Published in final edited form as:
Cancer J. 2014 ; 20(3): 181–189. doi:10.1097/PPO.0000000000000048.

Microbiome, Inflammation and Cancer

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Abstract

Inflammation has long been suspected to play a major role in the pathogenesis of cancer. Only recently however, have some mechanisms of its tumor promoting effects come to light. Microbes, both commensal and pathogenic, are critical regulators of the host immune system, and ultimately, of inflammation. Consequently, microbes have the potential power to influence tumor progression as well, through a wide variety of routes, including chronic activation of inflammation, alteration of tumor microenvironment, induction of genotoxic responses, and metabolism. In this review, we will provide a general overview of commensal microbiota, inflammation and cancer, and how microbes fit into this emerging field.

Introduction

The human body quickly becomes inhabited by microorganisms shortly at birth[1]. Microbes colonize areas that are directly exposed to the air and surroundings, including the mouth, nostrils, skin, stomach, and the gastrointestinal and urogenital tracts[2]. Each environment favors the survival and growth of particular bacteria, and each bacterial niche thus harbors a characteristic collection of microbes. Nevertheless, there is a large variation in the bacterial composition of sites within each organ system between individuals, and the variability is influenced by genetics, diet, antibiotic and medications intake, and other external environmental factors[1-3]. Additionally, the immune system affects types and localization of microbiota, through complex regulation of immune tolerance and inflammation.

The composition (quality and quantity) of microbes in the human body is critical to human health. These ecosystems help the body maintain a number of key processes including digestion of complex plant matter, production of high energy metabolites (for example, short chain fatty acids), immune homeostasis, and protection against pathogenic bacterial species[2,4,5]. Commensal bacteria can outcompete potentially hazardous bacteria by modulating the local environment. The microbiota is thus metabolically active; it exerts its beneficial effects by producing toxins to destroy pathogenic strains of similar species, altering the pH of the local environment[6], metabolizing key nutrients to starve their competitors[7,8], maintaining mucosal layers and epithelial integrity[5,9], and by activating the host immune system[10].

Microbiota diversity is site specific and varies depending on the location in the body, and this diversity (or lack thereof) can correlate with human health. For example, a wide range

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of commensal microbes in the colon is linked to better health[11], while less variety is more beneficial to the overall well being of the vagina[12]. A pathologic imbalance in a microbial community is referred to as dysbiosis[13]. Specific pathogens can also take advantage of the altered microbial ratios, or can cause dysbiosis themselves. The ratios of certain phyla of bacteria are known to be significantly shifted in disorders of the skin, colon, and lung[2] (Figure 1). For example, in psoriasis, a cutaneous inflammatory condition, there is an increased ratio of *Firmicutes* vs. *Actinobacteria*[14], and in spontaneous and maternally transmitted colitis mouse models, the numbers of *Enterobacteriaceae* species are elevated[15]. *Fusobacteria* species have been enriched in colon cancer adenomas and tumors[16,17]. Undoubtedly, dysbiosis is prevalent in diseases of mucosal areas and more research will need to be performed in order to understand the origins of dysbiosis, as well as the mechanisms involved.

Inflammation and cancer

More than 150 years ago Virchow made the first connection between inflammation and cancer by observing leukocytes in neoplastic tissues[18]. Recently, evidence of underlying molecular mechanism has been obtained suggesting that inflammation plays an important role in tumorigenesis and that chronic inflammation increases cancer risk[19]. Up to 10-20% of all cancers can be attributed to infections, often chronic. In more general developmental terms, up to 20% of all cancers are *preceded by chronic inflammation* at the cancer site as exemplified by hepatocellular carcinoma (HCC) and hepatitis, colon cancer (CAC) and inflammatory bowel disease (IBD), and gastric cancer and *H. pylori*-induced gastritis [19,20]. However, the role of inflammation is not limited to its action during tumor initiation and growth; inflammation can also be induced in growing tumor (*"tumor elicited inflammation"*) or as a response to anti-cancer therapy and cell death[19].

Inflammation preceding cancer development: "textbook" examples

IBD and colon cancer risk—IBD is an important risk factor for colon cancer (CRC) development[20], especially in the form called colitis associated cancer (CAC). Indeed, CRC is the third most common malignancy worldwide and is responsible for more than 600,000 deaths per year[21]. Many cytokines and growth factors up regulated in IBD are also highly expressed in CRC, which are vital for tumor growth. Chronic injury that accompanies IBD induces "wound healing like" reactions important to stimulate pre-neoplastic proliferation. Loss of tissue integrity causes stem cells to be more accessible to mutagens and promotes bacterial-driven inflammation in IBD, CAC, and CRC.

