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Animal symposia

A-1

Baculovirus Technology: Protein Expression, Drug Discovery, Vaccines, What Next? T. A. KOST. Molecular Discovery Research, GlaxoSmithKline R & D, Research Triangle Park, NC, 27709. Email: tom.a.kost@gsk.com

The baculovirus insect cell protein expression system has been used widely for over 25 years to produce recombinant proteins. Cervarix®, a vaccine for the prevention of cervical cancer, produced in this system was approved for clinical use by the FDA in 2009. More recently baculoviruses modified to express recombinant proteins in transduced mammalian cells (BacMam viruses) have been used for assay development and protein production in drug discovery programs, as immunogens and as potential gene therapy vectors. This presentation will describe the generation, production and benefits of these recombinant viruses together with examples of their application in biological research.

A-2

Use of a Fish Macrophage Cell Line, RTS11, to Study Virus/Macrophage Interactions. N. C. BOLS[1], P. H. Pham[1], P. P. Chiou[2], S. J. DeWitte-Orr[3], B. Dixon[1], J. S. Lumsden[4], and C. Tafalla[5]. [1]Dept of Biology, University of Waterloo, Waterloo, ON N2L 3G1 CANADA; [2]Academia Sinica Jiaosi, Yilan, TAIWAN; [3]Dept of Biology, Wilfrid Laurier University, Waterloo, ON N2L 3C5 CANADA; [4] OVC, Pathobiology, University of Guelph, Guelph, Ontario, N1G 2W1, CANADA; and [5] Ctr Invest Sanidad Anim, E-28130, SPAIN. Email: ncbols@sciborg.uwaterloo.ca

Macrophages can interact with viruses in diverse ways, from coordinating immune defenses to supporting the replication and/or spread of viruses within hosts. Macrophage cell lines are one approach to studying these interactions and RTS11 is a rainbow trout monocyte/macrophage cell line that allows them to be studied with fish viruses. RTS11was developed from spleen primary long-term hemopoietic cultures. These had a stromal layer of fibroblast-like and epithelial-like cells on which small phase-bright cells grew and were released into the medium. These floating cells were cultured independent of the stroma, giving rise to RTS11. Cultures of RTS11 consist of two cell populations, macrophages and monocytes or progenitor cells. The relative proportions of each shift with subtle changes in culture conditions. RTS11 has been used to study grouper iridovirus (GIV), Chum salmon reovirus (CSV), and viral hemorrhagic septicemia virus (VHSV). In RTS11 GIV rapidly induced apoptosis but did not replicate; CSV caused a unique cytopathic effect (CPE), homotypic aggregation, but replicated only slightly; VHSV entered and produced viral transcripts but failed to cause a CPE or produce all viral proteins. Exploring these interactions might reveal mechanisms responsible for a species being a non-host and for hosts having acute or persistent infections.

A-3

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

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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-4

Characterization of a Hepatitis E-like Virus from Fish. J. WINTON[1], W. Batts[1], S. Yun[2], and R. Hedrick[2]. [1]Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA 98115 and [2]Department of Medicine and Epidemiology, University of California, Davis, CA 95616. Email: jwinton@usgs.gov

Beginning in 1988, the Chinook salmon embryo (CHSE-214) cell line was used to isolate a novel virus from spawning adult trout in the state of California, USA. Termed the cutthroat trout (*Oncorhynchus clarkii*) virus (CTV), the small, round virus was not associated with disease, but was subsequently found to be present in an increasing number of trout populations in the western USA, likely by a combination of improved surveillance activities and the shipment of infected eggs to new locations. The full genome of the 1988 Heenan Lake isolate of CTV consisted of 7,269 nucleotides of positive-sense, single-stranded RNA beginning with a 5' untranslated region (UTR), followed by three open reading frames (ORFs), a 3' UTR and ending in a polyA tail. The genome of CTV was similar in size and organization to that of *Hepatitis E virus* (HEV) with which it shared the highest nucleotide and amino acid sequence identities. Similar to the genomes of human, rodent or avian hepeviruses, ORF 1 encoded a large, non-structural polyprotein that included conserved methyltransferase, protease, helicase and polymerase domains, while ORF 2 encoded the structural capsid protein and ORF 3 the phosphoprotein. Together, our data indicated that CTV was clearly a member of the family *Hepeviridae*, although the level of amino acid sequence identity with the ORFs of mammalian or avian hepeviruses (13-27%) may be sufficiently low to warrant the creation of a novel genus. Finally, we showed that CTV was able to form persistently infected cultures of the CHSE-214 cell line that may have use in research on the biology or treatment of hepevirus infections of humans or other animals.

