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Sporadic colorectal cancer: microbial contributors to disease prevention, development and therapy

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The gut microbiota has been hailed as an accessory organ, with functions critical to the host including dietary metabolic activities and assistance in the development of a proper functioning immune system. However, an aberrant microbiota (dysbiosis) may influence disease processes such as colorectal cancer. In this review, we discuss recent advances in our understanding of the contributions of the microbiota to prevention, initiation/progression, and treatment of colorectal cancer, with a major focus on biofilms and the antimicrobial and antitumoural immune response.

Colorectal cancer (CRC) is one of the leading causes of cancer deaths among both men and women in the world. The framework for our understanding of CRC is that a series of mutations and epigenetic changes accumulate slowly over the span of decades, leading to a gain of function in oncogenes and a loss of function in tumour-suppressor genes as healthy tissue is transformed along the adenoma–carcinoma sequence (Fearon and Vogelstein, 1990; Wood *et al*, 2007). Although inherited genetic susceptibility has a key role in a subset of CRC cases, the vast majority of CRC cases are sporadic and non-inherited. For example, a Western diet, smoking, obesity, diabetes, alcohol consumption, and exposure to carcinogens have been validated as important risk factors for the development of sporadic CRC. However, despite long-standing associations between diet, the microbiome, and CRC (Aries *et al*, 1969; Hill *et al*, 1971), only recently have we begun to appreciate the specific mechanisms by which the gut microbiome may influence not only the initiating events of carcinogenesis but also its progression. This review covers recent advances in our understanding of the various roles in which bacteria may initiate or alter the course of CRC and discusses some of the pressing questions in the field.

INTRODUCTION TO COLON STRUCTURE, BIOLOGY, AND IMMUNOLOGY

As our scientific understanding and interest in how the gut microbiota influences complex disease processes such as CRC

expands, it is important to first highlight biological differences along the colorectal axis with regards to the composition and function of the microbiota and the cognate inflammatory response.

Biological differences along the colorectal axis in CRC. The entire gastrointestinal tract spans a distance of 25 feet, although the largest burden of microbes resides in the most distal portion of the gut, namely, the colorectum. Features of CRC that differ along the axis of the colon are outlined in Figure 1. Traditionally, the conceptual division between the right and left colon has been located near the splenic flexure, based on developmental biology. However, recent findings by Dejea *et al* (2014) proposed redefining the right vs left colon based on the overwhelming burden of microbial biofilms proximal to the hepatic flexure. Thus, for the purposes of this review on CRC, we term the right colon to be the region from the caecum through the ascending colon and hepatic flexure and the left colon to contain the remaining distal portions consisting of the transverse colon, splenic flexure, descending and sigmoid colon, and rectum.

Genetic differences in CRC have been noted along the colon as well, including a high frequency of KRAS mutations specifically in the caecum and a gradient in the prevalence of CpG island methylator phenotypes, microsatellite instability (MSI), and BRAF mutations that is highest in the ascending colon but then gradually decreases as one moves distally along the colonic axis (Yamauchi *et al*, 2012). Given these major differences in the microbial organisation (e.g., biofilms) and genetic features of CRC as one moves along the colonic axis, one might expect to see similarly

