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The Impact of the Gut Microbiome on Colorectal Cancer

Charles Maisonneuve,[1] Thergiory Irrazabal,[1] Alberto Martin,[1] Stephen E. Girardin,[1,2,*] and Dana J. Philpott[1,*]

[1]Department of Immunology, University of Toronto, Toronto, Ontario M5S 1A8, Canada; email: stephen.girardin@utoronto.ca, dana.philpott@utoronto.ca

[2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada

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*These authors contributed equally to this article

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Abstract

Colorectal cancer (CRC) represents one of the leading causes of morbidity and cancer-related mortality in the world. While the etiology of CRC is believed to arise from genetic mutations, alterations in the gut microbiota composition also influence cancer incidence and progression. This review focuses on how gut microbiota and their relationship with the innate immune system link inflammation to genotoxicity and carcinogenesis.

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer mortality in Canada and represents the third most commonly diagnosed form of cancer worldwide (Can. Cancer Soc. 2017). More than 1.4 million new cases were diagnosed globally in 2012, accounting for nearly 700,000 cancer-related deaths (Arnold et al. 2017, Torre et al. 2015). This high incidence imposes a considerable economic burden on the healthcare system and stresses the need for novel treatments. While CRC rates are stabilizing or declining in developed countries, likely due to increased screening and removal of precancerous polyps, the occurrence of CRC continues to rise in the developing world (Stewart & Wild 2014). Despite the geographical disparities, CRC remains a life-threatening obstacle that deserves international attention. Indeed, the global prevalence of CRC is estimated to increase by 60% by 2030, representing over 2.2 million new cases and 1.1 million cancer deaths worldwide (Arnold et al. 2017, Torre et al. 2015).

The disease stems from a sequence of genetic mutations acquired over the course of many years, a process known as the adenoma-carcinoma sequence. Nonetheless, cancer is a multifaceted disease, and the etiology of CRC involves an association of host genetics and environmental factors. It is increasingly clear that the host microbiota also contribute to CRC initiation and progression. For instance, tumors preferentially develop in the distal colon and rectum, which likely reflects the differences in microbial composition along the gastrointestinal tract. Indeed, the colon is the most densely colonized organ and contains approximately 70% of the host's microorganisms (Gagniere et al. 2016). Studies in germ-free animals have also highlighted a role for the microbiota in various models of carcinogenesis (Schwabe & Jobin 2013). Moreover, patients with inflammatory bowel diseases (IBDs) are significantly more susceptible to the later development of colon cancer, supporting a causal relationship between the microbiota, inflammation, and CRC.

Individual microbes or microbial communities are present at mucosal sites and are therefore well suited to potentiate CRC incidence. Microbes and microbial products can infiltrate the tumor microenvironment and collectively contribute to carcinogenesis in many ways. Namely, the microbiota can alter cellular proliferation and cell death, shape the immune response toward cancer, modulate host metabolism, and influence therapy. This review discusses the complex interaction between the microbiota, innate immunity, and CRC, with a focus on the causal role of bacteria in cancer initiation and tumor progression.

MICROBIOTA CONTRIBUTION TO INTESTINAL STEM CELL HOMEOSTASIS: POTENTIAL LINKS TO COLORECTAL CANCER

The gastrointestinal tract is colonized by a myriad of microorganisms primarily dominated by bacteria but also includes archaea, fungi, protozoa and viruses. The coevolution of the microbiota with their larger host reflects an intricate relationship in which, for the most part, both the host and microbes benefit and thrive. Commensal microbes help maintain host homeostasis by regulating metabolic and immune processes and by establishing a network that prevents pathogenic infections. However, pathogens and pathobionts, which are potentially pathogenic microbes associated with chronic inflammatory conditions, can establish themselves within the host and promote disease.

The microbiome is segregated within the intestinal lumen by the intestinal epithelium, which consists of a cohesive monolayer of intestinal epithelial cells (IECs). Due to their proximity to the harsh luminal environment, IECs are continually regenerated to maintain the integrity of the intestinal barrier. It is estimated that the intestinal epithelium completely self-renews every 4–5 days (Cheng & Leblond 1974c, Stevens & Leblond 1947). Intestinal stem cells (ISCs) contained within the crypt niche fuel the constant cellular turnover of the intestinal epithelium and give rise to all the differentiated cell types, including absorptive enterocytes (ECs) and colonocytes, Paneth

cells, goblet cells, tuft cells, and enteroendocrine (EE) cells (Tetteh et al. 2015). The protective niche of the crypt is thought to shield the rapidly dividing ISCs from damaging luminal factors, such as pathogenic bacteria, bacterial metabolites, and genotoxic agents, while providing key host factors regulating ISC maintenance and differentiation. For instance, differentiated colonocytes metabolize butyrate, a microbial metabolite and potent inhibitor of proliferation, preventing it from reaching the ISCs and thereby maintaining crypt homeostasis (Kaiko et al. 2016). However, due to their high turnover rate, ISCs are particularly susceptible to malignant transformation. A pending question in the field is how the microbiota can influence ISC function and thereby initiate or promote carcinogenesis.

