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Animal contributed papers

A-1000

Homobrassinolide Enhances Myogenic Potential of Satellite Muscle Cells. D. ESPOSITO[1,2], I. Raskin[2], and S. Komarnytsky[1,2]. [1]Plants for Human Health Institute, NC State University, 600 Laureate Way, Kannapolis, NC 28081 and [2]Rutgers University, Plant Biology Department, 59 Dudley Road, New Brunswick, NJ 08901. Email: esposito@aesop.rutgers.edu

Muscle satellite cells are widely accepted as the resident stem cells of skeletal muscle, supplying myoblasts for growth, homeostasis and repair. Activation of these cells in response to muscle mechanical change or injury involves activation of the muscle-specific transcription networks. Here we present evidence suggesting that oral administration of brassinosteroid to rat resulted in upregulation of muscle-specific genes to ensure proper expression during muscle fiber hypertrophy observed in the previous study. Gene expression profiling revealed that two groups of myogenic transcription factors MyoD and Pax3/7, calcineurin (Cnaa), adrenergic alpha-1d receptor (Adra1d), and androgen receptor (Ar) transcripts were increased in muscle of the treated animals fed either standard chow or high protein diet. Animals consuming standard diet showed a moderate increase in muscle atrophy/remodeling-related signaling that was abolished by high-protein diet. In L6 rat myoblast lineage cells, brassinosteroid treatment accelerated differentiation, expression of structural proteins, and fusion into multinucleated myotubes. Taken together, our results suggest that therapeutic modulation of myogenic activity by brassinosteroid could represent a viable future approach for repairing damaged muscle.

A-1001

IkappaB-Kinases Modulate the Activity of the Androgene Receptor in Prostate Carcinoma Cell Lines. GARIMA JAIN and Ralf B. Marienfeld. Institute of Pathology, University of Ulm, Albert-Einstein-Allee 23, 89070 Ulm, GERMANY. Email: garima.jain@uni-ulm.de; ralf.marienfeld@uni-ulm.de

Background: Whether canonical NF-κB signalling plays a role for initiation and progression of prostate cancer (PCa) is still a matter of debate. For instance, a class of PCa cell lines (PC3 and DU-145) with low AR expression depend on constitutive IKK and NF-κB activity and enhanced nuclear localization of NF-κB was observed in PCa samples. However, the majority of PCa depend on the activity of the androgen receptor (AR) and the role of NF-κB in these PCa remains unclear. Objective: This prompted us to analyze the role of canonical NF-κB signalling in AR expressing PCa cell lines. Method: Inhibition of NF-κB was achieved by both pharmacological IKK inhibitor BMS345541 and IKKsiRNAs. Results and Discussion: BMS345541 attenuated proliferation and induced apoptosis in a panel of PCa cell lines. Furthermore, AR activity and target gene expression was distinctively reduced while AR protein levels remained unaltered upon BMS345541 treatment. Similar effects on PCa cell proliferation and AR target gene expression were observed particularly after siRNA-mediated knockdown of IKK1. Further biochemical analysis of the AR signalling pathway revealed a reduced nuclear translocation of AR by BMS345541 treatment. In accordance, overexpression of IKK1 augmented the hormone-induced nuclear translocation of AR in COS7 cells and IKK1 increased AR activity in LNCaP cells. Finally, reduced in vivo AR phosphorylation after BMS345541 treatment and in vitro AR phosphorylation by IKK1 or IKK2 imply that AR constitutes a novel IKK target. Conclusion: Taken together, our data suggest that IKK1 affects the activity of AR possibly by phosphorylation-dependent augmentation of its nuclear translocation.