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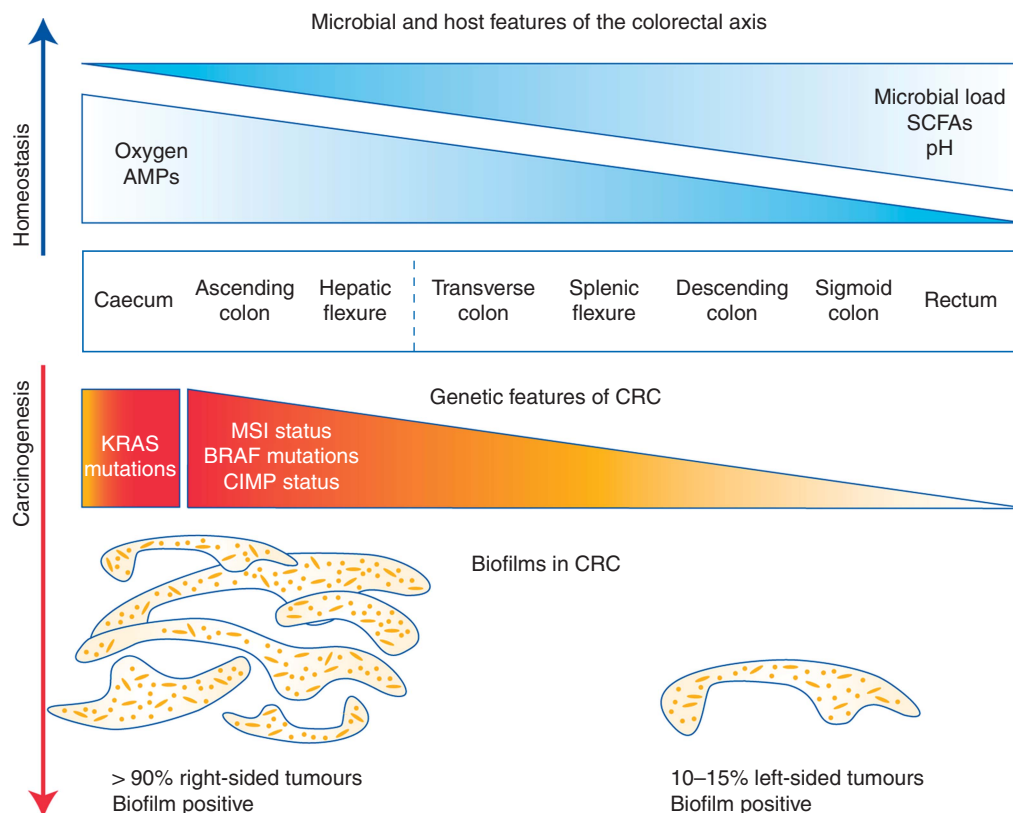


Figure 1. Molecular features of the colon during homeostasis and carcinogenesis. (Top) General biological differences among healthy tissues along the colorectal axis are depicted in blue. (Bottom) Differences in the prevalence of genetic features and biofilms among tumours along the colorectal axis are summarised in orange and red. The dashed line represents the transition between right and left colon based on the overwhelming burden of biofilms in tumours proximal to the hepatic flexure as reported in Dejea *et al* (2014).

divergent changes in the microbiome along this axis. Surprisingly, the composition of the mucosal microbiome is largely consistent within a healthy adult (Eckburg *et al*, 2005). The faecal microbiome, however, is much richer in diversity than the mucosal microbiome, but, for reasons that yet remain unclear, not all of the mucosal species in a given patient are necessarily detected in stool (Eckburg *et al*, 2005; Mira-Pascual *et al*, 2015). Thus stool samples thus far have been poor indicators of the mucosal microbiome. Furthermore, adherent microbes are more likely to be key players in CRC pathogenesis than those passing fleetingly through the gut via the stool, given that adherent microbes are the only organisms that have direct access to the colonic epithelial cells (CECs) for any extended period of time. Thus the prognostic use of stool samples for CRC detection may be limited.

Symbiotic and antimicrobial immune defenses in the colon.

Because all CRCs exhibit excess, subclinical inflammation, it is also important to note the unique characteristics of the gut-associated lymphoid tissue (GALT), as the interplay between the GALT and the gut microbiome likely impacts the inflammation involved in CRC initiation, progression, and treatment. Whereas interaction with a pathogen in the spleen or other central lymphoid organs is rare and triggers an immediate and robust inflammatory response, the gut has evolved to coexist with commensal bacteria as it depends on these microbes for not only digestive health but also for the development of a properly functioning mucosal and systemic immune system. In the colon, a thick, mucus bi-layer serves as the primary innate defense barrier, with some bacteria colonising the thin outer layer but the inner, Muc-2-dominated layer being largely sterile (Johansson *et al*, 2008). Tonic stimulation of several pattern recognition receptors (PRRs) on hematopoietic

cells and intestinal epithelial cells by commensals appears to be instrumental in the maintenance of this innate mucus barrier, as disruption of these PRRs results in a dramatic thinning of the mucus layer and increases susceptibility to colitis in mouse models (Philpott *et al*, 2014; Wlodarska *et al*, 2014). Early mutagenic events, including loss of APC and/or activation of β -catenin signalling, may also alter Muc-2 and tight junctional proteins and allow for infiltration of protumorigenic microbial products (Grivennikov *et al*, 2012). Secreted antimicrobial peptides (AMPs) and IgA serve as additional innate immunity factors in the colon but can also be manipulated by microbial players (Levy *et al*, 2015; Moon *et al*, 2015).

