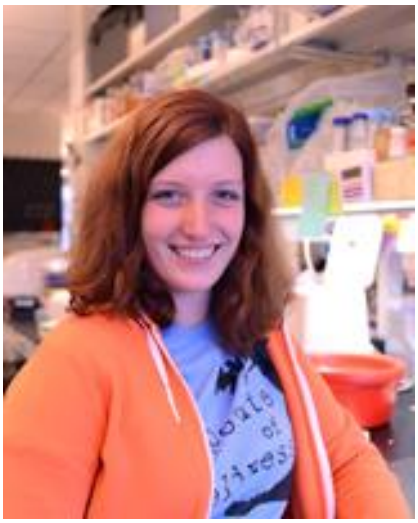

Modeling Human Colorectal Cancer Initiation Using *in vitro* Directed Differentiation of Pluripotent Stem Cells
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As a rising senior sub-matriculating in Chemistry, this upcoming year will be a busy year. I will be conducting independent research that will culminate in a master's thesis in April. My research in Dr. Christopher Lengner's laboratory this summer laid the foundation for my thesis work in modeling human colorectal cancer (CRC) using directed differentiation of pluripotent stem cells into intestinal organoids.

The majority of human colorectal cancers have loss of function mutations in the Adenomatous Polyposis Coli (APC) protein, an important regulator of the Wnt signaling pathway. Wnt signaling molecules bind to cell surface receptors and activate a signaling cascade that stimulates cell division. In the absence of Wnt signals, APC coordinates the degradation of β-catenin, the transcriptional effector of the Wnt pathway. When APC function is lost, β-catenin, which can no longer be degraded, accumulates and results in uncontrolled cell proliferation. This is thought to be the initiating event in colorectal cancer, as APC loss can subsequently lead to the accretion of additional oncogenic mutations and ultimately CRC.

Because APC loss is the most frequent mutation in CRC, and families carrying a heterozygous APC mutation (only one of the two copies of the gene are mutated) invariably will lose the second functional copy through a process called loss-of-heterozygosity (LOH), it is thought that APC loss is the initiating event in CRC. My goal is to develop a human organotypic cell culture model to study APC LOH in the intestinal epithelium.

Because there is tremendous selective pressure for APC heterozygous cells to undergo LOH in culture, I need to take a conditional genetic approach to study LOH with precise temporal control. To achieve this, I start by inserting LoxP sites, small DNA sequences, around one of the two APC alleles in human pluripotent cells (embryonic or induced pluripotent stem cells). The introduction

of the LoxP sites allows me to inactivate this copy of APC at any time through the introduction of Cre recombinase, which excises the DNA between the two LoxP sites.

Once the LoxP sites are introduced, the pluripotent cells will be directed to differentiate into human intestinal tissue in cell culture. Pluripotent cells can be coaxed to form intestinal "organoids" that are the closest approximation of primary human tissue that have been generated to date. These organoids will be functionally wild type throughout differentiation. Inactivation of one APC allele will be induced by addition of Cre recombinase in mature intestinal organoids. The remaining APC allele will spontaneously undergo LOH in culture, enabling us to study the parameters that govern LOH and screen for factors that influence loss of heterozygosity and tumor formation.

Over the summer I learned how to direct differentiation into intestinal organoids. I also used molecular cloning techniques to prepare the constructs necessary for LoxP site insertion. Finally, I designed methods of screening for correctly mutated clones. Currently, I am in the process of inserting the LoxP sites and will continue to direct the differentiation of the correctly targeted cell lines throughout the school year.