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Response of Rosiglitazone, UAB 30, and Atorvastatin in the Human Melanoma Prevention Assay. E. ELMORE[1,2], A. Jain[1], L. Kopelovich[3], F. L. Meyskens[2], V. E. Steele[3], and J. L. Redpath[1,2]. [1]Department of Radiation Oncology, University of California, Irvine, CA, 92697, [2]Chao Family Comprehensive Cancer Center, University of California, Irvine, CA, 92697, [3]Chemopreventive Agent Development Research Group, Division of Cancer Prevention, NCI, Bethesda, MD, 20892. Email: eelmore@uci.edu

Due to the increasing incidence of malignant melanoma, which was projected to reach over 59,000 cases in 2005 (http://seer.cancer.gov), and the poor prognosis for patients with late stage disease, we have developed a screening assay for identifying melanoma prevention agents. The assay measures chemopreventive agent induced changes in melanoma-related biomarkers in radial growth phase human melanoma cells (WM3211). We report data on rosiglitazone—a PPARγ agonist used to treat Type II diabetes, UAB 30—a retinoid that is selective for RXRα, and atorvastatin—an HMG-CoA reductase inhibitor used to lower cholesterol. The assay incorporates an exposure to UVB (25 mJ/cm^2) with both pre- and post-treatment with potential preventive agents. Biomarkers used to measure agent efficacy include: induction of annexin V, an early marker for apoptosis; induction of E-cadherin, a biomarker that is expressed in melanocytes and WM3211 cells but is lost in metastatic melanoma cells; inhibition of n-cadherin, a biomarker that is expressed in melanoma cells but not in melanocytes. E-cadherin plays a key role in the communication between melanocytes and keratinocytes, which is important in the control of melanocyte growth *in vivo*. N-cadherin expression allows the fibroblasts to control the growth of melanocytes. The following are some of the important findings from our study. Rosiglitazone was positive for E-cadherin induction and strongly positive for N-cadherin inhibition.UAB 30 and atorvastatin induced E-cadherin at multiple concentrations. All three agents demonstrated a positive effect on the N-cadherin/E-cadherin expression ratios relative to the ratios with cells treated with UV-B alone. Rosiglitazone and atorvastatin were active at clinically achievable concentrations. The activities of these agents in the assay were: rosiglitazone > atorvastatin >UAB 30. The assay data suggests that rosiglitazone, UAB 30, and atorvastatin have potential to prevent melanoma. Supported by NCI contract No. N01-CN-43300.

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Lineage Specificity and Interspecies Variation in Hematopoietic Toxicity Testing. Carla Pereira, Jackie Damen, CINDY MILLER, and Emer Clarke. Contract Services, StemCell Technologies Inc, Vancouver, BC V5Z1B3, CANADA. Email: emer@stemcell.com

In the search for efficient and cost effective ways to screen lead compounds for hematotoxicity, the use of Colony Forming Cell (CFC) assays has received a great deal of attention. These robust standardized assays allow the detection of toxicity on hematopoietic progenitor subsets (erythroid, myeloid, megakaryocytic) to evaluate potential cytopenic conditions, as well as mesenchymal progenitors to evaluate potential damage to bone and connective tissue. The toxic effects of three antineoplastic compounds were tested on erythroid, myeloid and mesenchymal progenitor growth. Results indicate that each compound displays a unique spectrum of toxicity on each progenitor lineage showing different relative susceptibility to toxicity depending on the compound tested.

IC_{50} Values for Various Bone Marrow Derived Progenitors

Progenitor	5-Fluorouracil	Hydroyurea	Paclitaxel
Erythroid	2.4 ug/mL	75 uM	5 ng/mL
Myeloid	0.5 ug/mL	30 uM	5 ng/mL
Mesenchymal	0.4 ug/mL	148 uM	4 ng/mL

In addition, up to 10-fold differences were seen between human and murine progenitor sensitivity to each compound. Our data highlights the multifaceted nature of primary cells from bone marrow and unique specificity of action of individual compounds.

