In Vitro Tissue-Engineered-Based Model to Mimic Obese and Non-obese Colorectal Cancer Microenvironments

Grace E. Hester, Iman Hassani, Benjamin Anbiah, Bulbul Ahmed, Michael W. Greene, and Elizabeth A. Lipke

According to the CDC, 42% of adults in the United States are considered obese. Obesity is a growing public health concern, especially in Western and Western-influenced countries. Obesity-linked insulin resistance remains a risk factor for certain types of cancer including colorectal cancer (CRC). The precise molecular mechanisms involved in obesity-induced tumor growth remain unclear partly due to a lack of relevant experimental models. The purpose of this research was to develop an *in vitro* model for examining the link between obesity and CRC.

In this study, we have developed an *in vitro* three-dimensional (3D) engineered CRC (3D-eCRC) tissue model using HT-29 colon cancer cells co-cultured with insulin sensitive (IS) and insulin resistant (IR) adipocytes to recapitulate the native non-obese versus obese microenvironment. Mature adipocytes were obtained by differentiating 3T3-L1 fibroblasts over a period of 10 days. The mature adipocytes were either left untreated or treated with hypoxia (HYP) or/and TNF-α (TNF) for 24 hours to generate IS and IR models. These adipocytes were then co-cultured with engineered tissues to investigate the impact of non-obese and obese microenvironments on cancer cells. Phase contrast images of the tissues were taken on days 8 and 15 to monitor and quantify tissue growth. Images were analyzed using ImageJ [1] software to measure tissue area and protrusiveness. To investigate the increase in CRC cell number, 3D-eCRC tissues were dissociated, and the viable cells in each tissue were counted using a hemocytometer and trypan blue staining.

Results for this model demonstrated its ability to mimic non-obese and obese CRC microenvironments. Oil Red O staining showed that the adipocytes lost lipid content over 3 days when treated with HYP and TNF as compared to the control group, whereas untreated adipocytes did not. This loss of lipid content is indicative of an IR cell phenotype (Figure 1). Expression of the insulin sensitive gene markers, *adipoq* and *Slc2a4*, was significantly downregulated in adipocytes treated with HYP and TNF as compared to untreated adipocytes, which is indicative of IR conditions. Interestingly, the 3D-eCRC tissues cocultured with IR adipocytes demonstrated significantly higher tissue area, tissue protrusiveness, and viable cell numbers on Day 15 as compared to those cocultured with IS adipocytes, thereby mimicking the obese and non-obese CRC microenvironments (Figure 2).

In conclusion, we have developed an *in vitro* 3D-eCRC tissue model cocultured with IS or IR adipocytes and demonstrated the ability of our model to mimic non-obese and obese CRC microenvironments. We plan to use our *in vitro* model in the future to answer mechanistic questions on the link between obesity and CRC.

Statement of Research Advisor

Grace has been instrumental in driving our *in vitro* obesity model project forward. Grace has demonstrated a high capacity for problem solving and critical thinking in the lab environment. She asks thoughtful questions about her research and is consistently furthering her understanding of the work being done. Even with the changes to her project mandated by the COVID-pandemic, Grace persisted and adapted. Her contributions were critical to developing this novel *in vitro* obesity-linked CRC model.

-Elizabeth Lipke, Chemical Engineering, and Michael Greene, Nutrition, Dietetics, & Hospitality Management