A-1002

Inhibition of 32D Myeloid Progenitor Cell Differentiation by the *SALL1* Gene. MARIA R. STUPNIKOV[1,2], Jerell Aguila[1], Jian Zhong[1], and Yupo Ma[1]. [1]Department of Pathology and [2]Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY. Email: maria.stupnikov@stonybrook.edu

Adult human hematopoietic stem cell (HSC) research is an innovative area in genetic engineering and stem cell biology and is of great interest in recent times. HSCs have been found in bone marrow, umbilical cord blood, and other places in the body. The therapeutic potential for these cells

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is great, possible therapies include organ regeneration and cures for diseases such as diabetes mellitus. There are several drawbacks to using adult stem cells in vitro. Besides a limited number of sources, in vitro HSCs age rapidly in culture and committed stem cells cannot expand. Additionally, HSCs have different properties in vitro and in vivo, where in vitro, unipotent characteristics are observed, whereas in vitro yields multipotent stem cell characteristics. The SALL1 gene is a stem cell gene and zinc-finger transcriptional factor which is a member of the SALL family, originally cloned based on its DNA-sequence homolog to Drosophila homeotic gene, sal. Recently, our group and others have shown that the embryonic stem cell (ESC) factor, SALL1, plays a vital role in maintaining ES cell pluripotency and in governing decisions affecting the fate of ES cells. We extended our studies on this stem cell gene in hematopoietic stem cells using 32D murine progenitor cells as a model. These are myeloid progenitor cells and are interleukin-3 (IL-3) dependent for sufficient proliferation. The 32D cells differentiate rapidly in culture in the presence of G-CSF without IL-3. IL-3 assists in 32D cell culture and prevents these cells from differentiating, however it has been found that IL-3 negatively affects the expansion of HSCs (therefore there is a need to replace it). The goal of this project is to determine if SALL1 is able to block myeloid differentiation and permit proliferation of cells. Production of a lentivirus with the SALL1 gene was implemented to transfect the 32D cells. The cells were then culture to determine SALL1's effect on 32D cell proliferation. Preliminary results imply SALL1 is a potential proliferative factor and differentiation repressor for myelopoiesis.

A-1003

Characterization of Bovine Herpesvirus Type 1 as a Novel Oncolytic Virus. B. CUDDINGTON[2] and K. Mossman[1, 2]. Departments of [1]Biochemistry & Biomedical Sciences and [2]Pathology and Molecular Medicine, Institute for Infectious Disease Research, McMaster University, Hamilton, ON, CANADA. Email: cuddingb@gmail.com, mossk@mcmaster.ca

Oncolytic viruses are an attractive avenue of cancer therapy due to the absence of toxic side effects often seen in current treatment modalities. HSV-1 has been studied extensively as an oncolytic virus due to the many advantages it possesses for use in virotherapy; however, there are also a number of disadvantages to this approach. To this end, we are interested in evaluating BHV-1 as an oncolytic herpesvirus. A preliminary screen measuring both virus replication and cellular viability showed that BHV-1 is cytotoxic to various human immortalized and transformed cell lines, while being relatively restricted in normal primary cells in vitro. In addition, the NCI60 panel of human cancer cell lines has been screened with BHV-1. Results of the screen show that 94% of tumour cell lines were permissive to BHV-1 with 79% showing a decrease in cellular viability. These results suggest that BHV-1 holds promise as a broad spectrum oncolytic vector that is able to infect tumour cells from a variety of histological origins. In contrast to many oncolytic viruses, cellular sensitivity does not correlate with type I IFN signaling. Furthermore, non-permissive cell lines are susceptible to infection with wild type HSV-1, which utilizes the same cellular receptors as BHV-1 for viral adsorption and penetration, indicating that the block to BHV-1 infection occurs downstream of entry. However, it is not yet known which mechanisms dictate cellular sensitivity to BHV-1 infection. Additional studies will evaluate the factors determining permissivity to BHV-1 infection, the mechanism of cell death in permissive cell types, and the in vivo tumour killing ability of BHV-1. In the future, these results will contribute to optimizing effectiveness of BHV-1 for use in the oncolytic platform as an ideal cancer therapy. These studies will also provide important insights into the mechanisms that govern virus-host interactions which are crucial for understanding how viruses cause disease and for the development of clinically relevant antiviral treatments.

