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Thesis

THE ROLE OF MICROBIOTA IN GASTROINTESTINAL CANCERS

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NORBERT SMIETALO

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Approved by

First Reader

Oscar Dominguez, Ph.D.
Assistant Professor of Medicine

Second Reader

Olga Kaczocha, Ph.D.
Associate Professor of Biochemistry
Stony Brook University

Third Reader

Flore Bogdan, Ph.D.
Support Specialist
Stony Brook University

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To my friends, family, and mentors who have supported me every step of the way.

THE ROLE OF MICROBIOTA IN GASTROINTESTINAL CANCERS

NORBERT SMIETALO

ABSTRACT

The human microbiome plays an integral role in a healthy gastrointestinal (GI) tract. Interestingly, the GI tract is a location where microbes and their byproducts, host epithelium, and host immunity each interact with one another. As a result of this complex interplay, a signal imbalance of one may result in dysfunction. Recently, microbiota have been implicated in many disease states involving the GI tract, including chronic inflammatory states and cancer development. Their role in promoting or deterring against chronic inflammatory states is difficult to assess as their number within an intestinal population is exceedingly large, as well as the variation of microbial species found within human individuals. NSAIDs, antibiotics, and, more recently probiotics, have begun to increase in popularity for the treatment of inflammatory bowel states. Understanding the difference between “good” bacteria and “bad” bacteria can help research preventive and treatment measures for patients suffering from a microbial imbalance.

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LIST OF ABBREVIATIONS

APC.....	adenomatous polyposis coli
CAC	colitis associated cancer
CD.....	Crohn's disease
CDC	C. difficile associated disease
COX.....	cyclooxygenase
CRC.....	colorectal cancer
DSS	dextran sulfate sodium
GI	gastrointestinal
GSK3 β	glycogen synthase kinase 3 beta
HB-EGF	heparin binding EGF-like growth factor
IBD.....	irritable bowel disease
IFN	interferon
IL.....	interleukin
LPS.....	lipopolysaccharide
LTA.....	lipoteichoic acid
MALT	mucosa associated lymphoid tissue
NFATC2	nuclear factor of activated T cells 2
NF- κ B	nuclear factor kappa-light-chain-enhancer of B cells
NSAID	non-steroidal anti-inflammatory drug
PI3K	phosphoinositide 3 kinase

RNI.....reactive nitrogen intermediate
ROS.....reactive oxygen species
STAT3..... signal transducer and activator of transcription 3
TLR.....toll-like receptor
TNF.....tumor necrosis factor
UC.....ulcerative colitis

INTRODUCTION

An important aspect of the digestive tract is the interaction between the microbiota and the host immune system. Quickly after birth, the mucosal surfaces exposed to air become colonized by microbes, including the gastrointestinal tract, the urogenital tract, skin, and lungs [1, 2]. Microbes established within the intestinal system are crucial to the health and stability of the tract. Normal function of gut microbes includes extraction of nutrients and energy from the diet, partly by catalysis of indigestible plant product as well as production of short chain fatty acids, contribution to host immune function, and protection from exogenous pathogens [2,3]. Additionally, the gut microbiota can alter the local pH, activate host immune cells, starve invaders by metabolizing available nutrients, and maintain mucosal barriers and epithelial integrity [3-7]. The importance of a healthy gut microbiota cannot be understated and it has been shown that increased microbial variation is linked with better health [8]. Dysbiosis can be defined as the opposite, an imbalance of the microbial population, which is correlated with poor health [9].

Dysbiosis of the gut has been shown to be associated with multiple disorders, including obesity, ulcerative colitis (UC), Crohn's disease (CD), irritable bowel disease (IBD), and multiple cancers [10-12]. Specifically, this dysbiosis may be due to change in number, diversity, and stability of the microbial ecosystems [13]. These imbalances cause concern because chronic bowel inflammation has been shown to

result in elevated risk of colorectal cancer (CRC) [14]. Colorectal cancer is the 3rd most prevalent form of cancer worldwide. As only 20% of CRC cases are attributed to genetic disorders, other factors, including effects of the microbiome, are important to our understanding of the cause and progression of this disease [15, 16]. A specific form of CRC known as colitis associated cancer (CAC) has been shown to be specifically linked with chronic inflammatory bowel states including UC, IBD, and CD. Specifically, CAC risk tends to increase by 18-20% in UC patients and 10% in CD [17, 18]. The exact increase in risk varies depending on the patient, with factors including severity and duration of the inflammatory process, efficacy of chosen therapy and management of the inflammation, and variability among patients [19]. The difference between CRC and CAC is simple, CRC tumors, by definition, do not arise in the context of preceding inflammation, whereas CAC is always preceded by a chronic inflammatory state [20]. For example, a single injection of the carcinogen azoxymethane, a common inducer of colon cancer, into mouse models with colitis resulted in malignancy whereas wild type mice required multiple injections [21]. It has been shown that in CAC, inflammatory and oncogenic pathways are intertwined [20]. Clearly, inflammation plays an important role in cancer risk and formation.

The purpose of this study is to analyze the literature to understand the great microbial variation between populations and what constitutes a favorable and an unfavorable environment. This will help us understand the impact gut microbes have on

chronic inflammatory processes and how specific microbial populations may promote or inhibit chronic inflammation. Furthermore, the exact inflammatory mechanisms microbes may interact with will be linked with the oncogenic pathways commonly implicated in CAC cases. Lastly, treatment and prevention options will be researched to evaluate the candidacy of targeting microbial inflammatory pathways.

PUBLISHED STUDIES

Microbial Variation Among Population

With over 1000 species of microbes found within a typical gut and about 10^{13-14} cells comprising 90% of the DNA found within the human body, it can be daunting to characterize the variation found within the microbiome [22]. However, it is crucial to understand in the context of IBD and CAC development as certain microorganisms have been found to induce pro-inflammatory pathways, through cytokines, including IL-17 and IFN γ , and others found to induce suppressive pathways, including IL-10. [23, 24]. For example, certain genera elevated in CD and UC patients include Chlamydia, Mycobacterium, Candida, and various E. coli species [25]. Conversely, certain species of the Firmicutes and Bacteroidetes phyla have been found to be decreased in IBD [26]. This indicates there may be certain species that fall within the “bad” and “good” groups of bacteria.

