ANNUAL REVIEWS

Annual Review of Medicine

Microbiota Effects on Carcinogenesis: Initiation, Promotion, and Progression

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Annu. Rev. Med. 2021. 72:243–61

First published as a Review in Advance on October 14, 2020

The *Annual Review of Medicine* is online at med.annualreviews.org

https://doi.org/10.1146/annurev-med-080719-091604

Keywords

carcinogenesis, colorectal cancer, microbiota, colibactin, *B. fragilis* toxin, *Fusobacterium*

Abstract

Carcinogenesis is a multistep process by which normal cells acquire genetic and epigenetic changes that result in cancer. In combination with host genetic susceptibility and environmental exposures, a prominent procarcinogenic role for the microbiota has recently emerged. In colorectal cancer (CRC), three nefarious microbes have been consistently linked to cancer development: (*a*) Colibactin-producing *Escherichia coli* initiates carcinogenic DNA damage, (*b*) enterotoxigenic *Bacteroides fragilis* promotes tumorigenesis via toxin-induced cell proliferation and tumor-promoting inflammation, and (*c*) *Fusobacterium nucleatum* enhances CRC progression through two adhesins, Fap2 and FadA, that promote proliferation and antitumor immune evasion and may contribute to metastases. Herein, we use these three prominent microbes to discuss the experimental evidence linking microbial activities to carcinogenesis and the specific mechanisms driving this stepwise process. Precisely defining mechanisms by which the microbiota impacts carcinogenesis at each stage is essential for developing microbiota-targeted strategies for the diagnosis, prognosis, and treatment of cancer.

INTRODUCTION TO THE MICROBIOTA

At birth, humans are seeded with a diverse collection of microbes, including bacteria, viruses, fungi, archaea, protozoa, and helminths (1–3). Humans and microbes are in symbiosis, supporting host physiology, immunological development, and metabolism, among other essential functions (4–6). This human-associated microbial consortium is termed the microbiota. Bacteria are the best-studied gut microbiota members, due to advances in sequencing technology and bioinformatics pipelines that distinguish bacterial taxa by polymorphisms in the ubiquitous bacterial *16S* ribosomal RNA genomic sequence (7–9). By scrutinizing the genomic content of these microbes (the microbiome), research has uncovered some general principles regarding the gut microbiota.

Microbial communities are finely tuned to fit the ecology and function of each body site, likely benefiting from the nutrients in each microenvironment (3, 10). Many of our microbial species evolve with us, acquiring traits through mutation and horizontal gene transfer over our lifetime that may influence health and disease (11). The gut microbiota is the best-studied human-associated microbial community, due to its diversity, abundance, and ease of sample collection (feces/stool). The gut microbiota community composition and function differ longitudinally down the gastrointestinal tract from mouth to anus, depending on the physiological needs of each niche (12). Interestingly, microbiota are more similar between the guts of different individuals than between different body sites of one individual (e.g., skin versus gut) (5). However, the composition of our individual gut microbiota significantly differs (3).

Researchers have not found a core microbiota. However, when we consider the capabilities—genes and pathways—harbored in individual microbiomes, we see strong similarities among people (3). Thus, it appears that the function of the microbiota may be more important than the presence or absence of species within the community (13). Therefore, individual microbiota hold a potential to impact human health and disease that may be overlooked when simply classifying by taxonomy. The procarcinogenic microbes discussed in this review all harbor specific genes and capabilities absent from the core genome of their particular species (i.e., *Escherichia coli*, *Bacteroides fragilis*, and *Fusobacterium nucleatum*) (14–16). Therefore, we have an obligation to look deeper than community structure to evaluate the procarcinogenic capability of the microbiota.

We previously explored this body of knowledge using Hanahan & Weinberg's "hallmarks of cancer" (17) as a framework to classify specific mechanisms by which microbes, microbial communities, and microbial metabolites may impact cancer development (15). These ten hallmarks comprise key biological capabilities acquired by normal cells as they develop traits of cancer cells and progress toward tumor development. Here, we review and expand upon mechanisms by which specific members of the microbiota influence the development of cancer and speculate upon what stages of carcinogenesis they principally impact.

