In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-014-9764-z

Animal Contributed Papers

A-1000

Development and Characterization of a Honey Bee (*Apis mellifera*) Cell Line. M. GOBLIRSCH, M. Spivak, and T. Kurtti. University of Minnesota, Department of Entomology, 219 Hodson Hall, 1980 Folwell Ave, St. Paul, MN 55108. Email: goblirmj@umn.edu

The relationship that humans have with honey bees is multi-facetted. For example, growers and beekeepers realize economic gains through pollination services that honey bees provide to over 100 commercial crops. Honey bees are the subject of broad studies in development, aging, and the evolution of complex traits such as social behavior. Moreover, beekeeping for leisure has increased in popularity, especially in urban settings where minimal space is needed to set one or two hives. Unfortunately, the future of our longstanding relationship with honey bees is uncertain. Annual levels of colony death are well-above normal attrition, and there remains to be identified a definitive cause(s) to explain these atypical losses. Hindering investigation into widespread colony death is the lack of *in vitro* systems derived from honey bee cells to provide a controlled, homogenous environment for the input of factors suspected to have a negative impact on bee health. Here, we report the establishment of the first continuous honey bee cell line, which we have named AME-711. AME-711 was isolated from embryonic tissues, and is characterized as adherent, fibroblast-type cells according to morphology. Cells from the line are mainly diploid (2n = 32) by karyology, and sequence analysis of the mitochondrial-encoded cytochrome c oxidase subunit I gene confirms that it is of *Apis mellifera* origin. The population doubling time is approximately 4 days and the line is passaged every 10-14 days. The AME-711 line has remained in continuous culture for at least 2.5 years and survives cryopreservation in liquid nitrogen. As a demonstration of the utility of the line, we also show that AME-711 cells are susceptible to infection with the emerging fungal pathogen, *Nosema ceranae*, a contributing factor to honey bee decline. The AME-711 honey bee cell line will serve as a powerful tool for future studies in transgenesis, host-pathogen interactions, toxicology, and nutrition.

A-1002

Regulation of RNA Polymerase III Transcription by Daidzein in MCF-7 Cells. J. KOO and N. Nole. St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439. Email: jana.koo05@stjohns.edu

RNA polymerase III (RNA pol III) synthesizes small untranslated RNAs involved in metabolic processes such as translation (tRNA, 5SrRNA) and mRNA splicing (U6 snRNA). Also, deregulation of RNA pol III activity has been directly linked to cancer cell transformation. In particular a subunit of TFIIIB, termed Brf2 required for a proper initiation by RNA pol III, behaves as an oncogene in certain lung cancers. Polyphenols, including EGCG and daidzein have been shown to possess a number of chemopreventive properties. Our lab has previously demonstrated that EGCG negatively regulates RNA pol III transcription in cancer cells via the TFIIIB subunit Brf2. We hypothesized that daidzein also regulates RNA pol III transcription. MCF-7 cells were treated with increasing concentrations of daizein (0, 1, 3 and 10 mM) and analyzed for its effect on RNA pol III transcription. Low daidzein concentrations decrease the activity on the RNA pol III promoters U6 and tMet. Interestingly, treatment of MCF-7 cells with a 10 mM dose of daidzein leads to an increase in the U6 and tRNA transcription. Additionally, daidzein treatment alters Brf1 and Brf2 expression levels in a dose dependent manner. This data suggests that Brf2 and Brf1 could be the targets for regulation of RNA pol III transcription by daidzein. Subsequently, we investigated possible mechanisms by which daidzein regulates the expression of the Brf2 and Brf1. Using methylation sensitive restriction enzymes, we demonstrate that daidzein alters the Brf2 promoter methylation profile. Our data indicate that daidzein regulates RNA pol III transcription by regulating the expression of TFIIIB subunits. Mechanistically, changes in Brf2 expression levels may involve epigenetic regulation of the Brf2 promoter.

Published online: 14 May 2014

A-1003

Profiles of Secretory Mature hsa-miR-22-3p and DNMT1 Transcript Abundance During Hepatogenic Differentiation of Mesenchymal Stem Cells (MSCs). C. WU[1], J. B. Alley[5], S. A. Samy[5], L. Fitzgerald[5], A. C. Trecartin[4], M. L. Shuler[2], and M. Ariza-Nieto[3]. Cornell University, [1]526 Campus Road, [2]115 Weill Hall, and [3]305 Weill Hall, Cornell University, Ithaca, NY 14850 and Guthrie Clinic, Ltd., [4]323 B. Hayden St. and [5]One Guthrie Square, Sayre, PA 18840. Email: cw674@cornell.edu

