruman1@uncc.edu

Heat shock protein 70s (Hsp70s) are a highly conserved class of chaperone proteins involved in cellular processes such as the stress response, homoeostatic maintenance, and cell cycle progression. The starlet sea anemone *Nematostella vectensis* is found in a variety of environments that cause organisms physiological stress through abiotic factors such as temperature, UV radiation, salinity, and oxygen concentration. *N. vectensis* is found throughout a range of latitudes and elevations that may promote the evolution of divergent functions of Hsp70 isoforms among different populations. Preliminary data shows dramatic differences among expression profiles of the NvHsp70 isoforms under stress. To bypass the lack of in vivo protein technologies for marine invertebrates, we expressed the 3 major NvHsp70 isoforms in yeast lacking native Hsp70. These cells while viable grow at substantially different rates and display altered tolerance to a variety of cell stressors including hydroxyurea, cadmium, copper, hydrogen peroxide and high temperature. Going forward, chimeras of these isoforms in yeast will be created and expressed in order to pinpoint the specific amino acid differences that determine cellular tolerance to stress. In addition, we intend to isolate the individual isoforms and their respective complexes (‘interactomes”) to characterize the molecular cause of isoform-specific stress responses.

A-1002

Enhanced Directional Axon Outgrowth of Peripheral Nerve Fibers Using Submicron Topographic Cues and Live Cell Imaging. R. GARCIA[1], H. Sharthiya[2], S. C. Veen[1], C. Liu[3], N. Patel[1], K. Kristjansdottir[1], M. Fornaro[2], P. F. Nealey[3], and J. Z. Gasiorowski[1]. [1]Department of Biomedical Sciences, Midwestern University, 555 31st Street, Downers Grove, IL

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Peripheral nerve injury is a debilitating disease characterized by a loss of sensation and/or motor function at the affected site. Unlike the central nervous system, the peripheral nervous system spontaneously regenerates after injury. In many cases, however, full functional restoration is not achieved. One likely explanation is the propensity of axons to migrate in undesired directions in the absence of proper biochemical and biophysical cues. Therefore, the aim of this study is to optimize directional axon outgrowth using surfaces with nano- to microscale anisotropic topographic patterns as a biophysical guide. Previous work in our lab has shown that mouse dorsal root ganglia (DRGs) cultured ex vivo on surfaces with repeating groove widths of 700nm or 2000 nm had longer and more controlled axon outgrowth than a flat surface. Our hypothesis is that the speed and directionality of axon growth parallel to the grooves will be enhanced when observed in time-lapse and at specific time points, both on tissue culture plates and tube-like structures. We harvested cervical and thoracic DRGs from mice and cultured them on chemically identical surfaces, with groove widths of 700nm or 2000nm as well as a flat control. Axon growth was observed for 24 hours in time-lapse 1–2 days and 3–4 days after initial plating. Our analysis indicates that DRGs grown on topographic surfaces exhibit significantly more directional axon growth parallel to the grooves compared to the flat control, as well as a marginally faster axon growth speed. In addition, DRGs were cultured in 5 mm diameter half-tube structures with similar topography on the inner wall, and imaged 6 days after initial plating. Analysis showed that DRGs grown on half-tubes with inner-wall topography exhibited longer axon outgrowth than control half tubes with no topography. In conclusion, our results may be used to better understand the various mechanisms of peripheral nerve regeneration and applied towards the fabrication of implantable biomaterials with specialized topography to ultimately restore nerve function.

A-1003

In Bioassays, Blind Faith Is a Fool's Errand—Run the Controls Every Time. JOHN W. HARBELL. JHarbell Consulting LLC, Dallas, TX. Email: johnharbell@sbcglobal.net