FOUNDATIONAL ASSOCIATIONS BETWEEN INFLAMMATION, CANCER, AND THE MICROBIOTA

In 1984, Drs. Barry Marshall and J. Robin Warren performed an unprecedented experiment in which self-colonization of Dr. Marshall with patient-derived *Helicobacter pylori* rapidly induced gastritis that was ameliorated by eradication of *H. pylori* with antibiotics (18, 19). Others in the gastric cancer field had been skeptical that bacteria could survive the acidic environment of the stomach and attributed gastric cancer development to genetic susceptibility or other host physiological causes. *Helicobacter* is now recognized as a group 1 carcinogen and the primary cause of gastritis, peptic ulcers, and gastric cancer (20). *Helicobacter* deploys the cytotoxin-associated gene

A (CagA) toxin as the predominant oncoprotein that hijacks multiple epithelial signaling pathways and initiates carcinogenesis with chronic inflammation fueling cancer progression (20). With their infamous experiment, Marshall & Warren (19) had not simply fulfilled Koch's postulates for *H. pylori* and gastritis—they piqued interest in the nuances of this complex relationship between host and microbe(s), and a new field of research emerged to explore microbial-induced chronic inflammation and carcinogenesis.

With the advent of high-throughput sequencing and an explosion of knowledge about our intestinal microbiota, it is now clear that our gut microbiota influences cancer development, most notably colorectal cancer (CRC) (21–23). Microbiota impact host metabolism, inflammation, immunity, and cellular proliferation, which are all processes that when dysregulated can promote tumorigenesis (24). Furthermore, ample evidence suggests that the microbiota can directly impact tumor formation. Fecal transplants from human patients with CRC promote carcinogenesis in germ-free (devoid of any microbiota) and conventional mice administered the colon-specific carcinogen azoxymethane (AOM) (25). Transferring the microbiota of tumor-bearing mice versus non-tumor-bearing mice accelerates the development and severity of tumorigenesis in the AOM/dextran sulphate sodium mouse model (26). The structure and physiological state of the microbiota also influence procarcinogenic effects, as biofilm-associated communities from both CRC patients and healthy individuals induce more tumorigenesis than non-biofilm communities in mouse models (27). These studies demonstrate a causal relationship between the microbiota and CRC development and provide a rationale for further mechanistic studies.

THE MICROBIOTA IN CARCINOGENESIS: INITIATION, PROMOTION, AND PROGRESSION

Over the past decade, preclinical and clinical evidence connects the microbiota and its metabolites to carcinogenesis. The conventional paradigm proposes that microbial eubiosis (balanced flora) is positively health associated, while a change in microbial diversity or functionality (dysbiosis, unbalanced flora) can promote development of disease, including various cancers (14, 28). Dysbiotic triggers include changes in genetics, environment (e.g., inflammation, medication, diet), or pathogenic infection. However, it is still debated whether microbial community alterations are a cause or effect of carcinogenesis.

Data suggest that microbial pathogens drive cancer formation in 15–20% of cancer cases (29). Currently, the International Agency for Research on Cancer classifies 10 microbial species as group 1 human carcinogens. Four of these, namely *H. pylori*, hepatitis B virus, hepatitis C virus, and human papillomavirus, drive 90% of infection-associated cancers (14, 21, 29). Despite pathogen-triggered carcinogenesis being the focus for the past 10 years, association studies and studies with selectively colonized (gnotobiotic) mouse models clearly demonstrate the procarcinogenic capability of commensal microbes (**Table 1**).

Carcinogenesis can be divided into three stages: initiation, promotion, and progression (30). Initiation is defined by spontaneous or induced genetic alterations, such as exposure to a carcinogenic agent; this alters the responsiveness of cells to their environment and provides a proliferative advantage. Promotion is a period of preneoplastic cell proliferation and accumulation, inducing additional genetic damage and amplifying mutations. Progression is marked by further neoplastic expansion, with enhanced tumor growth rate, invasiveness, and metastasis. The microbiota has the potential to impact carcinogenesis at all stages (**Table 1**). In the following sections, we discuss three prominent microbes and the mechanisms by which they initiate, promote, and enhance progression of carcinogenesis in CRC.

