In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-013-9609-1

Animal Contributed Papers

A-1000

Development of Cell Lines from the Walleye Caudal Fin and Their Ability to Survive and Support Viral Hemorrhagic Septicemia Virus (VHSV) Group IVb Replication at Low Temperatures. A. W. BENDER[1], N. T. K. Vo[1], P. A. L. Rivers[1], J. S. Lumsden[2], B. Dixon[1] and N. C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON, CANADA and [2]Fish Pathology Laboratory, Ontario Veterinary College, University of Guelph, Guelph, ON, CANADA. Email: aaronwbender@gmail.com

A unique Northern American genotype (IVb) of viral hemorrhagic septicemia virus (VHSV) emerged over the last 10 years in the Great Lakes, causing significant mortality in a wide range of fish, including walleye. However, cell lines from commercially important species native to this region that could be used to study the virus in these species are few. Therefore, we sought to develop cell lines from the walleye caudal fin and have developed one (WECF11-f) with a fibroblast-like morphology and another (WECF11-e) with an epithelial-like morphology. These survive at temperatures from 4 to 32°C and provide an opportunity to compare VHSV IVb replication at 4°C, which walleye experience through winter, with 14°C, which is usually considered optimal for VHSV production. At 14°C, VHSV IVb was produced in both cell lines and caused cell death. At 4°C, the expression of VHSV IVb transcripts was slightly delayed in both cell lines. However, viral production and cell death were greatly delayed at 4°C but after 2 weeks had occurred in WECF11-f cultures but still had not happened in WECF11-e cultures. Possibly VHSV IVb has a tissue tropism that is most prominently expressed at low temperatures.

A-1001

The Susceptibility of Walleye Cell Lines from the Spleen Stroma and Caudal Fin to Viral Hemorrhagic Septicemia Virus (VHSV). NGUYEN T. K. VO[1], Hina A. Bandukwala[1], Aaron W. Bender[1], John S. Lumsden[2], Lucy E. J. Lee[3,4], Brian Dixon[1], and Niels C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON, CANADA; [2]Ontario Veterinary College, University of Guelph, Guelph, ON, CANADA; [3]Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA; and [4]Faculty of Science, University of the Fraser Valley, Abbotsford, BC, CANADA. Email: ntkvo@uwaterloo.ca

Viral hemorrhagic septicemia virus (VHSV) is a rhabdovirus that causes a significant disease (VHS) with high mortality in many fish species including walleye (*Sander vitreus*), leading to economic losses in aquaculture. In a susceptible fish species, severely infected individuals hemorrhage and die, whereas chronically infected individuals are often anemic. The anemia suggests that the virus could be impairing hematopoiesis, which in fish occurs in the spleen and head kidney. Hematopoiesis is supported in these tissues by stromal cells. One way of studying viral pathogenesis is to examine their impact on spleen stromal cells. Therefore, we developed a cell line (WES6S) from the walleye spleen and compared this cell line to a fibroblast cell line (WECF11f) from the walleye caudal fin for susceptibility to VHSV IVb. Both cell lines produced the virus and showed a cytopathic effect (CPE), which was a loss of cell viability that was measured by fluorescent dyes Alamar Blue and CFDA-AM. However, virus production began earlier in WES6S as indicated by the earlier accumulation of viral proteins, development of CPE, and increase in viral titers. The induction of the antiviral polypeptide, Mx, by VHSV IVb was seen in WECF11f but not in WES6S. This result shows that the antiviral mechanisms are affected but regulated differently in response to VHSV infections in the caudal fin and spleen stroma of *S. vitreus*.

A-1002

Effects of Differential Trk Expression on Neuroblastoma Cells Cultured in the Microgravity Rotary Bioreactor. ROBERT A. REDDEN[1], Jane E. Minturn[2], Garrett M. Brodeur[2], and Edward J. Doolin[1]. [1]Department of General and Thoracic Surgery and [2]Division of Oncology. The Children's Hospital of Philadelphia. Philadelphia, PA. Email: redden@email.chop.edu

Background. Neuroblastoma features confounding biological and genetic heterogeneity that drives diverse, often unpredictable, clinical behavior. Differential expression of the Trk family of neurotrophin receptors strongly correlates with clinical behavior: TrkA expression is associated with favorable outcome, and TrkB with unfavorable outcome. Objective. Neuroblastoma cells cultured in a microgravity rotary bioreactor spontaneously aggregate into tumor-like structures, called organoids. We evaluated the effect of TrkA or TrkB expression on aggregation kinetics and organoid morphology in neuroblastoma cell lines. Methods. SY5Y cells (Trk-null) were stably transfected to express either TrkA or TrkB. Short-term aggregation kinetics were determined by counting the number of single (non-aggregated) viable cells in the supernatant every hour. These data were plotted, and the area under the curve calculated. Organoids were harvested after 4 or 8 days of bioreactor culture, stained, and analyzed morphometrically. Results. SY5Y and SY5Y-TrkB aggregated significantly faster than SY5Y-TrkA cells. SY5Y and TrkB cell lines formed irregularly shaped organoids, featuring stellate projections. In contrast, TrkA cells formed smooth (non-stellate) organoids. SY5Y organoids were slightly smaller on average, but had significantly larger average perimeter than TrkA or TrkB. Conclusion. TrkA expression alone is sufficient to dramatically alter the behavior of neuroblastoma cells in vitro. This pattern is consistent with both clinical outcome and in vivo tumorigenicity, in that SY5Y-TrkA represents a more differentiated phenotype. The microgravity bioreactor is a useful in vitro tool to rapidly investigate the biological characteristics of neuroblastoma and, potentially, as a prognostic assay.

