Student: James Swan

Title: Does Diet Switch a Bacterial Commensal to a Colon Carcinogen?

Supervisor(s): Dr. Jacqui Keenan, Professor Frank Frizelle

Sponsor: Canterbury-West Coast Division of the Cancer Society of New Zealand

Introduction: Colorectal cancer is the third most diagnosed cancer in New Zealand causing approximately 1 300 deaths annually. This disease is becoming increasingly common in younger people (under the age of 50) and this may, in part, reflect dietary changes both here and around the world. Diet is known to have a direct influence on our gut health, as well as the microorganisms that live there. It is becoming more apparent that the effects of diet on gut microbiota may be just as significant as the direct effect(s) that diet has on the gut epithelium.

Bacteroides fragilis is a common bacterial species that colonises the gut, and for the most part, aids in digestion and the general health of the colon. However, a small percentage of people are colonised with enterotoxigenic strains of *B. fragilis* (known as ETBF). The *B. fragilis* toxin (BFT) is thought to increase the risk of a person developing colorectal cancer through cleavage of an essential cell adhesion protein known as E-cadherin that helps maintain barrier function in the gut. Loss of E-cadherin can lead to irregular cell growth and an associated increased risk of cancer. Heme iron (from red meats) and butyrate (a metabolite produced by the breakdown of plant fibers) are introduced into the body via our diet and are two examples of dietary components considered to have the potential to respectively negatively and positively influence gut health.

Aim: To determine whether heme iron and/or butyrate can influence the expression of BFT by an enterotoxigenic strain of *Bacteroides fragilis*, and consequently, to see how the presence of heme iron and/or butyrate affects the growth and development of colonic epithelial cells with and without the presence of ETBF.

Impact: To aid in developing potential prevention strategies (relating to diet) for the onset of colorectal cancer.

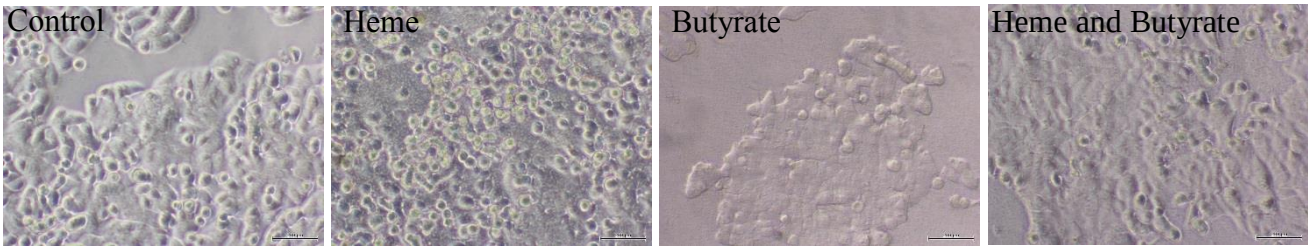
Methods: An enterotoxigenic strain of *Bacteroides fragilis* was grown in the presence of increasing concentrations of heme iron or butyrate. Change in absorbance over time was used as a measure of bacterial growth and this was confirmed by counting the number of viable bacteria in the supernatants after 48 h. RNA was extracted from the bacteria and analysed to determine the effect of heme iron and/or butyrate on the molecular expression of *bft*.

The HT29 colonic epithelial cell line was used to model the potentially toxigenic effect of BFT on the gut epithelium. Cells were grown for 48 h before the addition of heme iron, butyrate and/or ETBF for a further 24 h. Cell counts were used to determine cell growth under the different conditions while changes in cellular morphology were visualized by microscopy. Additionally, RNA was extracted from treated and untreated HT29 cells for molecular analysis of E-cadherin expression.

Results: Heme iron (0µM to 15µM) had a concentration-dependent effect on ETBF growth as determined by absorbance and the number of viable bacteria in the supernatants. In contrast, increasing butyrate concentrations (0-16 mM) had no significant effect.

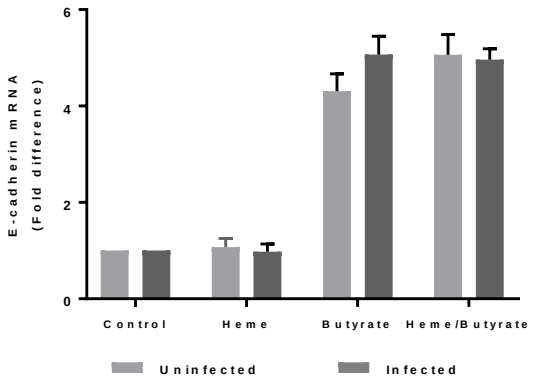
Attempts to measure the relative expression of BFT toxin under the different growth conditions were unsuccessful and further experiments are required before any conclusions related to the effects of heme iron and butyrate on toxin expression levels can be made.

It was evident by visualising the HT29 cells (see below) that the ETBF had an effect on their growth/development. Compared to the control, the heme-treated samples clearly showed that more cell rounding occurred indicating E-cadherin cleavage, suggesting that higher toxin concentrations were present. In marked contrast, the butyrate and heme/butyrate-treated cells showed little evidence of cell rounding.



HT29 cell counts showed no significant variance in cell abundance after 72 hours of growth in the presence of heme iron and/or butyrate. This was the case for both infected and uninfected cells.

When compared to the control, the molecular analysis of the HT29 cells showed a highly significant increase in E-cadherin expression from HT29 cells grown in the presence of butyrate, with and without the addition of heme. This was apparent for both the infected and uninfected samples.



Conclusion: There is evidence suggesting that heme iron promotes ETBF growth, and notably more cell rounding when infected HT29 cells are grown in the presence of heme iron suggests toxin production may be increased. It is anticipated that the measurement of *bft* toxin expression levels under the different growth conditions will support this observation.

The highly significant increase in E-cadherin expression levels in cells treated with butyrate (even when heme iron and/or ETBF is also present) strongly suggests that butyrate may promote the healthy growth of colon epithelium. This looks promising for future studies, as well as the future development of prevention strategies for colorectal cancers.