Intestinal Stem Cells

The location and identity of ISCs within the small intestine were first suggested in the 1970s and remained, until recently, only descriptive (Barker 2014; Cheng & Leblond 1974a,b,c; Potten 1977). The recent advent of long-term lineage-tracing transgenic mice has furthered our understanding of the molecular signature of these ISC populations, leading to a unifying theory of ISC hierarchy. The current model suggests that alternative ISC populations coexist and contribute to crypt homeostasis and regeneration. Specifically, Lgr5 marks actively cycling ISCs that contribute to the daily homeostatic regeneration of the epithelium, while a subset of Bmi1$^+$, mTert$^+$, and Lrig1$^+$ quiescent ISCs are mobilized following tissue damage and acquire properties of functional ISCs (Barker et al. 2007, Breault et al. 2008, Buczacki et al. 2013, Li & Clevers 2010, Li et al. 2014, Montgomery et al. 2011, Powell et al. 2012, Sangiorgi & Capecchi 2008, Sato et al. 2009, Tetteh et al. 2015, Tian et al. 2011, Yan et al. 2012). However, despite the identification of several markers for the two ISC populations, the true identity of ISCs remains controversial. In line with the ambiguous distinction between the ISC populations, the expression patterns of Lgr5$^+$ and quiescent ISCs are partially overlapping, and both ISC populations have been shown to interconvert (Buczacki et al. 2013, Itzkovitz et al. 2011, Munoz et al. 2012, Takeda et al. 2011). For instance, Bmi1$^+$ ISCs have been shown to express Lgr5, Paneth cells, and EE markers, including Mex3a and Prox1, suggesting that they could rather be preterminal endocrine cells capable of cellular plasticity during homeostasis and following the loss of Lgr5$^+$ ISCs (Buczacki et al. 2013, Jadhav et al. 2017, Yan et al. 2017). The existence of distinct and heterogeneous lineages offers an additional level of epithelial diversity and plasticity, where IECs can rapidly adapt to extrinsic and intrinsic factors and adopt alternate cellular fates to restore ISC function and intestinal homeostasis.

The colon epithelial architecture differs from the small intestine in terms of both structure and cellular composition. The colon contains crypts and Lgr5$^+$ ISCs but lacks villi at the mucosal surface and does not contain Paneth cells or Bmi1$^+$ populations. However, long-lived and quiescent ISCs have recently been identified in the colonic crypt, including Lrig1$^+$, Krt19$^+$, and Dclk1$^+$ populations (Asfaha et al. 2015, Powell et al. 2012, Westphalen et al. 2014). Comparable to the Bmi1$^+$ ISCs found in the small intestine, these ISCs proliferate and divide upon injury and contribute to the replenishment of damaged crypts. The Paneth cell equivalents in the colon crypt niche have also recently been identified. Reg4$^+$ deep crypt secretory cells are found intermingled with Lgr5$^+$ ISCs and support the colon ISC niche through Wnt activation and Notch inhibition (Sasaki et al. 2016).

Cancer Stem Cells Within the Intestine

Plasticity represents a fundamental aspect of stem cell dynamics and hierarchy. The epigenetic similarities between ISCs and their crypt progenitor cells allow them to respond to niche stimuli and shift between different functional states, including quiescence and proliferation

(Vermeulen & Snippert 2014). Given that cancer is essentially a disease of uncontrolled cell proliferation, we can apply the stochastic model of ISC dynamics to study cancer development. Indeed, ISCs are believed to be the cells of origin for CRC. Furthermore, CRC has been regarded as a paradigm of sequential tumorigenesis, where tumor initiation and progression result from the gradual acquisition of genetic mutations that confer a competitive advantage over neighboring cells. Since normal ISCs share key hallmarks with cancer stem cells, including self-renewal capacity, it is likely that ISCs preferentially gain the initial mutations required for malignant transformation (Visvader 2011). This guiding principle supports the earlier framework of CRC, known as the adenoma-carcinoma sequence, where genetic mutations are acquired across different stages of colorectal tumor development (Kreso & Dick 2014, Vogelstein et al. 1988).

In line with the discussion above, evidence from several murine experiments and human samples has demonstrated that ISCs are preferentially transformed during intestinal and colon cancer initiation (**Figure 1**). The conditional deletion in Lgr5$^+$ ISCs of the tumor suppressor and negative Wnt regulator *Apc* results in their rapid transformation, fueling the growth of adenomas (Barker et al. 2009). Similarly, Lgr5 labels a subpopulation of adenoma cells and promotes the growth of established intestinal adenomas by generating additional Lgr5$^+$ cells and progenitor cells (Schepers et al. 2012). Accordingly, Lgr5 is overexpressed in murine and human adenomas compared to matched normal tissue and correlates with poor prognosis (Becker et al. 2008, Han et al. 2015, Hsu et al. 2013, Liu et al. 2014, Takahashi et al. 2011). The cancer-initiating properties of ISCs in human and murine CRC are also dependent on Bmi1 (Kreso et al. 2014, Yanai et al. 2017). Importantly, inactivation of *Apc* in more differentiated cells along the villi rarely leads to the formation of adenomas, indicating that the activation of the Wnt pathway in these cells is insufficient to induce tumor formation (Barker et al. 2009).

There is supporting evidence that colonic stem cells are also preferentially transformed and represent the cancer-initiating cells of tumors. Loss of *Apc* results in the expansion of the Lgr5$^+$ ISC population, progenitor cell hyperproliferation, and CRC initiation. ISC expansion during tumor initiation is dependent on Rac1-driven reactive oxygen species (ROS) production and NF-κB activation. ROS are also rapidly generated in response to microbial stimuli and injury, thereby having the potential to activate ISC signaling and promote colonic tumorigenesis (Myant et al. 2013). The conditional knockdown of *Apc* in either Lrig1$^+$ or Dclk1$^+$ cells also results in colonic adenoma initiation (Asfaha et al. 2015, Westphalen et al. 2014). Because of their quiescent state, these ISCs may maintain mutations for prolonged periods and initiate CRC once activated following mucosal injury.

Intestinal tumors can also arise from the dedifferentiation of nonstem cells, according to their intrinsic genetic variations and their microenvironment. Indeed, the dedifferentiation of Lgr5$^-$ progenitor cells as a result of aberrant Wnt activation and constitutive NF-κB activity can lead to tumor initiation (Schwitalla et al. 2013). The properties of cancer ISCs are also partly governed by the tumor microenvironment; progenitor cells gain features of cancer ISCs following cytokine stimulation produced by tumor-associated cells (Kryczek et al. 2014, Todaro et al. 2014, Vermeulen et al. 2010). Given the pivotal role of the microbiota in the etiology of CRC, microbes may influence the cancer-initiating functions of ISCs, as explored below. Overall, the diverse nature of cancer-initiating cells could explain the broad heterogeneity of CRCs seen among patients.