A-1003

Comparison of Human Colonic Crypts and Abnormal Crypts from Adenoma in Culture: A Multi-stage Model to Assess Colon Chemoprevention. Y. JIANG[1], M. K. Dame[2], D. K. Turgeon[1], H. Appelman[2], M. N. Aslam[2], K. Copley[2], D. Attili, D. Brenner[1], and J.Varani[2]. [1]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 and [2]Department of Pathology, University of Michigan Medical School, Med. Sci. 1, 1301 Catherine St., Ann Arbor, MI 48109–5602. Email: yanjian@med.umich.edu

A primary human colonic tissue culture model is needed to define efficacy and mechanisms of action of colon cancer preventive interventions. We developed methods for the isolation and in vitro maintenance of intact colonic crypts from histologically-normal human colon and adenomatous polyps. Crypts or crypt-like structures were collected from samples of both normal and adenoma tissue and maintained in 3-D culture with low Ca^{2+} (0.15 mM) media. Cell proliferation and differentiation were assessed by immunostaining with markers Ki67 and E-cadherin. After 24 hours in culture, crypts from normal colonic tissue showed strong Ki67 expression at the crypt base, with this gradually decreasing over time until by day-4 Ki67 was not evident. The differentiation marker E-cadherin (and Cytokeratin-20) increased over the same period. Other markers such as Chromogranin A and Mucin-2, for enterocytes and goblet cells, were expressed in cultured crypts. As in situ, the crypts isolated from normal tissue gradually shed apical cells until by day-7 most of the structures had disappeared. A similar culture system preserved characteristics of adenomatous tissue, with well-defined structure and expanding buds/tubules. Both Ki67 and E-cadherin were expressed strongly, lacked spacial organization and did not ablate after extended culture. After 15 days the adenoma structures were Ki67 and E-cadherin positive. The cultured abnormal crypt-like structures were harvested and maintained through multiple passages. Intact colonic crypts from normal human mucosa were viably maintained in 3-D culture for up to 7-days, while abnormal crypt-like structures from adenoma tissue could be maintained for several generations (up to months). This provides a valuable platform for colon chemoprevention research.

A-1004

Voluntary Exercise Prevents Obesity and Significantly Alters Major Gut Bacteria Phyla. JEFF KWAK[1], Christian C. Evans[2], Samantha Laskowski[3], Joseph Dougherty[1,4], Kathy J. LePard[5], and Mae J. Ciancio[1]. Midwestern University, [1]College of Health Sciences, Department of Biomedical Sciences, [2]College of Health Sciences, Department of Physical Therapy, [3]College of Osteopathic Medicine, [4]College of Dental Medicine Illinois and [5]College of Osteopathic Medicine, Department of Physiology, 555 31st St., Downers Grove, IL 60515. Email: jkwakx@midwestern.edu (presenting author), mcianc@midwestern.edu (corresponding author)

Background: Diet induced obesity (DIO) is a significant health concern that has been linked to changes in the enteric microbiome. Objective: Determine the effects of voluntary exercise (Ex) and high fat (HF) DIO on the relative fecal content of *Bacteroidetes* (B) and *Firmicutes* (F). Methods: Male C57BL/6 littermates (5 weeks) were distributed equally into 4 groups (n=6/group): low fat (LF) sedentary (Sed; LF/Sed), LF Ex (LF/Ex), HF Sed (HF/Sed) and HF Ex (HF/Ex). Mice were individually housed in rat cages. Ex cages were equipped with an Ex wheel attached to an odometer. Bacterial DNA was isolated from fecal pellets

