Production of TNF-α and IL-10 by HepG2 and THP-1 cells differs depending on whether ethanol conditioned media is derived from 3D vs. 2D cultured HepG2 cells.

Joshua Hood,1 Alexander Southern,2 and Luke Schroeder2

1Univ of Louisville; and 2University of Louisville

Abstract ID 16411 Poster Board 244

Background: One of the difficulties in cancer research is bridging the gap between human and animal tumor models. One proposed method of improving the screening of cancer-promoting toxins and novel cancer therapies is the development of preclinical 3D human culture models. We are currently developing 3D human hepatocellular carcinoma (HCC) culture models using the standardized HepG2 liver cancer cell line as a template. Ethanol is being used as a test agent to generate conditioned media (CM) from 2D adherent vs. 3D HepG2 suspension spheroid cultures. The conditioned media is then applied to human monocytes to determine differences in TNF-α (proinflammatory) and IL-10 (anti-inflammatory) cytokine induction.

Objectives: The experimental goals were to determine differences in the production of TNF-α and IL-10 between 2D and 3D cultured HepG2 cells, and by THP-1 monocytes treated with conditioned media (CM), +/- ethanol, derived from 2D and 3D HepG2 cells.

Methods: HepG2 cells were grown in both 2D (adherent) and 3D (suspension) cultures to produce two different patterns of cell growth (Fig. 1). The cells were then switched to fetal bovine exosome-free media and grown with and without 100 mM ethanol to produce CM. Human monocytes (THP-1 cells) were then cultured in the presence of CM. Cell viability and production of TNF-α and IL-10 were measured using PrestoBlue™ and Lumit™ immunoassays.

Results: Changes in THP-1 morphology were not observed following treatment with HepG2 CMs derived from 2D or 3D cultures with or without 100 mM ethanol. Assessment of TNF-α in HepG2 CM demonstrated a trending decrease in TNF-α production for all CMs evaluated particularly for CMs derived from ethanol treated 2D and 3D HepG2 cells. In contrast, IL-10 content tended to increase in CMs obtained from ethanol treated or untreated 3D cultured HepG2 cells. In general, induction of TNF-α production by THP-1s post HepG2 CM treatments hovered around baseline, while induction of IL-10 by THP-1s post HepG2 CM treatments decreased. These findings did not relate to cell viability since no significant difference in cell viability for any CM treatment was observed. Tracking the ratio of TNF-α to IL-10 production in THP-1s treated with HepG2 CMs revealed significant differences +/- ethanol during CM production. Baseline exofree media with ethanol increased the ratio of TNF-α to IL-10. In contrast, the ratio decreased for 2D and 3D CMs. CM +/- ethanol from 3D HepG2 cells produced a less dramatic increase in the ratio of proinflammatory TNF-α to immunosuppressive IL-10 production.

Conclusion: A 3D vs. 2D HepG2 model may be more suitable for pre-clinical studies requiring a better approximation of an immunosuppressive HCC microenvironment. Future investigations will explore the individual contributions of CM media components to these findings to facilitate development of new biomarkers and therapeutic targets for HCC.

Research was supported by the University of Louisville Cancer Education Program NIH/NCI (R25-CA134283), University of Louisville faculty start-up funds to J. L. Hood, and UofL Hepatobiology and Toxicology COBRE NIH NIGMS P20GM113226.

Figure 1. A comparison of 2D vs. 3D HepG2 cell culture morphology. A) 2D adherent cells. Image obtained of HepG2 cells growing in a T-300 culture flask. B) 3D suspension spheroid. Image obtained of 3D HepG2 cells in a U-shaped 96 well plate at 48 hours post incubation of 10,000 cells.