Intestinal Stem Cell–Microbiota Interactions: Lessons from *Drosophila*

Studies in *Drosophila* have provided some of the earliest insights into the interaction between the microbiota and ISCs. The gut epithelia of metazoan organisms share similarities with their mammalian counterparts. Structurally, ISCs reside directly underneath epithelial ECs and possess self-renewal properties allowing for ISC maintenance and EC generation (Micchelli & Perrimon

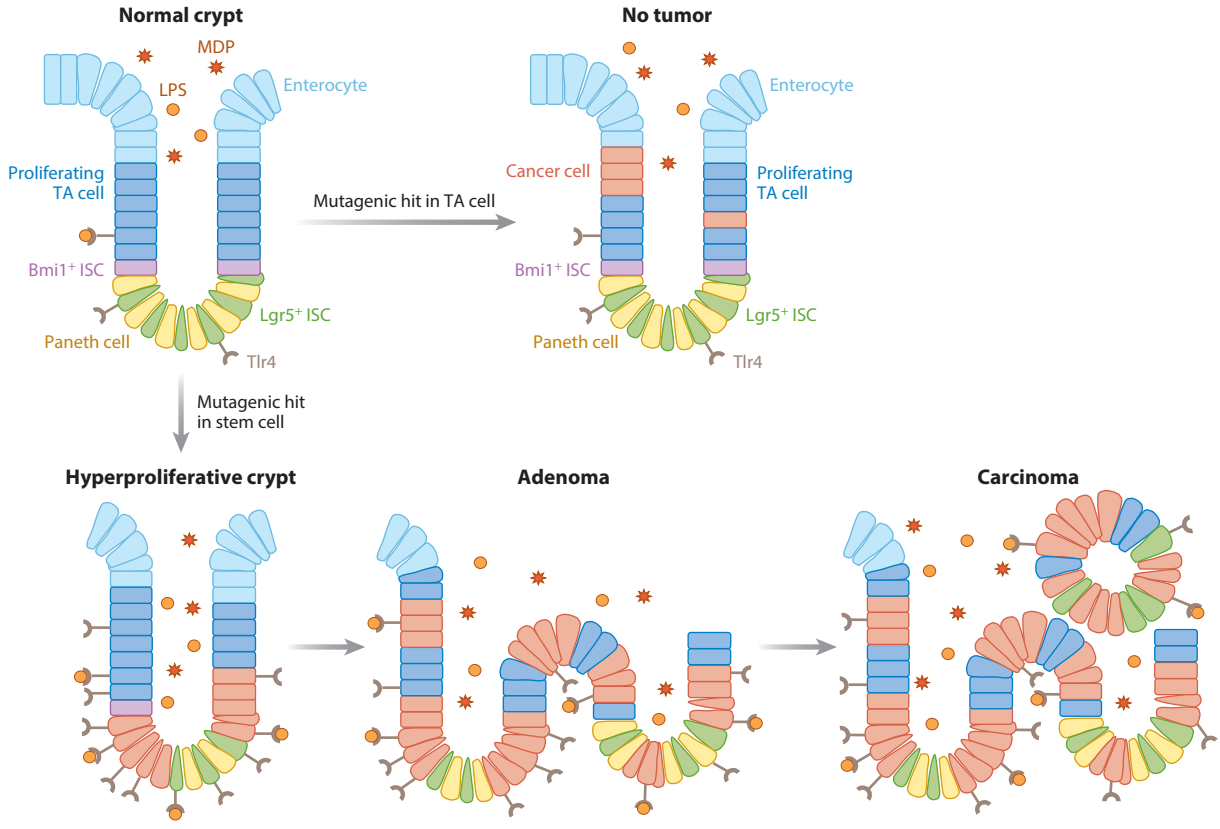

Figure 1

The development of colorectal cancer (CRC) from a normal crypt toward carcinoma, known as the adenoma-carcinoma sequence. (*Bottom panels*) Loss of Apc or β-catenin mutation in intestinal stem cells (ISCs) typically represents the first step toward adenoma formation. The transformation of Lgr5$^+$ ISCs (*green*) results in increased Wnt activation and a higher proliferative index associated with the expansion of the ISC compartment (Barker et al. 2009). The transformation of Bmi1$^+$ ISCs (*purple*) can also result in adenoma formation. Bacteria and bacterial metabolites can support the growth of adenomas. Lgr5$^+$ ISCs can upregulate Toll-like receptor 4 (Tlr4) (*brown*), and its activation with lipopolysaccharide (LPS) (*orange*) further promotes cell proliferation and the expansion of cancer cells (*red*) (Santaolalla et al. 2013). Other bacterial products, such as muramyl dipeptide (MDP) (*red star*), likely influence ISC survival and CRC progression. Mutated ISCs can acquire other genetic mutations and spread toward an invasive carcinoma. (*Top panels*) The initiation of adenomas by more differentiated cells along the villi, such as the proliferating transit-amplifying (TA) cells (*dark blue*) and enterocytes (*light blue*), depends on their intrinsic genetic variations and tumor microenvironment (Barker et al. 2009, Schwitalla et al. 2013).

2006). Importantly, the fly gut also harbors complex microbial communities that provide a dynamic environment influencing ISC activity (Hooper & Gordon 2001). Indeed, several groups have demonstrated that infectious and indigenous bacteria dramatically impact ISC function (Buchon et al. 2009, Cronin et al. 2009, Jiang et al. 2009). Under homeostatic conditions and following tissue damage or infection, bacteria provide a physiological signal that activates the JAK-STAT signaling pathway, thereby promoting ISC proliferation and wound repair. JAK-STAT activity was also shown to induce Delta/Notch signaling, resulting in EC differentiation and midgut regeneration (Jiang et al. 2009).