A-5

Diabetes-accelerated Vascular Disease – In Vitro and In Vivo Studies. KARIN E. BORNFELDT. Departments of Medicine and Pathology, Diabetes and Obesity Center of Excellence, University of Washington, Seattle, WA 98109. Email: bornf@uw.edu

Diabetes accelerates cardiovascular disease by promoting formation and progression of atherosclerotic lesions through mechanisms that are incompletely understood. We investigate these mechanisms by using a combination of in vitro studies of the effects of factors associated with the diabetic environment on vascular cells, and in vivo experiments in genetically engineered diabetic and non-diabetic mice. These studies have demonstrated that monocytes and macrophages take on an inflammatory phenotype in mouse models of type 1 diabetes, and that this phenotype is associated with an increased expression of acyl-CoA synthetase 1 (ACSL1), an enzyme that catalyzes the conversion of primarily arachidonic acid into arachidonoyl-CoA in macrophages. This ACSL1-associated inflammatory phenotype can be mimicked by exposure of macrophages in vitro to endotoxin (lipopolysaccharide), but not to elevated glucose concentrations or to saturated fatty acids. Furthermore, knocking ACSL1 expression down prevents the inflammatory phenotype of macrophages exposed to endotoxin in vitro. In vivo studies confirm these findings. Thus, diabetic mice deficient in myeloid ACSL1 expression are protected from the diabetes-induced inflammatory monocyte and macrophage phenotype as well as from diabetes-accelerated atherosclerosis. Conversely, increased glucose uptake in monocytes and macrophages does not mimic the effect of diabetes on inflammation or atherosclerosis in vivo. These studies suggest that a combination of in vitro and in vivo studies provides a fruitful approach to understanding the molecular and cellular mechanisms of diabetes-accelerated vascular disease.

A-6

Genomic Comparison of In Vivo and In Vitro Adipose Insulin Resistance Models. E. FRAENKEL. Department of Biological Engineering, 77 Massachusetts Ave., Building 16, Room 241, Massachusetts Institute of Technology, Cambridge, MA, 02139. Email: fraenkel-admin@mit.edu

Diet-induced obesity predisposes individuals to insulin resistance, and adipose tissue has a major role in the etiology of disease. Adipose insulin resistance can be induced in cultured adipocytes by a variety of treatments, but it is unknown what aspects of in vivo adipose insulin resistance are captured by the different in vitro models. We used global RNA-sequencing (RNA-Seq) to investigate the gene expression changes of four different in vitro insulin resistance models induced by treatments with TNFα, hypoxia, dexamethasone, and high insulin. We analyzed these changes in parallel with those from three independent microarray studies of white adipose tissue from diet-induced obese (DIO) mice, examining the transcriptional effects on a genome-wide level and with a focus on a set of 1,319 adipogenesis-related genes. We further explored the differences in transcriptional regulation among the in vitro models using DNase I hypersensitivity followed by massively parallel sequencing (DHS-Seq). In this talk, I will discuss which aspects of the in vivo changes are captured by each model and our insights into the regulation of the transcriptional changes.

A-7

Mechanisms of Oxidative Damage Studied with In Vitro Models of Non-alcoholic Fatty Liver Disease. MARTHA C. GARCIA and Thomas J. Flynn. Division of Toxicology, FDA Center for Food Safety and Applied Nutrition, 8301 Muirkirk Road, Laurel, MD. Email: Martha.garcia@fda.hhs.gov

