

Animal Contributed papers

A-1000

Identification of a Homogeneous Adult Stem Cell Population, miR Signatures, and miR-Dependent Differentiation. F. O. COPE¹ and M. S. Blue². ¹The Ohio State University Comprehensive Cancer Center and James Cancer Hospital, Columbus, OH 43210 and ²Clue Genomics, LLC, Powell, OH 43065. Email: fred.cope@osumc.edu

The overriding aim of our effort has been to define and temporally stratify the factors and mechanisms controlling the differentiation of a novel progenitor cell population heretofore uncharacterized, and unappreciated as a commercial clinical potential source of stem cells. We have now identified a source of adult mesenchymal stem cells that bear a remarkable stem cell signature composite similar to embryonic stem cells. These cells are highly homogeneous, provide $>3 \times 10^7$ cells upon initial isolation, can be cultured for >20 d without loss of initial stemness, and bear a relative temporal stemness at least twice as early as hematopoietic precursor cells, and, based on our preliminary findings, are only marginally diminished in stemness from some embryonic cell lines. Our prior experience with culture systems suggests that the development of such a differentiation system would be remarkably advantageous from several aspects, not the least of which is ease of commercial manipulation, reproducible phenotypes, and minimal regulatory/safety concerns. We have focused on defining the signatures of microRNAs (miRs) in these cells as they exist in the stem cell state, the modulation of miRs through the differentiation process of these stem cell precursors to altered phenotypic states, e.g., hematopoietic, pulmonary, or vascular phenotypes, and the utilization of miRs and antagomiRs as the differentiating agents, thus eliminating the need for xenobiotic materials and providing a defined commercial process that is dependent on the application of small synthetic molecules for production cycles. Based on our already characterized stem cells, and their miR signatures that control both lung phenotypes and hematopoietic phenotypes (normal and malignant), we believe we have a significant point of entry toward providing copious amounts of both stem cells and clinically valuable tissues derived from them.

A-1001

Expansion of Pancreatic Stem Cells from Human Islet of Langerhans Preparations. L. H. CAMPBELL¹, Nancy L. Parenteau², and Kelvin G.M. Brockbank¹. ¹Cell & Tissue Systems, Inc., North Charleston, SC and ²Parenteau BioConsultants, LLC, New England. Email: lcampbell@celltissuesystems.com

The feasibility of allogeneic Islet of Langerhans transplantation is limited to less than 1% of the insulin dependent Type I diabetes mellitus patients due to inadequate numbers of suitable pancreas donors for islet isolation. Many investigators have attempted to identify and cultivate the stem/progenitor cells from the adult pancreas. However, lineage fidelity, commercial expansion and differentiation capability in adult human pancreas cells has been difficult to demonstrate. The underlying hypothesis of the progenitor cell expansion method presented here is that tissues capable of regeneration can, if given a permissive environment, recapitulate that process in vitro. Human pancreatic islets from research tissue donors were cultured in an animal product-free culture medium with low or no calcium minus growth factors that failed to support the maintenance of differentiated cells. After about 3 wk, cell colonies were observed, which were subsequently expanded by passage. These cells demonstrated expression of markers indicative of their progenitor lineage such as Ngn3 and their pancreatic lineage, such as PDX1. These markers were retained over numerous cell doublings, and they were able to form islet-like structures when cultured with MatrigelTM. Assuming that the in vitro expansion seen thus far correlates with the ability to form new islet tissue, this level of progenitor cell proliferation would be consistent with the goal of treating multiple patients with cells derived from a single donor pancreas. Obviously, expansion of a putative endocrine progenitor cell alone is not a cure for diabetes; the cells must differentiate and function appropriately. Any immune response to allogeneic islets is also a significant clinical hurdle. In conclusion, we have developed a method for obtaining a pancreatic islet stem/progenitor cell population that is capable of extensive proliferation in vitro. Research in progress is focused on optimizing this method and improving terminal cell differentiation.