Underneath this innate defense barrier lies an adaptive immune compartment dominated by the anti-inflammatory cytokines TGF β and IL-10, which are largely secreted by epithelial cells, regulatory T cells (Tregs), and stromal cells (Smythies *et al*, 2005; Smythies *et al*, 2006). TGF β , in particular, is critical for a number of different functions, including the induction of class switch recombination in B cells from IgM to secretory IgA (by both T-cell-dependent and -independent pathways), for the induction of and maintenance of a tolerogenic phenotype in dendritic cells, macrophages, and Tregs, and for the induction of specific immunosuppressive pathways, such as indoleamine 2,3-dioxygenase (Smythies *et al*, 2005; Contractor *et al*, 2007; Belladonna *et al*, 2009; Pallotta *et al*, 2011). Importantly, this suppressive milieu can be overcome by IL-6 in the gut, which in combination with TGF β triggers differentiation of naive CD4+ T cells into proinflammatory Th17 cells (Hu *et al*, 2011) that in turn lead the adaptive immune response against invading microbial pathogens.

Despite the critical role of Th17 cells in antimicrobial defense, these cells are thought to be causally related to the chronic,

subclinical, unbalanced mucosal inflammation revealed to be of importance to the pathogenesis of colon carcinogenesis in recent years. A Th1-dominated inflammatory state seems to be protective in CRC patients (Tosolini *et al*, 2011) and boosts antitumoural T-cell responses via the production of IFN γ . In contrast, Th17 (or IL-17 produced by other cell types) has been shown to be a key driver of CRC in multiple intestinal neoplasia (Min) mouse models, including the spontaneous tumorigenesis *Apc*^{Min/+} mouse model (Chae *et al*, 2010) and in *Apc*^{Min/+} mice colonised with enterotoxigenic *Bacteroides fragilis* (ETBF) (Wu *et al*, 2009). Furthermore, a Th17 signature is associated with a worse prognosis in CRC patients (Tosolini *et al*, 2011). Thus the balance between Th1/Th17 inflammatory responses may have a key role in patient outcomes.

The Treg/Th17 balance may also be critically important to intestinal homeostasis, as Tregs are one of the pivotal cell types responsible for keeping colon inflammation in check. Paradoxically, two recent reports have shown that Tregs are necessary for early Th17 responses to pathogens, including ETBF in the distal mouse colon of Min mice (Geis *et al*, 2015) and *Candida albicans* in the mouse oropharyngeal tract (Pandiyani *et al*, 2011). Mechanistically, Tregs, which are highly IL-2 dependent but incapable of producing their own, were shown to deplete exogenous IL-2, creating an IL-2-deprived environment that favoured Th17 development at the expense of Th1 (Chen *et al*, 2011; Geis *et al*, 2015). In the ETBF model, the depletion of Tregs reduced early microadenoma formation in the colon by limiting Th17 cell differentiation despite the persistence of marked Th1-skewed inflammation. The dependence of Th17 responses on Tregs was only required during early events, showing that not only is the type of inflammation important (Th1 vs Th17) but also the timing (early vs late infection) following challenge with specific pathogens.

MICROBIAL INITIATORS AND PROGRESSORS OF TUMORIGENESIS

Theories on how the microbiota may contribute to the initiation and progression of CRC can be divided according to evidence for individual bacteria, evidence for aggregate changes in the microbiota as a whole largely through metabolism and diet, and finally the effects of differential microbial organisation as in the case of biofilms. These theories are summarised in Figure 2.