The liver plays an important role in lipid metabolism, and the excessive accumulation of lipids in hepatocytes (steatosis) causes cellular dysfunction (lipotoxicity) that increases the sensitivity to further liver injury. Steatosis is the relatively benign form of a spectrum of conditions known as Non Alcoholic Fatty Liver disease (NAFLD) or fatty liver that can progress to more advance inflammatory forms like non alcoholic steatohepatitis (NASH) or liver cirrhosis. NAFLD is strongly associated with obesity and the metabolic syndrome. The elevated levels of circulating free fatty acids, as is observed in patients with NAFLD, is predominantly from the dietary monounsaturated fatty acid, oleic acid (C18:1, OA), and the saturated fatty acid, palmitic acid (C16:0, PA). Previous studies suggest that the type of fatty acid in excess may play a role in the progression of the disease; PA induces apoptosis, and OA is more efficiently accumulated in triglycerides. This presentation will describe our work on the development of cellular in vitro models of steatosis. We have cultured liver cells with an excess of OA and PA, and evaluated oxidative stress status by measuring lipid peroxidation and the cellular content of the antioxidant glutathione. Since epidemiologic data suggest sex-associated differences in the prevalence and progression of NAFLD with estrogen having a protective effect, we have also evaluated the role of sex hormones on oxidative stress and the risk of NAFLD development.

A-8

Utilization of Direct Receptor Visualization for Endocrine Activity Tests in Non-targeted Environmental and Chemical Screening. RAYMOND A. LEWIS. Thermo Fisher Scientific, Life Science Research - Cellomics, 100 Technology Dr., Pittsburgh, PA 15219. Email: raymond.lewis@thermofisher.com

Endocrine active chemicals are thought to produce endocrine disruptive affects in humans and wildlife by interfering with steroid hormone signaling. Environmental contaminants and industrial pollutants that interact with estrogen and androgen receptors can profoundly affect normal development and hormonal homeostasis and are suspected to produce numerous health and reproductive problems in both humans and wildlife. Regulatory mandates requiring endocrine disruption risk assessment for consumer products present major obstacles to current testing procedures that are expensive, time consuming, and require large numbers of animals. These obstacles can be addressed by automating procedures to assess a chemical's endocrine activity in vitro as a high content pre-screen prior to investigating potential organismal and environmental impact. Thus, utilization of in vitro screens for potential endocrine active chemicals (EACs) reduces animal testing by categorizing and prioritizing chemicals based on their ability to alter endocrine receptor activity. Two cell-based assays were used to efficiently test chemicals for their ability to activate GFP-tagged steroid receptors estrogen receptor alpha (ERα) and androgen receptor (AR). These biosensors form nuclear foci in response to stimulation that can be easily quantified by automated fluorescence imaging. Additional outputs for individual cells are simultaneously measured resulting in direct assessment of compound toxicity and comparison to positive controls, thus providing insight into the dynamics of receptor activation while simultaneously monitoring cell cycle perturbations and toxicity. Compared to currently validated in vitro endocrine disruption assays, these cell-based functional assays resulted in higher specificity and sensitivity (>80%) against a panel of compounds that included pesticides, phytoestrogens, and plasticizers. Bisphenol A was detected by the ERα assay at ~66 ppb, which is more sensitive than the current allowable intake limits from several regulatory agencies. These assays were developed following EPA and ICCVAM guidelines for endocrine disruption assays and provide functional in vitro determination of receptor activity, therefore resulting in a more thorough assessment of the potential for in vivo endocrine disruption.

A-9

Stably Transfected Estrogen Receptor Alpha Transactivation Assay Using HeLa9903 Cell Line as In Vitro Method to Screen the Endocrine Disruption Potentials of Chemicals. A. ONO. Division of Risk Assessment, National Institute of Health Sciences, JAPAN. Email: atsushi@nihs.go.jp

There is increasing evidence that some synthetic chemicals in our environment may interfere with our endocrine system and induce various types of adverse health effects. The OECD initiated a high-priority activity in 1998 to revise existing, and to develop new Test Guidelines (TG) for the screening and testing of potential endocrine disrupting chemicals. The disrupting effect on the estrogen receptor (ER) downstream gene transcription by the chemicals is one of the important mechanisms of endocrine disruption. In vitro transactivation assays, i.e. reporter gene assay, are based on a direct or indirect interaction of the chemical with a specific receptor that regulates the transcription of a reporter gene product. The HeLa9903 cell line, which is derived from a human cervical tumor, with two stably inserted constructs, i.e ER alpha and the luciferase reporter plasmid, has been developed to detect the ER alpha (anti-)agonist activity of chemicals. "The Stably Transfected Human Estrogen Receptor Transcriptional Activation Assay (STTA) using the humanERalpha-HeLa-9903 (HeLa9903) cell line for the detection of estrogenic agonist activity of chemicals" was established as an OECD TG No.455 (TG 455) in 2009. TG 455 is first OECD TG base on the reporter gene assay, which evaluates molecular mechanisms of toxicity 'endocrine disruption', and is not alternative for any existing test method. Although, the way to use the results in regulatory decision is still need to discuss because the results from TG 455 are not directly match with any in vivo endpoints. The test methods to evaluate the toxic mechanism like TG455 are important to the next generation of toxicology.