A-1002

Growth Factors and Extracellular Matrix Components Induce Formation of Membranes in Mouse Embryonic Stem Cells. A. R. CALABRO and F. A. Barile. St. John's University College of Pharmacy and Allied Health Professions Department of Pharmaceutical Sciences, Toxicology Division, Queens, NY 11439. Email: barilef@stjohns.edu

Mouse embryonic stem (mES) cells (ES-D3, ATCC) are pluripotent cells derived from the inner cell mass of mouse blastocysts. The cells are capable of forming embryoid bodies (EBs) as an intermediate stage of differentiation. In vitro culture of EBs on culture inserts in the presence of various growth factors (GFs) and extracellular matrix (ECM) components influences their differentiation toward specific cell lineages. We examined the effect of five GFs (EGF, KGF, amphiregulin, TGF beta-1, LIF), known to influence differentiation, on EBs grown on filter inserts coated with ECM proteins (collagen types I, IV; fibronectin; laminin). Differential gene expression was detected using RT-PCR of mRNA purified from EBs grown between 1–16 d on the inserts. Concurrently, transmonolayer electrical resistance (TMER) was measured as an indicator of tight junction formation. In addition, time-lapsed videos monitored the morphological changes of mES cells in culture with or without feeder layers. Results suggest that undifferentiated mES cells and EBs preferentially express Oct-4 and Afp, respectively. Differentiated cells from EBs show greater expression of Arnt and Egfr, suggesting differentiation toward epithelial and/or epidermal lineages. EGF- and KGF-supplemented media on C-IV matrix induced significant increases in TMER. The data supports previous paracellular permeability and acute cytotoxicity studies that specific GFs and ECM proteins induce formation of epithelial/epidermal-like membranes with increased TMER. Thus, the cell culture insert model represents a novel approach for inducing biological membrane formation for cytotoxicity testing and drug screening protocols (supported in part by grants from ARDF, 35336-985; IFER, 35262-985; NIH, R15-ES012170-01).

A-1003

Melanin Synthesis Inhibiting Activity of MC20 Isolated from a Marine Red Alga, *Calliarthron yessoense* in B16 Melanoma Cells. Y. KAMEI and Y. Ohtsuka. Coastal Bioenvironment Center, Saga University, 152-1 Shonanchō, Karatsu, Saga 847-0021, JAPAN. Email: kameiy@cc.saga-u.ac.jp

We attempted to screen the melanin synthesis inhibiting activity in vitro from marine algae collected from Hokkaido Island, Japan in order to develop the new type of whitening cosmetics for the prevention of skin pigmentation. We evaluated the melanin synthesis inhibiting activity of the extracts prepared from 104 species of marine algae against B16 mouse melanoma cells. As the MeOH-extract of marine red alga, *Calliarthron yessoense* showed potent melanin synthesis inhibiting activity, we tried to isolate and determine the chemical structure of its active substance, MC20. Several spectrometric analyses showed MC20 is 3-methylindole. When 3-methylindole was evaluated for the melanin synthesis inhibiting activity comparing to three known ingredients containing in the commercial whitening cosmetics as the melanin synthesis inhibitors, arbutin, ascorbic acid, and kojic acid, 3-methylindole showed the activity in dose-dependent manner at its intoxicating concentrations and had no cytotoxicity at up to 200 µg/ml based on MTT assay. Its activity was higher than those of arbutin, ascorbic acid, and kojic acid at the lower concentrations below 40 µg/ml. The biochemical analyses with RT-PCR and northern hybridization indicated that the mechanism of melanin synthesis inhibition by 3-methylindole is neither to directly inhibit the key enzyme, tyrosinase nor both tyrosinase and tyrosinase-related protein 1 syntheses in the cells, but is to inhibit tyrosinase-related protein 2 synthesis in the cells.

A-1004

Responses of Pineapple Stem Bromelain in a Human Cell Screening Assay for Melanoma Prevention. EUGENE ELMORE^{1,2}, Aarti Jain¹, Vernon E. Steele³, and J. Leslie Redpath^{1,2}. ¹Department of Radiation Oncology, University of California, Irvine, CA 92697; ²Chao Family Comprehensive Cancer Center, University of California, Irvine, CA 927973; and ³Chemopreventive Agent Development Research Group, DCP, National Cancer Institute, Bethesda, MD 20892. Email: eelmore@uci.edu