More recently, commensal bacteria were demonstrated to influence ISC activity through the generation of ROS. Specifically, introduction of members of the genus *Lactobacillus* into the murine or *Drosophila* gut resulted in oxidative stress responses and Nox1-dependent ROS production and

subsequent ISC proliferation (Jones et al. 2013). Taken together, these results provide strong evidence for host-microbiota interactions acting as physiological signals that control ISC activity and gut homeostasis.

Impact of Bacterial Products on Mammalian Intestinal Stem Cells

Bacteria are present in the mouse proximal colonic crypts and could provide a major source of stimuli to the ISCs (Pedron et al. 2012, Swidsinski et al. 2005). There is supporting evidence from comparative studies between germ-free and conventionally raised animals that microbes can influence crypt homeostasis (Alam et al. 1994, Reikvam et al. 2011). However, the specific role that the microbiota play on ISC activity has only recently been explored, and it remains unclear whether bacteria can directly stimulate ISCs via innate pattern recognition receptors (PRRs). PRRs selectively recognize distinct microbial products conserved and shared among different microorganisms, and their engagement typically results in a proinflammatory response. Several classes of PRRs have been identified, including Toll-like receptors (TLRs) and NOD-like receptors (NLRs).

In mice, *Tlr4* expression is mainly associated with cells within the crypt base of the small intestine. Neal et al. (2012) demonstrated that *Tlr4* is expressed in Lgr5$^+$ ISCs. By engineering a mouse in which *Tlr4* was selectively deleted in Lgr5$^+$ ISCs, they showed that Tlr4 regulates their proliferation and apoptosis. Conversely, the overexpression of *Tlr4* in the intestinal epithelium (villin-*Tlr4*) results in the expansion of colonic Lgr5$^+$ ISCs associated with increased epithelial proliferation (Santaolalla et al. 2013). Of note, villin-*Tlr4* mice develop spontaneous duodenal dysplasia over time and are more susceptible to colonic adenoma formation when treated with the genotoxic agent azoxymethane (AOM) (Santaolalla et al. 2013). In agreement with these findings, *Tlr4*-deficient mice are protected from colitis-associated carcinogenesis (CAC), and *Tlr4* is overexpressed in human and murine CRC (Fukata et al. 2007). Together, these results reveal a previously uncharted relationship between Tlr4 signaling and ISC maintenance, which could impact tumor initiation.

More recently, the cytoplasmic innate immune receptor Nod2, which detects the bacterial cell wall fragment muramyl dipeptide (MDP), was shown to be expressed in Lgr5$^+$ ISCs (Nigro et al. 2014). Nod2-mediated MDP stimulation promotes Lgr5$^+$ ISC survival and epithelial regeneration, highlighting another mechanism by which bacterial products can influence ISCs. Accordingly, Nod2 regulates epithelial regeneration following T cell–mediated enteropathy of the small intestine, and MDP stimulation protects mice from experimental colitis (Watanabe et al. 2008, Zanello et al. 2016). The role of Nod2 in the gut is particularly important, as genetic variants within the *NOD2* gene have been associated with increased susceptibility to Crohn disease, a chronic inflammatory disorder of the gastrointestinal tract (Hugot et al. 2001, Ogura et al. 2001). The causal relationship between chronic inflammation and cancer is strongly supported by the fact that patients with IBD show an increased risk for CRC development (Itzkowitz & Yio 2004, Khor et al. 2011). However, the role of *Nod2*-expressing ISCs in CRC development remains unexplored and warrants further investigation. It would be worthwhile to determine if Nod2 activity can also protect cancer stem cells and drive adenoma formation. Intriguingly, polymorphisms in *NOD2* have been associated with the onset of CRC in humans, and as discussed below, *Nod2*-deficient mice are predisposed to CRC development (Couturier-Maillard et al. 2013).

ASSOCIATION OF SPECIFIC MICROBES WITH COLORECTAL CANCER: DRIVERS OF TUMORIGENESIS?

The recent advent and widespread availability of next-generation sequencing technology have revolutionized microbiota studies. Investigators are now able to readily analyze the composition

of commensal microbiota and identify differences between disease states by sequencing part of or entire bacterial genomes. Specifically, bacterial taxa are identified by sequencing the variable regions of the gene encoding 16S ribosomal RNA (rRNA) (Tringe & Hugenholtz 2008). While 16S rRNA gene amplicon sequencing is useful in measuring microbial community structures and broad shifts in community diversity over time, it does have limited resolution and sensitivity when compared to metagenomic data obtained from standard shotgun sequencing protocols (Poretsky et al. 2014). Nonetheless, 16S rRNA gene sequencing analysis has revealed differences in gut microbial composition between CRC patients and healthy controls. For instance, decreased microbial diversity was observed in the feces of CRC patient when compared to healthy controls (Ahn et al. 2013). The same was true at the mucosal level when comparing dysplastic mucosa to neighboring control tissue (Chen et al. 2012). Several studies have also proposed that specific bacterial groups are either more or less commonly associated with CRC (Ahn et al. 2013, Chen et al. 2012, Kostic et al. 2012, Mira-Pascual et al. 2015, Monira et al. 2013, Nicolson et al. 2013, Shazali et al. 2014, Sobhani et al. 2011, Wang et al. 2012, Weir et al. 2013). However, the disparity between studies, in part due to the lack of taxonomic and classification consistency, renders it nearly impossible to characterize a common cancer-associated microbiome.

