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Joint Symposium

J-1

2D and 3D Primary Human Breast Tissue Culture Models to Understand Environmental Impacts on Stem Cells in Cancer. JUSTIN A. COLACINO[1,2,3], Evan Hill[1], Tasha Thong[1], Sabrina Rocco[1], Chanese Forté[1], Yutong Wang[4], Katelyn Polemi[1], Lauren Middleton[1], Nicholas Polakowski[1], Anagha Tapaswi[1], Michael Brooks[5], and Max Wicha[5]. [1]Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI; [2]Department of Nutritional Sciences, University of Michigan School of Public Health, Ann Arbor, MI; [3]Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; [4]Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI; and [5]Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI. Email: colacino@umich.edu

Approximately 85–90% of breast cancers are estimated to be sporadic and due to environmental exposures. In parallel, epidemiological studies show that the number of stem cells in a tissue is a strong predictor of cancer risk. Here, we discuss using primary human breast tissue culture models in both 2D and 3D to characterize the impact of environmental risk factors on normal breast stem cells. Both models provide various advantages – 2D models are conducive to high throughput screening while 3D models maintain the complexity of the cellular environment. We have adapted the conditional reprogramming (CR) methodology of Liu et al., 2017 to establish a living biobank of normal breast epithelial cells from racially diverse donors in 2D. Single cell RNA-seq analyses comparing samples before and after CR reveal that CR maintains luminal and myoepithelial lineages, while depleting stromal fractions. CR enriches for cells expressing stem cell markers *ALDH1A3* and *ITGA6*, as well as cells co-expressing the epithelial and mesenchymal markers *EPCAM* and *VIM*. Adapting the methods of Sokol et al., 2016, we cultured normal human breast cells as organoids in 3D hydrogels which resemble the breast extracellular matrix, including laminin, hyaluronic acid, collagen, and fibronectin. By exposing organoids to stressors in these conditions, we can model the effects of environmental factors on stem cell development and differentiation. Treatment of organoids with the known carcinogen cadmium led to reduced branching morphogenesis, linked to inhibition of HIF-1α and downregulation of mesenchymal genes including *ZEB1* and *VIM*. These culture methods provide new ways to assess the impact of environmental stressors on stem cells in non-transformed human tissues from diverse genetic backgrounds.

J-2

Differentiation in the Colonic Mucosa with a Mineral Supplement Derived from the Marine Algae *Lithothamnion sp*: Clinical Trial Outcomes Compared to Response in Human Colonoid Culture. J. VARANI[1], S. McClintock[1], D. Attili[1], M. K. Dame[2], D. K. Turgeon[2], and M. N. Aslam[1]. Departments of [1]Pathology and [2]Internal Medicine-Gastroenterology, The University of Michigan, Ann Arbor, MI 48109. Email: varani@umich.edu

Thirty healthy human subjects were enrolled in a three-arm, 90-day interventional trial in which Aquamin (a calcium- and magnesium-rich, multi-mineral product) providing 800 mg of calcium/day was compared to calcium alone and placebo for effects on the colonic mucosa and colonic microbial / metabolomic profiles. Prior to intervention and at the completion of the study, subjects underwent unprepped sigmoidoscopy. Replicate 2-mm colonic biopsies and stool specimens were obtained. Quantitative immunohistochemistry (IHC) and proteomic analysis was used to assess proteins involved in growth and differentiation in a pre-post intervention comparison model. 16S rRNA sequencing was used to obtain microbial profile and liquid chromatography-mass spectroscopy was used to assess levels of bile acids and short chain fatty acids. In parallel, biopsies obtained prior to intervention were established in colonoid culture, treated ex vivo with the same interventions and analyzed by IHC and proteomics. In the ex vivo study, Aquamin treatment strongly up-regulated

multiple proteins associated with differentiation and with barrier formation / tissue integrity as compared to control. Barrier formation were improved as seen by trans-epithelial resistance (TER) increase. In the in vivo study, Aquamin up-regulated numerous proteins (including those involved in differentiation) as compared to placebo. Microbial sequencing revealed a significant shift in the communities and reduced levels of major bacterial phyla in colon and stool specimens from Aquamin-treated subjects relative to placebo. This was accompanied by a modest (but statistically significant) reduction in certain bile acids and an increase in acetate. Calcium showed intermediary effects in some assays, but was indistinguishable from control in others. Taken together, these data demonstrate that a multi-mineral approach can produce beneficial changes in the colonic epithelium and has the potential to improve barrier function in the colon. These data also indicate that utilization of colonoid culture as part of the analytical approach enhances data acquisition over that achievable with the direct biopsy approach alone.

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A better understanding of biological systems is necessary to ensure security of our nation. Through the innovations being developed by investigators in the Insect Allies and Advanced Plant Technologies program these systems are being leveraged for increased national security.