The human cell melanoma prevention assay is used to identify agents that alter biomarkers with relevance to melanoma development. The assay uses the radial growth phase-like melanoma cell line, WM3211, and co-exposure to 25 mJ/cm² UVB irradiation. Biomarkers were selected based on relevance to the control normal melanocyte growth and differentiation. E-cadherin induction and N-cadherin inhibition were included as biomarkers based on the normal homeostatic controls and the changes in the N-cadherin/E-cadherin ratios during melanoma development. The importance of the E-cadherin/N-cadherin homeostatic tissue controls appear to be very relevant for identifying possible melanoma prevention activity. Caspase 3 induction was included as a biomarker for apoptosis. The induction of

HLA-ABC expression, which is reduced during the conversion to melanoma and facilitates immune surveillance system, was also selected. The human cell assay for melanoma prevention was used to evaluate a lyophilized extract from the pineapple plant stem, bromelain, which is reported to have anticancer activities both *in vitro* and *in vivo*. Bromelain was tested in the assay at six concentrations ranging from 0.3 to 100 $\mu\text{g/ml}$. Bromelain induced E-cadherin at all concentrations evaluated. N-cadherin was inhibited by five of the six concentrations evaluated. Caspase 3 was induced by five of six concentrations and HLA-ABC was induced by two of six concentrations. Bromelain compared favorably to other agents with strong responses in the assay. The biomarker responses observed in the human cell melanoma prevention assay suggest that bromelain may have the potential to prevent melanoma. Supported by NCI Contract #N01-CN-43300.

A-1005

Anti-cancer Effect of Enzyme-digested Fucoidan Extract from Seaweed *Mozuku*. SANETAKA SHIRAHATA¹, Kii-chiro Teruya¹, Sakiko Matsuda¹, Ayumi Nakano¹, Takuya Nishimoto¹, Masashi Ueno¹, Kenji Shirouzu¹, Makiko Yamashita¹, Hiroshi Eto², and Yoshinori Katakura¹. ¹Department of Genetic Resources Technology, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, JAPAN and ²Daiichi Sangyo Co. Ltd., Osaka 530-0044, JAPAN. Email: sirahata@grt.kyushu-u.ac.jp

Fucoidan is viscous fucose-rich polysaccharide derived from brown algae. Recently, the abalone enzyme-digested fucoidan extract derived from seaweed *Mozuku* of *Cladophora novae-caledoniae* *kylin* (fucoidan extract) draws much attention because of its clinical anti-cancer effect in Japan. The fucoidan extract suppressed the growth of mouse colon cancer cells *in vivo*. Here, we report the cancer cell-specific apoptosis inducing effects of the fucoidan extract. The fucoidan extract suppressed the growth of various anchorage-dependent and independent cancer cells. The fucoidan extract contained low molecular weight components, which induced apoptosis of human leukemia HL60 cells but not of human lymphocytes. It has been known that sugar chain expression on the membrane surface of cancer cells changes dependent upon their malignancy. The analysis on sugar expression profiling using eight kinds of FITC-labeled lectin (Con A, DBA, LCA, PHA-E4, PNA, RCA120, UEA-I, WGA) revealed that the expression of Con A binding sugar chain was enhanced by the treatment of human lung adenocarcinoma A549, human uterine carcinoma HeLa, and human fibrosarcoma HT1080 cells with the fucoidan extract. Con A-induced apoptosis of cancer cells was stimulated in a dose- and time-dependent manner by the

treatment with the fucoidan extract but not of human normal fibroblast TIG-1 cells.

A-1006

Multiendpoint Mechanistic Profiling of Hepatotoxicants in HepG2/C3A Human Hepatoma Cells and Comparison of Statistical Methods for Development of a Prediction Model for Acute Hepatotoxicity. THOMAS J. FLYNN¹ and Martine S. Ferguson². ¹Division of Toxicology, FDA, Center for Food Safety and Applied Nutrition, Laurel, MD 20708 and ²Division of Biostatistics, FDA, Center for Food Safety and Applied Nutrition, College Park, MD 20740. Email: thomas.flynn@fda.hhs.gov

