Understanding the Role of Microbes in Intestinal Tumor Pathogenesis

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Abstract

Colorectal cancer (CRC) is the third most deadly cancer worldwide, with 1/2 million deaths yearly. The gut microbiome and its metabolites have been implicated as contributors and regulators of CRC pathogenesis. Treatment with celecoxib, a NSAID and selective COX-2 inhibitor, has been found to reduce the formation of polyps in the gastrointestinal tract of humans and mice. Furthermore, celecoxib has been demonstrated to reduce the rate by which Lgr5-positive stem cells gave rise to differentiated cell types in intestinal crypts through alterations to the gut microbiome. Mice treated with broad-spectrum antibiotics also developed significantly fewer, smaller tumors than untreated mice, hinting that tumor incidence and penetrance were dependent on the gut microbiome.

In this study, methods of immunohistochemistry for Ki-67 and PCR were employed to study the role of celecoxib and antibiotics in modulating microbes and intestinal tumor development in $Apc^{Min/+}$ mice. A combination of celecoxib and broad-spectrum antibiotics significantly decreased tumor size and number in the mice, more effectively than celecoxib or antibiotics alone. Under antibiotics, the administration of celecoxib resulted in significantly reduced basal proliferation of normal intestinal crypt stem cells. Further experiments exposing HCT116 cells to varying concentrations of murine fecal extract demonstrated that the gut microbiome could directly manipulate inflammation within the gut. This study provides insight into the understanding of the role of gut microbiota in CRC development. Targeting the microbiome as a strategy to prevent inflammation-based CRC development has the potential to aid the development of chemopreventive strategies.

Introduction

Colorectal cancer (CRC) results in half a million deaths annually and is the third leading cause of cancer mortality in the world (Jemal et al., 2011). Lifestyle-related factors such as obesity, physical inactivity, diet, and smoking have been linked to a greater chance of developing CRC. Familial adenomatous polyposis is a condition that is diagnosed when one develops more than 100 adenomatous colon polyps (Soravia et al., 1998). Two-thirds of all colonic polyps in the general population are adenomatous. These adenomas grow into the lumen of the colon and intestines and have malignant potential. If left untreated, adenomas carry a 100% risk of being precursor lesions to CRC. *Apc*$^{Min/+}$ mice, an established genetic model for familial adenomatous polyposis, are often used in CRC laboratory research.

Intestinal crypts surround the base of each villus and host stem cells that replenish the epithelial cells, along with frequently regenerating cells involved in host defense and signaling, such as Paneth cells. The colonic epithelium lacks villi, so the crypts penetrate the underlying submucosa. As CRC develops, crypts become polyps. Stem cells at the base of the crypts have characteristics of self-renewal and the potential to differentiate. When they become cancerous, they are highly tumorigenic, critical in the recurrence of colorectal cancer and therefore disease-free survival (Zhou et al., 2018). Stem cells have evolved to manage a constantly changing luminal microenvironment.

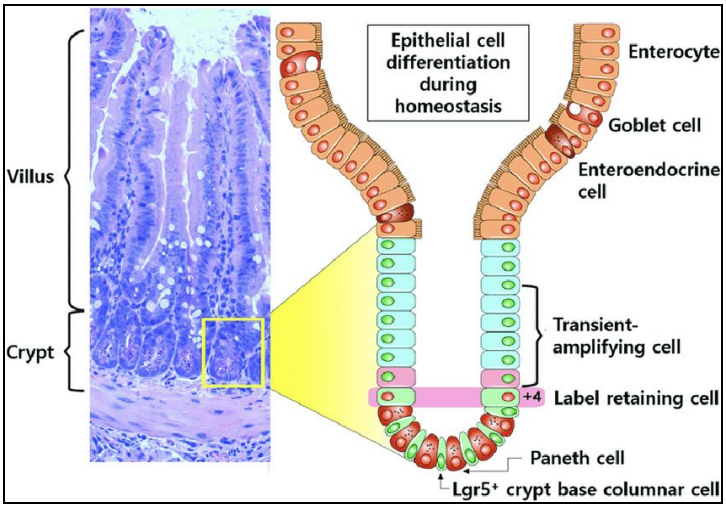

Fig. 1. (Hong, Dunn, Stelzner, & Martin, 2016). Intestinal crypt. Lgr5+ crypt base columnar cells are intercalated with Paneth cells.

One significant risk factor for CRC is the chronic inflammation of the gastrointestinal tract. A combination of heredity, genetic, and environmental factors may cause the development of inflammation. Various inflammatory cells produced during chronic inflammation lead to the formation of a microenvironment that provides nutrients and secreted factors that support tumorigenesis (Kaur et al., 2018). The chronic inflammation of the gastrointestinal tract is also closely associated with changes in the composition of the community of microorganisms that inhabit our gastrointestinal tract (Montrose et al., 2016). This community, the gut microbiome, has ~10^{14} microorganisms and has the ability to promote physiological functions associated with cancer, including cell proliferation and apoptosis (Sears & Garrett, 2014). This microbial ecosystem lives in close proximity to the intestinal epithelium and contributes essential functions involved in the maintenance of host homeostasis. Furthermore, the microbiota has the ability to adhere to the epithelium.

