Title: DNA hypomethylation inhibits colitis-associated colorectal cancer

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Background: Colorectal cancer is the second leading cause of cancer death in Canada. A major risk factor for the development of colorectal cancer is chronic inflammation leading to colitis-associated cancer (CAC). We previously described a CAC mouse model in which tumors arise from DCLK1+ tuft cells following loss of the tumor suppressor adenomatous polyposis coli (APC) and induction of colitis. Interestingly, both colitis and CAC display epigenetic changes that modulate gene expression. However, the impact of DNA methylation on colonic tumorigenesis is unknown. Thus, we hypothesize that inhibition of DNA methylation in DCLK1+ tuft cells reduces colonic tumorigenesis. We investigated this hypothesis by inhibiting DNA methylation by genetic and pharmacologic means.

Method: Dclk1-CreERT2/Apc$^{f/f}$ mice were crossed to Dnmt1$^{f/f}$ mice to knock-out DNMT1 in DCLK1+ tuft cells. Dclk1/Apc$^{f/f}$ and Dclk1/Apc$^{f/f}$/Dnmt1$^{f/f}$ mice were administered three doses of tamoxifen followed by 2.5% dextran sodium sulfate (DSS) for five days to induce colitis. Fourteen weeks later, we assessed colonic tumor number and size. In a separate cohort of Dclk1/Apc$^{f/f}$ mice, we induced colitis and treated with six doses of 5-AZA-2'-deoxycytidine (5-AZA) or vehicle and assessed colonic tumor number. To examine DNA methylation changes, we treated WT mice with 5-AZA and DSS and isolated intestinal epithelial cells. From the intestinal epithelial cells, we isolated DNA and ran the Infinium Mouse Methylation BeadChip Array.

Results: Deletion of DNMT1 in DCLK1+ cells significantly inhibited the number and size of colonic tumors. Treatment with 5-AZA decreased global and gene specific DNA methylation levels, and significantly reduced the number of mice with tumors and the average colonic tumor number and size per mouse.

Conclusions: Our findings demonstrate that DNA hypomethylation by 5-AZA treatment or loss of DNMT1 reduces CAC formation suggesting that altered DNA methylation plays a critical role in colonic tumorigenesis.