The roles of individual microbes in CRC initiation. The carcinogenic potential of individual bacterial species has recently been covered in depth by others, with two major theories emerging for how these bacteria may initiate CRC (Irrazabal *et al*, 2014; Sears and Garrett, 2014). The first and perhaps most direct is that certain bacteria have direct DNA mutagenesis capabilities and/or interfere with the host DNA repair machinery, as seen with ETBF that express the *B. fragilis* toxin (BFT), superoxide-producing *E. faecalis*, and the polyketide synthase (*pks*)-expressing clade of *E. coli*. The second is that many of the implicated bacteria share a common ability to enhance Wnt-mediated signalling pathways or other specific proinflammatory pathways that are commonly mutated and/or overexpressed in CRC, as seen in the above three species as well as *F. nucleatum*. Importantly, however, none of the bacteria have consistently been demonstrated to be enriched in all tumour specimens compared with healthy controls, suggesting that no single organism is likely responsible for all CRC cases. Additionally, all of these mouse models rely on genetically predisposed mice with mutations in tumour-suppressor genes such as *Apc* or mice that have been administered chemicals to help drive carcinogenesis such as dextran sodium sulphate (DSS), which

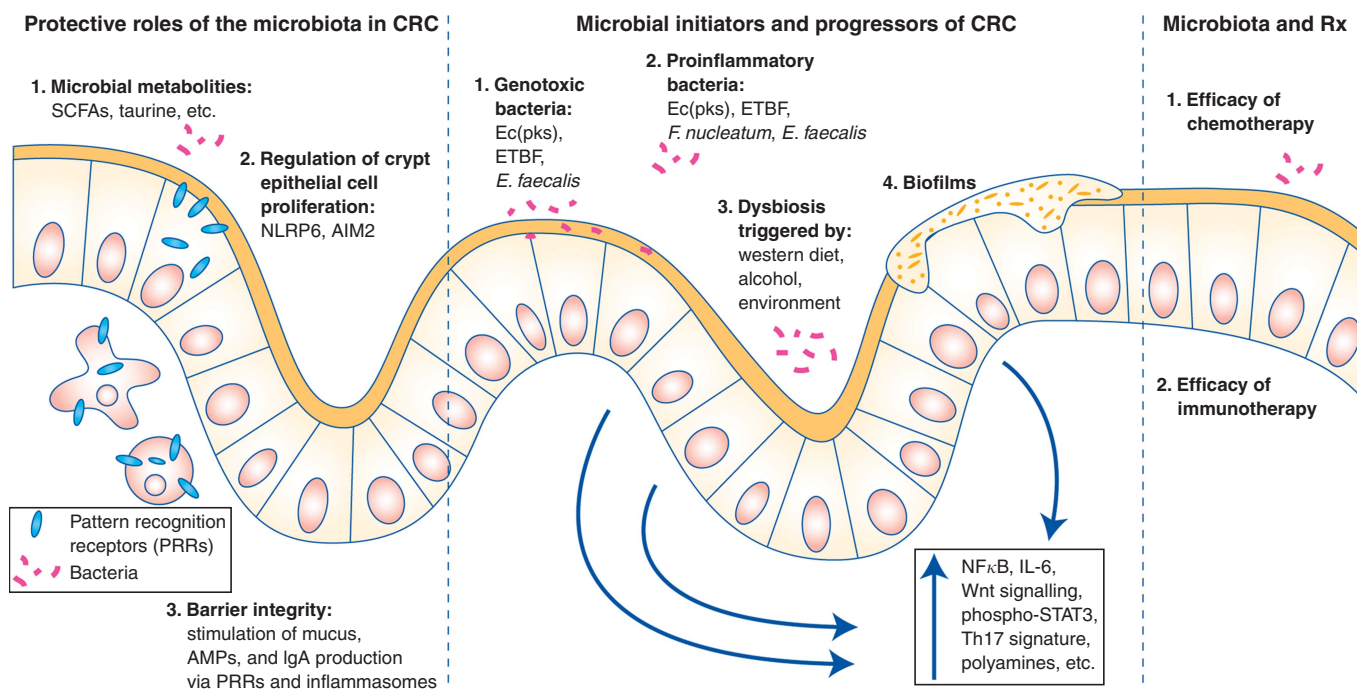


Figure 2. Roles of the microbiota in CRC prevention, initiation, progression, and therapy. Colonic epithelial cells are depicted with the mucus layer (dark yellow) facing the gut lumen. (Left) Bacteria have multiple protective roles against CRC, including production of anti-inflammatory metabolites and regulation of crypt epithelial cell proliferation. Most notably, bacteria have integral roles in the maintenance and repair of the colonic epithelial barrier, by triggering controlled innate immune responses through PRRs on host cells. Disruption of PRR signalling leads to breaches of the epithelial barrier and excessive inflammation that may instead promote tumorigenesis. (Middle) Multiple avenues by which bacteria may initiate or promote CRC tumorigenesis are also depicted, including the direct genotoxicity of specific bacteria, as well as the proinflammatory effects triggered by either specific microbes, a dysbiotic microbiota as a whole, and/or colonic biofilms. (Right) Immune responses to commensals have also been proposed to be essential for the efficacy of multiple chemotherapies and immunotherapies.