Liver inflammation and risk of cancer—Hepatocellular carcinoma (HCC), the most common form of liver cancer, is the third leading cause of cancer deaths worldwide[22]. Infections with Hepatitis B (HBV) or C (HBC) viruses increase the risk of HCC by up to 15-17 fold respectively[22]. Other major risk factors that contribute to HCC are obesity and alcohol consumption[23] and 30% of US adults are now estimated to be obese[22]. While HBV and HCV infections are set to decrease, obesity is clearly on the rise. Obesity results in non-alcoholic fatty liver disease (NAFLD), further progresses to nonalcoholic steatohepatitis (NASH), which leads to cirrhosis (chronic liver disease) and HCC, with obesity overall increasing HCC risk by 5-7 times[22]. A recent study also showed that translocation of

intestinal microbes contributes to hepatic inflammation and fibrosis, in which intestinal microbiota and Toll-like receptors (TLRs) promote HCC[24].

Bacteria, stomach inflammation and gastric cancer—*Helicobacter pylori* is a type of bacterium found in the stomach of about two thirds of the world's population[25] and has long been associated with gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma[26]. *H. pylori* infection is one of the causes of global cancer mortality with 1-3% occurrence in chronically infected individuals[26]. However, *H. pylori* is not acting alone to promote gastric cancer. Some studies have shown that *H. pylori* mono-associated mice developed fewer tumors compared to their germ-free and antibiotic-treated mice[27,28]. In addition, *H. pylori* can produce virulence factors such as *CagA* (cytotoxin-associated gene A), as well as its pathogenic islands (*Cag*PAI) and *VacA* (vacuolating cytotoxin A), which may dysregulate host intracellular signaling pathways and lower the neoplastic transformation threshold[29],. It is known that *CagA* can interact with host proteins to activate downstream signaling pathways, including MEK/ERK pathway[30], NF-κB pathway[31], and β-catenin pathway[32]; thus activating host inflammatory responses and cell proliferation[33]. Contrasting with to its tumor-promoting effects, *H. pylori* infection has been associated with lower risks of some other cancers, including esophageal adenocarcinoma in humans[2] and gastric cardia cancer[25].

Tumor-Elicited Inflammation (TEI)

Even seemingly ‘non-inflammatory’ solid tumors possess a remarkable ability to recruit immune cells and up-regulate pro-inflammatory cytokines and growth factors, which further influence tumor progression and metastasis[19,34]. This process may be important for further malignant progression and spread of tumors, as well as for regulation of resistance to anti-cancer therapies, even if the initial tumor emergence and growth were not controlled by inflammation. The inflammatory mediator CSF-1, has in particular been demonstrated to be critical in the acceleration of tumor development and in the acquisition of metastatic potential via recruitment of a massive amount of macrophages to pre-malignant areas[35]. Additionally, tumor expression of oncogenic Ras is thought to be responsible for the up-regulation of the pro-inflammatory cytokine IL-8, which leads to increased tumor size, immune cell infiltration, and angiogenesis in nude mouse models[36]. Other groups have demonstrated that tumor production of cytokines recruits myeloid cells to the tumor, which in turn secretes IL-6, activating STAT3 and its subsequent downstream pro-oncogenic signaling in tumor cells[37,38]. We have found that damaged epithelial junctions in colon cancer, due to lack of mucin production and decreased cadherin expression, results in a robust “Th17-like” inflammatory response (IL-23 and its downstream cytokines IL-17, Il-22 and Il-6), exacerbating tumor growth and progression[39]. Another study highlights that the loss of tumor suppressor p120-catenin, vital to E-cadherin stability and thus to epithelial junctional integrity[40] increases expression of GM-CSF, M-CSF, MCP-1, and TNF[41], due to disrupted barrier homeostasis. This induces an influx of immature myeloid cells and activated fibroblasts, which continue to support tumor growth.

Not only does inflammation promote primary tumor development, it can also create a metastatic niche in the tumor microenvironment. In mouse model of lung metastasis, lung

cancer cells were shown to cause induction of cytokines from bone marrow derived macrophages that promoted metastasis, through a TLR-2 inflammatory mechanism[42]. Similarly, in an orthotopic breast cancer model, the chemokine CCL2, was found to be a major chemoattractant for inflammatory monocytes, and was critical for the development of a metastatic niche in the lungs, but not for primary tumor development[43,44]. Blockade of Fas signaling, better known for its role in apoptosis, was recently demonstrated to reduce tumor size and metastatic burden in an orthotopic breast cancer model, by reducing tumor production of IL-6, which inhibited immature myeloid cell accumulation into tumors[45]. Taken together, inflammatory cytokines and chemokines produced by cancer cells can attract immature myeloid cells or pro-inflammatory T-helper cells into tumors, creating a pro-tumorigenic microenvironment, stimulating cancer cell growth. Simultaneously, the inflammatory signals can foster a metastatic niche in distant organs, paving the road for secondary tumor development. These are just a few examples of the progress made in this resurgent and exciting field known as “TEI”.

