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Abstract

The gut epithelium is a renewing tissue that must balance cell differentiation and cell stemness to promote a secure barrier. The stem cell niche depends on Wnt/β-catenin signaling, a tightly regulated pathway that controls the gut epithelial organization and promotes stemness in the crypts. Lack of accurate Wnt/β-catenin signaling in colonic stem cells can lead to gastrointestinal (GI) diseases. Despite its prominence in pathologies, it is difficult to therapeutically target major components of the Wnt pathway. There is a need to illuminate new mechanisms of Wnt regulation and uncover potential therapeutic targets. I recently found CDC2-Like-Kinase 3 (CLK3) is a kinase to be involved with the canonical Wnt/β-catenin pathway. Our research shows CLK3 plays a role in cell proliferation due to its downstream effect in the Wnt/β-catenin signaling pathway, and I observed decreased levels of CLK3 resulted in decreased levels of colonic cell proliferation. Little is known about the function of CLK3 in the Wnt pathway, but our preliminary data suggests it can directly affect signaling via its regulation of the Wnt transcriptional complex and alternative splicing of developmental genes. Additionally, high-resolution microscopy shows that the majority of CLK3 expression resides in the stem cell population of the crypt. **I hypothesize that CLK3 kinase activity induces alternative splicing of Wnt/β-catenin target transcripts to promote stem cell fate in the intestinal epithelium.** For my first aim I will be using RNA-sequencing data to observe splicing differences between cell lines of varying CLK3 expression and potentially discover novel isoforms of target genes important for stem cell state. Aside from understanding the role of CLK3 in alternative splicing, I will investigate the phenotypic impact on organoid organization and differentiation. I am conducting loss-of-function studies in human colonic epithelial cells (HCECS) to monitor the changes in mRNA splicing that affect the Wnt transcriptional complex and stem cell identity. In my second aim, I will genetically engineer mice with an inducible CLK3 KO via the CRE-lox system to explore its function in vivo. Additionally, I will use patient-derived colon organoids to study CLK3's role in supporting the stem cell population. Together, our work will test CLK3's function in promoting stemness in gut epithelium through stimulating alternative splicing to stabilize the Wnt transcriptional complex, thereby maintaining robust Wnt signaling. My work will take place in the Curtis Thorne lab at the University of Arizona, and I will uncover CLK3 as a critical kinase regulator of cell fate in the gut and lead to opportunities to therapeutically target CLK3 in GI diseases.