Background Studies have shown a direct relationship between obesity and the incidence of cancer, and the dysregulation of adipokines is a common abnormality in both conditions. Adiponectin is an antiangiogenic, antiatherogenic, anti-inflammatory, insulin sensitizing and anticancer biomarker (Barb D, et al 2006). Irisin is a newly discovered peptide hormone, mainly expressed in response to exercised and physical activity. MicroRNAs are non-coding important regulatory molecules that target specific mRNAs. Adiponectin, irisin and microRNAs are secretory biomarkers useful both in vivo and in vitro. The goal is to develop a human in vitro model using differentiated mesenchymal stem cells (MSCs) to study regulatory signaling. Methods This in vitro work is complemented with a clinical prospective observational study (IRB number 1207-27). Donors (ongoing n=38 and n=24 controls) undergoing weight lost surgery sign consent prior joining the study. Total adiponectin, irisin and secretory microRNAs are assayed on fasting plasma, collected at baseline and 12 weeks post-op. Bone marrow, omental adipose tissue and mononuclear cells in circulation are used for the isolation, expansion and differentiation of MSCs. Hepatogenic differentiation is achieved following the protocol by Stock P et al 2010. Time course demethylation with 5'- azacytidine is monitored with qPCR for DNMT1, adiponectin and FNDC5. Additionally profiles of adiponectin, irisin and hsa-miR-22 3p, are done on media. Results Our observations suggest that MSCs isolated from bone marrow, omental adipose tissue or mononuclear cells are consistent. Hypoadiponectinemia, insulin sensitivity and T2D are resolved 12 weeks post-op; however, the profiles of mature secretory microRNAs as well as the levels of irisin and the qPCR transcript abundance are donor dependent as well as the microRNA profiles assayed on the isolated MSCs. Conclusion These results suggest the interactions between hsa-miR-22-3p and DNMT1 could hold clues in understanding adipokine dysregulation and the outcome of weight lost interventions.

A-1004

The Role of Viral DsRNA and Host Scavenger Receptors in the Innate Antiviral Response in Fish. S. DEWITTE-ORR, A. Aloufi, J. Weleff, and A. Soares. Wilfrid Laurier University, 75 University Avenue, West Waterloo, ON, N2L3C5, CANADA. Email: sdewitteorr@wlu.ca

Viral dsRNA is an important pathogen associated molecular pattern (PAMP). Not only is it able to induce protective innate antiviral responses in virus-infected cells, but when extracellular, it is able to stimulate innate immune responses in neighboring cells as well. Almost nothing is known of viral dsRNA produced by fish viruses in fish cells. We have found that fish viruses, those with either RNA or DNA genomes, do indeed produce dsRNA molecules during their replicative cycle in fish cells and that the location of dsRNA production appears to correlate with the location of viral genome replication. We have also found that rainbow trout cell lines from the gill (RTgill-W1), gut (RTgut-W1) and gonad (RTG-2) express functional class A scavenger receptors (SR-As). As in mammals, we have found that these receptors mediate the uptake of classic SR-A ligands such as modified low-density lipoproteins (ex. AcLDL) as well as synthetic dsRNA molecules (ex. poly IC) in fish cells. These findings suggest that fish viruses produce the potent PAMP, dsRNA, and that SR-As may function as a pattern recognition receptor (PRR) for extracellular dsRNA in fish. Both of these findings contribute to a better understanding of virus-host interactions in fish, providing foundational knowledge for the development of better antiviral therapies and vaccines in this economically important animal.

A-1005

Differential Regulation of RNA Polymerase III Transcription by EGCG. N. NOLE, J. Koo, J. Jacob, and L. Schramm. St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439. Email: nnole87@gmail.com; nicole.nole05@stjohns.edu

RNA polymerase III (RNA pol III) transcribes small untranslated RNAs used for essential metabolic processes such as mRNA processing (U6 snRNA) and translation (tRNA), thereby regulating cell growth. Accurate initiation by RNA pol III requires TFIIIB. TFIIIB has been shown to be regulated by a number of tumor suppressors and onocogenes, and its deregulation has been shown to play a role in oncogenesis. The TFIIIB subunit Brf2 has been characterized as an oncogene in

lung squamous cell carcinomas. Our lab has previously demonstrated that oral administration of 0.01% EGCG for three weeks could regulate RNA pol III transcription in C57/BL6 mice. Specifically we previously demonstrated that oral administration of 0.01% EGCG for three weeks in C57/BL6 altered RNA pol III transcription in lung tissue from treated mice as compared to controls. Additionally, we demonstrated that the changes in RNA pol III transcription in the lungs of treated mice was, in part, due to changes in the expression levels of TFIIIB subunits. Recently, total RNA was isolated from lung and kidney tissue of C57/BL56 EGCG treated mice. Using qRT-PCR, we determined that in lung tissue, oral administration of 0.01% EGCG significantly down regulated the expression of the TFIIIB subunits Brf2, Brf1 and Bdp1. Interestingly, in kidney tissue from the same EGCG treated mice, the expression of Brf1 is unchanged whereas Brf2, TBP, and Bdp1expression increased in response to treatment with 0.01% EGCG. EGCG is known to induce epigenetic changes; we investigated the methylation status of the TFIIIB promoters. We cloned the mouse TFIIIB promoters in silico and CpG islands identified for each mouse TFIIIB promoter. Lung and kidney tissue from control and treated mice was isolated and theBrf1 and Brf2 promoters were analyzed for alterations in methylation status in response to treatment. We report that the methylation profiles of the TFIIIB subunit Brf2 in both tissues remains unchanged in response to EGCG, suggesting an alternative mechanism for the negative regulation of RNA pol III transcription in the lung.