A-1004

Establishing and Characterizing an Endothelial Cell Line from the Bulbus Arteriosus of Juvenile Walleye, *Sander vitreus,* for Physiology, Toxicology and Virology Research. NGUYEN T. K. VO[1], Ci Chen[1], John S. Lumsden[2], Brian Dixon[1], Lucy E. J. Lee[3], and Niels C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON, CANADA N2L 3C5; [2]Ontario Veterinary College, University of Guelph, Guelph, ON, CANADA N1G 2W1; and [3]Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA N2L 3C5. Email: ntkvo@uwaterloo.ca

As few fish endothelial cell culture models are available, the goal was to develop a teleost endothelial cell line with properties that would allow its use for a wide number of purposes, befitting the critical roles of endothelial cells in tissue homeostasis, toxicology and infectious diseases. Cell cultures were sought from the bulbus arteriosus (BA) of walleye (WE), which is one of North America's most important commercial and recreational freshwater fish. The BA is between the ventricle and ventral aorta of the teleost circulatory system and has an inner wall of endothelial cells. Unlike other potential sources of fish endothelial cells, the BA was large, visually distinct, and excised cleanly, permitting the easy preparation of explants. From these developed a cell line, WEBA1F. WEBA1F was maintained for at least 20 passages in L-15 medium with 10% fetal bovine serum at 26°C. The cells had cobblestone morphologies at

confluency and formed capillary-like tubes in superconfluent cultures. As one physiological function of some fish endothelial cells is to 'cleanse' blood, WEBA1F were examined for scavenger activities. The cells highly phagocytized polystyrene beads, took up acetylated low-density lipoproteins, and responded to the scavenger receptor ligand, polyinosinic: polycytidylic acid. As the endothelium in several vertebrates is the target for dioxins, inducing CYP1A1 and endothelial dysfunction, WEBAIF were exposed to a wide range of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) concentrations. Ethoxyresorufin-O-deethylase (EROD) activity, which is a catalytic measure of CYP1A, was induced in a dose-dependent manner. Viral hemorrhagic septicemia virus (VHSV) genotype IVb has recently been detected in Great Lakes fish and appears to replicate in endothelial cells and impair their functions. Therefore WEBA1F cultures were inoculated with VHSV IVb. WEBA1F underwent cytopathic effect (CPE) and produced VHSV IVb. In summary WEBA1F is a novel endothelial cell line that should be a useful tool in studying fish endothelial physiology, toxicology and virology.

A-1005

Evaluation of Aurora Kinase B as a Potential Therapeutic Target for Human Liposarcoma. T. LAVALLIE[1], L. C. Alt, M. J. Fay[1], and S. Noronha[2]. [1]Biomedical Sciences and [2]Physician Assistant Programs, Midwestern University, 555 31st Street, Downers Grove, IL 60515. Email: taylorlavallie@me.com

In 2012 it is estimated that 11,280 new cases of soft tissue sarcomas will be diagnosed in the United States and 3,900 Americans will die due to soft tissue sarcomas. Liposarcoma represents one of the most common types of soft tissue sarcomas. Current chemotherapeutic options for sarcomas include ifosfamide and doxorubicin; however, these drugs have not significantly increased survival rates for patients with sarcoma. In a pilot study we recently demonstrated that Aurora kinase B is overexpressed in intermediate and high grade soft tissue sarcomas compared to normal tissues. The goal of this research was to investigate Aurora kinase B expression in cultured human adipocytes versus the SW-872 human liposarcoma cell line, and to determine the effects of an Aurora kinase B inhibitor, AZD1152, on cellular proliferation. To evaluate Aurora kinase B expression, human mesenchymal stem cells were differentiated into adipocytes, and differentiation was assessed by oil red O staining and by examining the expression of the late differentiation marker, peroxisome proliferator-activated receptor gamma. Expression of Aurora-kinase B mRNA was evaluated by RT-PCR and was found to be increased in the SW-872 liposarcoma cells compared to the mesenchymal stem cells or the differentiated adipocytes. Treatment of the SW-872 liposarcoma cells with AZD1152 (0–300 nM) resulted in a growth inhibition and the presence of multi-nucleated cells. These studies suggest that Aurora kinase B may represent a novel chemotherapeutic target for human liposarcoma.