Inflammation and the microbiome have a bidirectional relationship. Driven by the host's genetic makeup and environmental exposures, the gut microbiome and its metabolites have been implicated as contributors and regulators of CRC pathogenesis through pro-inflammatory response and microbial metabolites. Data suggest that individuals with colorectal cancer have a distinct bacterial profile compared to healthy controls (Marchesi et al., 2011). Tumors consistently formed a niche for *Coriobacteria*, while potentially pathogenic *Enterobacteria* were underrepresented in tumor tissue, suggesting that CRC-associated metabolic changes may recruit commensal bacteria that have the ability to invade and colonize solid tumors (Leschner & Weiss, 2010). Preclinical studies have suggested that bacteria can modulate intestinal tumorigenesis by secreting specific metabolites, such as short-chain fatty acids (Rooks & Garrett, 2017).

Non-steroidal anti-inflammatory drugs are one method to treat CRC through cyclooxygenase (COX). COX is a common target for anti-inflammatory drugs and is usually specific to inflamed tissue. Prolonged use of non-selective NSAIDs have been found to reduce the formation of adenomatous polyps by 30%–50%. However, non-selective NSAIDs have a broad range of side effects (Tsioulias, Go, & Rigas, 2015). COX-2 is an enzyme in the COX system selectively induced by proinflammatory cytokines at the site of inflammation. Excess amounts of COX-2 are associated with adenomas and colon cancer. Elevated COX-2 expression

was found in approximately 50% of adenomas and 85% of adenocarcinomas and is associated with lower survival rates among CRC patients (Marnett and DuBois, 2002; Ogino et al., 2008).

Celecoxib is a selective NSAID used as part of anti-CRC chemotherapy to directly target COX-2. With less side effects than non-selective NSAIDs, treatment with celecoxib has been found to significantly reduce the number of colon polyps in people with familial adenomatous polyposis. Patients receiving celecoxib twice a day for six months experienced a 28% reduction in the mean number of colorectal polyps and a 30.7% reduction in polyp burden (Steinbach et al., 2000). Celecoxib has been demonstrated to reduce the rate by which stem cells gave rise to differentiated cell types in the crypts (Marnett & DuBois, 2002). Celecoxib, in addition to its ability to reduce polyps, can exhibit antimicrobial activity against Gram-positive pathogens from a variety of genera, including *Staphylococcus* and *Mycobacterium.* Celecoxib's anti-inflammatory and antibacterial properties may both play a role in its chemotherapeutic abilities. CRC treatment with celecoxib has altered luminal bacterial populations in $Apc^{Min/+}$ mice and wild-type mice in the form of decreased *Lactobacillaceae* as well as increased *Coriobacteriaceae*. Moreover, metabolomic analyses demonstrated that celecoxib reduced cell proliferation in the base of normal-appearing ileal (intestinal) and colonic crypts of $Apc^{Min/+}$ mice. A metabolomic analysis on the feces from mice used for microbiota analysis showed that changes are caused by a strong reduction in fecal metabolites that are linked to carcinogenesis, such as glucose and lipids (Montrose et al., 2016).

To determine whether tumor incidence and penetrance were dependent on the gut microbiome, Zackular et al. (2013) treated mice with a broad-spectrum antibiotic cocktail before and during AOM injection and DSS treatment. Broad-spectrum antibiotics act on a wide range of bacteria within the gut microbiome. Antibiotic-treated mice had significantly fewer, smaller tumors in the colon than untreated mice. Zackular et al. also examined stool samples of mice treated with AOM/DSS. One round of DSS administration caused a significant decrease in microbial diversity in the murine microbiome through tumor development. A recent matched case-control study concluded that a single dosage of antibiotics boosts the risk of colon cancer development ten years later (Zhang et al., 2019). It seems that when antibiotics are used during one's lifetime (not for cancer specifically), they negatively affect the gut microbiome.

Inflammation-linked carcinogenesis is a concept often observed within the gastrointestinal tract, but the underlying mechanisms driving microbially mediated tumorigenesis remain to be elucidated. Despite recent research, there are significant gaps in the understanding of how antibiotics increase the risk of colorectal cancer. The goals of this study were to examine the mechanisms by which the administration of antibiotics reduces tumor burden, whether the attenuation of the proliferation-suppressing effects of celecoxib under antibiotics is mediated via the stem cells, and whether the bacterial waste and the gut microbiome could directly manipulate inflammation within the gut. Comparing celecoxib in the presence and absence of antibiotics could provide information as to whether celecoxib within the gut microbiome more as an anti-inflammatory, an antibacterial, or both.

Methodology

Mouse Treatments

All of the mouse treatment and tissue isolation was done by a member of the lab. To equilibrate the gut microbiota across mice by microbial exchange, 2 mice were housed together until 6 weeks of age, then individually housed until 16 weeks of age. Mice were given a purified chow diet with 1,000 ppm celecoxib or a control chow diet. After initial fecal collections, mice were individually housed and either continued on purified diet or given the same diet supplemented with 1,000 ppm celecoxib for 10 weeks. At 6 weeks of age, a broad-spectrum antibiotic cocktail of metronidazole, vancomycin, and streptomycin was given to Groups 3 and 4 through their drinking water. Mice were sacrificed at 16 weeks of age. Their ileal content was collected, then intestines were flushed with phosphate-buffered saline, and fixed for quantification of polyp burden.