Table 1 Host-associated microbes impact carcinogenesis at all stages

Stage of carcinogenesis	Microbe	Microbial carcinogenic agent	Proposed host effect	Site of cancer	IARC classification	References
Initiation	Adherent-invasive *Escherichia coli*	Colibactin produced from the polyketide synthase (*pks*) gene cluster	DNA damage	Colon	N.C.	32, 33, 37–39, 42, 48, 49
	Campylobacter jejuni	Cytolethal distending toxin (CDT)	DNA damage	Colon	N.C.	104–106
	Enterococcus faecalis	Reactive oxygen species	DNA damage, genomic instability, cell cycle arrest	Colon	N.C.	91, 92
	Helicobacter hepaticus	Cytolethal distending toxin (CDT)	DNA damage and impairment of DNA repair	Biliary tract, intestine, breast	N.C.	107–109
Initiation and promotion	*Chlamydia trachomatis*	Unknown	DNA damage, impaired DNA repair, cell survival and proliferation	Urogenital	N.C.	110–112
	Helicobacter pylori	Cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA)	DNA damage, cell survival, cell proliferation	Stomach	1	18–20
	Neisseria gonorrhoeae	Restriction endonucleases	DNA damage, cell survival, cell proliferation	Prostate	N.C.	113–115
	Salmonella spp.	AvrA and cytolethal distending toxin (CdtB)	DNA damage, cell survival, cell proliferation, cell cycle arrest	Intestine and hepatobiliary	N.C.	105, 116–118
Promotion	*Clonorchis sinensis*	Parasitic excretory-secretory products	Cell proliferation	Bile duct	1	119–121
	Enterotoxigenic *Bacteroides fragilis* (ETBF)	*B. fragilis*–derived toxin (BFT)	Cell proliferation and tumor-promoting inflammation	Colon	N.C.	56, 59–61, 63, 105
	Hepatitis B virus (HBV)	Hepatitis B virus X (HBx) and hepatitis B surface protein (HB)	Genetic instability, cell survival, cell proliferation	Liver	1	122

(*Continued*)

Table 1 (*Continued*)

Stage of carcinogenesis	Microbe	Microbial carcinogenic agent	Proposed host effect	Site of cancer	IARC classification	References
	Hepatitis C virus (HCV)	Core and non-structural protein 3 (NS3), and NS5a	Genetic instability, cell survival, cell proliferation	Liver	1	123
	Human papillomavirus (HPV) types 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	E6 and E7 proteins	Genetic instability, cell survival and proliferation	Urogenital and oropharynx	1	124–126
	Human T cell lymphotropic virus 1 (HTLV-1)	HTLV-1 transactivator protein (Tax) and HTLV-1 basic leucine zipper factor (HBZ)	Cell proliferation	Adult T cell lymphoma	1	127, 128
	Opisthorchis viverrini	Parasitic excretory-secretory products	Cell proliferation and procarcinogenic inflammation	Liver	1	121, 129
	Porphyromonas gingivalis	Gingipain	Cell survival	Esophageal, oral, and pancreatic	N.C.	130, 131
	Schistosoma haematobium	Excretory-secretory products	Cell proliferation and procarcinogenic inflammation	Bile duct and bladder	1	121
Promotion and progression	Epstein-Barr virus (EBV)	Epstein-Barr virus nuclear antigens (EBNA) and latent membrane proteins (LMP)	Cell survival, proliferation, differentiation, migration	Burkitt's lymphoma, nasopharynx, Hodgkin's lymphoma	1	132, 133
	Fusobacterium nucleatum	*Fusobacterium* adhesin A (FadA) and *Fusobacterium* autotransporter protein 2 (Fap2)	Tumor binding, invasion, cell survival, cell proliferation, immune evasion	Colon and breast	N.C.	70–72, 75–81
	Kaposi sarcoma-associated herpesvirus (KSHV or HHV8)	K1, K15, and vGPCR	Cell survival, cell proliferation, cell differentiation	Kaposi sarcoma and primary effusion lymphoma	1	134, 135

Abbreviations: IARC, International Agency for Research on Cancer; N.C., not classified.

COLIBACTIN-PRODUCING *PKS+ ESCHERICHIA COLI* INITIATES CARCINOGENESIS

During carcinogenesis, normal host cells acquire mutations that confer growth and survival advantages. Cancer formation is often initiated by a chemical carcinogen, which induces genotoxicity or DNA damage (30). The microbiota is predicted to produce hundreds of unique small molecules and secondary metabolites that may influence host health and disease (31). These metabolites are often synthesized by complex enzymatic assembly lines encoded by biosynthetic gene clusters. One cancer-associated genotoxic molecule is colibactin, produced from the polyketide synthase (*pks*) gene cluster present among certain strains of *E. coli* (32, 33). *Pks+ E. coli* strains are prevalent in the microbiota of CRC patients (33, 34), induce CRC in mouse models (33, 35–37), and leave a distinct mutational fingerprint in human colorectal tumors that signifies former exposure and points to a role in cancer initiation (38, 39) (**Figure 1*a***).