induces inflammation and disruption of colonic mucosa, and azoxymethane (AOM), a DNA alkylator. Likewise, while germ-free mice have proven invaluable in our understanding of specific pathogens, the absence of commensals hinders development of the immune system and further complicates their relevance to understanding human disease. Thus, while murine studies have demonstrated strong evidence for the procarcinogenic activity of certain bacteria, they have not proven that bacteria can provide the first hit to initiate cancer nor has clear relevance to human disease been established.

The roles of individual microbes in CRC progression. All of the specific microbial mechanisms above that may have a role in CRC initiation likely also have a role in CRC progression. Additionally, observational studies on patient outcomes have given us clues as to which microbes may be associated with CRC progression. For example, a trend towards increased prevalence of BFT toxin (particularly BFT-2) was recently demonstrated in more advanced CRC cases (100%) vs early-stage CRC (72.7%) (Boleij *et al*, 2015). There is also evidence for an association of fusobacteria with worse prognoses, although the data are more complex. For example, a high level of *F. nucleatum* DNA in the tumour tissue was associated with increased lymph node metastases (Castellarin *et al*, 2012) and a worse outcome in CRC patients (Mima *et al*, 2015a) but was not associated with worse mortality in a separate study (Mima *et al*, 2015b). Likewise, while the presence of *F. nucleatum* DNA in tumours was associated with MSI status (Mima *et al*, 2015a), which is typically associated with better patient outcomes, in another study, *F. nucleatum* was associated with a decrease in CD3+ T cells within the tumour in CRC patients (Mima *et al*, 2015b), a feature that is typically associated with MSS status and worse outcomes in patients (Kawakami *et al*, 2015). These observational studies clearly need to be validated by prospective studies in patients to clarify the role of these bacteria in CRC progression, as these microbes may merely be taking advantage of a compromised host defense/mucus layer, rather than contributing to actual tumorigenesis.

The aggregate microbiota and microbial metabolites in CRC initiation. The gut microbiota as a whole may also alter cancer susceptibility, largely via interactions with host diet and metabolism. Louis *et al* (2014) recently reviewed in depth the potential procarcinogenic roles of bacterial products, including toxins, hydrogen sulphide (H₂S), polyamines, secondary bile acids, and reactive oxygen species (ROS). Elevations in many of these factors have been linked to diets rich in saturated fats and sugars and low in fibre. For example, high levels of saturated fats leads to increased production of bile acids by the liver, and these, in turn, are metabolised by bacteria into secondary bile acids, such as lithocholic and deoxycholic acid, which have proinflammatory and procarcinogenic properties (Sears and Garrett, 2014). In addition, these high fat/high sugar/low fibre diets may also result in a less diverse microbiota overall, which is also associated with an increased risk of CRC.

Conversely, the vast metabolomic potential of the gut microbiota can also be harnessed to prevent CRC. For example, short chain fatty acids (SCFA) have potent anti-inflammatory properties, including the promotion of colonic Treg expansion (Arpaia *et al*, 2013; Smith *et al*, 2013), and several studies have shown a protective effect of alternative bacterially produced bile acid products such as urodeoxycholic acid, as reviewed by Louis *et al* (2014). Most recently, the microbial metabolites taurine, histamine, and spermine were found to alter host inflammasome processes in mice, either positively or negatively, and may represent other easily modifiable targets (Levy *et al*, 2015).