collected at 0, 6 and 12 weeks of diet and Ex. Quantitative PCR was performed using a universal primer that recognized all bacteria and primers specific to the phyla B and F. Bacterial DNA was also amplified using the V4 region of the16S rRNA genes with a 515F and 806R barcoded primer set and then 151 base pairs were sequenced from both ends of the insert using the Illumina MiSeq platform (Argonne National Laboratory). Results: Results are mean±standard deviation (*p<0.05 vs. HF/Sed). Ex significantly prevented HF DIO weight gain (LF/Sed=27.1*±0.51 g; LF/Ex=25.3± 0.22 g*; HF/Sed =33.1±1.2 g; HF/Ex=27.9±1.3 g*). Oral glucose tolerance (OGTT), expressed as area under the curve (mg/dL × min), was significantly improved by Ex in the HF group: HF/Sed=32500±1440; HF/Ex=24100±2500 (p= 0.001). Quantitative PCR demonstrated a significant decrease in the delta Ct ratio of B:F in Ex compared to Sed and groups (LF/Sed=2.29±0.16; LF/Ex =1.54±0.18; HF/Sed =2.07± 0.22; HF/Ex=1.23±0.26, p=0.001). Sequencing data corroborated the quantitative PCR results, demonstrating a trend for a significant change in B:F ratio for the Ex groups (p=0.08, data not shown). Final body weights were positively correlated to the delta Ct B:F ratio (R^2 =0.16, p<0.05). Conclusion: Results suggest that Ex alters the gut microbiota at the phyla level and that this may play a role in Ex attenuation of DIO and glucose intolerance.

A-1005

The p53 Inhibitor, Pifithrin-μ, Induces Apoptosis in Rainbow Trout Gill Cell Line, RTgill-W1. F. ZENG[1], J. P. Sherry[2], B. Dixon[1], B. P. Duncker[1], and N. C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, CANADA and [2]Aquatic Ecosystem Protection Research Branch, Environment Canada, Burlington, ON, L7R 4A6, CANADA. Email: fzeng@uwaterloo.ca

Understanding the functions of p53 in fish will provide insights into how the functions of this critical cellular regulator evolved in vertebrates. Among p53's functions in mammalian cells is the regulation of cell death. A similar role might occur in lower vertebrates because of the high degree of conservation at the molecular level for the p53 of fish and mammals. However, knowledge of cell death mechanisms, such as apoptosis, and p53 is limited in fish. Therefore, in this study the actions of p53 have been explored in the rainbow trout gill cell line, RTgill-W1, through the use of a specific inhibitor of p53, pifithrin-μ. Exposure of RTgill-W1 cells to pifithrin-μ induced apoptosis but the anti-oxidant N-acetylcysteine (NAC) blocked this action. Treatment of cultures with pifithrin-μ also caused the accumulation of insoluble protein and of HSP70. These results suggest that pifithrin-μ caused oxidative stress and the unfolded protein response in RTgill-W1. Collectively these actions of pifithrin-μ have yet to be seen in other cells, suggesting that in fish cells the 'off target' actions of pifithrin-μ are either unique or some of the regulatory functions of p53 are different in fish.

A-1006

Using Fish Cell Lines to Study How Engineered Nanomaterials Influence the Survival of Viruses from Lower Aquatic Vertebrates. Y. J. HUANG[1], P. H. Pham[1], A. Hu [2], K. Oakes[1], S. X. Tang[3], and N. C. Bols[1]. [1]Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, CANADA; [2]Department of Mechanical Engineering and Mechatronics, University of Waterloo, 200 University Avenue West, Waterloo, CANADA; and [3]Department of Chemistry, University of Waterloo, 200 University Avenue West, Waterloo, CANADA. Email: yj3huang@uwaterloo.ca; ncbols@uwaterloo.ca

Nanomaterials, such as titanium dioxide (TiO_2) and carbon nanotubes (CNTs), are becoming increasingly used in the manufacturing of commercial products. TiO_2 is commonly used in sunscreen and CNTs in sporting equipment. This results in greater exposure of land and aquatic environment to contamination by manufactured nanomaterial. The inactivation of bacteria and bacterial phage by nanomaterial is well documented but few studies have examined the effect of nanomaterial on aquatic viruses of lower vertebrates. Our research examined the interaction of nanomaterials with three viruses of differing size, structure and composition. Two of the viruses, viral hemorrhagic septicemia virus (VHSV) and chum salmon reovirus (CSV), are fish pathogens, while Frog virus 3 (FV3) mainly infects amphibians. Our research showed that single-walled CNT functionalized with NH_2 can significantly inactivate VHSV while other functional groups and mutli-walled CNTs cannot. The effect of TiO_2 on these viruses differs depending on the virus and treatment condition. In suspension test, TiO_2 is capable of reducing the titer of VHSV and FV3 but not CSV. When the viruses were exposed to TiO_2 under long wavelength UV (L_{UV}), the L_{UV} enhanced the inactivation of VHSV but not FV3 or CSV. Interestingly, when VHSV was exposed to TiO_2 under short wavelength UV (S_{UV}), the TiO_2 protected VHSV from the more damaging effect of S_{UV}. These results suggest TiO_2 in the environment could protect viruses from S_{UV} inactivation by the sun. In addition, TiO_2 combined with L_{UV} shows promising application as a method to disinfect viruses in water.