The *pks* island was first described in 2006 as a 54-kb genomic island of 19 genes (*clbA* to *clbS*) that encodes a large and sophisticated nonribosomal peptide and polyketide synthase assembly line (32, 40). Not all *E. coli* harbor the *pks* island, but those that do are restricted to *E. coli* phylotype B2 and represent both commensal and pathogenic strains (41). Among human microbiota, *pks+ E. coli* are highly prevalent in CRC patients (33, 34), with one study estimating carriage among 66.7% of CRC patients, 40.0% of inflammatory bowel disease patients, and only 20.8% of healthy patients (33). These correlative findings suggest that *pks* may play a role in disease promotion.

Early studies demonstrated that *pks* was responsible for inducing cell cycle arrest and activation of DNA repair machinery in mammalian cells exposed to *E. coli*, suggesting that *pks* products were microbially derived genotoxins (32, 42). More specifically, epithelial cells that encounter colibactin-producing *E. coli* exhibit DNA double-strand breaks and are characterized by γ-H2AX foci, G2/M cell cycle arrest, megalocytosis, and activation of ATM/CHK/CDC25/CDK1 DNA damage signaling cascades (32, 33, 42). The *pks+* island was first demonstrated to enhance tumor multiplicity and invasion in the AOM/interleukin 10–deficient ($Il10^{-/-}$) colitis-associated CRC mouse model (33). These procarcinogenic effects were validated by multiple groups in additional mouse models; that later work also defined the role of various *pks* genes and proteins required for colibactin's genotoxic effects (reviewed in 41).

While the precise chemical identity of bioactive colibactin has remained elusive, chemical and structural analyses have defined inactive precolibactins and stable colibactin-DNA lesions that can lead to mutation and tumorigenesis (43–45). Briefly, inactive precursors are synthesized in the bacterial cytoplasm and then deacetylated in the periplasm by the peptidase ClbP (41, 46, 47). In the mammalian cell nucleus, colibactin alkylates DNA with a so-called double warhead composed of a cyclopropane ring conjugated to an α,β-unsaturated imine, creating adenine-colibactin adducts and DNA crosslinks (48–50). Although bacterial:mammalian cell contact is required (32) for genotoxicity, beyond that it is currently unknown how bioactive colibactin is released from the bacteria and enters the mammalian cell to cause DNA damage.

It was predicted that colibactin-DNA lesions lead to mutations in oncogenes or tumor suppressors that drive cancer. Indeed, two recent studies defined unique mutational signatures caused by colibactin exposure (38, 39). Both studies repeatedly exposed mammalian cells to *pks+ E. coli* in culture and identified single base pair substitutions contained in specific AT-rich motifs that are structurally and chemically consistent with the effects of previously identified adenine-colibactin adducts. The single base pair substitution (SBS) signature was termed SBS-*pks* and includes ATA, ATT, and TTT with the middle base mutated (38). An additional signature contained single T deletions at T homopolymers, with enrichment of adenines upstream of the insertion/deletion site (termed indels), and was termed ID-*pks* (38). Importantly, the mining of established whole-genome