The aggregate microbiota in CRC progression. It is also becoming increasingly clear that an aberrant microbiota, also

known as dysbiosis, may have an important role in tumour progression. Although dysbiosis may take many forms and a consistent signature in all CRC patients is unlikely to be unveiled, the protumorigenic mechanism of dysbiosis appears to involve enhanced microbial stimulation of the Toll-like receptor (TLR)/MyD88 pathway, as knockout of MyD88 in both the *Apc^{Min/+}* and *AOM-IL10^{-/-}* colitis-associated cancer models abrogates tumorigenesis, as does treatment with antibiotics or the use of germ-free mice (Rakoff-Nahoum and Medzhitov, 2007; Uronis *et al*, 2009; Song *et al*, 2014). Most recently, this elevated TLR/MyD88 signalling was found to enhance calcineurin-mediated activation of NFAT signalling that, in turn, enhanced intestinal stem cell proliferation (Peucker *et al*, 2016), providing a probable mechanism of action.

Biofilms as CRC initiators. An emerging concept in the role of the microbiota in CRC initiation is that it is not just the composition of the microbiota that is important but that complex community structures such as bacterial biofilms can also dramatically alter both host and microbial function in CRC. Bacterial biofilms along the colorectal axis have been shown to be present in approximately 15% of healthy patients upon colonoscopy (Swidsinski *et al*, 2009) but were recently shown to be a feature of nearly 100% of right-sided CRC (Dejea *et al*, 2014). Biofilms are massive bacterial invasions of the mucus layer, are encased in a polymeric matrix, and often consist of many different types of bacteria and even fungi. They are commonly found in the environment but have also been reported to be a feature of dozens of problematic medical infections, particularly those involving artificial medical devices such as catheters as well as with living tissue as in the case of endocarditis (Costerton *et al*, 1999). How and why they form in the colon, where they involve invasion of the protective mucus layer adjacent to the CECs, is largely unknown. However, one hypothesis is that biofilm formation is, in part, a defense mechanism of the microbiota against the host. For example, biofilms may provide a degree of stability to the gut microbiota in the face of the rapid turnover of the gut mucus layer. Biofilms may also protect luminal microbiota from host factors such as IgA, AMPs, and even antibiotics, which may be inactivated by or do not diffuse well through the biofilm matrix (Costerton *et al*, 1999).

The bacterial species that make up colorectal mucosal biofilms are clearly a small subset of what is found in the faecal microbiota, with only approximately 100 species present in colon biofilms compared with the nearly 1000 species detected in stool. The class Bacteroidetes (encompassing *Bacteroides* and *Prevotella*) is typically the predominant constituent of the biofilms, although proteobacteria (including enterobacteria such as *E. coli*), *Clostridium* spp. (e.g., eubacterium), actinobacteria (e.g., bifidobacteria), bacilli (including the putatively protective lactobacilli), and fusobacteria are also commonly detected (Macfarlane *et al*, 2004; Swidsinski *et al*, 2005; Dejea *et al*, 2014). Although colon biofilms are observed sporadically in healthy controls (~15%), those biofilms tend to be much thinner and more disjointed as compared with the thick, continuous biofilms seen in inflammatory bowel disease and CRC patients (Swidsinski *et al*, 2005; Dejea *et al*, 2014). Importantly, 100% of the CRC patients examined to date who had biofilm-positive tumours also had biofilms on their paired, normal tissue. Tissues underlying the biofilms in CRC patients and in healthy individuals were demonstrated to have decreased or altered E-cadherin, enhanced IL-6 and Ki67 expression (a measure of CEC proliferation) alongside in the tumour hosts, phospho-Stat3, suggesting that the biofilms were eliciting a procarcinogenic effect (Dejea *et al*, 2014). This is perhaps not surprising, given that a feature of biofilms is the invasion of the mucus layer by bacteria, which allows for bacteria to directly interact with colonocytes and potentially trigger inflammatory responses as

well as oncogenic mechanisms in the CEC layer. Conversely, CECs and/or leukocytes can be seen invading the biofilms, again suggesting that the biofilms are immunogenic and involve highly dynamic bacterial: host interactions (Dejea *et al*, 2014).