Multiple attempts at characterizing the human microbiome are currently underway, including the metaHIT project and the Human Microbiome project [27]. Both share the goal of characterizing what is defined as a healthy microbiome and what differences may occur that result in disease. Their focus include the diversity amongst different human populations as well as factors that may cause diversity throughout a human’s lifespan. One question arises when classifying the microbiome regarding whether to focus on classifying taxonomically or functionally. Studies have shown that

>1000 phylotypes, or clusters of sequences with as much diversity within the small subunit rRNA genes in a single species, can be found within an individual's gut [28]. The most dominating phyla have been found to comprise less than 0.5% of the microbial population, resulting in difficulty defining a core set of species found amongst many humans. On the other hand, one confirmed study showed 18 females shared greater than 93% of enzyme functional groups, but few genus phylotypes [29], indicating a core set of functional groups exists amongst individuals despite stemming from different species of bacteria. Unsurprisingly, many of these shared metabolic pathways involved carbohydrate and amino acid metabolic pathways, pathways which are vital for survival [29]. Therefore, it becomes much more feasible to classify differences in enzyme and function rather than taxonomic classification.

Shortly after birth, the human microbiome is colonized by bacteria and remains dynamic during the first few years of life. Stability and diversity tend to increase rapidly during the first three years, marking this as a crucial period for microbiome development [30]. Factors which may have wide bearing affects during this time period include antibiotic administration, breastfeeding, and mode of childbirth delivery [31]. The implications of each are not well understood, though they are believed to be linked in childhood asthma and eczema.

A key player in the development, variety, and stabilization of an individual's microbiome is the intestinal environment resulting from the diet. For example,

individuals with a high fiber diet from areas of Africa, Venezuela, and Malawi have been shown to have gut microbiome dominated by the genus *Prevotella*, whereas individuals with diets rich in protein and saturated fats are found to have guts dominated by species of the genus *Bacteroides* [32]. Interestingly, IBD is more common in these Western diets comprised of protein and saturated fats when compared with high fiber diets [33]. An additional variation among populations is found when considering the digestion of soy products. (S)-equol is a bacterial byproduct from the digestion of soy and is implicated in positive outcomes for various disorders, including vasomotor symptoms, osteoporosis, prostate cancer, and cardiovascular disease [34]. However, when a Western population is fed soy, 25-30% of the population is found to produce (S)-equol, while 50-60% of the Chinese, Korean, and Japanese populations tested produced (S)-equol when fed soy [35]. This indicates soy may not be as beneficial to different populations while also suggesting other compounds may have different effects depending on the population tested.

Acetaminophen is one example of microbial byproducts altering metabolism. In populations with gut microbes which produce p-cresol, substrate competition occurs. P-cresol competes with sulfonation of acetaminophen, resulting in decreased concentrations of acetaminophen sulfate and increased concentrations of acetaminophen glucuronide through a different pathway, altering overall efficacy and toxicity [36]. However, the populations with which elevated p-cresol is found is not well understood. This finding indicates that many compounds when ingested may have differing effects when compared between different populations.

Understanding that an individual's microbiome has effects, both positive and negative, on the GI tract, one may ask if it is possible to alter the microbial population. Once established, an individual's gut remains in a steady state, where the dominant microbial population resists change and outcompetes exogenous microbe, both beneficial and harmful. Perturbation can be defined as the amount of stress required to result in a different, stable community within the gut [37]. In a human trial, two courses of ciprofloxacin were given over 10 months. By the end of the trial, the microbiota found within stool samples for most individuals had been found to return to the original state, though the speed, extent of change, and recovery did vary between individuals [38]. With one individual in the study, the first treatment resulted in a slow, unstable, microbiome recovery while the second treatment resulted in fast and stable recovery, suggesting the first treatment may have resulted in antibiotic resistance development. Another individual in the same study differed with the first treatment resulting in a quick, stable microbiome recovery to original state, but the second treatment resulted in stabilization to a different state, suggesting decreased resistance to perturbation. In another study, three individuals suffering from dyspepsia were given 1 week metronidazole, clarithromycin, and omeprazole treatment, which resulted in a microbiome state shift lasting 4 years with no additional antibiotic treatment [39]. Given these findings, it seems perturbation using antibiotics varies among individuals. Rather than using antibiotic treatment, additional bacteria may be introduced into the system. Phylogenetically similar bacteria have been found to promote each other's survival and not compete with each other [40]. This

finding is thought to be due to each group promoting an environment which is mutually beneficial. However, in mouse models with relatively homogenous gut microbes, susceptibility to colonization by exogenous pathogens or exogenous commensal bacteria was found to be higher than mice with more diverse microbiota [41]. This finding indicates that a highly diverse microbiota is beneficial in defending against pathogenic invaders.

Key Inflammatory Pathways

Before studying the effects that microbial misbalance has on the mucosal layers and its immunologic defense, it will be useful to understand the key pathways involved. Within the gut, the β -catenin is the main pathway involved in epithelial renewal [42]. Hyperactive activation of the pathway results in enhanced cell proliferation from the crypt and reduces cell shedding [43]. Common mutations which cause CAC include mutations in genes encoding APC, a tumor suppressor, and GSK3B, a kinase, which controls APC and β -catenin activity [42]. Early mutations in APC are found in over 30% of human CRC cases [44]. However, in CAC mouse models, mutations in p53 and k-ras are found initially while up-regulatory β -catenin and inhibitory APC mutations are found later in tumor development [45]. The question arises, why is β -catenin and APC crucial for the initiation of sporadic CRC development, whereas in CAC these mutations occur much later? Inflammation may be a cause of up-regulation of the pathway without the need for oncogenic alterations. This is supported by the finding that mice lacking IL-10

may form colitis and resulting colorectal tumors, which have elevated β -catenin activity but lack APC mutations [46]. Additionally, soluble mediators, including prostaglandin E2 are found to be elevated during colitis induced inflammation [47]. These soluble mediators act on the AKT, PI3K, and NF- κ B pathways which also enhance β -catenin activity in the absence of APC mutations. [48, 49]. These findings indicate that oncogenic mutations are not required to promote hyperactive cell proliferation, but inflammation may be a driving force as well. Pro-inflammatory immune cells, including neutrophils and activated macrophages can also have inflammatory and oncogenic effects through the production of reactive nitrogen intermediates (RNIs) and reactive oxygen species (ROS) [50]. Specifically in regards with ROS, oxidative inactivation of mismatch repair and other DNA repair mechanisms have been linked to CAC development [51].