Aspirin and NSAIDs-inhibiting TEI—Several studies on the non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, on CRC risk have demonstrated that their regular use can reduce CRC incidence by up to 50% [46,47]. Additionally, a recent long term study, with 20 years of follow up data, revealed that people who took aspirin (at least 75 mg) regularly had 40-50% reduction in CRC risk, and a 70% reduction of CRC risk was observed if taken for 5 or more years[46]. Furthermore, in a meta-analysis of four aspirin trials, overall adenoma risk was decreased by 17% over a 3-4 year trial period, while advanced lesions were decreased by 28%. Numerous studies have also determined that non-aspirin NSAIDs reduced CRC risk as well, by as much as 56%, depending on the location of the cancer and the duration of the therapy[48], illustrating that inhibition of inflammation is the key.

Presumably, aspirin and other NSAIDs prevent/treat colon cancer by inhibiting the COX-2 (cyclooxygenase-2) enzyme. The major mechanisms by which NSAIDs treat cancer are by either limiting tumor promoting inflammation, or by directly acting on tumor cells, via reduced proliferation and migration. Indeed, selective COX-2 inhibitors, such as Celecoxib, reduced the number of intestinal polyps in patients with FAP (Familial Adenomatous Polyposis) and reduced CRC risk[49].

Microbiota and Cancer: Important Mouse Models

While it is well established that inflammation can promote cancer development and progression, and that microbiota is an essential regulator of inflammatory response, a potentially more direct link between microbiota and cancer is incompletely understood. Studies of germ free (gnotobiotic) mice, and various mice with defective immune pathways, have yielded great insight into the role of microbiota and cancer. A study on the IL-10 knockout mouse reveals that the mutant mice develop spontaneous colitis under conventional conditions, but the disease is less severe when mice are housed in specific pathogen free (SPF) conditions[50]. In a follow up study, the same group discovered that an uncontrolled Th1 response, most likely in response to microbiota, exacerbates colitis in IL-10 deficient mice and results in adenocarcinoma formation in older mutant mice[51].

Similarly, mice with conditional deletion of STAT3 from macrophages and neutrophils develop chronic colitis, likely through disrupted IL-10 signaling and over active Th1 responses[52]. These studies suggest that resident commensal bacteria can trigger exaggerated immune responses (colitis) when key components of immune tolerance are broken. Germ free IL-10 KO mice fail to develop colitis, and have no evidence of abnormal immune system activation[53], but when colonized with a pathogenic NC101 *E. coli* strain, they develop tumors much more readily, possibly due to increased DNA damage[54].

Additional studies on toll-like receptor (TLR) signaling in mice have also contributed to our understanding of microbes and cancers[55]. Mice deficient in TLR-4, the major receptor for LPS are much less susceptible to colitis-associated cancer[56]. Overexpression of TLR-4 in the intestinal epithelium of mice results in hyper-proliferation of crypts and expansion of the stem cell population[57]. Administration of azoxymethane (AOM) to TLR-4 mice increases β-catenin activation and results in more spontaneous tumors compared to WT mice. Moreover, multiple mouse studies involving the knockout of a key TLR adaptor protein, MyD88, further illustrates the connection between host microbial sensing and the development of cancer[58]. $Apc^{Min/+}$ mice devoid of MyD88 have a delayed progression of spontaneous intestinal tumors and reduced expression of inflammatory mediators[59]. In a skin papilloma and a fibrosarcoma model, MyD88 has also been shown to promote tumorigenesis, presumably through inflammatory cytokines as well[60]. In all of these models, the over-amplification of inflammation is the promoter of carcinogenesis, and microbiota can also elicit pro-tumorigenic responses. Elevated TLR-4 and MyD88 correlate with poor prognosis in human colon cancer as well[61]. Thus, microbial sensing through TLR/MyD88 can promote tumor development.