A-10

EDSP21: The Incorporation of In Silico Models and In Vitro High Throughput Assays in the Endocrine Disruptor Screening Program (EDSP) for Prioritization and Screening. S. G. LYNN. Office of Science Coordination and Policy, U.S. Environmental Protection Agency, Washington DC. Email: lynn.scott@epa.gov

The U.S. EPA developed the Endocrine Disruptor Screening Program (EDSP) in response to the Federal Food, Drug, and Cosmetic Act (FFDCA) of 1996 which requires EPA to use *"appropriate validated test systems"* to determine endocrine effects. EPA adopted a two-tiered screening and testing strategy, utilizing a mix of in vivo and in vitro assays, and expanded the program to include the androgen and thyroid hormonal pathways of the endocrine system and to address both human and ecological effects. In September 2011, the EPA outlined the EDSP21 Work Plan which describes an approach for using in silico models and molecular-based in vitro high-throughput (HTP) assays to prioritize and screen chemicals to determine their potential to interact with the estrogen, androgen or thyroid (E, A, or T) hormonal systems in an incremental progressive step forward. The EDSP21 Work Plan proposes three stages of implementation: prioritization, screening and data replacement. In general, prioritization (near-term<2 years) is expected to use existing data, in silico models and in vitro HTP assays to determine the order in which chemicals are screened in the existing EDSP Tier 1 battery of assays. Screening (intermediate-term 2-5 years) is expected to initially involve integrating and eventually replacing current Tier 1 in vitro assays, that are relatively low throughput, with validated in vitro HTP assays. In addition, as the program continues to evolve, Tier 1 screening is expected to utilize a targeted approach for in vivo assays to reduce the use of animals. Replacement (long-term >5 years) of the current Tier 1 in vitro and in vivo assays with a comprehensive and validated suite of HTP in vitro assays will be a longer term effort but will result in eliminating the use of whole animals for screening purposes. This talk will provide an overview and update on the efforts and challenges to implement the EDSP21 Work Plan in a transparent manner that will require periodic scientific peer review and consideration of input from the public and respective stakeholders. *This abstract does not necessarily reflect U.S. EPA policy.*

A-11

Validating High-throughput Test Methods for Tox21; the Technology Has Changed, but the Objective Remains the Same. WARREN CASEY. NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, National Institute of Environmental Health Sciences, Mail Code K2-16, PO Box 12233, Research Triangle Park, NC 27709. Email: warren.casey@nih.gov

As we move closer to the vision of Toxicology in the 21st Century (Tox21), the issue of test method validation in the context of high-throughput screening (HTS) needs to be addressed. Much of the data generated using HTS will initially be used for prioritizing chemicals for further testing in pre-existing validated test methods, but the ultimate goal of Tox21 is to use HTS data directly to make regulatory decisions. Using such data to make regulatory risk assessment decisions will require validation to demonstrate that

the proposed decision strategies can provide equivalent or improved protection of public health. Flexibility in the validation of HTS methods is essential, and will vary depending on the intended purpose, applicability domain, and existing data for the proposed tools. ICCVAM recently completed a multi-laboratory international evaluation of the BG1Luc ER TA test method, and concluded that the accuracy of this assay is at least equivalent to U.S. EPA OPPTS 890.1300/OECD Test Guideline 455. The BG1Luc ER TA test method has recently been adapted to a high-throughput format using 1536-well plates by the NIH Center for Translational Therapeutics (NCTT; formerly the NIH Chemical Genomics Center). Preliminary results are promising, and it is expected that this method will be incorporated into the Tox21 screening paradigm in 2012. This talk will use the BG1 ER TM as a model to similarities and difference in the validation of low throughput versus high-throughput test methods.