A-1002

Computer-aided Tissue Engineering: Predicting Self-assembly of Prostate Cancer Spheroids. K. O'CONNOR, H. Song, and S. Clejan. Tulane University and Health Sciences Center, New Orleans, LA 70118. Email: koc@tulane.edu

Computational methods that predict tissue assembly aid in the production of in vitro constructs that mimic native tissue. In particular prostate cancer cells self-assemble on an attachment-limiting substrate into spheroids that resemble micrometastases. These tissue constructs exhibit drug resistance that approaches clinical levels and have application to high-throughput drug testing and design of patient-specific treatments. Two mathematical models of spheroid formation have been developed based on collision theory and Monte Carlo technique. The models accommodate a variety of size populations in the inoculum: single cells, spheroids of different sizes, and combinations of cells and spheroids. Model simulations provide an excellent fit to experimental concentrations of spheroids as measured by the residual error between these two data sets. Collision theory predicts spheroid size distributions over a 5-fold range of cell concentrations in the inoculum. Also it accurately predicts trends in the adhesion properties of DU 145, LNCaP and PC 3 cells, including an up-regulation in the expression patterns of E-cadherin and other adhesion molecules upon spheroid formation. Monte Carlo simulations predict long-range interactions between aggregating cells on the order of several cell diameters. This study provides experimental evidence that cancer cells, which have deficient gap junctions, communicate with intercellular bridges that transport membrane vesicles (1 to 3 microns in diameter) between cells. The bridges contain tubulin and can extend at least 100 microns in length. The computational methods presented here have proven exceptional robust in predicting the physical assembly of spheroids and underlying biological phenomena. Since the composition of spheroids is dependent on their size, the models may be able to predict both spheroid size and composition from the properties of the inoculum. In addition, spheroids may be useful in the study of intercellular adhesion and communication, which have prominent roles in metastatic progression of cancer.

A-1003

Characterization of Neuroblastoma Cells Cultured in Three-dimensional, Microgravity Rotary Bioreactor: Organoid Formation and Free Cell Dynamics. R. A. REDDEN and E. J. Doolin. The Children's Institute for Surgical Science, The Children's Hospital of Philadelphia, Philadelphia, PA 19104. Email: redden@email.chop.edu

Neuroblastoma, one of the most common and deadly pediatric tumors, features clinical, genetic, and biologic heterogeneity that defies simple risk assessments and demands more extensive characterization. The three-dimensional rotary bioreactor offers a unique low-shear, microgravity culture environment in which many cancer cell lines form small tumor-like organoids. The bioreactor allows analysis of inherent cellular characteristics and behavior, without confounding influences seen in animal models and traditional 2D culture. Materials and Methods. A suspension of human neuroblastoma cells (CHP-212, ATCC) was seeded into the rotary bioreactor (Slow-turning lateral vessel, Synthecon) at 5 x 10^5 cells/ml. Two aspects were examined: 1) growth, morphology, and composition of organoids, and 2) the number and viability of 'free' cells. Organoid samples were taken at 12 hours, and at day 1, 2, 5 and 8; and were stained with hematoxylin and eosin. Digital microscopic images were analyzed using SigmaScan software. Media samples for cell counts—both viable and nonviable—were taken hourly for the first 12 hours and daily afterwards. Results/Discussion. Heterogeneous cell aggregates formed spontaneously within 6 hours. Over time, the aggregates became larger homogeneous spheroids composed of hundreds of viable cells, with characteristic inner necrotic regions, presumably due to limited diffusion of nutrients. Furthermore, the thickness of the viable zone likely indicates inherent cellular resistance to hypoxia. The number of free cells decreased exponentially, plateauing at less than 5% of original seeding density by 12 hours. After day 1, nonviable cells began to increasingly appear, presumably due to shedding. The free cell profile curves, both the initial disappearance of viable free cells and the later appearance of nonviable cells, may be an indirect measure of cell-cell adhesive capability and organoid metabolism. Identification of inherent cellular factors intrinsic to specific clinical presentations of neuroblastoma could provide invaluable information regarding characterization and treatment of this devastating disease.