Antibiotic treatment in mouse models has solidified the role of microbiota in cancer, by ameliorating inflammation and limiting cancer progression. This, however, does not imply that prolonged usage of antibiotics may be somehow beneficial for the prevention and treatment of human cancers, apart from those where eradication of single pathogen/carcinogen (i.e. *H. pylori*) may actually prevent tumor development. Antibiotic depletion of microbiota reduces tumor burden in $Nod1^{-/-}$ mice, which are more prone to developing colitis-associated tumors[62]. In patients with gastric mucosa-associated lymphoid tissue (MALT) lymphoma, eradication of *H. pylori* with antibiotics greatly improves the outcome of many patients, and, in some cases, cures them[63]. In a large analysis of antibiotic trials in patients with IBD, it was demonstrated that broad-spectrum antibiotic treatment ameliorated disease[64], confirming that microbiota play an essential role in promoting pro-tumorigenic inflammation. In a colon adenoma mouse model, antibiotic ablation of commensal bacteria reduced tumor burden and inflammatory signature significantly[65].

From Microbiota to Cancer Progression: Inflammation as the Link

So how do microbes truly promote cancer development and progression? Do they initiate a protumorigenic microenvironment or are they simply a consequence of cancer? Presumably, the answer lies somewhere in the middle. One commonality across many diseases in which microbiota contribute to progression is the disruption of the mucosal/epithelial layers of organs, allowing bacteria (or bacterial products and their metabolites) to enter compartments

that are not normally in close proximity to microbes. This can trigger a local chronic inflammatory response, due to perpetually injured tissue and thus a constant stream of infiltrating microbes/microbial products. For example, in IBD and CRC, the underlying mucosal barrier is disrupted, either by genetic defect or by rapidly expanding tumor cells, exposing the colon tissue and resident immune cells to large amounts of microbial antigens and products[65,66]. This, in turn, accelerates tumor progression through pro-tumorigenic cytokines and chemokines that can act as growth factors, activate wound-healing programs, induce migration, and promote angiogenesis. A recent study has demonstrated that commensal microbiota induces IL-23 and IL-17, IL-22 and IL-6 signaling in colon adenoma mouse models, due to defects in colon barrier integrity, and antibiotic treatment or genetic ablation of IL-23 abrogates tumorigenesis[39]. Along these same lines, barrier defects in the intestines of HBUS mice (HB-EGF transgenic mice, predisposed to polyp formation) were shown to allow microbes to induce neutrophil accumulation and inflammation, which promoted cancer development[67]. Antibiotic treatment could reverse polyp formation, and reintroduction of stool from polyp bearing mice could re-induce polyp formation, indicating the importance of microbiota in neoplastic transformation.

Control of IL-22 signaling has proven to be important in CRC models. IL-18 was shown to down-regulate IL-22BP during injury to the colon, which allowed an increase in IL-22 signaling, that if left unchecked, promoted tumorigenesis[68]. Similarly, inhibition of IL-22 signaling was shown to reduce inflammation and tumor burden in a microbial driven CRC model[69]. Antibiotic depletion of commensals results in normalization of colon morphology, increased mucin production, and reduction of infiltrating inflammatory cells, reversing the effects of matriptase depletion[70]. Commensal *E. coli* up-regulate IL-17C expression in $APC^{min/+}$ mice, as well as in colitis associated cancer mouse model, which increased tumor cell growth through suppression of apoptosis, by induction of BCL_{XL}, and recruitment of tumor promoting lymphocytes[71]. Ablation of inflammasome proteins, such as NLRP6, selects for “colitogenic” microbes that cause colon inflammation and advanced CAC development, and this is mediated through bacterially induced up-regulation of CCL-5 from epithelial cells, resulting in an influx of IL-6 producing immune cells and increased epithelial proliferation[72]. Inhibition of IL-6 signaling significantly reduces inflammation and tumor burden, and blocks the effect of transferred colitogenic microbes. These studies support the notion that bacterial localization is critical in the regulation of inflammation in the colon and the breakdown of epithelial or mucosal layer integrity is a major physiological mechanism by which microbiota can promote carcinogenesis.

Clearly, commensals can exacerbate CRC progression, as demonstrated by these studies, antibiotic treatment in cancer mouse models, and inhibition of the microbial sensing pathways (TLR/MyD88)[39,58]. Recently, several studies have explored the contribution of immune tolerance and commensal microbiota in the colon. T-cell derived IL-10 protects DSS treated $APC^{\Delta 468}$ mice from microbe-induced intestinal polyp formation[73]. Mice with T-cell specific ablation of IL-10 have reduced pro-tumorigenic infiltrating eosinophils, and thus fewer numbers of polyps. Commensals were also shown to help prevent inflammation and, therefore, inflammation-associated tumorigenesis, by promoting a normal wound healing program, characterized by acute inflammation and then epithelial normalization[74].