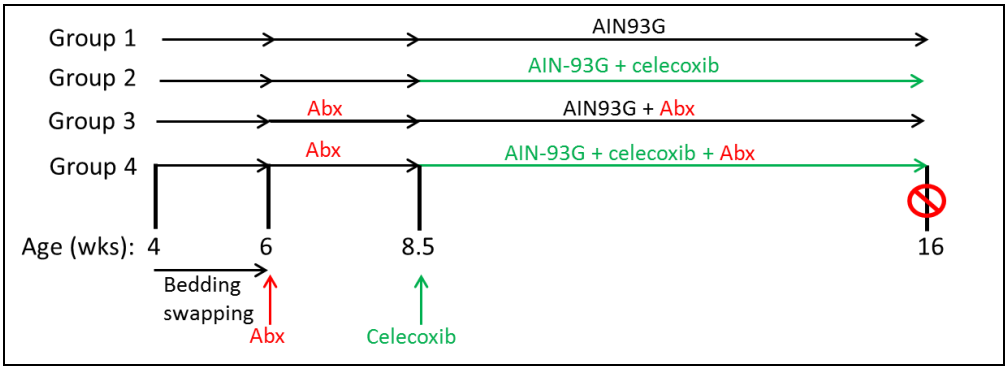

Fig 2. Schematic representation of the experimental design.

Immunohistochemistry for Ki-67

Cellular proliferation in intestinal crypts was evaluated using immunohistochemistry for Ki-67. Paraffin-embedded murine tissue sections were deparaffinized and incubated with 1% hydrogen peroxide at 25°C for 20 minutes. Sections were boiled in a pressure cooker filled with sodium citrate (pH 6.0), then blocked with 10% goat serum for antigen retrieval. Sections were then incubated overnight at 4ºC with anti-Ki-67 antibody in a 1:200 dilution (Cell Signaling Technology). Sections were washed, blocked, and incubated with biotinylated anti-rabbit secondary antibody (Vector Laboratories, Inc.) and incubated with avidin-biotin complex reagent (Vector Laboratories, Inc) for 30 minutes at 25°C. Tissue sections were analyzed for well-oriented crypts with open, straight lumina and a clearly visible base. The epithelial cells in the crypt were counted and the percentage of cells that showed positive staining Ki-67 in both basal and non-basal regions was recorded. In the ileum, the crypt base was defined as the horizontal portion in which epithelial cells proliferated perpendicular to the surface of the lumen. The lateral aspects of the crypt were considered non-basal regions. The assessment of tumors was performed in a blinded fashion. To determine tumor number and size, tumors were stained with methylene blue. Then tumor number and size were quantified under a dissecting microscope using a ruler capable of discerning size differences of 0.5 mm to determine tumor size. A negative binomial regression and general linear hypothesis test were used to analyze the data for tumor number and size.

qRT PCR for HCT116 Cells

Human colon cancer HCT116 cells were used as the cell line in this study. Cells were cultured as adherent monolayers in flasks with pre-warmed McCoy's 5a medium supplemented with 10% fetal bovine serum in a humidified incubator containing 5% CO_2 at 37°C. qRT PCR was used to determine the relative expression of COX-2 in HCT116 cells. After harvesting 1 x 10^7 HCT116 cells as a cell pellet, RNA was purified using the RNeasy Plus Mini Kit (Qiagen). The concentration of total RNA for each sample was measured using a Nanodrop spectrophotometer (Thermo Fisher) and used to calculate the amount needed to be put into each PCR plate well. Samples were loaded in a 96 well PCR plate and spun down before use. B-actin was used as an internal control. CT values, the PCR cycle numbers where signals become discernible above background noise, were used to calculate the change in the CT value of COX-2 relative to the change in the CT value of β-actin, the calibrator. The relative expression of the COX-2 gene was calculated from CT values using the formula below.

$$\text{Relative Expression} = 2^{-\Delta\Delta CT}$$

Results

To correlate changes induced by celecoxib in the intestine of *Apc*$^{Min/}$ mice with changes in intestinal polyp burden, the effects of celecoxib and broad-spectrum antibiotics on intestinal polyp burden were first determined. Intestines were stained, and tumor number and size were quantified under a dissecting microscope using a ruler. The administration of broad-spectrum antibiotics and celecoxib caused a trend for reduction in tumor number, as well as a significant reduction in tumor size (Figure 3A/B). Regarding tumor number, the average for control mice without antibiotics was 6, and the average for celecoxib-fed mice without antibiotics was 5. The average for control mice with antibiotics was 5, and the average for celecoxib-fed mice with antibiotics was 4. Regarding tumor size, the average for control mice without antibiotics was 1.65 mm, and the average for celecoxib-fed mice without antibiotics was 1.3 mm. The average for control mice with antibiotics was 1.4 mm, and the average for celecoxib-fed mice with antibiotics was 1 mm. The difference in tumor number between mice given a control diet and mice given celecoxib was reduced by 33% when mice were given water with antibiotics. The

difference in tumor size between mice given a control diet and mice given celecoxib was reduced by 20% under antibiotics.