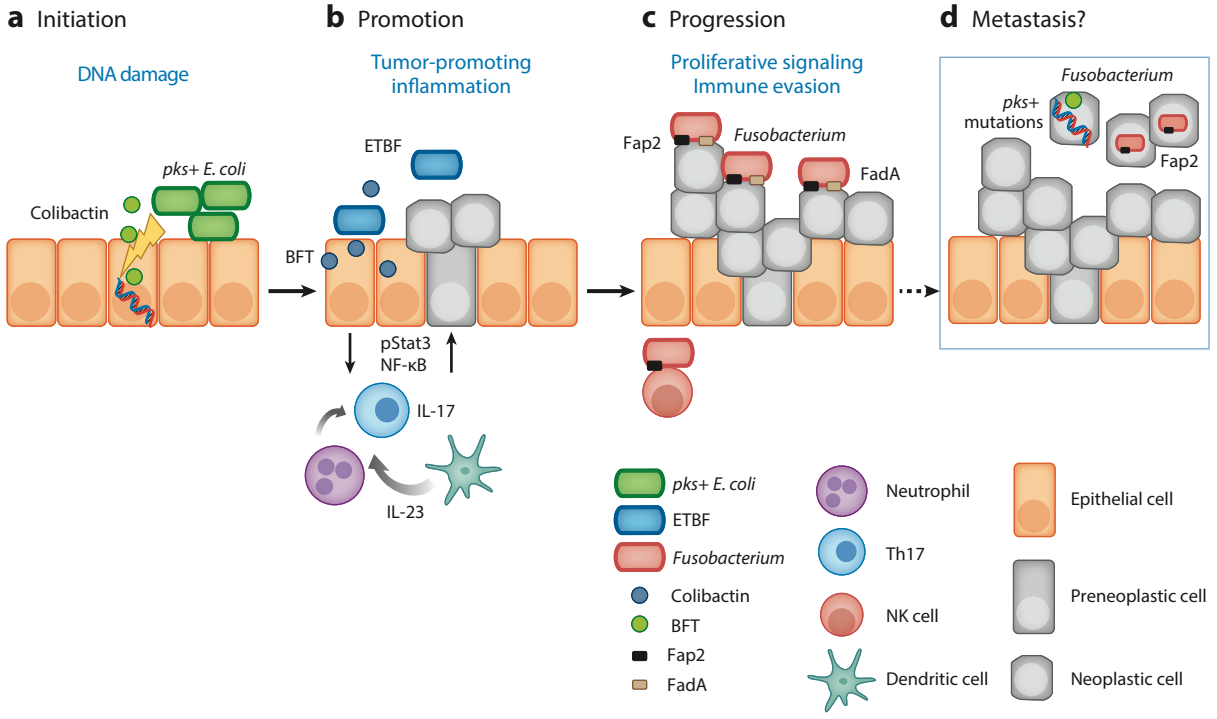

Figure 1

Microbiota impact all stages of carcinogenesis. (*a*) Initiation, the first stage, is characterized by DNA alterations to normal cells. Colibactin, a specialized metabolite produced by *pks*+ *Escherichia coli* (*green*), has genotoxic activity that damages DNA and leads to mutations. (*b*) Promotion, the second stage, is characterized by proliferation of transformed cells. BFT produced by ETBF (*blue*) damages the colonic epithelium and barrier integrity. This disruption leads to procarcinogenic Th17-dominant inflammation. Epithelial cells, neutrophils, and dendritic cells produce cytokines that activate T cells to promote Th17 inflammation, including dendritic cell–derived IL-23. IL-17-producing T cells signal back to the epithelium and induce epithelial cell proliferation driven by pStat3 and NF-κB pathways. (*c*) Progression, the final stage, is characterized by tumor growth and invasion, leading to metastases. *Fusobacterium* (*red*) uses adhesins FadA (*brown*) and Fap2 (*black*) to bind to E-cadherin and Gal-GalNAc, respectively, on tumor cells to promote proliferative signaling. Fap2 also binds TIGIT on NK cells to enhance immune evasion. (*d*) Although there is not yet strong evidence that these bacteria promote metastasis, *Fusobacterium* and a *pks*+ mutagenic signature have been found in metastases. Abbreviations: BFT, *Bacteroides fragilis* toxin; ETBF, enterotoxigenic *Bacteroides fragilis*; FadA, *Fusobacterium* adhesin A; Fap2, *Fusobacterium* autotransporter protein 2; IL, interleukin; NF-κB, nuclear factor κB; NK, natural killer; Th17, T helper 17; TIGIT, T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains.

sequencing datasets revealed that these signatures predominated in CRC tumors and metastases relative to other cancer types (38, 39). SBS-*pks* and ID-*pks* signatures were positively correlated, suggesting that they derived from a common origin—colibactin exposure (38). Both studies linked the location of these *pks* signatures to CRC mutational hotspots, with the adenomatous polyposis coli gene *APC* [the most commonly mutated gene in CRC (51, 52)] harboring the highest number of mutations with the SBS-*pks* or ID-*pks* mutational signatures (38). These signatures can serve as biomarkers of past colibactin exposure, and the findings clearly link the mutational signature of colibactin exposure to known CRC driver mutations.

Intriguingly, a separate study examining non-neoplastic colon tissue detected SBS-*pks* and ID-*pks* in 29 of 42 healthy individuals, and data modeling revealed that these signatures were likely acquired before 10 years of age (53). Thus, early exposure to *pks*+ *E. coli* and a prominent *pks* mutational signature found early in life may indicate a greater risk for CRC. In combination with

genetic susceptibility and other risk factors, the presence of colibactin-derived mutational signatures may inform new prognostic algorithms for CRC. It will be important to better understand how the genomic location and/or abundance of colibactin-DNA adducts may relate to future cancer risk.

ENTEROTOXIGENIC *BACTEROIDES FRAGILIS* PROMOTES CARCINOGENESIS

Almost every neoplastic lesion contains immune cells. Once thought to be solely an antitumoral response, inflammation can enhance tumor promotion and progression (14, 54, 55). The close proximity of the microbiota and mucosal immune system provides opportunity for resident microbes to elicit protumorigenic immune responses. *Bacteroides* spp. are normal inhabitants of the intestinal microbiota, representing approximately 30% of the gut community members, and help shape mucosal immune responses (56).

