Understanding the role of Turicibacter/Butyrate in intestinal tumorigenesis associated with obesity

[1]Department of Nutrition, School of Public Health and Health Sciences, and [2]Department of Food Science, University of Massachusetts, Amherst, MA

MATERIALS AND METHODS

- MTT assay was used to examine the Caco-2 cell viability at various concentrations placed in 96-well plates with 50 µL of a 1 mg/mL solution of MTT in PBS.
- To examine the effects of butyrate, we treated Caco-2 cells (colorectal cancer cells) with varying concentrations of butyrate. We started off by using a general cell culturing protocol in which Caco-2 cells were maintained in 100mm petri dishes at 37℃ incubator with a humidified atmosphere of 5% CO2. The cells' monolayers were grown in cell culture media, freezing media, and, 0.25% Trypsin-EDTA solution. Caco-2 cells were maintained in 100mm petri dishes at 37℃ incubator with a humidified atmosphere of 5% CO2. The cells' monolayers were grown in cell culture media (w/ ~10% fetal bovine serum, FBS & 1% Pen/Strep), freezing media (usually culture media w/ 5~10% dimethylsulfoxide, DMSO), and, 0.25% Trypsin-EDTA solution (1X).
- To determine the protective effect of butyrate on tumorigenesis, the activation of tumorigenic Wnt-signaling was observed through immunoblotting analysis.

RESULTS #1: Our cell viability assay indicates a concentration of 5 mM butyrate showed an acceptable modest cytotoxic response (< 30%), therefore in the following up cell culture, 5mM dosage were used.

Caco-2 cells were seeded on a 96-well plate and cultured for 48 hours. The 96-well plates were replaced with fresh medium and different concentrations of sodium butyrate (1, 2, and 5 mM) and were further incubated for 72 hours. By the end of the treatment, the wells were added with 50 µL of a 1 mg/mL solution of MTT in PBS and were incubated for another 4 hours at 37°C. The supernatant was discarded and the colored formazan crystals were solubilized with 100 µL/well of DMSO. The absorbance of the 96-well plate was measured with a microplate spectrophotometer at 570 nm.

RESULTS #2: At a concentration of 5mM, butyrate displayed inhibited proliferation along with induced apoptosis in vitro.

Results show that cell viability decreases as concentration of butyrate increases. (Fig. 1A) Apoptosis was assessed by western blot using a cleaved caspase-3 antibody. Results showed that butyrate induced apoptosis by increasing expression of cleaved caspase-3. (Fig. 1B)

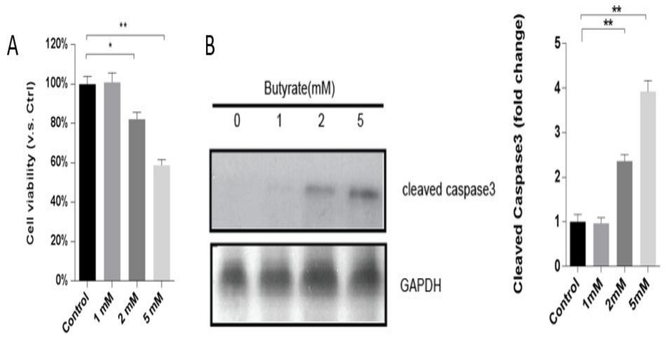

Fig. 1

RESULTS #3: Wnt-signaling was activated by the addition of butyrate.

The effect of butyrate supplementation on signaling pathways involved in CRC were studied by immunoblotting analysis, also known as Western Blot analysis. The levels of phospho-GSK3β (Ser9), the inactive form of GSK3β, and the dephosphorylated β-catenin (Ser37 or Thr41), the active form, which are two key molecules in the Wnt pathway were measured and further examined through the mRNA expression of Wnt pathway downstream oncogenes, Axin 2, c-Jun, c-Myc, and cyclin D1. It was found that apoptosis was significantly reduced when the Axin 2 gene was silenced through the regulation of mitochondrial apoptosis signaling pathway and therefore enhanced proliferation.

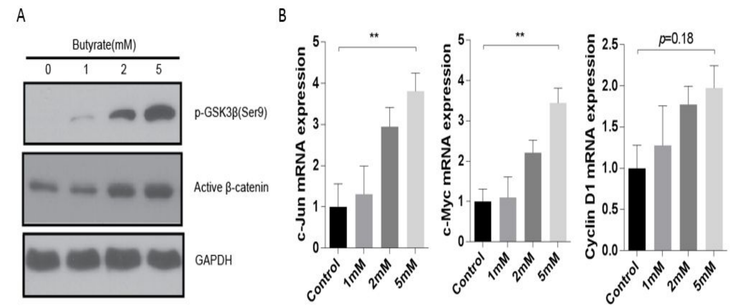

Fig. 2

CONCLUSIONS

- Butyrate possesses the ability to inhibit proliferation and induce apoptosis in-vitro through the activation of the Wnt-signaling pathway.
- Though I was unable to finish project on time due to it being an extensive and long-term project, I have gained not only experience in lab techniques and skills, but meaningful connections with a diverse group of individuals within the Nutrition Department here at UMass Amherst.

REFERENCES

1. Siegel, Rebecca L., et al. "Colorectal Cancer Statistics, 2020." *CA: A Cancer Journal for Clinicians*, vol. 70, no. 3, 2020, pp. 145–164., doi:10.3322/caac.21601.
2. Maki, Joel J., et al. "Complete Genome Sequence and Annotation for Turicibacter Sanguinis mol361 T (Dsm 14220)." *Microbiology Resource Announcements*, vol. 9, no. 25, 2020, doi:10.1128/mra.00475-20.
3. Canani, Roberto Berni, et al. "Potential Beneficial Effects of Butyrate in Intestinal and Extraintestinal Diseases." *World Journal of Gastroenterology*, Baishideng Publishing Group Co., Limited, 28 Mar. 2011, www.ncbi.nlm.nih.gov/pmc/articles/PMC3070119/.

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