Germ free mice have delayed epithelial proliferation, but after roughly one month, exhibit hyper proliferation and no apparent repair of the epithelial layers, while specific pathogen free (SPF) mice are protected from this damage. Interestingly, knockout of TLR/MyD88 in these germ free mice alleviates colitis and stunted tumor growth, suggesting that the TLR/MyD88 pathway may have both microbe dependent and independent mechanisms (danger signals). Therefore, tumor promoting inflammation can be induced in the absence of microbes (in a chemical model) and still promote inflammation associated tumorigenesis, indicating that the requirement for inflammation for tumor growth trumps the potential direct involvement of microbes stimulating tumor growth, i.e. that in many instances, microbes are needed to induce inflammation and do not act directly on the cancerous cells. Therefore, the delicate balance between microbes, host immune system, and inflammation is critical to the development or prevention of cancer.

Pathogenic Bacteria and Cancer

In addition to an imbalance in commensal bacterial composition, pathogenic bacteria play a large role in many diseases, including colorectal cancer. There are a large number of pathogenic microbes known to promote CRC including certain strains of Escherichia coli Streptococcus bovis (now S. gallolyticus), *Helicobacter pylori*, *Bacteroides fragilis*, *Enterococcus spp*, and some members of the Enterobacteriaceae family[75,76]. These microbes can attach to epithelial layers of the target tissue (colon for example), and directly induce proliferation of epithelial cells, which can lead to hyperplasia. In addition, they can produce toxins that can disrupt the integrity of the epithelial barrier, damage cells, and cause inflammation. As this topic has been highlighted in several recent reviews[77-79], we will briefly discuss a few key examples of how pathogenic bacteria can influence cancer progression. *E. coli* is one of the most extensively studied microbes in the context of CRC. A direct link between *E. coli* and its attachment and infiltration of tumors has been established, which correlates with poor prognosis in humans[80-82]. *E. coli* can promote tumor progression through attachment to colonic epithelial cells, causing hyper-proliferation and inflammation[82]. In addition, key virulence factors exert pro-tumorigenic effects by damaging DNA or the mucosal layer/epithelial barrier. One major source of toxicity is produced by the polyketide synthase (*pks*) genotoxicity island. *E. coli* that are pks^{+} promote colon cancer development in IL-$10^{-/-}$ murine models and are highly enriched in colon cancer patients[54,83]. Enterotoxigenic *bacteroides fragilis* (ETBF) is another bacteria that generates a toxin, leading to CRC promotion and progression[84]. The *B. fragilis* toxin can induce a variety of potentially pro-tumorigenic responses, including cleavage of E-cadherin and activation of β-catenin signaling, stimulation of the NF-κB pathway, and induction of Th17 immune responses[85,86]. Clinically, these findings are relevant as increased Th17 cell infiltration and ETBF colonization into colon tumors correlates with cancer progression and poor outcome of colorectal cancer[87]. *E. coli* and ETBF are just two of the many important pathogenic microbes that exemplify the contribution that specific bacterial toxins make to CRC development and progression. In a nice mechanistic study, *Fusobacteria* were shown to promote colon cancer tumorigenesis by binding to E-cadherin on tumor cells through its FadA adhesin protein, causing stimulation of growth of cancer cells, as well as the ability of the bacteria to invade neighboring tissue, eliciting a pro-tumorigenic immune response[88]. Further work is needed to determine if the elimination of certain pathogenic isolates in

human CRC patients will yield therapeutic results. In the next section, we will discuss possible ways of modulating bacterial composition, virulence factors, and metabolism in an effort to restore microbial balance and alleviate tumor-promoting inflammation.

Therapeutics Targets: Pre/Probiotics, Diet, and Targeting Microbes

A better understanding of cancer development has afforded investigators the rationale to explore the novel mechanism-based targeted and systemic therapies (Figure 2). Among potential approaches that are not yet fully explored are the usage of probiotics, which aims to "normalize" or "skew' host microbiome to influence cancer development.