A-13

Efficient Genome Engineering with Zinc-finger Nucleases. D. CARROLL. Department of Biochemistry, University of Utah School of Medicine, 15 N. Medical Drive, Room 4100, Salt Lake City, UT 84112-5650. Email: dana@biochem.utah.edu

Classical genetics consists of identifying a mutant phenotype, then finding the gene responsible. Reverse genetics starts with a gene and generates mutations in order to discover gene functions. The latter approach has gained prominence with the advent of whole-genome sequencing, and it has been facilitated by the introduction of targetable gene-editing nucleases. Zinc-finger nucleases (ZFNs) consist of a nonspecific DNA-cleavage domain linked to a DNA-binding domain made up of zinc fingers that can be manipulated to recognize a wide range of genomic sequences with high specificity. Introduction of ZFNs into cells induces mutagenesis at the cleavage site and stimulates homologous recombination when an exogenous donor DNA is introduced. The per-cell efficiency of both processes can be in the range of ten percent or better. ZFNs have been used successfully in many different organisms and cell types, and clinical trials using them are in progress. In addition, this targeting process is also being employed with a novel DNA-binding domain that is simpler to design and shows great promise in constructs called TALENs.

A-14

RNA Interference: A Tool to Elucidate Gene Function in Mammalian Cells. ANNALEEN VERMEULEN. Thermo Fisher Scientific, 2650 Crescent Dr., Suite 100, Lafayette, CO 80026. Email: annaleen.vermeulen@thermofisher.com

RNAi technology has revolutionized basic biological and medical research strategies in recent years. Now a common molecular tool to reveal the gene functional interactions and pathways, we will review past and recent technological innovations that improve the quality and reliability of RNAi-based experiments. Key considerations will include design, delivery and detection which will be illustrated by an example study employing siRNA and shRNA tools to evaluate gene function.

A-15

Tubeless Microfluidic Systems for Personalized Chemotherapy. ALBERT FOLCH. University of Washington, William H. Foege Building, Rm. N430-N, 3720 15th Ave NE, Campus Box 355061, Seattle, WA 98195. Email: afolch@u.washington.edu

Cancer therapy has a critical unmet need, which is to identify the best therapy for individual cancer patients. There are currently over 250 FDA-approved drugs for cancer chemotherapy. Most of these were identified and are applied empirically, either alone or in combination, to treat cancer. Drug choice is guided largely by a) tumor type, location and stage, and b) efficacy of therapy as assessed in prior clinical trials using groups of patients. The response of individual patients to these 'standard-of-care' therapies often varies widely. Ineffective therapies extract an enormous toll on individual patients and their families as well as on the health care system. A more rational and cost-effective way to improve cancer therapy, maximize the likelihood of response or cure, and minimize toxicity, would be to assess tumor response to chemotherapeutic agents *before* starting therapy. Our goal is to identify the best therapy for individual cancer patients by using a multiplexed microfluidic assay. This assay utilizes intact cells in order to rapidly predict tumor chemosensitivity to a large panel of drugs prior to initiation of therapy. The assay complements current genetic characterizations of tumor variation between individuals and should greatly speed up the testing of new drugs in research settings.

A-16

Telomerase Tales from the 3rd Dimension. S. MUKHERJEE. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA. Email: smukherj@fhcrc.org

The telomerase protein is upregulated in almost all human cancers, where it synthesizes telomeric DNA and thereby endows cancer cells with limitless replicative potential. Telomerase has alternative telomere-independent functions,

which could promote tumorigenesis in a subset of tissues, especially in the breast. These novel functions include the ability of telomerase to enhance proliferation of primary human breast epithelial cells in mitogen-deficient conditions. I will present an overview of our studies that extend and elucidate these findings using a physiologically relevant three-dimensional (3D) cell culture system. This culture system models the hollow terminal ductal units of the breast that are called acini, and unlike monolayer culture, allows the study of oncogenic events such as breast lumen filling, invasion, and changes in cell-cell junctions and polarity. I will elaborate how these advantages of the 3D culture system have enabled us to demonstrate that telomerase can alter breast morphogenesis to form structures that share properties of early stage breast cancer. Further, I will present data which indicates that telomerase can cooperate with known breast cancer genes to cause the formation of aberrant acini, and that its alternative functions are required for this cooperation. Finally, I will discuss how the 3D culture system has enhanced our understanding of the mechanistic underpinnings of these novel pro-proliferative telomerase roles. Together, these studies will highlight the critical role that the 3D cell culture system can play in answering basic biological questions and in potential translational studies, by demonstrating its application in elucidating the alternative functions of telomerase and thereby in uncovering new targets for the development of anti-telomerase treatments in breast cancer.