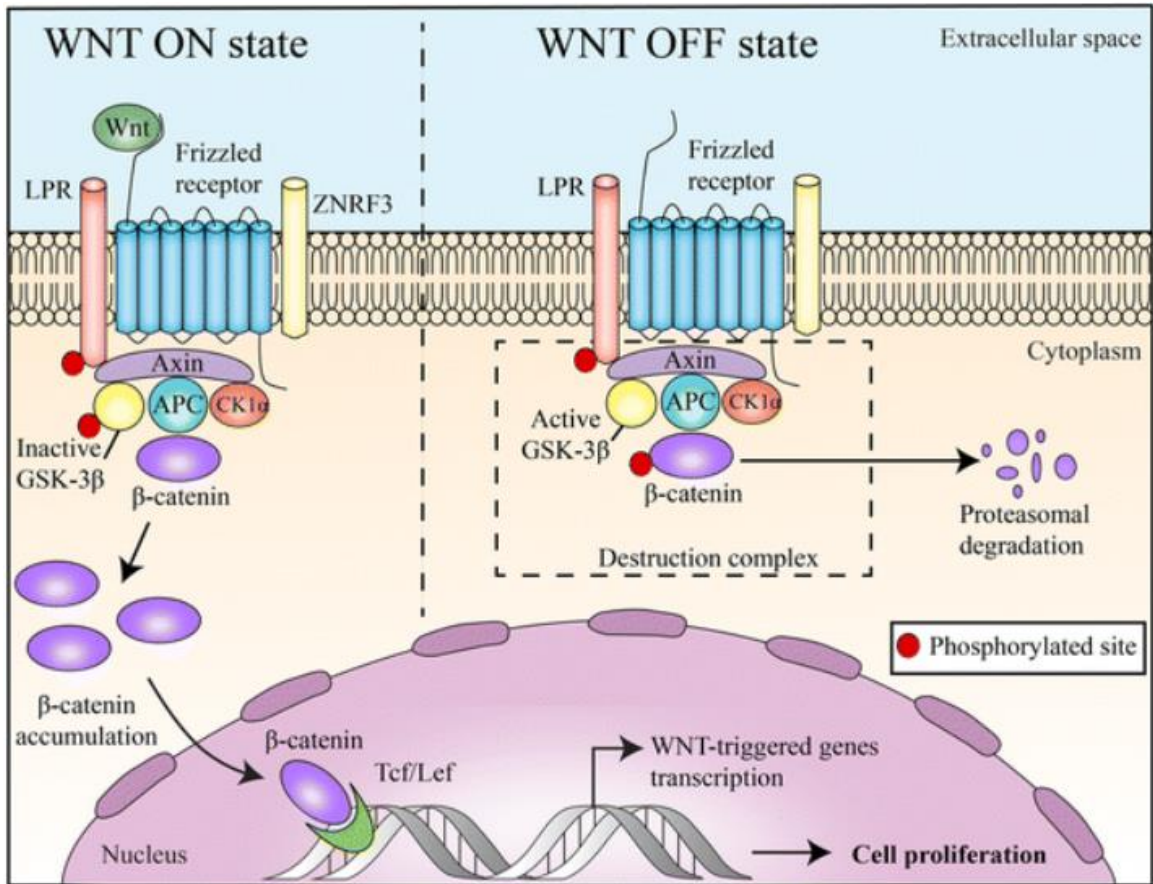


Figure 1: The role of WNT signaling in intestinal epithelial renewal. Active WNT promotes activated β -catenin accumulation, which subsequently translocates to the nucleus and activates transcription of proliferative proteins [132].

A commonality between guts afflicted by IBD and guts afflicted by CAC is elevation of NF- κ B activity, a transcription factor regulating inflammatory pathways [52]. Patients suffering CAC have increased NF- κ B activity within immune cells as well as within malignant epithelial cells [53]. Many IBD treatments act by targeting NF- κ B either directly or indirectly, resulting in symptom improvement, with one

study utilizing antisense phosphorothioate oligonucleotides which bind and inhibit NF- κ B activity [54]. In a different study, mouse CAC models lacking NF- κ B expression within epithelial cells were treated with pro-inflammatory compound DSS, resulting in increased inflammation but fewer numbers of tumors [55]. This is thought to be due to NF- κ B control of anti-apoptotic gene expression and in these mice, premalignant cells were able to produce an apoptotic response in the absence of NF- κ B activity. In another study, mouse CAC models with myeloid cells lacking NF- κ B were shown to have reduced tumor number and size, thought to be due to pro-inflammatory cytokine production being crucial to tumor initiation and growth which were lacking in these mice [56]. Clinically, TNF and IL-1 are two of these pro-inflammatory and tumor-promoting cytokines successfully being targeted for the treatment of IBD [57]. However, in the case of untreated and chronic inflammation, pathways between pro-inflammatory and pro-tumorigenic pathways will interweave.