A-1006

Integrating Omics Data for the Better Understanding of Cancer. HONGXING LEI, Yuyun Pan, Yuchao Pei, and Jiajia Wang. Beijing Institute of Genomics, Chinese Academy of Sciences, No.7 Beitucheng West Road, Chaoyang District, Beijing 100029, CHINA. Email: leihx@big.ac.cn

In the era of systems biology, cancer has been investigated by many high throughput technologies. However, interpreting these data as a whole has been a grand challenge to the cancer research society. In this work, we collected many different types of omics data from public resources, including genomic mutation, transcriptome, proteome, epigenome, miRNA and metabolome. For each type of omics data, we analyzed the perturbation of the systems network at the level of individual genes and functional categories. For example, from transcriptome analyses, the perturbed functional categories included the well-recognized signal transduction, metabolic pathways, cell cycle, DNA replication, and system development, as well as the less-recognized complement cascade and fatty acid metabolism. The transcriptional perturbation of important transcription factors, kinases, house-keeping genes and tissue-specific genes was also analyzed, some of which may play critical role in the development of cancer. Due to the similar procedure applied in the data analyses, the perturbed systems network from different types of omics data can be compared with each other and a holistic view of cancer disease mechanism can be revealed.

A-1007

Cullin-5 Knockdown Alters Protein Expression in MDA-MB-231 Breast Cancer Cells. J. W. KWAK[2], L. A. C. Alt[2], and M. J. Fay[1,2]. [1]Department of Pharmacology and [2]Department of Biomedical Sciences, Midwestern University, 555 31st Street, Downers Grove, IL 60515. Email: jeff.wak@mwumail.midwestern.edu, mfayxx@midwestern.edu

Breast cancer remains one of the major causes of cancer-related mortality in women in the United States. Cullin-5 (Cul5) may play a tumor suppressor role in breast cancer since the gene is located on a region of chromosome 11q22-23 that is frequently associated with loss of heterozygosity. Cul5 functions at the cellular level as an E3 ubiquitin ligase scaffold protein, and these E3 complexes target selected

substrate proteins for ubiquitin-mediated degradation. To investigate the effects of decreased Cul5 expression in breast-derived cells, an in vitro Cul5 knockdown model using small interfering RNAs was established in MDA-MB-231. The efficacy of Cul5 knockdown was monitored using PCR, real-time PCR, and Western blot analysis. A non-targeting siRNA that does not target any known mammalian RNA was used as a negative control. Isolated protein from the Cul5 knockdown and the negative control samples were screened for Differential Protein Expression using 2D difference gel-electrophoresis (2D DIGE) followed by mass spectrometry to identify the differentially expressed proteins. Cul5 knockdown was confirmed at the mRNA and protein levels in the siRNA transfected cells versus the negative control transfected cells. 2D DIGE analysis identified 76 proteins that were differentially expressed in the Cul5 knockdown sample versus the negative control sample. 21 of these 76 proteins that demonstrated a 2-fold or greater differential expression were selected for further analysis by mass spectrometry. The results of the 2D DIGE and mass spectrometry for selected proteins are being validated by Western blot analysis. The identification of proteins that are differentially expressed in association with Cul5 knockdown may provide insight into the tumor suppressor functions of Cul5 in breast cancer.

A-1008

Human Colon Mucosal Crypts and Epithelial Cells Isolated in Culture: A Model to Access Differentiation and Proliferation in the Epithelial Component. M. K. DAME[1], Y. Jiang[2], K. Copley[1], D. Brenner[2], and J. Varani[1]. [1]Department of Pathology, University of Michigan Medical School, Med. Sci. 1, 1301 Catherine St., Ann Arbor, MI 48109-5602 and [2]Department of Internal Medicine, University of Michigan Medical School, University of Michigan Comprehensive Cancer Center, 1500 East Medical Center Dr., Ann Arbor, MI 48109-5930. Email: mdame@med.umich.edu