Inherent in the development of a bioassay is the expectation of consistency in the test system and assay execution that will produce a reproducibility in the endpoint response to a given test material over time. Such stability is essential to developing and using a prediction model for interpretation of the endpoint data. The prediction models are generally quantitative (rather than qualitative) and so the magnitude of the response is critical. This consistency is required whether the test system is in vitro or in vivo. Even under ideal conditions, there is some variation over time with each assay. It is important to understand the amount of "random" variation around a "historical average" response (assay precision) and to detect time-dependent drift (overall increase or decrease in sensitivity) or acute assay failure (damage to the test system or errors in assay execution). This presentation will focus on approaches to measure the consistency of an assay over time and steps address breakdowns in this consistency. In particular, the use of concurrent positive and negative control materials is essential. The negative (or solvent) control provides the baseline measure of the test system (i.e., 100% viability or undamaged tissue) against which the endpoint measure of the treated test system in compared. The positive control should provide a quantitative measure of the response to a known material. It provides a link to the historical performance of the assay to measure both drift and acute assay failure. Thus, it is essential to include the positive control each time the assay is performed. Its purpose is not to show that the test system will respond but to show that the response is quantitatively consistent with the historical performance. Several examples will be provided to show the folly of blind faith in assay performance without quantitative substantiation.

A-1004

An Adventure in Serum-Free Cell Culture. J. DENRY SATO. Manzanar Project Foundation. Email: denrysato@aol.com

An unexpected finding in a side project stimulated the most intellectually satisfying research of my career. Whilst attempting to generate monoclonal antibodies to growth factor receptors, my colleagues and I decided to devise a serum-free medium for mouse myeloma cells and their hybridoma derivatives. Our rationale for this side project was to increase the reproducibility of the hybridoma method and to simplify the downstream purification of monoclonal antibodies of interest. Several serum-free media formulations for hybridomas had been published, but none supported the survival of the commonly used mouse myeloma parent cell lines so we focused on the NS-1-Ag4–1 clone of the P3-X63-Ag8 myeloma cell line. Extensive cell growth assays led to the discovery that NS-1 cells had an absolute and unusual requirement for a single component of serum. Further investigation showed that other clonal derivatives of P3-X63-Ag8 shared this survival requirement, and we were able to ascribe this dependency to a defect in a single enzymatic step in a complex synthetic pathway. This finding led us to devise an improved and novel

method of selecting hybridoma cells from myeloma x splenocyte fusions. Surprisingly, our serum-free myeloma cell medium with some modifications was found to support the proliferation of undifferentiated mouse and human ES cells and human iPS cells.

A-1005

Gap Junctional Intercellular Communication, the Archetypal Cell Phone of Tissues, Is an Excellent Biomarker for Assessing Environmental Stressors and Chemopreventive Compounds. BRAD L. UPHAM[1], Iva Sovadinova[2], and Pavel Babica[2]. [1]Department of Pediatrics & Human Development and National Food Safety & Toxicology Center, Michigan State University, East Lansing, MI and [2]RECETOX - Research Centre for Toxic Compounds in the Environment, Masaryk University, Brno, CZECH REPUBLIC. Email: upham@msu.edu

Tissues are essentially a society of cooperating cells whereby communication is instrumental in their coordinated efforts to conduct their genetically predetermined functions as well as for repair and maintenance required throughout life. Intercellular communication occurs primarily through gap junctional channels, which epigenetically coordinate the cellular signaling pathways involved in tissue function and maintenance. Interruption of gap junctional intercellular communication (GJIC) has been linked with numerous adverse health effects depending on cell type and tissue. In stem and progenitor cell types, dysregulation of GJIC has been associated with chemical carcinogenesis, and many chemopreventive agents can avert the disruption of GJIC by environmental carcinogens. Thus, GJIC is an excellent biomarker to assess multiple environmental stressors that affect the health of humans and wildlife. In vitro assays of GJIC showed that persistent (POPs) and emerging organic pollutants (EPs) such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, endocrine disrupters, numerous pesticides (DDT, lindane, vinclozolin, methoxychlor, alachlor, malathion), and perfluorinated fatty acids dysregulates GJIC. Various natural products, such as cinnamic acid, curcumin, diallyl sulfide, emodin, indole-3 carbinol, metformin, resveratrol, silibinin, quercetin, thymoquinone prevent inhibition of GJIC by many of these POPs and EPs. In vivo assessment of GJIC has validated the in vitro assays for several contaminants, such as perfluorinated chemicals. These results indicate that in vitro assessments of GJIC can play a crucial role in understanding the impact of exposure to environmental contaminants on the health of humans and wildlife, as well as identifying natural products that potentially prevent environmentally-induced diseases. In conclusion, good communication between cell neighbors via the archetypal cell phone, gap junctions, is essential for the harmony of a tissue.