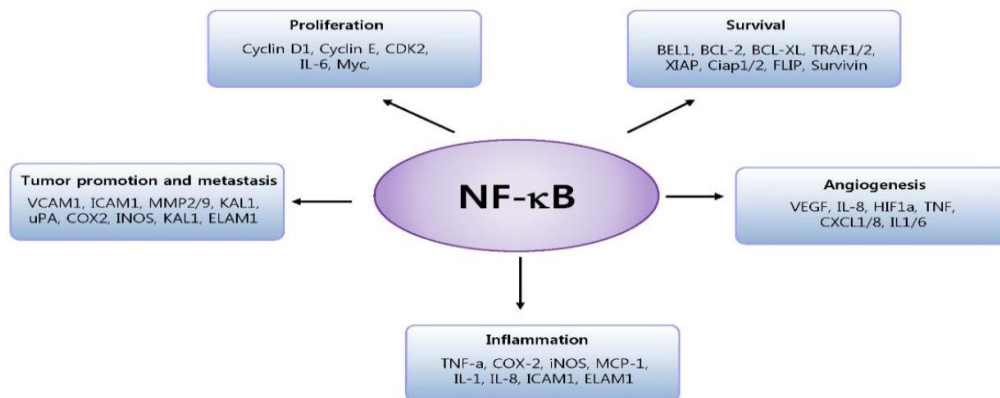
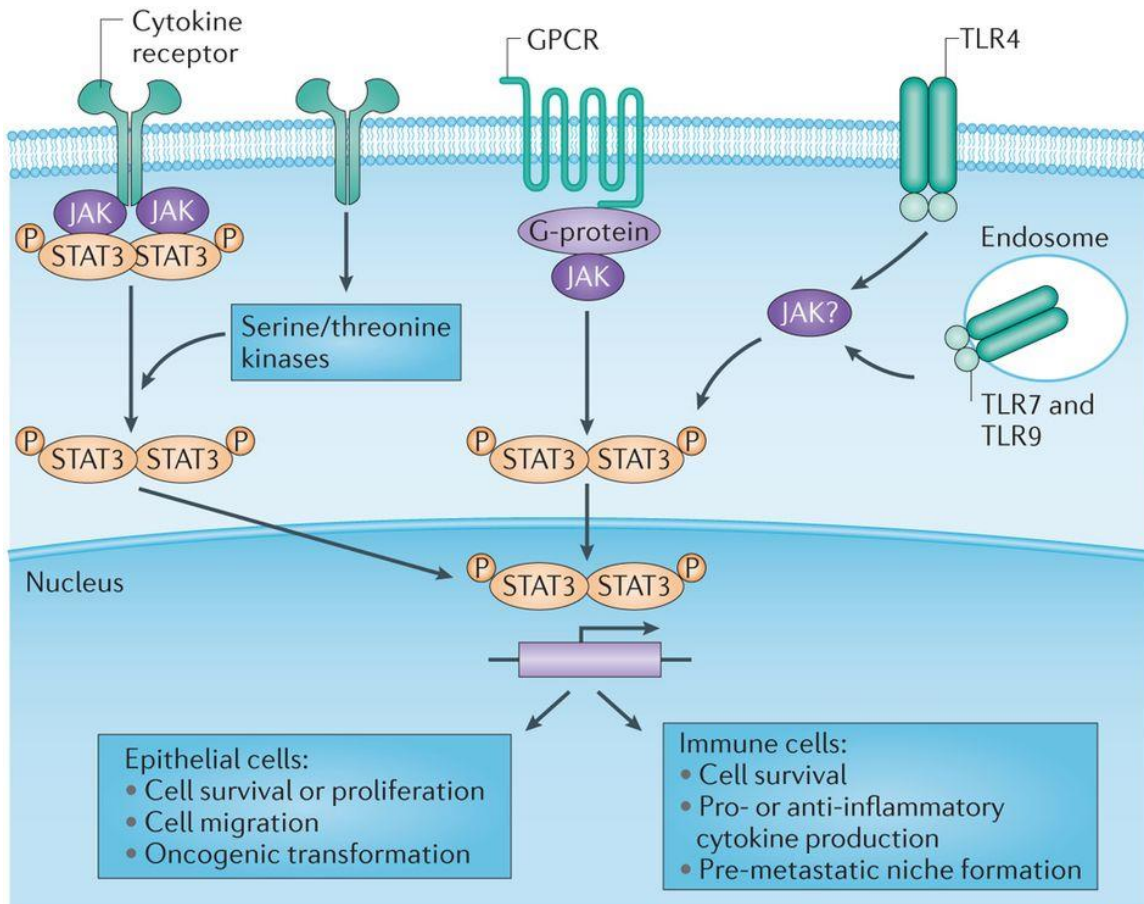


Figure 2. Summary of NF- κ B downstream effectors. NF- κ B is known to promote key pathways which result in neoplastic characteristics of epithelial cells [133].

Genetically, efforts have been made to detail the early events of CAC development. Initially, p53 mutations were found early in inflamed tissues [58]. Additionally, epigenetics plays a role, specifically with methylation of DNA and histones encoding APC, INK4a, and MLH were found in CAC. [59]. DNA methyltransferases were also found to be upregulated by inflammatory cytokines IL-6 and IL-22 [60]. Interestingly, miRNA silencing has also been shown to be activated by chronic inflammation and seen in CAC [61]. STAT3 and NF- κ B are crucial transcription factors implicated in their role in CAC development. Removal of STAT3 or NF- κ B expression in intestinal epithelial cells results in reduced expression of anti-apoptotic genes, including Bcl-2 and Bcl-xL, indicating their upregulation through inflammatory processes is crucial for hyperplastic cell survival. [62]. STAT3 mediated activation through cytokines is not only an important step in regulating these anti-apoptotic genes, but also cell cycle regulators, growth factor receptors, and resistance factors [63]. Therefore, it becomes important to understand the activators of STAT3 and its downstream effects. IL-6 is a potent activator of STAT3 which activates PI3K through JAK/STAT and Ras through GPCRs, each found on both malignant and normal epithelial cells [64]. One of the many downstream effects of Ras includes secretion of IL-8 which will result in increased immune cell infiltration, angiogenesis, and increased tumor size [65]. Immunologically, IL-6 has been shown to increase survival of T cells, promote differentiation to Th-17 cells, and suppress Treg cells within the gut [66]. Th-17 cell differentiation results in reduced mucin production as well as cadherin expression, crucial in tumorigenesis as