A-1006

Influence of the Extracellular Matrix on the Survival of Cryopreserved Adherent Cells. L. CAMPBELL and K. Brockbank. Cell & Tissue Systems, Inc., 2231 Technical Parkway, Suite A, North Charleston, SC 29406. Email: lcampbell@celltissuesystems.com

We have developed a convenient cell-based system for adherent cells cryopreserved on microtiter plates for testing in a wide variety of applications. The retention of cells and the effects of cryobiological conditions on the adherence of cells to the substrate are critical issues for the success of the CryoPlate™ concept. Individual extracellular matrix (ECM) components have been evaluated to determine if one specific component or some combination of one or more components preferentially promote cell attachment and viability during cryopreservation and thawing. Cell types that have been evaluated include differentiated endothelial cells and mesenchymal stem cells. ECM components that have been evaluated include: fibronectin, laminin and collagens I, III, IV, V. Optimal concentrations for each ECM component were determined by plating the cells using a range of concentrations of each component followed by cryopreservation and assessment of viability after thawing. Then combinations of 2 or more components were assayed. Viability after a one hour post-thaw recovery was assessed using a metabolic activity assay in which resazurin is reduced to fluorescing resorufin. Proliferation was assessed using the same assay at several days post-thaw. The results are presented as the mean relative fluorescence in percent of untreated controls ± 1 standard error of the mean. Results to date showed that while no single ECM component or combination of ECM components demonstrated the best viability and attachment after cryopreservation, certain trends were apparent. The presence of any ECM component versus none improved viability and combinations of 2 or more components provided better viability than using only one component. These results demonstrate that the presence of extracellular matrix components contributes to maintaining the attachment and viability of cells during cryopreservation, rewarming and post-cryopreservation cell culture.

Disclosure: Author was or is employed by Cell & Tissue Systems, Inc.

A-1007

Delineating the Metabolic Profiles of Skin Cancer Cells and Stromal Fibroblasts in Vitro. S. SAFFARI[1], R. Tay[1], J. Nolan[2], J. Baleja[2], and A. Alt-Holland[1]. [1]Tufts University School of Dental Medicine, One Kneeland Street, Boston, MA 02111 and [2]Tufts University School of Medicine, 136 Harrison Ave, Boston, MA 02111. Email: addy.alt_holland@tufts.edu; shawheen.saffari@tufts.edu

E-cadherin loss is one of the hallmarks of squamous cell carcinoma (SCC) development. However, how loss of E-cadherin in cancer cells and how tumor-stromal interactions between cancer cells and fibroblasts affect cellular metabolic pathways that can promote SCC or basal cell carcinoma (BCC) development in the human skin remain elusive. To that end, individual cell cultures and co-cultures of human E-cadherin-competent (II-4) or E-cadherin-suppressed (II-4-Ecad-) SCC cells and dermal fibroblasts were cultured for 24-48 hours. Extracellular metabolites in the growth medium were analyzed by Nuclear Magnetic Resonance (1H-NMR). Microscopy analysis showed that in individual monolayer cell cultures, fibroblasts presented elongated cell morphology, II-4

cells grew as compact colonies and II-4-Ecad- cells grew as single and randomly spread cells. In co-cultures, II-4 colonies were tightly surrounded by streams of fibroblasts, whereas in co-cultures of II-4-Ecad- cells and fibroblasts the tumor cells spread around the latter. NMR revealed that II-4 cultures consumed more glutamine, valine and leucine and secreted more pyruvate than II-4-Ecad- cultures that showed less metabolic activity. Notably, the overall energy metabolism of co-cultures of fibroblasts with either II-4 or II-4-Ecad- cells was lower than that of individual SCC cell cultures. This was evident by the decrease in consumption of glucose and production of lactate in co-cultures of SCC cells and fibroblasts. Thus, the cross-talk between epithelial tumor cells and dermal fibroblasts can alter the metabolic profile of these cell types upon their interaction with each other. These findings can shed light on the behavior of these cells in vivo during cancer progression. Furthermore, metabolites or their corresponding biochemical pathways can serve as targets for new mechanism-based therapeutic strategies or as biomarkers to monitor the progression of SCC, and potentially of BCC.