HepG2/C3A cells, a well-differentiated human hepatoma cell line, were exposed to serial concentration levels of seven chemicals of known acute hepatotoxicity for 48 h. Six endpoint assays were selected to model different known mechanisms of acute hepatotoxicity. Each compound produced a unique concentration-response pattern across the six endpoints. There was poor correlation across the six endpoints suggesting that each endpoint was, in fact, monitoring an independent cellular process. After standardization of the raw values, prediction models were developed using five different statistical methods. The models were developed using as the “gold standard” only the known hepatotoxicants. The zero concentration (control) and all concentration levels not significantly different from control were programmed as non-toxic levels and concentration levels significantly different from control as toxic levels. In this way, rather than a binary classification (i.e., toxic or non-toxic), the models gave a prediction of the concentration, if any, at which a compound showed behavior similar to known liver toxicants at their toxic concentration levels. The discriminant analysis model gave the best overall performance with a positive predictive value of 1.00 and a negative predictive value of 0.83. Ten additional compounds were tested using this prediction model. The model returned predicted liver active concentrations for each compound that were consistent with their known biologically active concentrations. This model system is amenable to high-throughput format, and it may be useful for predicting concentration levels at which unknown compounds would display undesirable liver activity.

A-1007

In Vitro Cellular Response to Nanoparticle Exposure. J. E. MORGAN and J. A. Jordan. Department of Natural Sciences, Clayton State University, Morrow, GA 30260. Email: jewelsmorgan@gmail.com

Nanoparticles are currently being used in numerous applications including drug delivery, pharmaceutical products, electrical components, and safety materials. However, there have been increasing concerns regarding possible dangers of nanoparticle exposure to human health. Due to the small surface area to volume ratio of these nanoparticles, they may cross biological membranes of cells causing cell toxicity and cell death. In this study, the cytotoxic effects of nanoparticle exposure were examined. Chinese Hamster Ovary (CHO) and African Green Monkey (COS-7) cells were cultured in the presence of fine and ultrafine (nano) blue-dyed latex particles. Cell cultures were monitored for changes in cell morphology, cytotoxicity, cellular localization of particles, and apoptosis. Fine latex beads exposed to CHO cells for 24 h resulted in clustered ballooned shaped cells with particles found in the cytoplasmic region of the cells. High dose exposure of fine and ultrafine beads resulted in a significant cytotoxic response as measured by trypan blue exclusion. Ultrafine and fine latex beads exposed to COS cells for 48 h resulting in only a slight change in morphology with beads clustered around the cells. Low doses of ultrafine and fine latex beads resulted in cytotoxicity. Cells were halted in metaphase to examine the cellular localization of particles. Both ultrafine and fine particles were localized to the cytoplasmic regions of the cells clustered around the nuclear area. Preliminary apoptosis studies suggest increase DNA fragmentation following ultrafine (nano) particles. In summary, these data suggest the importance of examining the cytotoxicity of nanoparticles and the potential effects on human health.

A-1008

Computational Tissue Engineering: Monte-Carlo Simulation of Restructuring Dynamics During Tissue Self-Assembly of Prostate Cancer Spheroids. K. O'CONNOR¹ and H. Song².
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Computational methods that predict tissue assembly aid in the production of biological substitutes that mimic native tissue. In particular, prostate cancer cells self-assemble on an attachment-limiting substrate into spheroids that resemble micrometastases and have application to in vitro drug testing. Our research group has developed mathematical models that predict cell aggregation kinetics in this culture system. Through an integrative experimental and computa-

tional approach, we are refining our models to account for the restructuring dynamics by which 2D aggregates form 3D multicellular spheroids. Our study investigates the mechanism of restructuring and the roles of cell adhesion and motility in this process for DU 145 and LNCaP prostate cancer cells. Irregularly shaped, 2D aggregates restructure through incremental cell movements into 3D spheroids that we have simulated with Monte Carlo techniques. Of the two cultures examined, restructuring is more pronounced for DU 145 aggregates. Motile DU 145 cells form spheroids with a minimum cell overlay of 30% for 25-mers as estimated by simulation vs. 5% for adhesive LNCaP cells in aggregates of the same size. Over 72 h, the texture ratio increases from 0.55 ± 0.05 for DU 145 aggregates with projected areas exceeding $2,000 \mu\text{m}^2$ to a value approaching 0.75 ± 0.02 ($p < 0.05$). For LNCaP aggregates of comparable size, the increase in texture ratio is more modest, less than 15% during the same time period ($p < 0.05$). These combined data suggest that motility events, rather than adhesion, govern the overall rate of spheroid restructuring. Since spheroid composition is size-dependent, our models may be able to predict both spheroid size and composition from properties of the inoculum.