Nonetheless, approximately 20% of the global burden of cancer is thought to stem from infectious agents (Zackular et al. 2013). The associations of *Helicobacter pylori* with gastric cancer and human papillomaviruses with cervical cancer are well-known examples of this infectious link to cancer development. Several bacteria are associated with CRC development (Arthur et al. 2012; Belcheva et al. 2014; Erdman et al. 2003, 2009; Wang et al. 2017; Wu et al. 2009), but it remains unclear whether such bacteria are CRC initiators or passengers (Tjalsma et al. 2012). CRC initiators induce DNA damage either directly through the production of genotoxins or indirectly by promoting inflammation that, in turn, leads to the production of genotoxic agents. Conversely, passenger bacteria exploit the unique tumor microenvironment to grow and outcompete other microbes. Therefore, bacterial enrichment at the site of tumors does not always signify that a particular microbe is driving tumor progression. Linking specific microbes to CRC is also hampered by the fact that individuals possess unique bacteriomes, influenced by constant horizontal gene transfer between unrelated bacteria (Lloyd-Price et al. 2016), presence or absence of bacteriophages, and strain differences among specific bacterial species (Scanlan 2017). Despite these caveats, there is evidence for the involvement of specific microbes in CRC (see **Table 1**). Two examples of these are described below.

Fusobacterium nucleatum Link with Colorectal Cancer: Initiator or Passenger?

Fusobacterium nucleatum is an invasive anaerobe that is associated with periodontal disease. However, recent work has shown that *F. nucleatum* is associated with CRC tissues compared to normal control tissues (Castellarin et al. 2012, Kostic et al. 2012). *F. nucleatum* positivity is also significantly associated with microsatellite instability status and shorter survival in patients with CRC (Mima et al. 2016, Nosho et al. 2016). Interestingly, *F. nucleatum* strains extracted from inflamed mucosa of IBD patients are more invasive than strains extracted from control patients, but whether this phenotype is related with carcinogenesis is unknown (Strauss et al. 2011). Moreover, genetic differences between pathogenic *F. nucleatum* strains have not been identified.

It has been suggested that *F. nucleatum* adhere to colonic IECs through the fusobacterial protein Fap2 binding to Gal-GalNAc, which is commonly overexpressed in cancer-associated colonocytes (Abed et al. 2016). Fap2 can also interact with the inhibitory receptor TIGIT, expressed in all human natural killer cells and on several T cell subsets, and thereby inhibit immune cell activation and act as an immunosuppressive agent (Gur et al. 2015). Accordingly, there is an inverse

Table 1 Human enteric bacteria associated with CRC[a]

Bacteria	Evidence in animal models	Evidence in humans	Proposed mechanism(s)	Conclusion
Helicobacter pylori	Unknown	CagA and VacA seropositivity associated with CRC (Epplein et al. 2013, Shmuely et al. 2001)	Dysbiosis (Kanno et al. 2009) Cell hyperproliferation (Renga et al. 1997) Chronic inflammation[b] (Papastergiou et al. 2016) ROS production[b] (Chaturvedi et al. 2011, Tsugawa et al. 2012)	Theoretical initiator and possible passenger
Helicobacter hepaticus	AOM-treated BALB/c *Il10*$^{-/-}$ mice (Nagamine et al. 2008a) *Smad3*$^{-/-}$ mice (McCaskey et al. 2012) *Rag2*$^{-/-}$ mice (Erdman et al. 2003, 2009) BALB/c *Rag2*$^{-/-}$ *Apc*$^{min/+}$ mice (Nagamine et al. 2008b)	Not detected in human colon	Colitis induction (McCaskey et al. 2012) ROS production (Wang et al. 2017) DNA damage (Bezine et al. 2016, Fedor et al. 2013, Wang et al. 2017)	Theoretical initiator
Streptococcus bovis biotype I	AOM-treated rats (Biarc et al. 2004, Ellmerich et al. 2000)	Endocarditis bacteremia associated with CRC (Corredoira et al. 2005, Ruoff et al. 1989)	Colon epithelial cell hyperproliferation (Ellmerich et al. 2000) Inflammation (Biarc et al. 2004, Ellmerich et al. 2000)	Undetermined
Enterotoxigenic *Bacteriodes fragilis*	*Apc*$^{min/+}$ mice (Wu et al. 2009)	Colitis inductor (Sears et al. 2014) Increased prevalence in early stages of colonic dysplasia (Purcell et al. 2017)	Wnt signaling activation (Wu et al. 2003) Inflammation (Geis et al. 2015) DNA damage and ROS production (Goodwin et al. 2011)	Possible initiator
Fusobacterium nucleatum	*Apc*$^{min/+}$ mice (Kostic et al. 2013) Xenograft in BALB/c nude mice (Yang et al. 2017)	Increased *F. nucleatum* DNA in CRC tissue associated with shorter survival (Castellarin et al. 2012) Increased anti–*F. nucleatum* IgA and IgG in CRC patients (Wang et al. 2016)	Wnt signaling activation (Rubinstein et al. 2013) Immune inhibition (Gur et al. 2015, Kostic et al. 2013, Mima et al. 2015) Tlr4 activation and miR21 expression (Yang et al. 2017)	Possible passenger and tumor growth promoter
Escherichia coli	AOM-treated *IL10*$^{-/-}$ mice (Arthur et al. 2012)	Colitis inductor (Chen & Frankel 2005) Increased prevalence in CRC (Gagniere et al. 2017)	Cell cycle disruption (Fedor et al. 2013) Senescence (Cougnoux et al. 2014)	Possible initiator

[a]Abbreviations: AOM, azoxymethane; CRC, colorectal cancer; IgA, immunoglobulin A; IgG, immunoglobulin G; ROS, reactive oxygen species; Tlr4, Toll-like receptor 4.
[b]Noncolonic tissue.

correlation between *F. nucleatum* association and $CD3^+$ T cell density (Mima et al. 2015). In addition, $Apc^{Min/+}$ mice with *F. nucleatum*–positive adenomas have an increased number of infiltrating myeloid-derived suppressor cells, suggesting that *F. nucleatum* can induce an anti-inflammatory environment to support CRC development (Kostic et al. 2013). Indeed, *F. nucleatum* produces butyrate, a known inducer of regulatory T cell differentiation, which could interfere with antitumoral inflammatory responses (Furusawa et al. 2013). Moreover, *F. nucleatum* has a unique FadA adhesin, which binds E-cadherin and increases free β-catenin, thereby activating the Wnt pathway and stimulating cell proliferation (Rubinstein et al. 2013). This adhesin represents a potential diagnostic marker for CRC since the levels of the *FadA* gene in human colonic adenomas are >10–100 times higher compared to controls.