B. fragilis is a key *Bacteroides* community member that represents about 0.5–2% of the entire gut microbiota (56). Strain level differences render *B. fragilis* either beneficial or proinflammatory and procarcinogenic. Beneficial *B. fragilis*, referred to as non-toxigenic *B. fragilis*, promotes regulatory T cell development and suppression of inappropriate inflammation through the production of polysaccharide A (57, 58). In contrast, enterotoxigenic *B. fragilis* (ETBF) produces a proteolytic enterotoxin, termed *B. fragilis* toxin (BFT) or fragilysin (59). BFT is a heat-labile metalloprotease that is produced as a protoxin and activated by fragipain, a *B. fragilis* cysteine protease (56). ETBF promotes inflammation and CRC predominantly through BFT.

Genetically susceptible mouse models have been instrumental in demonstrating the inflammatory and tumorigenic effects of ETBF. APC is a chief tumor suppressor protein commonly mutated in CRC patients (51, 52). $Apc^{min/+}$ mice and mouse models with truncated *Apc* spontaneously develop numerous intestinal tumors, mainly localized to the small intestine. However, in mice colonized with ETBF, tumors develop in the colon within a month of inoculation (60). ETBF primarily resides in the colon, where it is thought to drive tumorigenic effects via local production of BFT. Colonization with non-toxigenic *B. fragilis* does not induce colonic tumors, demonstrating the reliance on BFT for *B. fragilis* procarcinogenic activities (60).

Upon exposure to epithelial cells, BFT damages colonic epithelial barrier integrity by inducing cleavage of the zonula adherens protein E-cadherin (61). Oncogenic β-catenin is released from E-cadherin and translocates to the nucleus, where it acts as a transcription factor and induces epithelial hyperproliferation (62). Normally, cytosolic β-catenin is restrained by the host APC protein and is continually targeted for proteasomal degradation (62). However, the APC gene is mutated in 70–80% of CRC patients (51, 52), diminishing APC tumor-suppressive function. Therefore, β-catenin oncogenic signaling is likely enhanced by microbial-derived BFT.

BFT-mediated E-cadherin cleavage not only induces proliferative signaling but also increases gut permeability, which enhances translocation of microbial products (56). The disruption of epithelial integrity triggers a proinflammatory cascade that leads to rapid and sustained interleukin-17 (IL-17) production by colonic T cells, the defining feature of T helper 17 (Th17) cell immune responses (59, 63). IL-17 production evoked by *B. fragilis* is a key driver of colon tumorigenesis, which is inhibited by IL-17 neutralization. Thus, ETBF promotes cancer development by invoking tumorigenic inflammation, in part through BFT (**Figure 1*b***).

Th17 immune responses, induced by microbes and their metabolites, are associated with worse CRC patient prognosis (54, 64). Under homeostatic conditions and exposure to epithelial-adherent commensals, Th17 immunity is trained as a protective host defense response (65). However, Th17 release can be maladaptive in the context of inflammation and cancer (63). At sites

of inflammation and on developing adenomas, epithelial barrier defects and defective mucin production permit microbial sampling by intratumoral dendritic cells that then produce IL-23 (54). Neutrophils, other local immune cells, and epithelial cells produce proinflammatory cytokines including IL-1β and IL-6 in this microenvironment (55). This intratumoral cytokine milieu including IL-23 and IL-6 causes recruitment and expansion of T cells producing IL-17 (IL-17A specifically), which signal to epithelial cells through the receptor IL-17RA (63, 64). Epithelial recognition of IL-6 and IL-17 activates a signaling cascade that involves phosphorylated Stat3, NF-κB, and MAPK (63). This signaling cascade induces antiapoptotic and pro-proliferative genes that promote cancer development (59). These data suggest that inappropriate exposure to BFT induces a coordinated response between epithelial, myeloid, and lymphoid cells, which establishes a microenvironment of tumor-promoting inflammation that enhances cancer development.

FUSOBACTERIUM NUCLEATUM ENHANCES CANCER PROGRESSION

Healthy tissues tightly control cellular signals to modulate growth and maintain homeostatic cell densities, tissue architecture, and function. Dysregulated cellular signaling can permit sustained and potentially deleterious cell proliferation. As discussed above in relation to ETBF, microbial-induced dissociation of β-catenin from E-cadherin drives proliferative pathways that support tumor promotion and progression (62). *F. nucleatum* is a normal inhabitant of the oral microbiota that can cause inflammation in the gingival tissue and infectious inflammatory conditions at multiple body sites (14, 28, 66–68).