Pre/Probiotics—The popularity of prebiotics (a nondigestible food ingredient that selectively stimulates growth of one or a limited number of beneficial colonic bacteria) or probiotics (live microorganisms which confer a health benefit on the host) usage in prevention and treatment of a variety of diseases has increased in the recent years. It is also becoming a progressively crucial part in every day diet, as their beneficial effects are being actively investigated [89,90]. One cross-sectional study reports on associations between self-reported dietary fiber intake and the presence of fecal butyrate producing bacteria in subjects with and without advanced colorectal adenomas, raising the possibility that diets low in fiber impact butyrate producing bacteria and short chain fatty acid synthesis and that the resultant alteration in the gut microbiota is related to the presence of colon adenomas[90]. In addition, consumption of a fiber-rich diet enhances microbial methanogenesis, leading to reduction in hydrogen-producing bacteria, which is remarkable because hydrogen excess in the colon damages NAD regeneration[89]. Another value of soluble fiber consumption is that it induces a beneficial shift in gut microbiota, particularly *Faecalibacterium prausnitzii*, a bacterium thought to have anti-inflammatory properties[91]. The most common probiotic strains used in such treatment are *Lactobacillus* and *Bifidibacterium* species. They increase the activity of detoxification of toxin metabolites and carcinogens in colon[92], stimulate the host anti-tumor immunity[93], produce anti-tumorigenic or anti-mutagenic compounds that interact directly with tumor cells and inhibit their growth[94,95] and produce short-chain fatty acids, such as butyrate, which are important for proper immune system regulation.

Targeting Microbes—Although probiotics are becoming an extensively studied field, much less work has been done in developing therapeutics to specifically target microbial pathogenic pathways. Nevertheless, there are some excellent potential targets that could inhibit specific bacterial proteins without upsetting the overall host-microbiota homeostasis. One nicely demonstrated example is in animals infected with lipoteichoic-acid (LTA)-deficient *Lactobacillus acidophilus* strain, in whom the development of colitis was ameliorated and cancer burden was reduced[96]. Another group of investigators similarly reported a reduction in colitis-associated colon cancer burden when animals were infected with *pks*-deleted *E. coli* strain[54]. These studies have demonstrated a proof of principle that depletion of a bacterial protein can alleviate disease symptoms. One research group took this a step further and affected host health by targeting a bacterial enzyme. The cancer chemotherapy drug irinotecan can cause severe diarrhea that some patients limits effective therapy. Wallace et al. generated specific inhibitors against the gut bacterial enzyme β-glucuronidase, which reactivates (deconjugates) the conjugated form of irinotecan and

causes diarrhea in patients[97]. The specific inhibition of bacterial β-glucuronidase reduced the toxic side effects associated with chemotherapy in a mouse model, while not harming commensal bacteria. It will be critical in the future to develop specific inhibitors against potentially oncogenic properties of commensal bacteria without disrupting the delicate balance between microbial families.

Conclusions and Unanswered Questions

All in all, the influence of microbes on human health is immense. The microbiome can truly be considered another "organ." Bacteria, both commensal and pathogenic, contribute to inflammation and cancer development (Figure 3). The external environment (diet, antibiotics, toxins) has an effect on the composition the human microbiome, by altering the bacterial niches that exist within each tissue. This can lead to dysbiosis and select for microbes that disrupt tissue homeostasis through a number of potential mechanisms, including over amplification of the immune response, activation of epithelial proliferation, and breakdown of the integrity of the barrier. Although we are beginning to unravel the complexities of microbial-induced inflammation that promotes cancer, there are questions that remain to be explored. First, can microbes directly induce carcinogenesis without the assistance from tumor promoting inflammation? Much of the evidence suggests that inflammation is required for tumor development. Second, while the recent microbiome projects have uncovered a great deal about the relative ratios of bacteria in distinct organs of healthy individuals vs. cancer patients, there is little mechanistic insight into how these ratios are maintained and ultimately shift in cancer patients. Are there clear patterns that develop? Can some classes of microorganisms functionally substitute for known beneficial microbes? How does dysbiosis occur? How does the host's genetics/environmental exposure factor into the equation? All of these questions require further investigation. Lastly, novel therapeutics must be developed to target these pathogenic and opportunistic microbes. Moreover, we could generate treatments that are designed to prevent microbes from promoting cancer in the first place, by modulating immune system responses, or by maintaining epithelial barrier integrity. While there is much more to be explored, this field has seen some exciting developments in recent years and we should expect significant progress in the near future. Hopefully, we will take care of our microbial communities as well as any other part of our body, as they are just as beneficial to our overall health, and this could reduce the overall cancer burden, saving millions of lives.

Acknowledgments

Supported by the NIDDK/NIH R00DK088589, CCFA CDA#2693, FCCC-Temple University Nodal grant and by the Pew Scholar in Biomedical Sciences Program to S.G.