Genotoxic *Escherichia coli* and Colorectal Cancer

While most *Escherichia coli* strains are harmless, the abundance of certain strains in patients with IBD or CRC can correlate with disease. These *E. coli* strains express virulence factors linked to disease pathogenesis, including the genotoxins, colibactin, and cytolethal distending toxin (CDT). Colibactin is encoded within the virulent gene island polyketide synthase (PKS) and promotes CRC in animal models through the induction of DNA damage and senescence, the latter of which is a consequence of increased p53 SUMOylation (Arthur et al. 2012, Cougnoux et al. 2014, Dalmasso et al. 2014). CDT induces DNA double-stranded breaks in S phase, which, when left unrepaired by homologous recombination, leads to G2/M cell cycle arrest in an ATM-dependent manner (Fedor et al. 2013). Both CDT and colibactin can induce DNA damage, suggesting that mice harboring mutations in DNA repair genes are likely more susceptible to infection with these *E. coli* strains. In fact, CDT accelerates anchorage-independent growth in p53-deficient human colonic IECs (Graillot et al. 2016). These results suggest that DNA repair–deficient cells might be more susceptible to the genotoxicity induced by CDT. Intriguingly, it has been suggested that colibactin plays diverse roles in human health since its gene cluster is present in both pathogenic and probiotic enterobacteria. Indeed, the probiotic *E. coli* Nissle 1917 strain harbors the PKS genotoxicity island; however, it has not been associated with CRC in mouse models (Olier et al. 2012). Clearly, the expression and regulation of these DNA-damaging virulence factors still need to be defined. Moreover, how the biological activity of these *E. coli* strains is modulated in the context of the human intestine and with the resident microbiota might not be captured in mouse models of CRC.

MICROBIOTA, INFLAMMATION, AND COLORECTAL CANCER PROGRESSION: BREACHING BARRIERS

A key mechanism for bacteria-mediated CRC progression following malignant transformation involves epithelial barrier failure and low-grade inflammation. As crypts become dysplastic, they begin to lose their architecture, which results in the disruption of the epithelial barrier. Perturbation of the barrier promotes bacterial translocation, exposing the tumor microenvironment to immunogenic microbial products. The invading microbial compounds can shape the immune landscape of tumors by triggering a proinflammatory or immunosuppressive environment that promotes carcinogenesis (Olier et al. 2012). Barrier disruption is clinically relevant, as it contributes to the pathogenesis of IBD and further increases the risk of cancer development (Itzkowitz & Yio 2004, Khor et al. 2011). Indeed, in a mouse model of inflammation-driven CRC, barrier deterioration results in increased bacterial invasion and tumor progression. Bacterial products accumulate within adenomas and induce the production of the inflammatory cytokines, IL-17

and IL-23, which in turn promotes cancer development (Grivennikov et al. 2012). Importantly, antibiotic treatment was able to reduce carcinogenesis in this model. Thus, bacterial translocation associated with epithelial barrier defects can potentiate inflammation and diseases, including CRC.

Several germ line–encoded PRRs have evolved to maintain epithelial barrier homeostasis by detecting invading bacteria. However, defects in or chronic activation of these innate immune receptors has been associated with inflammatory diseases and carcinogenesis. Namely, bacterial products are believed to infiltrate tumors and activate NF-κB signaling via PRR engagement, which establishes a proinflammatory microenvironment that allows tumors to grow and spread. Inflammation maintains cancer development by enhancing the rate of mutagenesis, angiogenesis, and cell proliferation, survival, and migration (Coussens & Werb 2002). Additionally, tumors can upregulate the expression of several PRRs to support their development. In humans, PRR expression in tumors has also been associated with cancer severity and poor prognosis (Pandey et al. 2015). Growing evidence from animal studies further supports the links between the microbiota, innate immune activation, and cancer.

Toll-Like Receptor Signaling and Colorectal Cancer

The AOM/dextran sulfate sodium (DSS) model is among the most popular model to study the role of PRRs in CAC. It involves an initial injection of AOM, a potent carcinogen, followed by successive cycles of DSS to induce chronic inflammation of the colon and increase the incidence and progression of colon cancer (Wirtz et al. 2007). In mice, the specific inactivation of NF-κB, a key transcription factor downstream of PRR engagement, reduces tumor development. Specifically, deficient NF-κB signaling in IECs decreases tumor burden, while inactivation of NF-κB in myeloid cells greatly reduces tumor size (Greten et al. 2004). These results reflect the intricate and cell-specific effect of NF-κB signaling in cancer progression. Additional research is required to understand the cell-intrinsic role of bacterial stimuli on the different cells composing the tumor microenvironment, including tumor-associated immune cells, stromal cells, and cancer cells.

Pioneering research has revealed a role for the adaptor protein MyD88, a downstream adaptor of most TLRs, in cancer development. $Apc^{min/+}$ MyD88-deficient mice are significantly protected from spontaneous tumor development (Rakoff-Nahoum & Medzhitov 2007). During CAC, *Tlr4*-deficient mice show equally reduced tumor development, and the constitutive activation of epithelial Tlr4 increases tumor prevalence (Fukata et al. 2007, 2011). However, Tlr2 and MyD88 signaling also protects mice from CAC (Lowe et al. 2010, Salcedo et al. 2010). The disparity between these studies could be explained by the fact that Myd88 is also the adaptor protein in signaling downstream of IL-1β/IL-18 receptor engagement, which could implicate the role of inflammasomes in CRC, as discussed below.