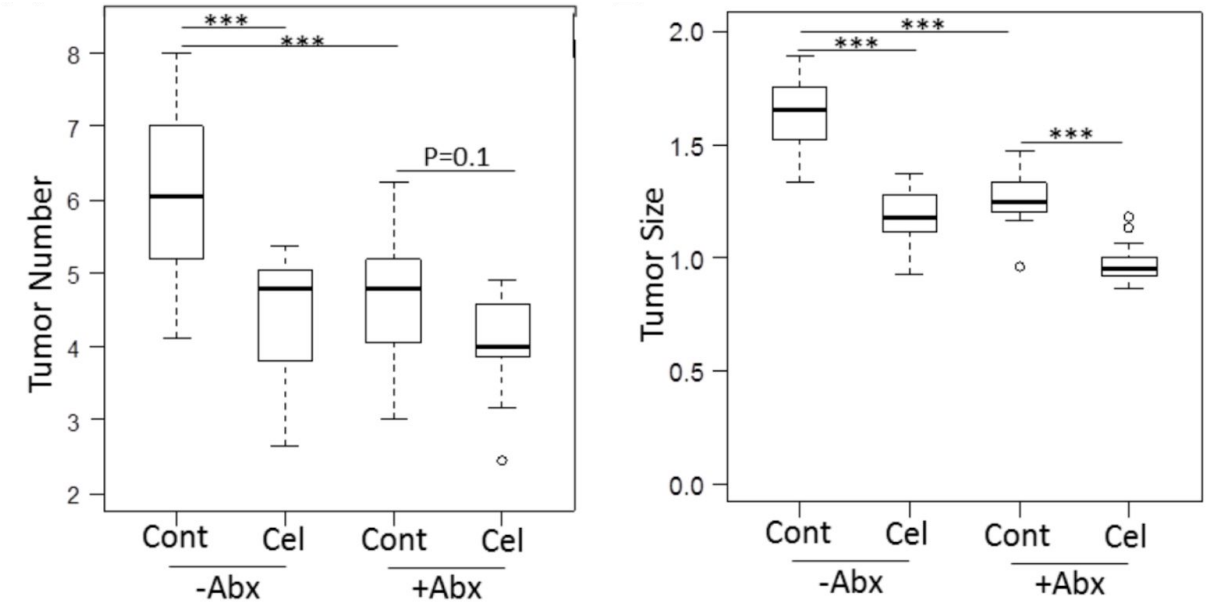

Fig. 3A/B. The tumor number and size were determined in the small intestine of *Apc*$^{Min/+}$ mice given a control or celecoxib containing diet and water with broad-spectrum antibiotics or normal water. Comparing the magnitude of the effect of celecoxib vs control when comparing the mice that did not receive antibiotics vs. those that did get antibiotics. *** represents statistical significance between groups. P (3A) = .1. P (3B) = .02. n=10 per group.

The attenuation of the proliferation-suppressing effects of celecoxib under antibiotics was examined to see if it was mediated via the stem cells located in the base of the crypt. Normal intestinal crypts of celecoxib and control fed mice given broad-spectrum antibiotics were stained for Ki-67 (a marker for proliferation expressed in all phases of the cell cycle except G_0) through immunohistochemistry. Overall, the intestinal polyps of *Apc*$^{Min/+}$ mice given water with antibiotics had a reduction in brown staining for Ki-67 than the polyps of *Apc*$^{Min/+}$ mice given normal water, demonstrating a decreased amount of proliferation (Figure 4).

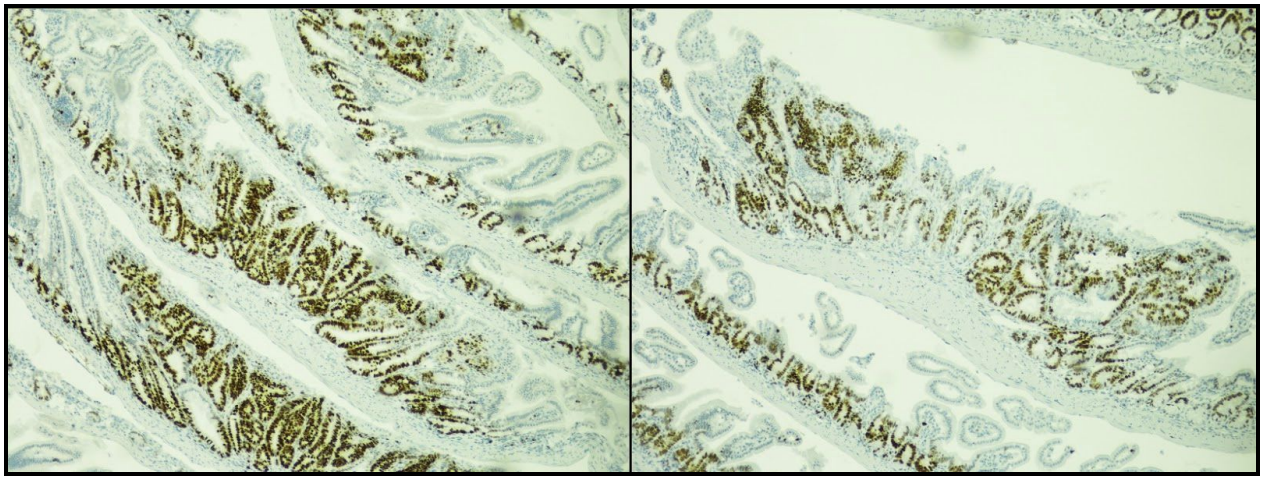

Fig. 4. Intestinal polyps in a representative *Apc*$^{Min/+}$ mouse (outlined in red) given water with broad-spectrum antibiotics (left) and normal water (right) stained for Ki-67 with immunohistochemistry. The regions stained dark brown are the regions with proliferating cells.