well as mucosal exposure to inflammatory cytokines and pro-inflammatory microbial byproducts [67]. With these findings in mind it is no surprise to see that inhibition of IL-6 has been shown to be effective in treating IBD in both mouse models and human trials [68, 69]. In the context of CAC, IL-6 is elevated systemically as well as locally within the gut, promoting cell proliferation [70]. In CAC, T-cell transcription factor NFATC2 and myeloid cell transcription NF- κ B are responsible for the production of IL-6 [71]. This finding illustrates the interplay between NF- κ B and STAT3 pathways. Interesting to note, deletion of STAT3 from neutrophils and activated macrophages within mice results in colitis development, likely due to an upregulated Th1 response [72]. An additional crucial cytokine for the development of chronic inflammation is IL-23. Polymorphisms in its promoter have been associated with increases and decreases in risk for chronic inflammation [73]. IL-23 is normally found within the colon and distal ileus, but in CAC, IL-23 is severely increased throughout the colon [74]. Suppression of IL23 and its receptor has been shown to reduce tumor size and number, as well as reduce concentrations of inflammatory cytokines in CAC mouse models. With chronically elevated pro-inflammatory cytokines and growth factors, premalignant epithelial cells will increase in proliferation and reduce in protective apoptosis. Once malignancy has been established, solid tumors are then able to continue upregulating these pro-inflammatory cytokines, propelling tumor progression and eventual metastasis [14]. These studies have shown that oncogenic mutations are not required for the initiation of tumor development. Prior chronic inflammatory states are sufficient in promoting hyperplasia, which then may lead

to oncogenic development if left untreated.



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Figure 3. Summary of STAT3 actions. Overactive STAT3 signaling may result in transcription of proteins which cause cell survival and proliferation [134].

Microbiota: The Link Between Inflammation and Tumorigenesis

Establishing multiple pathways of chronic inflammation begs the question of how the microbiome plays a role in tumor development. For example, one study showed IL-10 knockout mice are more likely to develop colitis under normal conditions, but when

grown in pathogen free conditions the colitis was less likely and less severe [75]. In a follow up study, an uncontrolled Th1 response resulted in worsening colitis and subsequent carcinoma formation in the same knockout mice [76]. In a similar study, gut sterilization in IL-10 knockout mice resulted in reduced colitis and CAC tumors, but chronic inflammatory processes were sufficient in promoting tumor initiation [77]. The study indicated chronic inflammation in IBD and colitis were sufficient in producing a genotoxic result. In the context of chronic inflammatory processes of the gut, many disruptions of the epithelial-mucus barrier may arise which provides an opportunity for bacterial infection to promote tumorigenesis [78]. Important to note, similar disruptions may be caused by colitogenic chemicals and infectious agents which cause colonic injury, including both DSS and enterotoxigenic bacteria, *Bacteroides fragilis* [79]. This barrier disruption will result in increased contact between the microbiome and the underlying epithelial and immune cells. Disruptions of the mucus layer or epithelial junctions is one example which increases risk of inflammation as was seen in a study done in mouse models in which a crucial component of the mucus layer, Muc2, was inactivated. Initially, the result was intestinal inflammation which progressed into CAC in the absence of additional carcinogenic factors [80]. Expectedly, when repeated in *APC^{-/-}* mice, the result was increased number and size of CAC tumors through supposed inflammation and overactive Wnt signaling [81]. Current IBD therapies are successful in treating symptoms, but unsuccessful in ensuring a full recovery of the mucosal barrier [82]. This may be the cause of IBD flare ups seen in patients, where a localized area of

the intestine is not adequately healed and protected, resulting in episodes of increased inflammation. This conjecture has been confirmed by endomicroscopy, which identified local barrier dysfunction within patients suffering IBD relapse [83]. With prolonged injury, induction of wound healing pathways can result in stem cell proliferation and expansion to repair the damaged mucosa. If these active cells contain oncogenic mutations, repetitive injury and proliferation may result in tumor formation [84]. Additionally, a pro-inflammatory Th-17 response will result in reduced mucin production and cadherin expression, further weakening the mucosal barrier [85]. In the case of established tumor growth, mutations in p120 catenin, a core component of E-cadherin stability, will result in reduced epithelial junction strength [86]. One strain in particular, *Fusobacteria*, has been shown to bind e-cadherin displayed on cancerous cells through FadA adhesion protein, resulting in growth stimulation in addition to infiltration of the bacterium to neighboring tissue, eliciting a tumorigenic immune response [86]. With a damaged mucosal barrier, the epithelial and immune cells are increasingly exposed to the environment rich in microbes and their byproducts. In this case, commensal bacteria are capable of eliciting an overactive immune response. Toll-like receptors (TLRs) are key mediators in activation of the immune response, with the ability to recognize exogenous substrates and subsequently activate pro-inflammatory downstream pathways discussed earlier [88]. For example, a deficiency in TLR-4 signaling, the receptor responsible for binding microbial antigen, lipopolysaccharide (LPS), has been shown to reduce the risk of CAC [89]. Likewise, overexpression of TLR4 will result in an overactive, hyper-

proliferative response and subsequent neoplasia [90]. Mouse models deficient in TLR adaptor protein, MyD88, also exhibit reduced intestinal tumor progression as well as reduced inflammation overall [91]. In humans, elevated TLR4 and MyD88 expression correlate with a poorer CAC prognosis [92]. The result of increased microbial recognition is increased cytokine production, activation of fibroblast infiltration, and further support of tumor growth [93]. Damage to the mucosal barrier within the gut establishes the opportunity for normally commensal bacteria to become harmful, by exposure to microbial antigen to the underlying immune cells and resulting inflammatory response.