Inflammasomes: Implicating Dysbiosis and Inflammation in Colorectal Cancer Progression

Inflammasomes are multiprotein complexes that induce inflammation and pyroptosis, an inflammatory form of programmed cell death, in response to microbial insults and damage-associated molecular patterns. Their structures typically include an NLR protein through which they can respond to environmental cues and activate the cysteine protease Capsase-1. Activation of Caspase-1 results in the cleavage and release of IL-1β and IL-18, which elicit local inflammation. Together, inflammasomes can respond to a wide range of stimuli. For instance, the Nlrc4 inflammasome is activated by bacterial components, whereas the Aim2 inflammasome responds to double-stranded

DNA (Fernandes-Alnemri et al. 2009, Franchi et al. 2006, Hornung et al. 2009, Mariathasan et al. 2004, Rathinam et al. 2010). While some of the mechanisms underlying inflammasome engagement remain unclear, their activation and production of inflammatory cytokines can influence many aspect of cancer biology, including tumor incidence, progression, and metastasis.

Inflammasomes and their effector proteins have also been shown to influence the gut microbiota composition, revealing another mechanism by which these multimolecular complexes can affect cancer cells (Chen et al. 2017, Hu et al. 2013, Man et al. 2015). *Nlrp6*-deficient mice develop a dysbiotic microbiome, which increases their susceptibility to colitis and adenoma formation. Importantly, the dysbiosis-mediated cancer risk was transmissible to wild-type (WT) mice in cohousing experiments (Hu et al. 2013). Nlrp6 was more recently shown to play a role in inflammatory monocytes and to protect from chemically induced gut injury and reduce tumor growth (Seregin et al. 2017). However, it remains unclear whether Nlrp6 also functions in monocytes to control the microbial composition. Deficiency for other members of the inflammasome or NLRs, including Nlrp3 and Nlrc4, has also been associated with CRC development, but their functional mechanisms in microbial-driven cancer remain elusive (Hu et al. 2010, Zaki et al. 2010).

The Nlrp12 inflammasome attenuates intestinal inflammation and tumorigenesis by suppressing both canonical and noncanonical NF-κB signaling (Allen et al. 2012, Zaki et al. 2011). Nlrp12 has also recently been shown to serve as a negative regulator of inflammation by maintaining and promoting protective gut microbial communities (Chen et al. 2017). Specifically, *Nlrp12*-deficient mice are associated with a dysbiotic microbial profile similar to that seen in IBD patients, characterized by lower abundance of the orders Bacteroidales, Clostridiales and Lachnospiraceae and a greater abundance of the family Erysipelotrichacea. However, it remains unknown whether the protective strains, such as those from the family Lachnospiraceae, contribute to the antitumor role of Nlrp12 in mice. The Lachnospiraceae family of bacteria is less abundant in the gut microbiota of CRC patients compared to healthy individuals and, therefore, could represent key regulators of microbial balance, namely, by preventing the outgrowth of opportunistic pathogens (Wang et al. 2012).

Aim2 is another inflammasome-related PRR involved in DNA sensing within the cytoplasm. Interestingly, Aim2 plays a role in suppressing CRC in the AOM/DSS model, but it does so in a manner independent of its inflammasome activity yet dependent upon microbial stimulation (Wilson et al. 2015, Man et al. 2015). Indeed, *Aim2*-deficient mice have altered proliferation within the ISC compartment due to aberrant Wnt signaling, independent of IL-1β/IL-18 signaling. Furthermore, *Aim2*-deficient mice exhibit an altered microbiome compared to WT mice, and cohousing these animals reciprocally enhanced tumor development in WT mice while decreasing the number of tumors in *Aim2*-deficient animals (Man et al. 2015). Taken together, these findings demonstrate the complex relationship between the host's genetics and microbial environment in promoting tumorigenesis.

NOD-Like Receptors in Colorectal Cancer Development

Nod2 is another NLR that is involved in bacteria-driven carcinogenesis. As mentioned previously, impaired Nod2 signaling is associated with CRC development in mice. The increased tumor burden observed in *Nod2*-deficient mice was transmissible to nonlittermate WT mice by cohousing, suggesting that the genotype-dependent cancer susceptibility is also mediated by microbial dysbiosis (Couturier-Maillard et al. 2013). However, these associations remain controversial, as the composition of intestinal bacterial communities does not differ between *Nod2*-deficient and WT littermates at homeostasis (Robertson et al. 2013). Moreover, the susceptibility of *Nod2*-deficient

mice to CRC was recently shown to occur independently of gut microbial dysbiosis and was dependent rather on Nod2-mediated suppression of the TLR pathways via Irf4 (Udden et al. 2017). Moreover, *NOD2* expression is increased in CRC patients compared to adjacent healthy control tissues, suggesting that NOD2 signaling plays a role in cancer progression (Liu et al. 2015). These discrepancies emphasize the complexity behind inflammation-driven tumor development and underscore the importance of using littermate controls to elucidate the role of the microbiota in CRC.

Nod1 is a member of the NLR family and a close relative of Nod2, which is likewise implicated in intestinal defense against bacteria. *Nod1* deficiency has also been associated with increased susceptibility to CRC in mice. The absence of Nod1 signaling is associated with greater inflammatory cytokine production following barrier disruption, which increases IEC proliferation and impacts tumor development (Chen et al. 2008). *Nod1*-deficient T cells have recently been shown to have impaired interferon-γ (IFN-γ) production, which is associated with increased tumor incidence. IFN-γ deficiency leads to increased CRC, and the adoptive transfer of *Nod1*- or *IFN-γ*-deficient T cells into T cell–deficient mice resulted in increased tumorigenesis (Zhan et al. 2016). These findings support the antineoplastic reputation of IFN-γ and suggest a novel role for Nod1 in tumor immunosurveillance through T cell–mediated mechanisms. Curiously, while IFN-γ is a potent inducer of T helper cell 1 (Th1) immune responses, Nod1 stimulation predominantly drives Th2 immune pathways (Fritz et al. 2007). Therefore, it would be interesting to determine how Nod1-mediated Th2 immunity impacts different immune cells found in the solid tumor microenvironment, including macrophages and other myeloid cells.