A-1006

Intrinsic Electrical Signal Characteristics of Rat Embryonic Cortex Tissue. NURDAN ÖZKUCUR and Valencia Joyner Koomson. Tufts University, Advanced Integrated Circuits and Systems Lab, Department of Electrical and Computer Engineering, 161 College Avenue, Medford, MA 02155. Email: nurdan.oezkucur@tufts.edu

Cell electrical activity is a fundamental part of normal cell development and function, and abnormal cellular electricity results in delayed- or abnormal development. The emergence of activity patterns and connectivity in the neonatal brain is incompletely understood. Currently there is no tool available to record electrical activity simultaneously at multiple locations at whole tissue level in vitro. We have developed a probe chip to map the neural activity at different brain regions simultaneously. Whole rat embryonic brain was placed into the well of the probe chip connected to an amplifier and a computer to visualize neural signals using a software. Each electrode was designed to align to a different region on the cortical hemisphere in a longitudinal orientation during recordings. The data we present is collected from a simple version of the probe chip with ten gold electrodes that enables to record intrinsic electrical signals at multicellular systems level from the different regions of rodent embryonic cortex in vitro. The probe chip was tested for the function after the fabrication to evaluate the signal to noise ratio. Custom MATLAB algorithms specific for correlation analysis, signal processing, and statistics were used.

A-1007

Function of rBC2LCN Lectin-recognizing Glycoprotein-positive Cells in Squamous Cell Carcinoma Cell Lines. HIROTAKA NAKATAO[1], Eri Akagi[1], Atsuko Hamada[1], Sachiko Yamasaki[2], Shigeaki Toratani[1, 2], andTetsuji Okamoto[1, 2]. [1]Hiroshima University Graduate School of Biomedical & Health Sciences, Molecular Oral Medicine and Maxillofacial Surgery; JAPAN and [2]Hiroshima University Hospital, Department of Oral and Maxillofacial Surgery. Email: nakatao@hiroshima-u.ac.jp

Introduction: A rBC2LCN is a lectin that recognizes carbohydrate structures that are specifically expressed in undifferentiated pluripotent stem cells such as ES and iPSCs, but not expressed in differentiated somatic cells. In this study, to develop a novel diagnosis and therapy targeting oral

squamous cell carcinoma (OSCC)-stem cells, we have studied whether OSCC cells express the carbohydrate recognized by rBC2LCN, and whether the positive cells have a function as cancer stem cells. Materials and Methods: Ho-1-N-1 and Ho-1-u-1 cell lines derived from OSCCs originally established in our laboratory, and A431 cells derived from the vulvar SCC were used. DME/F12 6F serum-free medium (1:1 mixture of DMEM and Ham F12 medium supplemented with 6 factors (insulin,transferrin,BSA-oleic acid,2-mercaptoethanol 2-aminoethanol and sodium selenite), were used. Lectin-recognizing carbohydrate-positive and -negative cells were separated by a Cell Sorter. The growth ability of the positive, negative and parental cells in monolayer culture, and in suspension culture were studied in serum-free culture. In addition, gene expression in these cells was analyzed by DNA microarray analysis. Furthermore, the properties and functions of exosomes in the culture medium produced by the positive cells were examined. Results: All the cell lines examined express rBC2LCN-recognizing carbohydrate, and the positive cell ratio among whole cell population were about 2%. There was no difference in the proliferation ability between the positive and negative cells in monolayer culture, but the positive cells exhibited high sphere forming ability. As a result of the microarray analysis, IGF-2 gene expression in positive cells was approximately 60 times higher than that in negative cells, and by Bio-Plex analysis, Akt signaling pathway in the positive cells was highly elevated compared to negative cells. Further, positive cell derived exosomes promoted the transition of negative cells to positive cells. Discussion: It has been revealed that pluripotent stem cell-specific carbohydrate chains recognized by rBC2LCN lectin were also expressed in OSCC cell lines, and the positive cells exhibited stem-cell like properties. Furthermore, IGF-2 highly expressed and produced by the positive cells might function as an autocrine growth factor to maintain stem cell property, and transition from negative to positive cells.