Reference

[1] Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. doi:10.1038/nmeth.2019

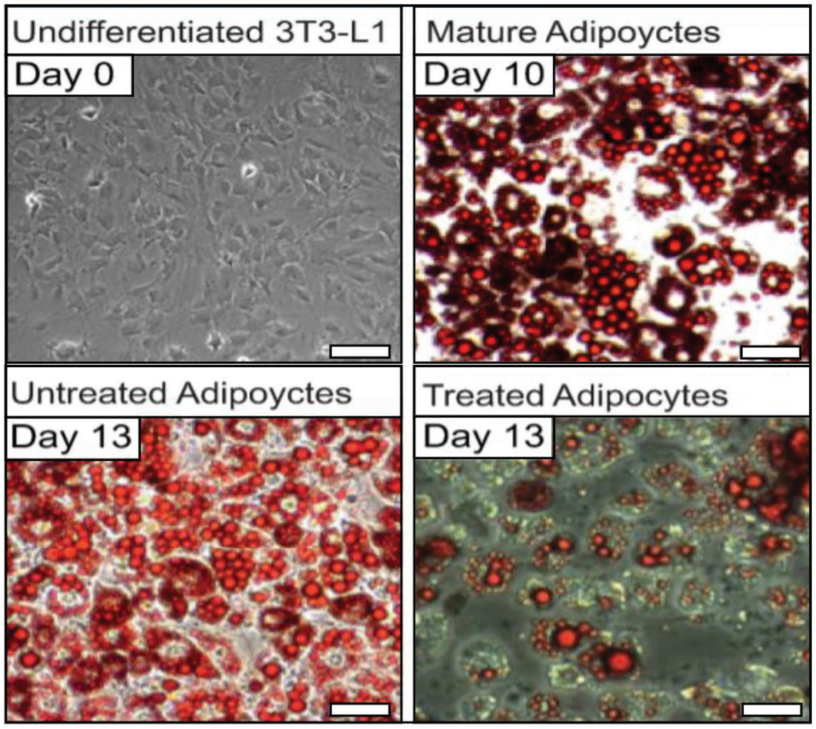

Figure 1: Insulin resistance induces a loss of lipid content in differentiated 3T3-L1 adipocytes. Undifferentiated 3T3-L1 fibroblasts (upper left panel) differentiate into mature adipocytes over a period of 10 days (upper right panel). The mature adipocytes were treated without (lower left panel) or with TNFα and hypoxia (lower right panel) and then stained with a red lipid dye and imaged (scale bar = 100μm) three days post-treatment. Lipid content was found to be lower in insulin resistant (treated) adipocytes than insulin sensitive (untreated) adipocytes, which indicates that the treatment induced insulin resistance.

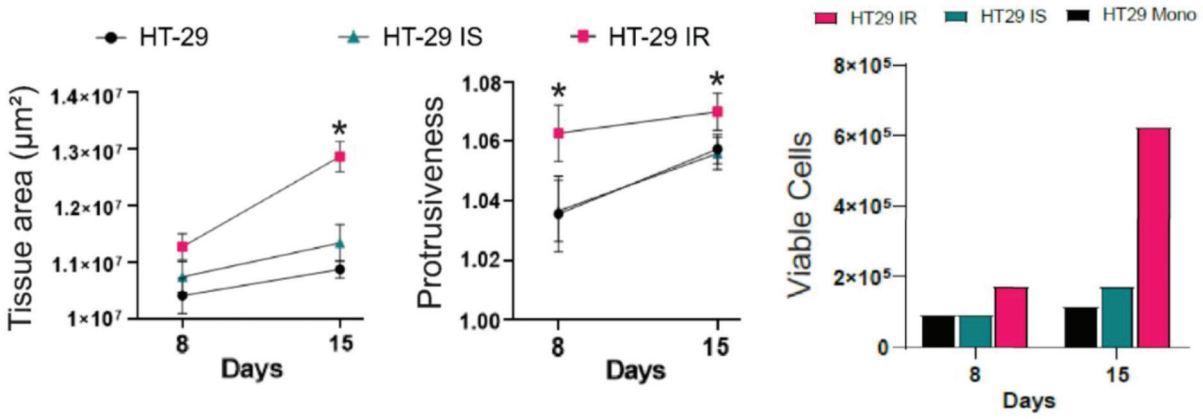

Figure 2: Insulin resistant adipocyte co-culture with 3D engineered colon cancer (HT-29) tissues stimulates colon cancer tissue growth. After 15 days of co-culture tissue area (left panel), tissue protrusiveness (middle panel), and viable cell numbers (right panel) were significantly higher for insulin resistant (IR) HT-29 as compared to insulin sensitive (IS) HT-29 tissues and HT-29 tissues cultured in the absence of adipocytes (Mono). *Indicates significant differences in theHT-29 IS group ($p < 0.05$).