While past studies have illustrated the effect of several NLRs and TLRs in microbial-driven cancer, the exact role these innate receptors play in specific cells associated with tumors remains elusive. Inflammation plays a complex role in cancer biology, as it can both potentiate cancer progression and influence cancer immune surveillance. It is therefore likely that bacterial stimuli in different cell types will exert different functions. Studies are beginning to unveil the cell-specific role of innate immune receptors in cancer development. For instance, Nlrx1 is known to play a role in tumorigenesis, but has only recently been shown to act as an epithelial-intrinsic tumor suppressor (Koblansky et al. 2016, Soares et al. 2014, Tattoli et al. 2016). Mice with an IEC-specific deletion of *Nlrx1* are more susceptible to CAC, which is associated with increased expression of factors involved in epithelial regeneration (Koblansky et al. 2016, Tattoli et al. 2016). Specifically, Nlrx1 was shown to regulate the proliferative response of IEC following TNF stimulation (Tattoli et al. 2016). Together, these studies reveal an epithelial-intrinsic role for Nlrx1 during wound repair, which ultimately influences cancer progression. Nevertheless, it is likely that Nlrx1 also exerts its tumor-suppressor role in different immune cell subsets. *Nlrx1*-deficient mice are more vulnerable to DSS-induced colitis, which correlates with increased inflammation. However, the increased inflammation associated with peak tissue damage was not observed in mice lacking *Nlrx1* specifically in IECs, suggesting that the innate receptor can downregulate inflammation in nonepithelial cells (Soares et al. 2014). These findings further reinforce the need to study the cell-intrinsic functions of PRRs in inflammation and carcinogenesis.

Appropriately, Karki et al. (2016) have recently unraveled the multifaceted role of Nlrc3 in cancer. Using mice lacking *Nlrc3* specifically in hematopoietic cells, the myeloid lineage, or IECs, the authors demonstrated that Nlrc3 functions predominantly in IECs as a tumor suppressor. Interestingly, hematopoietic-specific but not myeloid-specific deletion of *Nlrc3* also resulted in increased tumor burden, suggesting that Nlrc3 may function in lymphocytes to inhibit carcinogenesis. These recent data have expanded the concept that bacterial stimuli can influence inflammation and tumor progression at different levels. Understanding the cell-intrinsic mechanisms of innate

immune receptors involved in carcinogenesis will increase our knowledge of the heterogeneous nature of tumors and likely impact future cancer therapies.

PROSPECTIVES AND CONCLUSION

Given the relationship between the microbiome and CRC, there has been a lot of interest in the therapeutic potential of modulating the microbiome to treat or prevent cancer. Moreover, the recent discovery that the microbiome can alter therapeutic outcomes in cancer treatment has underscored the importance of microbial studies, with a particular interest in understanding how the gut microbiome composition might impact CRC therapy.

Dietary modulation, pro- and prebiotics, and fecal microbiota transplants (FMTs) are all measures that can influence gut microbiota composition and potentially impact CRC. In animal models, a Western diet, characterized by high fat or high carbohydrate intake, enhances tumorigenesis and is linked to altered gut microbiomes compared to normal-fed mice (Belcheva et al. 2014). Moreover, pro- and prebiotics are somewhat efficacious on CRC incidence in animal models through mechanisms that include modulation of the existing gut microbiome. Such measures can hinder procolitiogenic and procarcinogenic communities while encouraging a recalibration of a healthy homeostatic microbiome (reviewed in Ambalam et al. 2016). Despite these promising effects, however, translating animal studies into clinical trials in humans remains challenging. Finally, there has been a lot of recent interest in FMT for the treatment of IBD and prevention of CRC. Two recent randomized controlled trials of FMT in ulcerative colitis gave conflicting results, where the trial showing efficacy was donor dependent (Moayyedi 2016). These studies highlight the potential of this approach for the treatment of IBD; however, much research still needs to be carried out to determine dosage, the optimal route of administration, and, importantly, what defines efficacious donor microbiota. Furthermore, whether FMT would be a potential preventative strategy for CRC patients or individuals at risk for developing CRC needs to be explored.

The symbiosis that develops between the host and its microbiome is critical for health, and any imbalances in this relationship are a risk for disease development. Interestingly, recent evidence suggests that the gut microbiome can also influence the host's response to cancer treatment. Remarkably, the presence of specific gut microbes mediates the antitumor effects of two checkpoint inhibitors that block CTLA-4 or the PD-1/PD-L1 axis (Sivan et al. 2015, Vetizou et al. 2015). Moreover, cancer therapies can cause dysbiosis, which, in turn, can enhance or dampen the effects of chemotherapeutic agents (reviewed in Viaud et al. 2015). Finally, metabolism of chemotherapeutic agents by gut microbes might also modulate their actions. Indeed, microbial metabolism of the CRC therapeutic 5-fluorouracil mediates the cytotoxic effects of this drug in a *Caenorhabditis elegans* model (Garcia-Gonzalez et al. 2017, Scott et al. 2017). Thus, manipulating the gut microbiome might represent a new tool in combating tumorigenesis in CRC.

It is now clear that bacteria at the mucosal surfaces drastically influence CRC incidence and progression. Nonetheless, their contribution to carcinogenesis remains complex, as bacteria can both modulate cancer development and alter the immune response against cancer. Directions for future research will aim to understand the diverse contributions of bacteria and their metabolites to carcinogenesis. Indeed, the modulation of the microbiota has already proven to be of major clinical relevance and will continue to open new avenues for CRC prevention and treatment.

DISCLOSURE STATEMENT

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