The epithelial cells in the crypt were counted and the percentage of cells that showed positive staining for Ki-67 in both basal and non-basal regions was recorded. In the ileum, the crypt base was defined as the deep horizontal portion in which epithelial cells proliferated with their long axes perpendicular to the luminal surface. The lateral aspects of the crypt were considered non-basal regions. In the base of the crypt, there is stem cell differentiation that is not found in the non-basal regions of the crypt. Ki-67 is associated with tumor cell proliferation; regions with a large percentage of Ki-67+ (Ki-67 positive) cells have more proliferation. The control mice had a greater percentage of proliferating cells (25.25%) than celecoxib-fed mice (16.97%) in the base of the crypts (Figure 5). Celecoxib-fed mice showed 33% lower Ki-67+ cells in the base of crypts, showing that celecoxib and antibiotic treatment significantly reduced the number of Lgr5-positive stem cells that would be able to give rise to differentiated cell types within the crypts. Non-base cells were proliferating to a slightly greater extent in the crypts of celecoxib-fed mice (73.52% vs 71.65%). There was a negligible increase in the amount of proliferation of the overall crypts of celecoxib-fed mice when compared to the control fed mice. There were more proliferating cells than non-proliferating cells in both groups (59.07% for the crypts of celecoxib-fed mice, and 58.6% for the crypts of control mice).

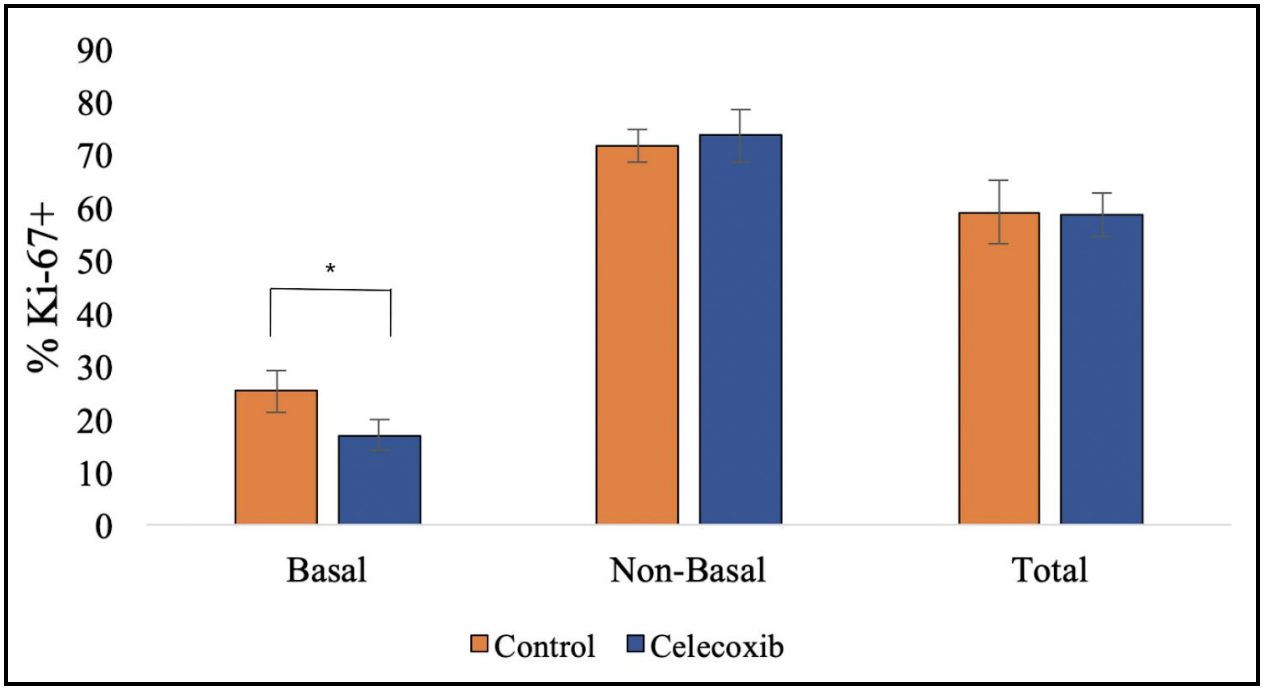

Fig. 5. The percentage of Ki-67-positive cells in the entire crypt, base, and non-base of the crypts was quantified in the ilea of *Apc*$^{Min/+}$ mice given broad-spectrum antibiotics and the control or celecoxib diet. The crypt base was defined as the deep horizontal portion in which epithelial cells proliferated with their long axes perpendicular to the luminal surface. The lateral aspects of the crypt were considered non-basal regions. n=9 (4 controls, 5 celecoxib-fed). 10 crypts were counted for each mouse. * = p<.05. Error bars: ±SD.

To provide further insight into the effects of broad-spectrum antibiotics on inflammation, the mechanisms by which the administration of broad-spectrum antibiotics reduces tumor burden were studied. Antibiotics alone did not have a pronounced effect on inflammation within the polyps of the mice (Figure 6). There was a negligible increase in the percentage of Ki-67+ cells in mice given antibiotics (57% in control vs. 63% in antibiotics).

