Nivetha Senthil Ramasamy
Department of Nutrition

ABSTRACT

Introduction: The growing incidence, prevalence, and burden of obesity and colorectal cancer are major public health issues today. Obesity creates an environment of chronic, low-grade inflammation in the body which is strongly correlated with the development of colorectal cancer. However, the mechanism by which obesity promotes tumorigenesis and tumor growth is unclear. Prior studies in the Hursting lab have discovered that IL-6 and leptin are produced in significantly increased quantities by adipocytes isolated from obese mice relative to adipocytes isolated from lean mice. IL-6 and leptin are known activators of the JAK2-STAT3 pathway, and the purpose of this Honors Thesis is to determine whether *in vitro* activation of the JAK2-STAT3 pathway by recombinant IL-6 and/or leptin influences the pro-cancer Wnt/β-catenin signaling pathway and/or epithelial-to-mesenchymal transition (EMT) process.

Methods: SW620 human colon carcinoma cells were treated with 20 ng/mL human recombinant IL-6 for 24 hours. Quantitative polymerase chain reaction (qPCR) was conducted on markers and targets of the JAK2-STAT3 pathway, Wnt/β-catenin pathway, and EMT process. MTT assays were conducted to analyze cell viability. MC38 murine colon carcinoma cells were treated with 20 ng/mL human recombinant IL-6 for four hours. Western blotting and qPCR were conducted on markers and targets of the JAK2-STAT3 pathway, Wnt/β-catenin pathway, and EMT process. MC38 cells were probed for the expression of leptin receptor through western blotting. MC38 cells were then treated for four hours with varying concentrations of recombinant leptin and subsequently treated with 100 ng/mL of leptin for varying time lengths to optimize leptin treatment conditions. MC38 cells were treated with 10 ng/mL IL-6 and 5 μM Stattic. Western blotting on markers of the JAK2-STAT3 pathway was conducted.

Results: IL-6 treatment of SW620 cells did not alter cell viability or significantly activate of the JAK2-STAT3 signaling pathway. IL-6 stimulation of MC38 cells significantly increased the relative expression of *Socs3* (JAK2-STAT3 target), *Sfrp4* (Wnt/β-catenin marker/EMT modulator), and *Ccnd1* (Wnt/β-catenin target). Leptin receptor was expressed in MC38 cells and 100 ng/mL of recombinant mouse leptin treatment for four hours was sufficient to activate the JAK2-STAT3 pathway. Treatment of MC38 cells with increasing concentrations of Stattic demonstrated a progressive decrease in the expression of phosphorylated STAT3. However, more optimization is required as these results were not reproducible with more replicates.

Conclusions: This study determined that *Ccnd1* and *Sfrp4* expression increased in MC38 cells after 4-hour treatment with human recombinant IL-6. These findings indicate that IL-6 secretion from obesity-associated adipocytes may activate the Wnt/β-catenin pathway and EMT process. Moving forward, gene expression analysis on four-hour 100 ng/mL leptin treatment and further optimization of Stattic concentrations for IL-6 treatment are needed.