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BNFO 300 Proposal

Introduction

Excluding skin cancer, colorectal cancer (CRC) is the third most common cancer diagnosed in both men and women in the US (cancer.org). CRC is associated with diets high in processed foods, red meat and chronic gastrointestinal inflammation (Hagland et al. 2015). So far, CRC has caused 49, 910 deaths this year (cancer.org). Twenty-five percent of patients diagnosed with CRC have a family history of this cancer (cancer.org).

The gut microbiome has been shown to be associated with the cell proliferation and apoptosis in cancer behavior (Zackular et al. 2013). Specifically, when comparing healthy gut microbiomes to the gut microbiomes of CRC patients, the CRC patients tended to have altered gut microbiomes (Zackular et al. 2013). The main type of alteration noted is a drop in gut microbiota diversity. A drop in diversity leads to increased competition among the bacterial colonies for space and food, which causes dysbiosis (an imbalance) (Coyte et al. 2015).

The role the gut microbiota plays in the formation of tumors can lead to finding therapies to prevent colon cancer. So far, there have been several studies that have associated several bacteria species with the development or prevention of CRC. Zackular et al. have observed that changing the gut microbiome can change the rate of tumor growth and the size of the tumor. Of the many bacteria they found in a cancerous gut microbiome, two species *Bacteriodes fragilis* (Bf) and *Bacteriodes thetaiotamicron* (Bt) were the most prominent. In an experiment to test how tumor growth was affected, Zackular et al. used 20 germ-free mice and split them up evenly into two groups. They took feces and bedding from healthy, untreated antibiotic mice and put it in with Group 1. They then took feces and bedding from tumor-bearing azoxymethane (chemical carcinogen, AOM)/dextran sodium sulfate (solvent, DSS) mice and put that with the second group (Group 2). They then split the two groups evenly again into five mice per cage (Group 1a & b, Group 2a & b). They then treated all the mice with AOM/DSS. In the figure, Group 1 is the “healthy community” and Group 2 is the “Dysbiotic community.” The group of germ-free recipient mice that had feces from AOM/DSS mice (Group 2a & b) had twice as many tumors as the group that had feces and bedding from healthy, untreated donor mice. The germ-free recipient mice that had feces from AOM/DSS also had a less diverse microbiome. There was a general increase in the populations of *Bacteroides and Erysipelotrichaceae* and less *Porphyromonadaceae*. The healthy germ-free mice (Group 1a &b) microbiome had an increase of *Odoribacter and Turicibacter,* which was not detected before and a decrease in *Prevotella and Porphyromonadaceae.* The authors observed that changing the microbiome changes the rate of tumor growth and the size of the tumor.

What is contained in Bf and Bt that is contributing to increased tumor growth? To answer this question, it is useful to use a combination of bioinformatics searches and *in vitro* experiments to identify what these two bacteria have in common. Now, this is a giant leap, nonetheless, it is a good place to start.

Experiment

For the bioinformatics portion, I am using Burns et al.’s model. They sequenced several gut microbiome bacteria looking for the genomic differences measured by the change in the abundance within normal microbiomes and CRC ones. I will use their method and data to test in the lab the predictions and see if there is an association between Bf and Bt. Burns et al. did a reciprocal BLAST comparison, where they used one bacteria as a query to blast against another bacteria’s genome. They then did a reciprocal blast in the other direction to make sure that the genes in the second bacteria were selected in the first bacteria.

Cario discusses using different vectors to stimulate or suppress proteins that turn on/off the inflammation response that leads to tumorgenesis. They used Polymerase chain reaction (PCR) to amplify and isolate each target gene. PCR uses DNA polymerase to synthesize a new strand of DNA complimentary to the target strand to get a specific region of target sequence that we want to amplify. In order to do this, we must first denature the target DNA at a high temperature, around 94-95 degrees Celsius. Add DNA polymerase, which is an enzyme that makes new strands of DNA complimentary to our target sequence. According to NCBI, when doing PCR, Taq (*Thermis aquaticus*) is the most common DNA polymerase used because it is heat resistant (NBCI 2016). Taq generates new DNA by using target DNA as a template and primers. Primers are short pieces of single stranded DNA that are complementary to the target sequence. For Bf and Bt, Matsuki et al found the primers were g-Brfa-F (forward primer) 5’-ATAGCCTTTCGAAA-3′ and g-Bfra-R (reverse primer) 3′-ATTTTAACGTCAACTATGACC-5′ (Matsuki et al. 2002). Ideally, these primers will bind to the specific genes we went to amplify, allowing Taq to bind and start. Taq will begin synthesizing new DNA from the end of the primer onward. Add deoxynucleotide triphosphates (dNTPs), which are single units of bases A, T, G, and C. Taq will use these dNTPs as the building blocks for the new DNA strand.

Through PCR, we can exponentially amplify the target sequence and make a larger quantity of gene. In this experiment, we are amplifying an expression vector as a plasmid for several genes that Bf & Bt have in common. A plasmid is a chromosomal DNA molecule found in bacteria that usually carry gene(s) that are beneficial but not necessary for the cell (Linott 2015). An expression vector is a synthetic plasmid that is created to express genes and a myriad of other things (Linott 2015). Here, we are adding it in the bacteria *Escherichia coli* so when the bacteria undergo replication, the plasmid undergoes transcription and translation and pop out a protein. We then lyse the cells with detergent and separate the plasmid DNA from the other cell components by centrifugation.

Next, we isolate the protein made by eluting with buffer and then feed the protein extract to colorectal tumor cells and see if the tumor size decreases.

Results

The results can tell us whether these bacteria have a gene or something that can be used to decrease tumorgenesis. It can also lead to more questions, like whether or not other bacteria have that gene, or is that gene the only one? Does it act alone or in tandem with others? Does a protein arise out of it that can be used? Some pitfalls are: if there is no decrease in tumor growth, we would have to look at other factors or something else common to the bacteria. The main limitation of the experiment is that this is a very controlled setting so it would be very difficult to state what would work in a variable environment like the gut microbiome.

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