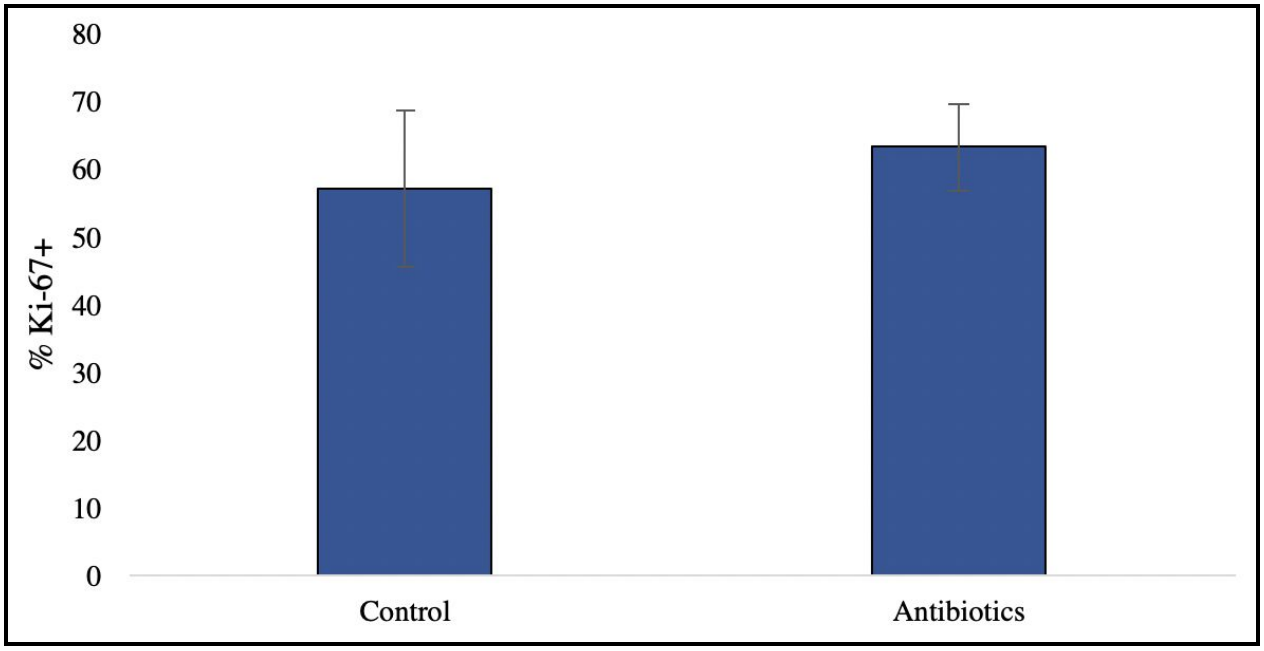

Fig. 6. The percentage of Ki-67-positive cells in polyps was quantified in the ilea of *Apc*$^{Min/+}$ mice given control water or water with broad-spectrum antibiotics. n=10 (5 controls, 5 given antibiotics). 10 crypts were counted for each mouse. Error bars: ±SD.

To study the idea that the bacterial waste and the gut microbiome could directly manipulate inflammation within the gut through COX-2, HCT116 cells were exposed to various concentrations of murine fecal extract (0%, .05%, .5%, and 5%) for 4 hours (Figure 7). qPCR was employed for the treated HCT116 cells to measure the relative COX-2 expression in mice using CT values. COX-2 regulates proliferation; relative COX-2 expression and proliferation have a direct relationship. Cycle threshold (CT) values were taken from the PCR, normalized and compared. A lower CT value corresponds to more PCR product present (a greater amount of mRNA). Relative expression of the COX-2 gene was calculated from CT values using the formula: relative expression = $2^{-\Delta\Delta CT}$. Compared to the 0% fecal extract, the .05% fecal extract caused a 1.45 relative fold increase in COX-2 expression, the .5% extract caused a 1.65 fold increase, and the 5% fecal extract caused a 2.6 fold increase. With an increase in fecal extract concentration (from 0% to .05%), a 27% increase in relative COX-2 expression was observed. With a tenfold increase in fecal extract concentration (from 0.05% to 0.5%), a significant 21% increase in relative COX-2 expression was observed. With a tenfold increase in fecal extract concentration (from 0.5% to 5%), a significant 108% increase in relative COX-2 expression was

observed. The 5% fecal extract was the most effective in increasing COX-2 RNA expression, with a normalized CT value of 2.72. When compared to the control (0% fecal extract), there was a 145% increase in relative COX-2 expression.