Although studies on the specific bacterial mechanisms rendering biofilms pro-oncogenic have only just begun, the polymicrobial make-up of the biofilms suggests a community mechanism is at play rather than one driven by individual bacteria. For example, biofilm-positive tissues from both the tumour region and histologically normal colon tissue display enrichment of bacterially synthesised acetylated polyamine N_1N_{12} -diacetylspermine compared with biofilm-negative tumours and healthy controls (Johnson *et al*, 2015). Polyamine synthesis is a near-universal feature of both bacterial and host cells and is a critical modulator of a multitude of both bacterial and host processes. Furthermore, polyamine synthesis is required for biofilm formation in at least one bacteria, *Yersinia pestis* (Di Martino *et al*, 2013). In CRC patients, N_1N_{12} -diacetylspermine was found to be highly localised to the luminal edge of the mucosa, consistent with bacterial production of this polyamine. Additional data suggested that both the host colon mucosa and the bacterial biofilms contributed to the production of N_1N_{12} -diacetylspermine. Whether this polyamine is required for the formation of biofilms in CRC patients remains to be examined, but it may be an important contributor to the increased cell proliferation and pro-oncogenic state seen in the biofilm-positive tissues.

PROTECTIVE ROLES OF THE MICROBIOTA IN CRC

PRRs, inflammasomes, and the aggregate microbiota in CRC.

Another emerging hypothesis is that loss-of-function mutations in PRRs may enhance CRC tumorigenesis by affecting the detection of pathogen- or cellular damage-associated molecular patterns (DAMPs). There are five major classes of PRRs: TLRs, C-type lectins (CTLs; e.g., DC-SIGN), RIG-I-like receptors, Absent in Melanoma 2 (AIM2)-like receptors (also known as the HIN-200 family of receptors), and NOD-like receptors (NLRs). These receptors detect diverse foreign antigens and trigger similarly diverse responses, including NF κ B activation, MAPK activation, and production of proinflammatory cytokines, that in turn modulate downstream adaptive immune responses. Several PRRs (e.g., AIM2 and the NLR family members NLRP1, NLRP3, NLRC4, and NLRP6) have also been shown to be sensor components of inflammasome complexes, cellular machinery designed to churn out copious amounts of mature caspase-1 that in turn proteolytically activates the proinflammatory cytokines IL-1 β and IL-18. Cytosolic PRRs have also been shown to detect DAMPs (e.g., extracellular ATP) (Mariathasan *et al*, 2006) and may be instrumental in priming antitumoural T-cell responses, highlighting the diverse nature of the PRR family.

Inherited mutations in these PRR families have been linked to a wide variety of autoimmune and inflammatory disorders. Given the key role of inflammation in CRC, it is not surprising that these genes are commonly mutated in CRC as well and in some cases have also been associated with CRC progression (Nonaka *et al*, 2011; Ungerback *et al*, 2012; Lu *et al*, 2013; Luddy *et al*, 2014; Philpott *et al*, 2014; Liu *et al*, 2015; Sharma and Jha, 2015). For example, loss-of-function mutations in the cytoplasmic PRR NOD2 is one of the strongest, heritable genetic links to Crohn's disease and has also been associated with an increased risk of CRC in patients (Tian *et al*, 2010). Although NOD1^{-/-} and NOD2^{-/-} deficiencies are not sufficient to induce spontaneous colitis in mice (Natividad *et al*, 2012), NOD2^{-/-} in combination with DSS resulted in a dysbiosis-mediated susceptibility to tumorigenesis that was transmissible via the microbiota and triggered tumours in wild-type

mice also treated with DSS (Couturier-Maillard *et al*, 2013). Similarly, mutations in NLRP6 and AIM2, which are the sensor PRR molecules of two separate inflammasome complexes, have also been shown to exacerbate chemically induced (DSS/AOM) tumorigenesis in mice via a microbially mediated mechanism that is transmissible to wild-type mice also treated with DSS and AOM (Hu *et al*, 2013; Man *et al*, 2015). Mutations in AIM2, similarly to NOD2, are frequently found in CRC and are associated with a worse prognosis (Dihlmann *et al*, 2014). Mutations in another inflammasome PRR sensor, NLRP3, are associated with an increased risk of developing inflammatory disorders such as Crohn's disease in patients as well as DSS-associated colitis in mice (Schultz *et al*, 2009), but direct links between NLRP3 and CRC initiation in patients have yet to be validated nor has the role of the microbiota in NLRP3-mediated attenuation of colitis been demonstrated.