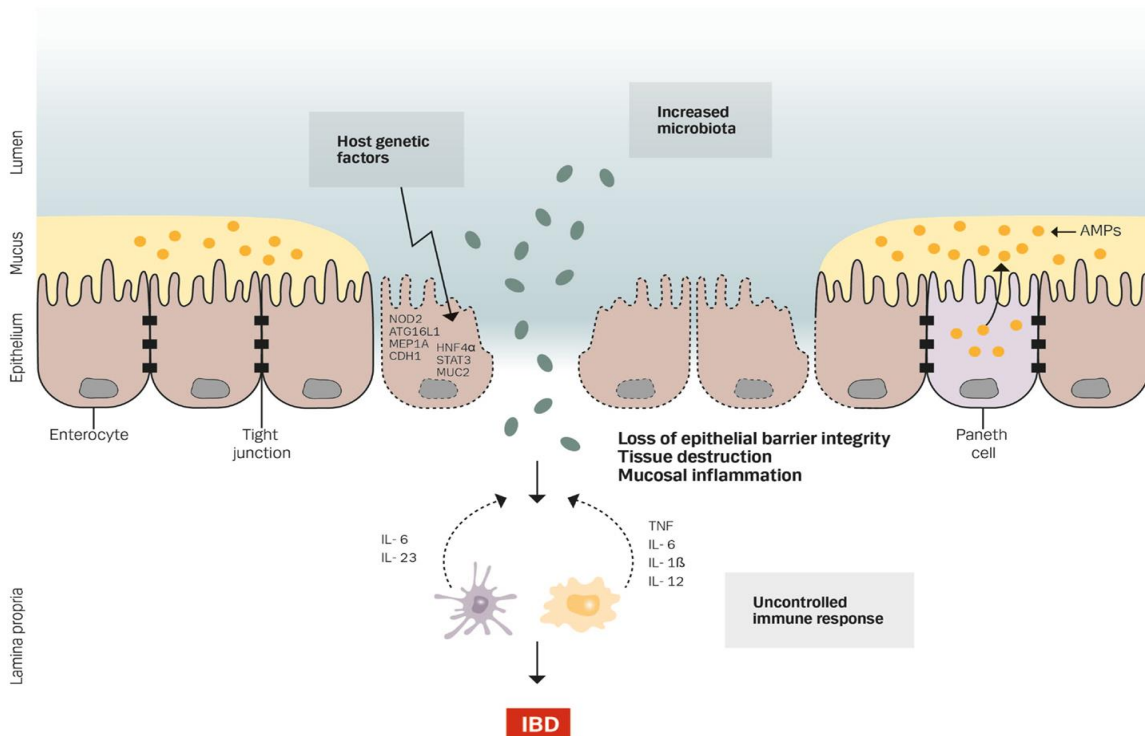


Figure 4. Effects of mucosal damage to underlying epithelium. In the context of sustained epithelial damage, commensal microbes may activate an inflammatory immune response [135].

With the underlying chronic inflammation established and the mucosal barrier exposed to increasing amounts of pro-inflammatory products, the cycle self-perpetuates until malignancy formation if left untreated. In the case of chronic inflammation, the underlying epithelium is exposed to microbial antigens and byproducts, resulting in further inflammation and oncogenic modifications [94]. The commensal microbiome will induce cytokines, including IL-23, IL-6, IL-22, and IL-17, resulting in signaling promoting barrier weakness and adenoma formation [95]. This is shown in HB-EGF transgenic mice, which have increased risk of polyp formation. Failure of the intestinal barrier in these mice allows microbial induction of neutrophil accumulation, subsequent inflammation, and ultimately tumor development [96]. Interestingly, treatment with antibiotics reduced polyp formation while reintroduction of stool samples from pre-treatment resulted in continued polyp formation, indicating bacteria played a role in tumor development. Additionally, when the colonic wall is injured, IL-18 has been shown to downregulate IL-22BP, resulting in an increase of IL-22 signaling and following tumor promotion. [97]. Likewise, inhibition of IL-22 resulted in reduction of inflammation as well as reduction in tumor burden in this microbial CAC mouse model [98]. Through multiple pathways, microbes enhance the pro-inflammatory response, resulting in the activation of multiple proliferative pathways.

One question which arises is if pro-inflammatory microbes are a requirement for IBD which then accelerates disease progression or if specific strains of microbes are

found to be elevated in cases of IBD which promote CAC. Multiple strains of bacteria have been shown to directly exacerbate CAC tumor development, including strains of *E. coli*, *Streptococcus bovis*, *H. pylori*, and *Bacteroides fragilis* [99]. These strains have been shown to attach to epithelial layers of colonic tissue and induce inflammation, proliferation, and resulting hyperplasia [100]. Additionally, toxins may be produced which directly disrupt barrier integrity, damage cells, and ultimately promote inflammatory processes. Specifically, in the case of *E. coli*, there has been a direct link between attachment and tumor infiltration with poor prognosis in humans [101]. It is believed *E. coli* achieves this by attachment to colonic epithelial cells and promotion of hyperplasia and inflammation. It has also been shown that normally commensal bacteria may translocate and subsequently trigger an immune response during intestinal wall injury. [102]. One example of this is a study which demonstrated commensal *E. coli* upregulating IL-17c expression in CAC mouse models, resulting in apoptotic suppression through induction of Bcl-xl and recruitment of lymphocytes, ultimately promoting tumorigenesis [103]. An increase in IL-6 producing lymphocytes and increased epithelial proliferation has been seen in CAC mouse models with inflammosomal protein removal [104]. This is thought to be due to selection of pro-inflammatory microbes which induce upregulation of epithelial CCL5. In the same study, inhibition of IL-6 resulted in reduced inflammation and reduced tumorigenic burden by blocking the effects of the selected pro-inflammatory microbes. In a different study, anti-inflammatory microbes were shown to induce IL-10 secretion in response to intestinal wall injury, resulting in protection from