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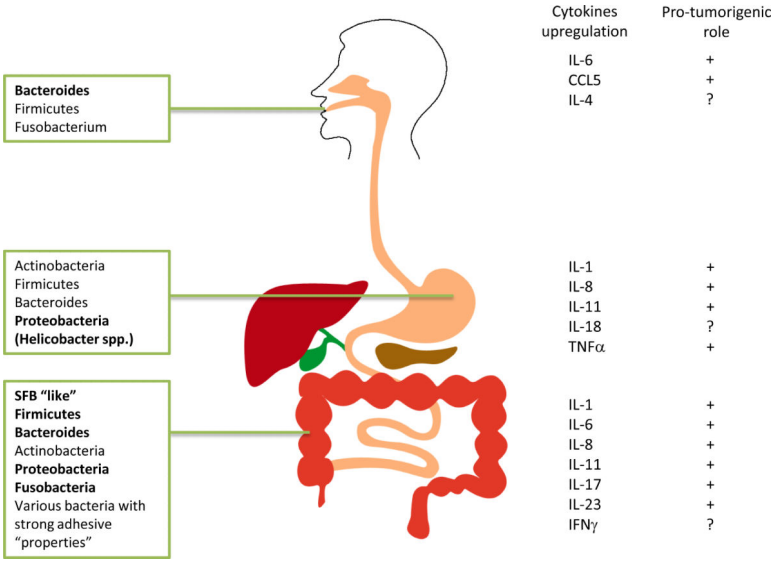

Figure 1. Microbiota distribution in the body and its influence on disease

Normal bacterial composition in various organs of the body (left). Bacterial population increased in dysbiosis are in bold. Cytokines and chemokines upregulated in the process of inflammation and cancer are shown in the right of the figure..

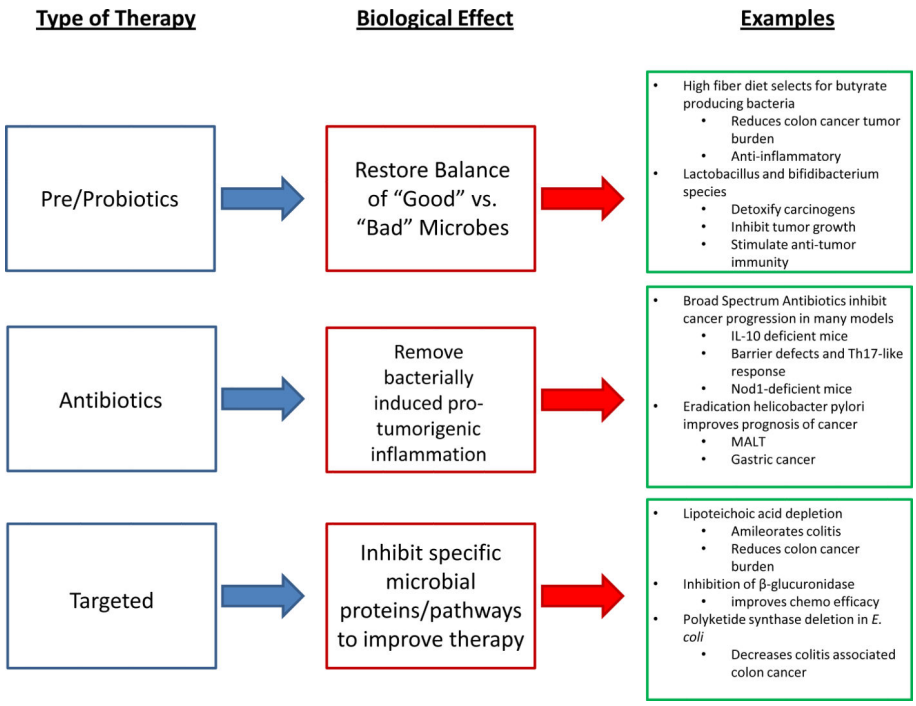

Figure 2. Microbes and Cancer: Therapeutic Avenues

Three potential intervention points to improve patient responses against cancer, along with what the desired effect, and important examples from the literature. (**Author: Need reference(s)?)**