A-17

3D Natural Polymer-based Matrices for Cancer Research and Drug Screening. MIQIN ZHANG, Forrest Kievit, Matthew Leung, and Stephen Florczyk. University of Washington, Dept of Materials Science and Engineering, 302L Roberts Hall, Seattle, WA 98195. Email: mzhang@u.washington.edu

There is an urgent need for development of engineered three-dimensional (3D) human tumor models using carcinoma cells to recreate microenvironmental characteristics representative of naturally occurring tumors for cancer research and drug screening. Standard two-dimensional (2D) cell line cultures provide researchers with a convenient in vitro platform for cancer research and drug development. However, cells cultured on flat petri dish surfaces behave far from in vivo 3D extracellular matrix (ECM) and multi-cellular conditions as these cells display dramatically reduced malignant phenotype compared to tumor cells in vivo. Thus, drug sensitivity data collected from 2D systems lack predictability and are often misleading, while animal models are expensive, time consuming, and present ethical dilemmas. Our research aims to develop a 3D tumor model built upon non-mammalian-sourced natural polymers. The platform is made from porous chitosan-alginate (CA) scaffold in which both chitosan and alginate bear proxy structures of glycosaminoglycans (GAGs), a major component of natural ECMs. Both chitosan and alginate are clinically preferable over synthetic polymers due to their biocompatibility, biodegradability, and minimum-immunogenicity. My talk will focus on our recent research in the development of the CA scaffolds for cancer research and drug screening, including design and characterization of these 3D scaffold systems, and their in vitro and in vivo performance.

A-18

Cell Transformation Assays: the ECVAM Study and Follow-up Activities. R. CORVI. Joint Research Centre of the European Commission, European Reference Laboratory for Alternative Methods to Animal Testing (EURL-ECVAM) 21027 Ispra, ITALY. Email: raffaella.corvi@jrc.ec.europa.eu

Among the various in vitro alternatives developed for carcinogenicity prediction, the cell transformation assays (CTAs) have been shown to closely model some key stages of the in vivo carcinogenesis process. The CTAs are faster and more cost efficient than the in vivo rodent carcinogenicity assay, providing a useful approach for screening of chemicals with respect to their carcinogenic potential. Although the CTAs have a long history of use, they have never undergone formal validation. Following past activities of the European Centre for the Validation of Alternative Methods (ECVAM) on the use of the CTA, and to complement the findings of the OECD Detailed Review Paper (DRP) on CTAs for the detection of chemical carcinogens, ECVAM coordinated a study on the Syrian hamster embryo (SHE) and BALB/c 3T3 CTAs. The study objective was to address issues of protocol standardization, within-laboratory reproducibility, test method transferability, and between-laboratory reproducibility. Three protocol variants (SHE pH6.7, SHE pH7.0 and BALB/c 3T3 CTAs) were evaluated in a multi-laboratory trial. The ECVAM Scientific Advisory Committee (ESAC) peer reviewed the study and concluded that the study succeeded in generating standardized protocols, which appear transferable and reproducible for the SHE CTAs. Although promising, further optimization of the BALB/c 3T3 protocol was recommended and use of the refined protocol was encouraged to expand the data on assay reproducibility. Based on the study results, the ESAC opinion and the extensive database summarized in the OECD DRP, ECVAM developed a recommendation, which supports the utility of the CTA for the assessment of carcinogenicity potential and further proposes the development of an OECD Test Guideline for the SHE CTA.

A-19

Bhas 42 Cell Transformation Assay for the Prediction of Chemical Carcinogenicity. AYAKO SAKAI, Kiyoshi Sasaki, and Noriho Tanaka. Laboratory of Cell Carcinogenesis, Food and Drug Safety Center, 729-5 Ochiai, Hadano, Kanagawa 257-8523, JAPAN. Email: sakai.a@fdsc.or.jp