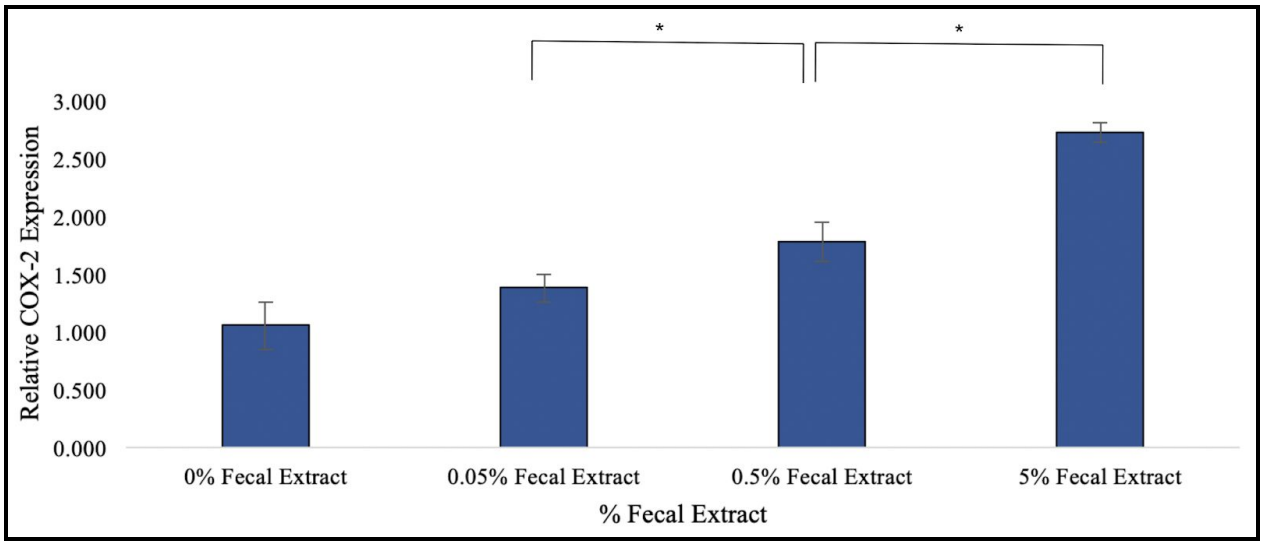

Fig. 7. Relative COX-2 expression generated from qRT PCR, for HCT116 cells treated with fecal water for 4 hours (0%, .05%, .5%, 5% fecal water). * = p<.05. Error bars: ±SD. n=6 for each treatment level.

Further experiments were performed to study the effects of 6, 12, and 24 hours of fecal water exposure on COX-2 induction (Figure 8). There were no significant effects on relative COX-2 expression when HCT116 cells were exposed to 5% fecal extract for various lengths of time. For the cells exposed to fecal water for 6 hours, the average relative fold change was 1.075. For the cells exposed to fecal water for 12 hours, the average relative fold change was 1.01. For the cells exposed to fecal water for 24 hours, the average relative fold change was 1.034.

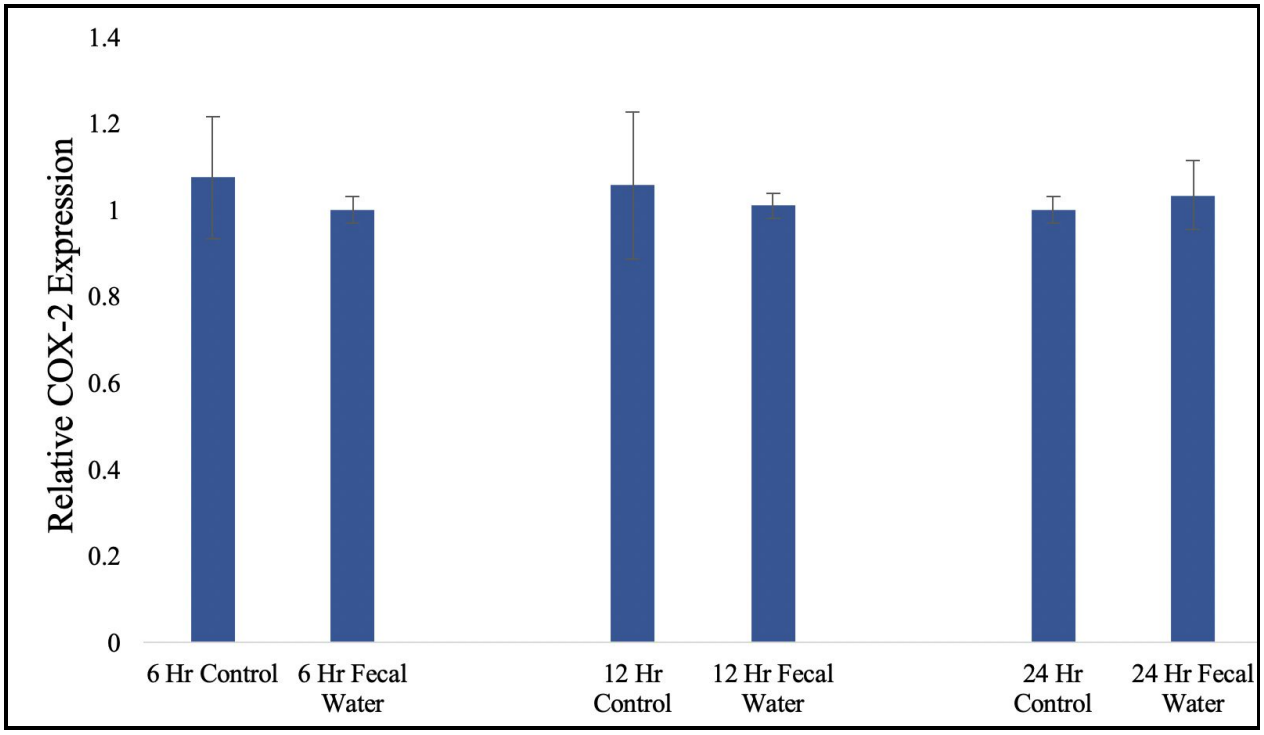

Fig. 8. Relative COX-2 expression generated from qRT PCR, for HCT116 cells exposed to 5% fecal extract for 6, 12, and 24 hours. Error bars: ±SD. n=6 for each treatment level.

Discussion

The experiments in this study were chosen to further understand the combined effects of celecoxib and antibiotics in *Apc*$^{Min/+}$ mice. The second set of experiments focused solely on antibiotics to determine the mechanisms by which the administration of broad-spectrum antibiotics reduces tumor burden and the third set of experiments studied the idea that the gut microbiome could directly manipulate inflammation within the gut through COX-2.