Mislocalization of *F. nucleatum* to the colon is associated with CRC. Although luminal spread seems possible from the oral cavity to the colon, evidence suggests that *F. nucleatum* reaches sites of inflammation and tumorigenesis via a hematogenous route (69, 70). *Fusobacterium* is prevalent in CRC patient tissue (71, 72), and its abundance positively correlates with cancer severity (73, 74), supporting a role for *Fusobacterium* in cancer progression.

As with other procarcinogenic bacteria, strain-specific differences in *F. nucleatum* drive commensal versus procarcinogenic behavior (67). An early study demonstrated that daily gastric inoculation of *F. nucleatum* into CRC-susceptible mice enhanced tumorigenesis, suggesting a causative role (71). Since early associations, the protumorigenic role of *F. nucleatum* has been supported by ample evidence (66, 71, 72, 75–79). Furthermore, we now understand that *F. nucleatum* carcinogenic effects are primarily mediated by the adhesins *Fusobacterium* autotransporter protein 2 (Fap2) and *Fusobacterium* adhesin A (FadA) (**Figure 1*c***).

A transposon screen revealed the importance of Fap2 in binding microbial and mammalian cells (80). Galactose-inhibited adhesion had been reported previously in studies involving various oral microbes and mammalian cells (66). Fap2 binding was blocked by galactose (80), whose partner was Gal-GalNAc, a disaccharide highly expressed on CRC tumors and metastases (81). Thus, *F. nucleatum* can home to developing and established tumors, contributing to cancer progression.

For tumorigenesis to progress, neoplastic cells must avoid immune detection and destruction. Natural killer (NK) cells comprise a key part of immune surveillance by killing nonself cells (e.g., virus-infected and tumor cells) via coordination of activating and inhibitory receptors. *F. nucleatum* Fap2 binds NK cell inhibitory receptor TIGIT [T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains], which inhibits NK cell activation and allows tumor cells to evade elimination (77). *Fusobacterium* also induces immunosuppressive myeloid-derived suppressor cells, which can boost tumor development by interfering with immune surveillance (77).

Another *Fusobacterium* adhesin, FadA, is implicated in carcinogenesis. FadA binds E-cadherin, activates β-catenin, and enhances experimental CRC tumor xenograft growth (78). FadA is

essential for active invasion of epithelial and endothelial cells (79, 82). A recent study demonstrated that FadA+ *F. nucleatum* invades HCT116 CRC cells and induces production of CXCL1 and IL-8, chemokines that then promote HCT116 migration (79). These data suggest that colorectal cell invasion by *F. nucleatum* may enhance metastatic potential. Interestingly, invasion of phagocytic cells—cultured neutrophils and macrophages—is FadA independent (79). *Fusobacterium* likely harbors additional factors that facilitate invasion and pathogenic interactions with mammalian cells, as invasive strains harbor large genomes predicted to encode multiple FadA-related adhesins and similar surface-associated proteins (83). Thus, FadA promotes proliferative signaling and, upon invading CRC cells, may enable cellular migration and metastasis.

Although it is unclear precisely what role the microbiota may play in metastatic growth, some evidence suggests that *F. nucleatum* plays a role in CRC metastases. One study found clonal *Fusobacterium* strains in a majority of primary CRC tumors and paired liver metastases (76). Furthermore, when *Fusobacterium* was found in CRC metastases, much of the primary tumor microbiome was present as well; this suggests that *Fusobacterium* may be a hub for multispecies procarcinogenic activities. *Fusobacterium*-containing patient-derived xenografts had viable *Fusobacterium* that appeared to be cancer cell invasive. In addition, tumor growth was reduced by treatment with metronidazole, an antibiotic highly effective against *Fusobacterium* (76). Interestingly, a recent study reported that *F. nucleatum* could accelerate experimental breast cancer and metastatic progression (70). Similar to findings in CRC, this procarcinogenic activity involved Fap2 binding Gal-GalNAc on breast cancer cells and suppression of tumor-infiltrating T cells. Metastatic progression was inhibited by antibiotic treatment with metronidazole (70). Overall, it is clear that *F. nucleatum* contributes to cancer progression, in part via FadA and Fap2 adhesins. While additional investigation is needed, evidence suggests a likely role for *F. nucleatum* in metastasis (**Figure 1*d***).

CARCINOGENESIS IS MEDIATED BY DIVERSE MICROBIAL FUNCTIONS

Our microbiota adapt to an array of microenvironmental shifts during our lifetime, shaping human development, health, and survival. At the same time, these diverse microbial communities can impact chronic disease and diseases of aging, including cancer (24). Carcinogenesis is a multistep process by which normal host cells acquire genetic and epigenetic changes that result in cancer (30). In combination with host genetic susceptibility and environmental exposures, a prominent procarcinogenic role for the microbiota has recently emerged (84).