tumor formation in mouse models [105]. These two studies illustrate that imbalance between pro-inflammatory and anti-inflammatory microbes can result in unfavorable, proliferative states. Virulence factors have also been found to exert tumorigenic effects by damaging DNA and damaging mucosal integrity. One example of this is a study researching strains of *E. coli* which express polyketide synthase, producing a genotoxic compound, which when induced in IL-10^{-/-} mouse models, greatly increased number of colonic tumors [106]. *Bacteroides fragilis* has also been shown to produce a toxin with a number of tumorigenic actions, including e-cadherin cleavage, upregulation of β -catenin activity, Th-17 cell differentiation, and upregulation of NF- κ B signaling [107]. The latter of which is clinically relevant as increased Th17 cell infiltration correlates with further tumor progression and poorer colorectal cancer prognosis [108]. Finally, strains of fusobacterium have been shown to be implicated in IBD and to be enriched in CACs. [109]. There are many examples of specific strains of bacteria enhancing tumorigenesis by disrupting epithelial integrity, activating inflammatory processes, promoting oncogenic mutations, and ultimately resulting in a hyper-proliferative states.

Prevention and Treatment

Understanding the effects of chronic inflammation promoted by microbial interaction with the enteric immune system, it is interesting to consider preventative measures as well as treatment options to reduce the risk of hyperplasia and malignancy formation. Multiple options may be considered, including treatment pathways targeting

microbes and microbial antigen as well as pathways targeting chronic inflammatory processes. Additionally, prevention of a pro-inflammatory state must be considered due to the long-term inflammation increasing risk of CAC development [15].

Antibiotic treatment is an obvious choice in depletion of a pro-inflammatory microbe population. Antibiotic treatment has been proven to improve colitis in both humans and mice who contain a complex microbiome [110]. In this study, IL-10^{-/-} mouse models infected with *Helicobacter* resulted in CAC development unless treatment with antibiotics, improving both IBD and CAC states. In a different colon adenoma mouse model, antibiotic reduction of commensal bacteria was found to reduce inflammation and tumor burden significantly [111]. In human patients with MALT lymphoma, reduction of *H. pylori* population through antibiotic treatment was found to significantly improve outcomes, and in uncommon circumstances, result in cancer remission. [112]. Meta-analysis of multiple antibiotic treatment trials in human IBD patients has shown that broad spectrum antibiotics were successful in improving disease outcomes [113]. Reduction of commensal bacteria using antibiotics has been shown to normalize colon morphology, while also providing increased mucin production and reduction of inflammatory cell infiltration [114]. Overall, antibiotic treatment appears to be a viable option for both treatment of chronic inflammation caused by microbes as well as reduction of tumor growth [114]. However, problems arise when considering consequences of abundant antibiotic usage, including resistance formation as well as

reduction in beneficial, anti-inflammatory microbes.

An additional treatment option may be to target inflammatory pathways themselves. One group has shown NSAIDs and specific cyclooxygenase inhibitors have been successful in reducing the incidence and severity of CRC as well as reduction of tumor growth [115]. Aspirin and NSAIDs have been shown to reduce CRC incidence by up to 50%, and has been shown that the inhibition of COX-2 resulted in reduced inflammation and a reduction in tumor cell proliferation and migration [116]. As previously mentioned, ROSs and RNIs induced by pro-inflammatory microbes are pathways leading to increased inflammation and subsequent proliferation. Expectedly, inhibition of ROS production has been shown to result in a reduction of IBD symptoms in humans [117]. Additionally, activation induced cytidine deaminase is an enzyme found to be overexpressed in many cancers and leads to genetic instability [118]. Targeting this enzyme, both pharmacologically and genetically, has been shown to disrupt colorectal tumorigenesis [119]. Lastly, inhibition of inducible NO synthase, responsible for the production of RNIs, has been shown to be an effective method of reducing DNA damage [120]. As discussed earlier, premalignant cells can be induced to produce ROS and RNI by pro-inflammatory cytokines. Inhibition of chronic inflammatory processes seem to be viable in ameliorating IBD symptoms and CAC development.

An emerging treatment option involves replacement of undesirable, pro-inflammatory bacteria with beneficial bacteria. Because species of *Fusobacterium* have

been implicated in IBD and enriched in CRC patients, depletion of these microbes followed by replacement with anti-inflammatory bacteria may be beneficial [121]. A distinction can be made between different treatment options. Prebiotics can be defined as indigestible foods which selectively promote growth of a desired microbe, while probiotics can be defined as an organism which will result in a health benefit when ingested [122]. Both forms have been increasingly used as treatments in recent years. [123]. One cross-sectional study studied the association between dietary fiber ingestion and the presence of bacteria producing butyrate within stool samples compared between a group with colorectal cancer and a group without colorectal cancer [124]. Their findings showed diets low in fiber did impact the number of butyrate producing bacteria and short chain fatty acid synthesis. Another study showed a diet high in fiber increased methanogenesis in the gut, which resulted in a reduction of hydrogen producing bacteria [123]. The researchers believed this to be impactful because excess colonic hydrogen resulted in decreased NAD regeneration. Ultimately, fiber rich diets have been proven to be beneficial in reducing inflammatory bacteria and promoting growth of anti-inflammatory microbes, including *Faecalibacterium prausnitzii* [125]. Examples of probiotics currently in use include *Lactobacillus* and *Bifidobacterium*, the former of which has been shown to reduce intestinal inflammation and CAC in animal models [126]. However, both have been shown to increase detoxification of toxic metabolites and carcinogens within the colon, promote anti-tumorigenic immune responses, and produce compounds which are anti-oncogenic and anti-mutagenic by interacting directly with

proliferative cells, resulting in growth inhibition [127]. Infection with LTA deficient *Lactobacillus acidophilus* has also been shown to reduce colitis and cancer development in mice [128]. Both prebiotic and probiotic treatment appears to be beneficial in the treatment of IBD and prevention of CAC. One final form of bacteriotherapy which shows promise has been fecal transplantation. The treatment includes the introduction of fecal homogenate from a healthy donor into the cecum of the patient. In otherwise untreatable cases of IBD, fecal transplantation has been shown to have positive outcomes in humans [129]. In another case, patients suffering from *C. diff* induced colitis, or CDAD, were found to contain stool dominated by *Veillonella* and *Streptococcus* species. When transplanted with donor stool dominated by species of *Bacteroides*, the disease state was resolved and *Bacteroides* were found to be the dominant species 1 month later [130]. In a study evaluating the efficacy of fecal transplantation, 92% of 317 cases were found to have disease clearance [131]. The importance of replacing pro-inflammatory microbes with anti-inflammatory microbes to treat chronic inflammatory disease is crucial, likely due to an established microbial population preventing further reinfection of the gut.