The carcinogenicity of chemicals has been predicted with genotoxicity assays, which address one of mechanisms involved in carcinogenicity, the induction of genetic damage. A cell transformation assay (CTA) can detect non-genotoxic as well as genotoxic carcinogens. Most of non-genotoxic carcinogens are considered to be tumor-promoters and many of them have been detected as promoters in a two-stage BALB/c 3T3 CTA, which mimics a in vivo two-stage carcinogenesis test. The Bhas 42 cells were established from the BALB/c 3T3 cells through transfection of v-Ha-*ras* gene and regarded as initiated cells in the two-stage carcinogenesis theory. Using the Bhas 42 cells, a short-term CTA was developed. Bhas 42 CTA is superior to conventional CTAs in cost and labor performance. It consists of an initiation assay and a promotion assay to detect initiating activity and promoting activity of carcinogens, respectively. We applied the Bhas 42 CTA to 98 chemicals to characterize the assay and evaluate its performance for the prediction of chemical carcinogenicity. The promotion assay could detect a considerable number of Ames-negative and Ames-discordant carcinogens, confirming that the Bhas 42 cells act as initiated cells in the CTA. The performance has been proved to be superior or equivalent to those of conventional genotoxicity assays. The above mentioned studies on the Bhas 42 CTA, development, improvement and application to 98 chemicals, were performed using 6-well micro-plates (6-well method). Meanwhile, the Bhas 42 CTA using 96-well microplates (96-well method) has been developed to be utilized for high throughput automated applications. The assay procedures are fundamentally the same between the 6-well and 96-well methods. In the intention to provide a basis for the development of Bhas 42 CTA into the OECD Test Guideline, formal validation studies of the assay have been performed. The results will also be presented. This work was supported by the New Energy and Industrial Technology Development Organization/the Ministry of Economy, Trade and Industry of Japan.

A-20

OECD Activities on the Cell Transformation Assays. H. KOJIMA[1,2]. [1]Japanese Society for Alternatives to Animal Testing, 5-3-13 Otsuka, Bunkyo, Tokyo, JAPAN and [2]Japanese Center for the Validation of Alternative Methods, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo, JAPAN. Email: h-kojima@nihs.go.jp

Cell transformation refers to the induction of phenotypic alterations in cultured cells that are characteristic of tumorigenic cells. This validated the use of phenotypic alterations in vitro as criteria for a carcinogenic potential in vivo. Since DNA damage and mutation are believed to be initiating events for carcinogenesis, a number of in vitro and in vivo tests for these events are commonly used to identify genotoxicants. Many chemicals that may not be detected by the these genotoxicity studies induce tumors through non-genotoxic mechanisms. The in vitro cell transformation assays (CTAs) have high sensitivity for detecting both genotoxic and non-genotoxic carcinogens. The term *non-genotoxic carcinogen* refers to a lack of direct chemical effect on DNA primary structure in the initiation of tumors; on the contrary, genotoxic carcinogens (or their metabolites) are defined as compounds able to initiate carcinogenesis through direct interaction with DNA. In the OECD working plan, four CTAs (SHE pH 6.7, SHE pH 7, Balb/c 3T3 and Bhas assays) are being evaluated to establish test guidelines for screening in vivo carcinogens. To date pre-validation studies on three CTAs (SHE pH 6.7, SHE pH 7, and Balb/c 3T3) were completed by ECVAM (European Center for the Validation of Alternative Methods) and reviewed by ESAC (ECVAM Scientific Advisory Committee). The validation study of the Bhas assay was completed by JaCVAM (Japanese Center for the Validation of Alternative Methods). In this session, updates of these assays will be presented by two speakers, and future directions will be discussed.

A-21

Introduction: New Therapeutic Approaches to Cancer from In Vitro Studies. KEN KATAOKA. Department of Cell Biology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikatacho, Kita-ku, Okayama 700-8558, JAPAN. Email: kenk@md.okayama-u.ac.jp

Cancer cells, like normal cells, are not free from environmental stresses including inflammatory, endoplasmic reticulum (ER) and oxidative stresses. It is known that inflammation contributes to tumor growth, progression, and metastasis. During tumorigenesis, high proliferation rates of cancer cells require increased activities of ER protein folding, assembly, and transport, a condition that can induce ER stress. Conditions of oxidative stress play an important role in both the initiation and the progression of cancer. Cancer cells must balance these stresses to survive. In this session the JTCA speakers will report on new therapeutic approaches to cancer based on the controlling these stresses from in vitro studies.