A-1009

Tools for Genetic Characterization and Identification of Cell Lines. M. R. FURTADO, R. Fang, J. G. Shewale, and F. Hyland. Applied Markets, Applied Biosystems, Foster City CA 94404. Email: furtadmr@appliedbiosystems.com

Proper characterization of cells is central to ensuring the integrity of studies using cell lines and to ensure absence of contaminating cells. Having a set of validated tools to monitor cell line integrity would be of great use to researchers. Characterization of genetic variation and specific expression profiles would be one way of ensuring cell line integrity. In this abstract, we will describe assays to analyze genetic variation in human cell lines including STRs, SNPs, and InDels and their application for cell identity testing. These markers have been used in forensic applications, paternity testing, and mass disasters. PCR based amplification methods and oligonucleotide ligation assay formats for detection of these genetic variations will be described. Data will be presented on the relative power of discrimination with each set of markers and their use in cell identity applications. Genetic profiling using these markers, of commonly used human cell lines will be presented.

A-1010

Mapping Signaling Pathways That Control Gap Junction Function Using Modern Proteomic Approaches. B. L. UPHAM¹, D. A. Whitten², C. G. Wilkerson², J. S. Park¹, I. Sovadinova¹, P. Babica¹, J. E. Trosko¹, and L. Blaha¹. ¹Dept Pediatrics & Human Development and Food Safety & Toxicol Ctr and ²RTSF Proteomic Core, Michigan State Univ, E Lansing, MI 48829. Email: upham@msu.edu

Gap junctional intercellular communication (GJIC) maintains tissue homeostasis and chronic interruption has been linked to cancer. To provide insight into the early upstream signaling events of toxicant-induced inhibition of GJIC, we used a novel proteomic approach that involved fractionating the proteins into membrane and cytosolic samples, followed by 2D gel separations using Invitrogen ZOOM[®] IEF fractionator, and then quantified using SILAC (Stable Isotope Labeling with Amino acids in Cell culture) in combination with traditional enzyme inhibitor studies. A pluripotent, F344 rat liver epithelial cell line was used for these studies. In response to low molecular weight polycyclic aromatic hydrocarbons (PAH) some of the early events observed is an immediate decrease of annexin-3 in the plasma membrane, activation of phospholipases, in which phosphatidylcholine specific phospholipase C modulated gap junction activity, and the release of arachidonic acid and activation of mitogen activated protein kinases. Based on literature, we hypothesize that annexin-3 inhibits phospholipases until removed from the plasma membrane in response to a PAH, and the subsequent phospholipase-induced events then regulate GJIC. Our study demonstrates advantages of recent proteomics techniques to identify the early signaling events that play an important role in tumor promotion processes such as inhibition of GJIC (support: NIEHS grants #R01 ES013268-01A2 to BLU).

A-1011

Online Monitoring of Physiological Parameters of Cell Cultures. RALF EHRET¹, Elke Thedinga¹, Sabine Drechsler¹, Axel Kob¹, Marcus Wego¹, Sebastian Rost¹, Steffen Fürst¹, Werner Baumann². ¹Bionas GmbH, Friedrich-Barnewitz-Str. 3, D-18119 Rostock, GERMANY and ²Biophysics Institute, Bioscience Department, University Rostock, Friedrich-Barnewitz-Str. 4, D-18119 Rostock, GERMANY. Email: elke.thedinga@bionas.de

The Bionas[®] 2500 analyzing system provides a cell based in vitro monitoring of dynamic cell behavior influenced by different supplements or compounds. It is a very useful tool for optimization of cell culture conditions as well as for the investigation of regeneration effects after treatment and removal of different compounds. A continuous monitoring of physiological parameters of adherent and suspension cells is possible. Our silicon chip based analyzing system is able to observe 3 different physiological parameters [acidification, respiration, adhesion (not for suspension cells)] per chip. Six chips can be measured in parallel. Changes of acidification activity, respiration rates, or adhesion behavior caused by supplements or compounds are hints of the effect on cell physiology and presumably cytotoxic property of the compound used. Generally, we have successfully established the using of cell lines or primary cells for the online monitoring of oxygen consumption, acidification activity and cell impedance (adhesion). An important advantage of our Bionas[®] 2500 is the continuous detection of all parameters from a few hours to several days. Our analyzing system is able to record in real time all actions on living cells until the endpoint of treatment and moreover subsequently the recovering effects after removing the compounds.