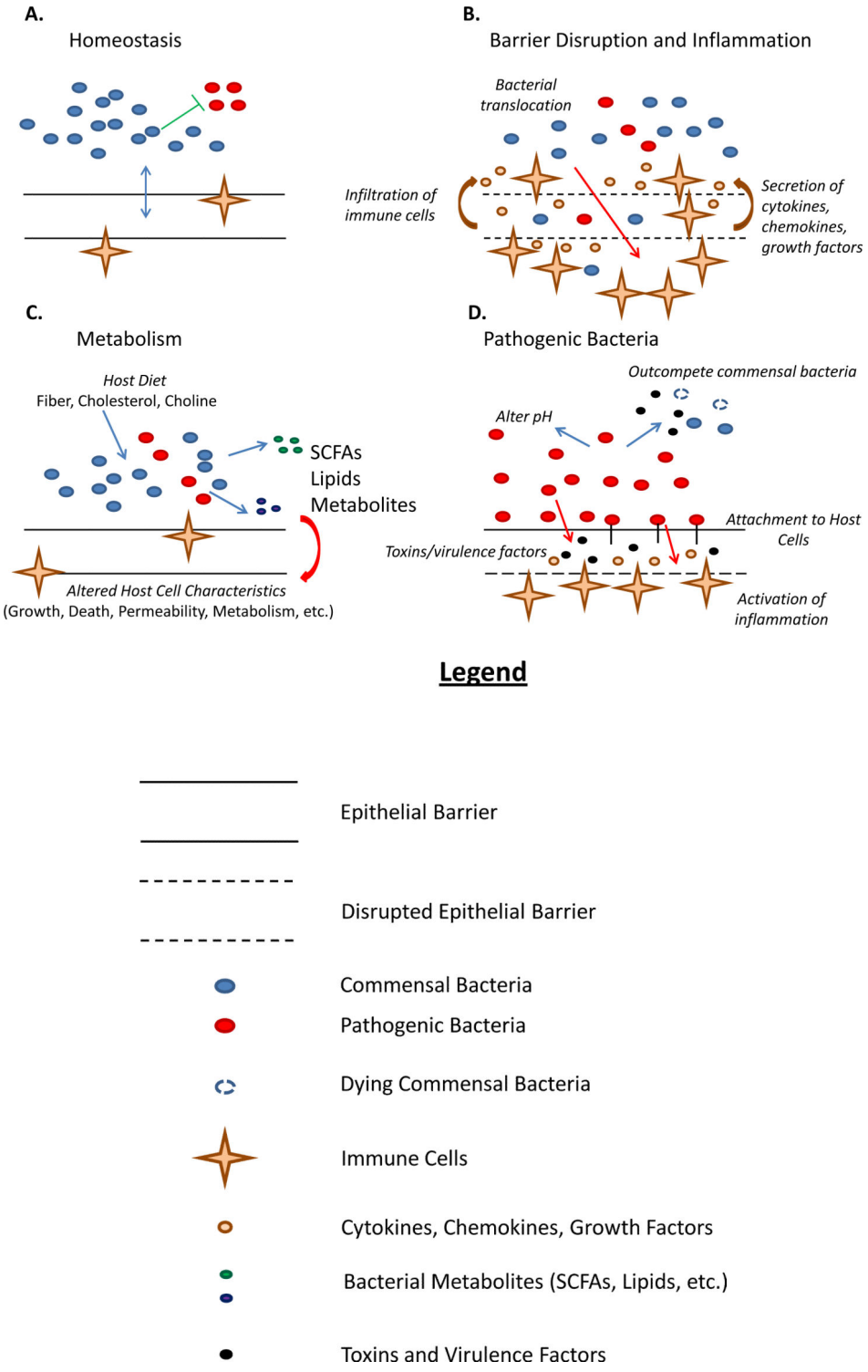

Figure 3. Summary of Microbial Influence on the Host

A.) *Homeostasis.* Beneficial bacteria occupy a dominant niche, inhibiting the growth of potentially pathogenic organisms. The integrity of the host organ is maintained and the immune system tolerates and limits bacterial expansion. **B.)** *Barrier Disruption and*

Inflammation. The barrier integrity of the host organ is compromised, due to tumor growth for example, allowing translocation of bacteria through the barrier, deeper into the organ. This elicits a robust immune response, as immune cells rush into the area, secreting a wide variety of cytokines, chemokines, and growth factors. This can lead to a chronic state of inflammation, and actually support tumor growth. **C.)** *Metabolism*. Microbes metabolize dietary intake from the host (fiber, cholesterol, and choline). These are converted into bacterial byproducts, such as short chain fatty acids (SCFAs: butyrate, acetate), lipids, and other metabolites, which modulate host cell behavior. **D.)** *Pathogenic Bacteria*. Here, dysbiosis occurs where pathogenic bacteria outcompete commensals by altering the pH and secreting toxins. Some bacteria can attach to the host epithelial layer, or even invade, and induce an immune response. Others actively secrete toxins or possess virulence factors that help break down host tissue and invade further into subsequent layers. The net result is a pro-tumorigenic microenvironment that can promote the progression of cancer.