DISCUSSION

The interplay between the human microbiome and the gastrointestinal system is complex. Interactions between microbes and the host immune system lies in a balance between pro-inflammatory and anti-inflammatory states. Researching these immunological interactions is crucial in understanding the chronic inflammatory dysfunctions which affect many today. Difficulty arises when attempting to classify a beneficial microbial ecosystem compared to a harmful ecosystem. Rather than classifying taxonomically, classifying by role in disease progression is a more feasible task. Many strains have been found to affect the intestinal ecosystem in negative ways while others have been found to promote ecological health within the gut. Groups have found diet plays a role in determining the overall health of an individual's microbial ecosystem. Specifically, diets rich in fiber have been shown to be advantageous while diets rich in saturated fats have been disadvantageous. However, due to the settlement of a dominating microbiome shortly after birth, it becomes difficult to assess which diet options are beneficial to one population or another. Regardless, once a chronic inflammatory disease process begins, it is crucial to combat before progression continues.

Previously stated, oncogenic mutations are not a requirement for colorectal cancer initiation. Overactive inflammatory pathways alone are sufficient in promoting hyperplasia, which may subsequently result in oncogenic mutations, further promoting tumorigenesis. Pathways involving NF- κ B and STAT3 result in increased cytokine

production, promoting growth, proliferation, lymphocytic infiltration, and an anti-apoptotic state. Through chronic activation of these inflammatory pathways, the risk of tumorigenesis occurring rises.

The microbiome itself can play a role in the chronic inflammatory state resulting in tumor development. Certain strains of bacteria have been known to produce inflammatory states by interacting with the immune system. Once damage to the mucosal wall is established, otherwise commensal bacteria become exposed to the underlying tissue. This exacerbates disease progression, as continued exposure to microbial antigen results in a chronic inflammatory state.

Multiple treatment options are currently being used and researched for the use of treatment and prevention of chronic inflammation. NSAIDs have been helpful in treatment of IBD patients through the inhibition of cyclooxygenase pathways. The resulting inhibition may ameliorate symptoms, but if mucosal wall integrity has been compromised, IBD flare-ups have been shown to return to the localized damage. Additionally, NSAIDs run the consequence of promoting gastric ulcers. Another treatment option involves antibiotic treatment. Previously stated, the microbiome is susceptible to perturbation. Antibiotics may be useful in exterminating harmful bacteria at the cost of reducing beneficial bacteria populations as well. Overuse of antibiotics is also a detriment as resistance is likely to form. One treatment option involves transplanting beneficial bacteria from a known host to a donor suffering from chronic

inflammation. The success rates with this method are good, especially if the exogenous microbes can colonize the intestine. Treatment with antibiotics beforehand may help perturb the pre-existing microbial population to allow for colonization by donor bacteria. Lastly, probiotic and prebiotic digestion has been gaining in popularity in recent years. With probiotics claiming improved gastrointestinal health with known strains of bacteria which deter inflammation, skeptics believe this method of prevention has challenges. First, the exogenous microbes must pass through the acidic environment of the stomach, thereby eliminating a number of the introduced microbes. Secondly, in a healthy, thriving, and established microbial population, exogenous microbes, both beneficial and harmful, may find difficulty with colonization due to competition for nutrition. The previously described fecal transplantation benefits in avoiding the acidic digestive tract. On the other hand, prebiotics promote a gastrointestinal environment which will favor beneficial bacteria. As previously mentioned, fiber rich diets will promote the growth of anti-inflammatory bacteria, while diets rich in saturated fats may promote the growth of pro-inflammatory bacteria. The distinction between prebiotics and probiotics lies within prevention and treatment. Prevention is preferred over symptom treatment as mucosal wall injury will result in susceptibility to further damage. Additionally, treatment with antibiotics and anti-inflammatory agents have secondary effects. Diet, on the other hand, can prevent the initiation of chronic inflammatory processes. However, in some cases IBD and CAC occur in cases where diet is not the primary promoter of the disease. Further research must be established in those cases to treat patients before tumorigenesis can establish.

LIST OF JOURNAL ABBREVIATIONS

Aliment Pharmacol Ther	Alimentary Pharmacology & Therapeutics
Annu Rev Ecol Evol Syst	Annual Review of Ecology, Evolution, and Systematics
Br J Cancer	British Journal of Cancer
Cell Host Microbe	Cell Host & Microbe
Comp Med	Comparative Medicine
Curr Opin Allergy Clin Immunol	Current Opinion in Allergy and Clinical Immunology
Curr Opin Pharmacol	Current Opinion in Pharmacology
Environ Microbiol	Environmental Microbiology
Genes Dev	Genes & Development
Infect Immun	Infection and Immunity
Inflamm Bowel Dis	Inflammatory Bowel Diseases
J Clin Gastroenterol	Journal of Clinical Gastroenterology
J Clin Invest	Journal of Clinical Investigation
J Exp Med	Journal of Experimental Medicine
J Leukocyte Biol	Journal of Leukocyte Biology
J Nutr	Journal of Nutrition
Mucosal Immunol	Mucosal Immunology
Nat Rev Cancer	Nature Reviews. Cancer
Nat Rev Genet	Nature Reviews. Genetics
Nat Rev Immunol	Nature Reviews. Immunology
Nutr Rev	Nutrition Reviews
PNAS	Proceedings of the National Academy of Sciences of the United States of America

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