A-22

Selective Cancer Cell Killing by Ashwagandha Leaf Extract Involves Activation of p53 and Oxidative Stress Signaling: Evidence from Cell Based Loss-of-Function Screenings. RENU WADHWA and Sunil Kaul. National Institute of Advanced Industrial Science & Technology (AIST), Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, JAPAN. Email: renu-wadhwa@aist.go.jp

Accounting for about 15% of total deaths, recently cancer has become a leading cause of worldwide mortality. Herbal remedies are increasingly being appreciated worldwide as easy, economic and safe way to combat a variety of cancers. Ashwagandha (*Withania somnifera*) is a tropical herb that enjoys more than 5000 years of history of use in Indian home medicine 'Ayurveda'. It is extensively used to promote physical and mental health. We generated alcoholic extract (i-Extract) from Ashwagandha leaves and investigated its anticancer activity in cell culture and nude mice models. i-Extract exhibited selective cancer cell killing activity; normal cells remained unaffected. In order to identify the anti-cancer components of i-Extract and its cellular targets in cancer cell killing, we used combined chemical and cell based loss-of-function screenings using human siRNA and randomized ribozyme libraries. These assays revealed that the i-Extract and its component 'withanone' kill cancer cells by at least two mechanisms involving selective activation of (i) tumor suppressor protein p53 and (ii) ROS signaling. Involvement of p53 and oxidative stress pathways was further confirmed by computational and experimental approaches. We demonstrate that whereas i-Extract and withanone induce cancer cell killing by induction of oxidative stress, the normal cells are protected against industrial chemical-, scopolamine- and glutamate-induced oxidative stress. These findings endorse that the i-Extract and withanone are strong candidates for interventions of age-related disorders including cancers and neurodegeneration that largely affect quality of life in old age.

A-23

Preparation and Culture of Spheriods Composed of Pure Primary Cancer Cells from Patients' Tumor Samples. MASAHIRO INOUE. Department of Biochemistry, Osaka Medical Center for Cancer and Cardiovascular Disease, 1-3-3 Nakamichi, Higashinari-ku, Osaka, JAPAN. Email: minoue 0112@gmail.com

Patients' tumor samples are critical source for understanding heterogeneity of cancer, although primary culture of cancer cells has been hampered by various technical issues. We established a novel preparation/culture system for primary cancer cells. The tumor tissue was dissociated, and the fragments retaining cell-cell contact were recovered by filtration. After culturing in suspension overnight, the fragments formed spheroids, which we named as CTOS, cancer tissue-originated spheroid. CTOS consisted of pure cancer cells. The cells in CTOS were quite stable compared with the cells dissociated into single cells. CTOS from colorectal cancer had polarity like as the original tumors, and formed tumor in NOD-SCID mice, in which pathological features were preserved. CTOSs from colorectal and lung cancers were able to grow in vitro in 3D culture with an embryonic stem cell culture medium. The CTOS culture was applied to the sensitivity assay for chemo-, radio-, and molecular targeting therapies. The results reproducibly correlated well with those of in vivo treatments. Thus, CTOS method provides platform to investigate personalized medicine, biomarkers, and nature of cancer cells.

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A Novel Tumor Suppressor, REIC/Dkk-3 Gene Identified by Our In Vitro Transformation Model of Normal Human Fibroblasts Works as a Potent Therapeutic Anti-tumor Agent. M. SAKAGUCHI[1], K. Kataoka[1], H. Murata[1], M. Namba[2], and N.H. Huh[1]. [1]Dept. of Cell Biology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikatacho, Kita-ku, Okayama 700-8558, JAPAN and [2]Niimi College, 1263-2 Nishigata, Niimi 718-8585, JAPAN. Email: masa-s@md.okayama-u.ac.jp

Reduced Expression in Immortalized Cell (REIC) was cloned by subtractive hybridization method as a gene whose expression is reduced in many human immortalized and neoplastic tumor cells. Current information based on structural and sequence homology has led to the recognition of REIC as Dkk-3 that is belonging to *Dickkopfs (Dkks)* gene family. Hence, REIC was designated as REIC/Dkk-3. Our studies have demonstrated that REIC/Dkk-3, when overexpressed by an adenovirus (Ad-REIC), exhibits a dramatic therapeutic effect on a wide variety of human cancers through a mechanism triggered by ER-stress-mediated JNK activation. In addition to its direct effect on cancer cells, Ad-REIC has also another arm against human cancers, an indirect host-mediated effect due to overproduction of IL-7 by mis-targeted normal cells. This "one-bullet two-arms" finding may lead to a powerful new therapeutic approach to the treatment of human cancers.