Despite the consistent role of the microbiota in transmitting susceptibility to CRC in many of the PRR knockout studies above, the actual microbial players suggested to be involved in the dysbiosis were quite different. For example, NOD2^{-/-} dysbiosis was associated with an overall loss of diversity as well as increased *Bacteroides* and a decrease in genera of the phylum Firmicutes (*Butyrivibrio* and *Lachnobacterium*); the NLRP6^{-/-} dysbiosis was associated with an increase in *Prevotella* and TM7 and reductions in *Lactobacilli* and Firmicutes; finally, the AIM2^{-/-} dysbiosis was associated with a decrease in *Prevotella* and an increase in *Akkermansia muciniphila* in one study but was recently associated with an increase in *E. coli* in another study (Hu *et al*, 2015). These studies suggest that more than one colitogenic phenotype may exist following PRR mutagenesis, which is perhaps not surprising given the diverse nature of the PRRs and the ligands that they recognise.

Additionally, our knowledge of how PRRs help regulate gut homeostasis in other ways continues to increase. For example, tonic sensing of commensals by NOD1 and NOD2 may be critical for mucus and AMP generation (Philpott *et al*, 2014). Similarly, NLRP6 helps promote goblet cell autophagy and mucus secretion (Wlodarska *et al*, 2014), while also regulating crypt epithelial cell proliferation (Normand *et al*, 2011). Thus, beyond protection against any individual pathogen, these PRRs have critical roles in maintaining general epithelial barrier integrity, heightening their importance to CRC. Clearly, further studies are needed in order to clarify how and why mutations in particular PRRs trigger dysbiosis, and whether mutations in PRRs in patients increase the risk of initial transforming events in CRC or are merely associated with CRC progression.

Microbial modulation of sensitivity to treatment. Finally, the microbiota may also influence the progression of a wide array of cancers in a positive manner by providing a critical boost to antitumoural T-cell responses following certain types of chemotherapy and immunotherapy. For example, treatment with cyclophosphamide was found to trigger the translocation of several Gram-positive bacterial species to the secondary lymphoid organs; surprisingly, this infiltration was required for the promotion of antitumoural Th1 and Th17 responses via a MyD88-dependent pathway, as germ-free mice and mice co-treated with either broad-spectrum antibiotics or antibiotics specific for Gram-positive bacteria in addition to the cyclophosphamide displayed larger tumours than mice with an intact microbiota (Viaud *et al*, 2013). Likewise, the antitumoural response following treatment with the ROS-inducing chemotherapeutic agent oxaliplatin was similarly reliant on functional MyD88 signalling triggered by microbes, as germ-free mice, mice treated with antibiotics, and MyD88^{-/-} mice did not demonstrate successful tumour regression (Iida *et al*, 2013). Most recently, efficacy of the immunotherapy checkpoint blockade inhibitors anti-PDL1 and anti-CTLA-4 were found to be reliant on commensal bacteria (*bifidobacteria* in the former, and

Bacteroides, among others, in the latter) (Sivan *et al*, 2015; Vetizou *et al*, 2015), though whether these antitumoural responses also relied on MyD88 signalling remain unclear. A critical point of all of these studies is that antibiotics, which are often life saving for cancer patients whose immune systems are suppressed by chemotherapy, should be administered with care due to the dependence of various cancer drugs on a functioning microbiota as well as to avoid the adverse consequences of antibiotic-resistant bacteria.

CONCLUSION

Overall, the interactions between the gut microbiota and CRC are complex and involve both positive and negative factors. Pathogenic roles of the microbiota involve aspects as varied as direct genotoxic activity, alterations of dietary metabolism, and triggering of proinflammatory immune responses. Alternatively, the gut microbiota may also have a protective role against CRC through maintenance of the epithelial/mucus barrier (largely through stimulation of PRRs) and production of protective metabolites, such as SCFAs. The potential for the microbiota to enhance antitumoural T-cell responses following chemotherapy and immunotherapy has added yet another exciting dimension to the interplay between the host and the microbiota. Emerging areas of research include delineating how different types of inflammation affect CRC, the role of biofilms and other complex bacterial structures in predicting and/or causing CRC, and the myriad roles of PRR sensors in maintaining innate defenses against not only microbes but also potentially early transformed cells. Prospective studies in patients will be absolutely critical in the validation of the proposed theories. All of these areas may provide novel targets for therapy or provide much-needed biomarkers of disease, as colonoscopy is unlikely attainable for all in resource-limited settings where CRC appears to be increasing in incidence.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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