The data demonstrate that suggest that individual treatments with celecoxib and antibiotics are equally effective in decreasing tumor number and size, but the combination of both celecoxib and antibiotics is more effective than individual treatments (Figure 3A/B). The broad-spectrum antibiotics that were used target *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Tenericutes* (Zackular et al., 2013). Celecoxib has been shown to decrease bacterial populations of *Lactobacillaceae* and *Bifidobacteriaceae* as well as increase *Coriobacteriaceae* (Montrose et al., 2016). Targeting different groups of bacteria may explain why the administration of celecoxib after broad-spectrum antibiotics further depletes the gut microbiota of mice, as well as

why the effects are increased in combination. Another possible explanation for the reduction in tumor size shown in Figure 3B is that the germ-free *Apc*$^{Min/+}$ mice have reduced vascularity within the small intestine. When the mice are colonized with microbiota, there is increased vessel density in the small intestine (Reinhardt et al., 2012). If bacteria are important for maintaining vascularity, then if they are removed, tumors could have fewer blood vessels.

Stem cells, which can contribute to therapeutic resistance, are believed to play an important role in the etiology of colorectal cancer. Within the gastrointestinal tract, stem cells were shown to be a critical place of origin for intestinal polyp development (Eun et al., 2019). Data in Figure 5 showed that celecoxib-induced alterations in the microbiota are associated with decreased proliferation selectively in the base of colonic crypts, where stem cells are found. This reduction in basal cell proliferation is likely to contribute to the observed decrease in polyp formation in celecoxib-treated, antibiotic-treated *Apc*$^{Min/+}$ mice. Changes in the gut microbiota causing a reduction in fecal metabolites linked to carcinogenesis are likely to explain why celecoxib inhibits crypt stem cell proliferation (Montrose et al., 2016). Although COX-2 is not expressed in most cells within the normal gastrointestinal tract, it is expressed in tuft cells, a small population of cells found in the normal murine small intestine (Bezencon et al., 2008). It is possible that celecoxib will inhibit COX-2 in this cell type, leading to reduced stem cell proliferation. Moreover, the diminishing of bacteria by antibiotics decreases the number of waste products that are produced by the bacteria, further decreasing proliferation.

The inconclusive results in Figure 6 do not support the possibility that reducing microbial abundance suppresses tumor development by decreasing tumor cell proliferation. It may be that future experiments must be carried out on more murine tissue sections before any conclusions can be made regarding the mechanisms behind antibiotics reducing tumor burden. Figure 7 demonstrated that there is a dose-dependent relationship between the concentration of fecal extract and COX-2 expression. As the concentration of fecal extract increased, the relative fold change in COX-2 expression increased. This supports previous research showing that the luminal contents of the gastrointestinal tract can induce COX-2 expression in HCT116 cells (Glinghammar & Rafter, 2001). There is a strong possibility that the gut microbiota in the fecal

extract that have been shown to contribute to inflammation-based CRC play a role in the increase in COX-2 expression.

The results of Figure 8 demonstrated that the amount of time that HCT116 cells were exposed to fecal extract did not have a significant impact on COX-2 expression. This may be due to the use of 5% fecal extract: although it had worked well when cells were exposed for 4 hours, 6-24 hours of exposure may have been harmful to the cells. Potentially pathogenic *Enterobacteria,* which has been shown to be in the feces of $Apc^{Min/+}$ mice, may have inhibited COX-2 expression in the human colon cancer cells after 4 hours (Leschner & Weiss, 2010).

Although there are several interesting observations in this study, there are also some limitations. It was difficult to accurately demarcate the polyps of mice for data collected in Figure 6; regions on the edges of the polyps were not clearly basal or non-basal. Therefore, the quantitative methods employed for the results in Figure 5 were unable to be used. Collaboration with a board-certified pathologist, Dr. Sui Zee, allowed for the estimation of the percentage of Ki-67 stained cells by eye. Although $Apc^{Min/+}$ mice are a well-established model to study the chemopreventive effects of NSAIDs, their polyps do not always progress to cancer. More relevant models should be explored to determine whether similar effects occur after celecoxib treatment, such as mice with *Apc* mutations in combination with models that have a greater tendency to develop colon tumors. Further experiments should be performed to analyze whether the differences in blood vessel density account for the attenuation of tumor size/number under antibiotics and celecoxib. Further work will also focus on immunostaining more tissues for Ki-67 to see if the difference in the percentage of Ki-67+ cells in control vs. antibiotic-fed $Apc^{Min/+}$ mice is a result of a small sample size.

Conclusion

The goals of this study were to examine the mechanisms by which the administration of antibiotics reduces tumor burden, whether the attenuation of the proliferation-suppressing effects of celecoxib under antibiotics is mediated via the stem cells, and whether the bacterial waste and the gut microbiome could directly manipulate inflammation within the gut. Methods of immunohistochemistry for Ki-67 and qRT PCR were employed to study two substances,

celecoxib and an antibiotic cocktail of metronidazole, vancomycin, and streptomycin, and their role in modulating microbes and intestinal tumor development in *Apc*$^{Min/+}$ mice. A combination of celecoxib and broad-spectrum antibiotics significantly decreased tumor size and number in *Apc*$^{Min/+}$ mice, more effectively than celecoxib or broad-spectrum antibiotics alone. Under antibiotics, the administration of celecoxib resulted in reduced proliferation of normal intestinal crypt stem cells. Further experiments exposing human colon cancer HCT116 cells to varying concentrations of murine fecal extract demonstrated that the gut microbiome could directly manipulate inflammation within the gut through COX-2.

This study highlights the possibility of targeting the gut microbiome as a strategy to prevent the development of inflammation-based colorectal cancer. The ideas of the combination of celecoxib and antibiotics decreasing tumor burden and celecoxib-induced alterations in gut microbiota leading to reduced stem cell proliferation are extremely applicable to the development of new chemopreventive strategies.

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