A-1008

Endogenous Epitope Tagging of Hsp70 Using CRISPR/Cas9 Genome Editing. NITIKA and A. W. Truman. Department of Biological Sciences, 9201 University City Blvd.,Woodward, University of North Carolina at Charlotte, NC. Email: nnitika@uncc.edu; corresponding author: atruman1@uncc.edu

Heat Shock Protein 70 (Hsp70) is an evolutionarily well-conserved molecular chaperone involved in several cellular processes such as folding of proteins, modulating protein-protein interactions and transport of proteins across membranes. In situations where refolding of proteins cannot be achieved, Hsp70 targets proteins for degradation by the proteasome. Binding partners of Hsp70 (known as 'clients') are identified on an individual basis as researchers discover their particular protein of interest binds to Hsp70. A full complement of Hsp70 interactors under multiple stress conditions remains to be determined. A promising approach to characterizing the Hsp70 "interactome' is the use of protein epitope tagging and then affinity purification followed by mass spectrometry (AP-MS/MS). Despite the power of this technique, AP-MS/MS suffers from several limitations. A major concern is the artifacts that arise from massively overexpressing tagged proteins from a strong promoter such as CMV. In addition, this overexpressed protein exists in addition to the native version protein leading to skewed protein interaction data. To address these shortcomings, we have used CRISPR/Cas9 genome editing to insert a FLAG-HIS epitope onto the N-terminus of Hsp70 at the genomic level. CRISPR-mediated DNA insertion into the genome is permanent and highly specific and minimizes the cell heterozygosity observed in traditional stable expression technologies that rely on random chromosomal integration. Genomic epitope tagging produces a fusion protein expressed at native levels allowing the expression of epitope-tagged protein as the sole version of the protein in the cell. Preliminary results suggest that this system will be highly useful in isolating and identifying global Hsp70 interactions.

A-1009

The Influence of Extracellular Topography on Mammalian Transgene Expression. A. J. YOUSSEF, S. C. Veen, and J. Z. Gasiorowski. Midwestern University, Department of Biomedical Sciences, 555 31st St, Downers Grove, IL 60515. Email: ayoussef99@midwestern.edu

Regeneration after wounding or traumatic injury can be a slow, complicated process that does not guarantee complete recovery. The extracellular matrix (ECM) plays a key role in mediating wound healing by providing structural and biochemical support to the surrounding cells. Importantly, the ECM consists of distinct chemical and physical cues. Previous studies have shown that biomimetic, physical ECM properties and cues can alter cell behavior and endogenous gene expression. Therefore, we hypothesized that transgene expression could also be influenced by the extracellular environment. Developing an implantable biomaterial that mimics the extracellular matrix, supplemented with therapeutic gene delivery could help promote controlled regeneration. The aim of this study is to examine how biophysical cues influence transgene expression. Our hypothesis is that transfected cells cultured on topographic surfaces with a repeating ridge and groove pattern of various pitch sizes will show significant changes in transgene expression compared to a chemically identical flat control. For our study, we used several cell types, including PC12 (pheochromocytoma), HEK293 (embryonic kidney), and C3H10 (mesenchymal) cells. Cells were

transfected with a plasmid that contains a CMV promoter driving green fluorescent protein (GFP) and then plated onto surfaces with 800nm, 1400nm, and 4000nm pitches (1:1 ridge: groove ratio) as well as a flat control. The cells were imaged with a microscope and macro-scale imager at 24 and 48 hours after being plated on the surfaces and macro-scale changes in GFP intensity were quantified. The differences in GFP expression on the different topographic substrates have led us to conclude that physical cues alter transgene expression. In particular, we observed increases in transgene expression when the cells were growing on sub-micron scale feature sizes. These results may be translationally applied to the development of novel treatments for controlled wound healing or nerve regeneration in the form of gene therapy with implantable biomaterials.