We have recently demonstrated that human colon tissue can be maintained in organ culture and that this system is a valuable model for the study of growth regulation and differentiation in the colonic mucosa. Maintenance of whole tissue required conditions which supported both the stromal and epithelial components. We believe that the health of the stromal tissue surrounding the colonic crypts is critical to the conservation of mucosal structure/function. Subsequently, we isolated mesenchymal cells from the stroma to access changes by the same agents employed to modulate whole tissue function. In our current work whole glandular crypts from human colon tissue were isolated. Immunostaining revealed preservation of histological structure and biochemical function, with Ki67 expression in the proliferating cells at the crypt base, and E-cadherin staining increasing towards the crypt apical surface. Further, epithelial cells from the isolated crypts were maintained in culture and formed colonies, which progressed through this progenitor cell condition to differentiation. Levels of MMP-1 and TIMP-1 were measured up to 3-5 d of cell culture. Finally, we propose to establish a 3-D tissue culture system that maintains the whole crypt structure and function. We will treat these models with a multi-mineral extract (red algal, *Lithothamnion calcerum*) that induced differentiation and reduced proliferation in the organ culture model, and suppressed colon polyp formation and inflammation in a concurrent mouse study. Interrogating processes at various levels of complexity – differential colonic cell types, to intact colon crypts, to whole colon tissue – will be an integral strategy to understanding the interrelationship between the epithelial and stromal compartments in the colon.

A-1009

E-cadherin Loss Drives Squamous Cell Carcinoma Tumor Cell Motility and Invasion Through Altered Expression and Localization of Dab2 and Beta 1 Integrins. S. Pore[1], J. Sun[1], A. Maione[2], J. Garlick[1,3], J. Baleja[4], and A. ALT-HOLLAND[1,5]. [1]School of Dental Medicine; [2]Sackler School of Graduate Biomedical Sciences; [3]Department of Oral and Maxillofacial Pathology, School of Dental Medicine; [4]Department of Biochemistry, School of Medicine; and [5]Department of Endodontics, School of Dental Medicine, Tufts University, Boston, MA 02111. Email: addy.alt_holland@tufts.edu

Advanced stages of squamous cell carcinoma (SCC) involve the loss of E-cadherin-mediated cell-cell adhesion, increased integrin-mediated cell-matrix adhesion, and cell invasion. However, it remains unclear how proteins that control intracellular trafficking are deregulated and cause mislocalization of cell surface adhesion receptors, such as beta 1 integrins, to promote the early stages of SCC development. The adaptor protein Dab2 - a putative tumor suppressor - is a candidate to regulate the behavior of SCC epithelial tumor cells through its interactions with Eps15-homology domain-containing proteins, and its involvement in integrin endocytosis, cell adhesion and motility. Here we investigated the effect of E-cadherin suppression on Dab-2 expression in monolayer cell cultures of human SCC tumor cells, and in bioengineered human skin tissues that mimic SCC development as it occurs in vivo. Immunostaining of E-cadherin-competent SCC tumor cells showed the localization of beta 1 integrin at cell-cell borders, and of Dab2 in numerous small endosome-like structures at the cells' periphery. In contrast, beta 1 integrin expression was elevated and localized at the perinuclear area of E-cadherin-suppressed SCC

tumor cells. Although overall Dab2 staining was markedly decreased, it was detected in patches at the periphery of these cells. Immunostaining of tissues harboring E-cadherin-competent SCC tumor cells demonstrated Dab2 expression throughout the epidermal compartment, and a continuous laminin 5 staining at the basement membrane zone. In contrast, tissues harboring E-cadherin-suppressed SCC tumor cells showed decreased Dab2 staining, tumor cell invasion into the dermis, and a patchy deposition of laminin 5. These findings suggest that the tumor-promoting effect of E-cadherin suppression is linked to Dab2 down-regulation and altered beta 1 integrin expression, endocytosis and localization that contribute, at least in part, to SCC tumor cell motility and invasion. The "Tufts Collaborates!" grant awarded to Drs. Alt-Holland and Baleja funded this study.