The microbiota comprises vast communities of microbes that inhabit most body sites. Although we generally exist in a healthy symbiotic relationship with our microbiota, a dysbiotic microbial community can contribute to the carcinogenic process. Links between carcinogenesis and ecological alterations to the microbiota are best exemplified by CRC, where there is intimate association between the host and a diverse community of microbes. Yet, it should be noted that the microbiota can impact extraintestinal cancers (e.g., breast, urogenital, liver) at all stages of carcinogenesis (**Table 1**). While human microbiota studies and experimental animal models of cancer have consistently highlighted several microbes that impact colorectal carcinogenesis, less is currently known about microbiota-mediated mechanisms that impact extraintestinal cancer (14, 28).

In this review, we have described mechanisms driving the procarcinogenic effects of three key gut bacterial species in CRC: *E. coli*, ETBF, and *F. nucleatum* (14, 28). Furthermore, we proposed that each of these microbes uniquely influences specific stages of carcinogenesis. Colibactin-producing *E. coli* initiate, ETBF promote, and Fap2+ and FadA+ *F. nucleatum* enhance progression of carcinogenesis. These microbes illustrate the stepwise procarcinogenic potential of the microbiota.

BIOTRANSFORMATION OF CHEMOTHERAPEUTICS BY THE MICROBIOTA

The microbiota can directly impact metabolism of xenobiotics, including chemotherapeutics (96, 97). Pancreatic ductal adenocarcinomas (PDACs) can harbor Gammaproteobacteria able to metabolize gemcitabine, a common chemotherapeutic for PDAC. Gemcitabine inactivation depends on expression of a particular isoform of the bacterial enzyme cytidine deaminase, common among Gammaproteobacteria (98). Although the host also produces cytidine deaminases, these results suggest that intratumor bacteria may contribute to PDAC resistance to gemcitabine. Irinotecan, a chemotherapeutic used to treat colorectal and pancreatic cancer, has limited efficacy due to gastrointestinal toxicity caused by reactivation of the drug in the colon by bacterial β-glucuronidases (99). Inhibiting bacterial β-glucuronidases prevents gastrointestinal toxicity and reduces gut epithelial damage, which may allow administration of higher effective doses (99–101). In the future, clinicians may consider individual variations in the microbiome to inform the most effective use of chemotherapeutics, an example of personalized medicine. Some approaches include building pharmacokinetic models to predict microbiome contributions to the metabolism and absorption of specific drugs and chemotherapeutics (102). Another way to capture the variability in drug metabolism across various patient microbiota is to employ *in fimo* screening (experimental examination of stool samples), inoculating patient-derived fecal samples with a drug of choice to determine the functional output of an individual's microbial community (103).

OUTSTANDING QUESTIONS AND FUTURE DIRECTIONS

In 2019, the International Cancer Microbiome Consortium published a consensus statement on the role of the human microbiome in carcinogenesis, stating that "the microbiome is one apex of a tripartite, multidirectional interactome alongside environmental factors and an epigenetically/genetically vulnerable host that combine to cause cancer" (84, p. 1624). As elaborated herein, microbiota have local effects on cancer formation and contribute to systemic effects through biotransformation of chemotherapeutics and immunotherapies (see the sidebar titled Biotransformation of Chemotherapeutics by the Microbiota). In this review, we have discussed recent evidence that human-associated microbes can impact each stage of carcinogenesis: initiation, promotion, and progression. However, many important questions remain.

What Other Microbial Factors Induce, Promote, or Progress Carcinogenesis?

The microbiota harbors a tremendous capacity for generating novel metabolites (31). Microbial-derived metabolites like short-chain fatty acids (SCFAs) and hydrogen sulfide (H_2S) can impact CRC (14, 28). The SCFA butyrate provides energy to healthy colonocytes and is less abundant in CRC patients. Administering butyrate or butyrate-producing microbes enhances mitochondrial respiration in healthy colonocytes and is tumor suppressive in a mouse model of cancer (85, 86). Conversely, H_2S is enriched in early-stage tumor samples and may promote inflammation/tumorigenesis (87). Various species, such as *Bilophila wadsworthia* and *Alistipes* spp., are abundant in CRC patients and produce H_2S that is toxic to epithelial cells and causes DNA damage (14, 28).

Does the Physiological State of Microbial Communities Impact Their Procarcinogenic Potential?

Biofilms have consistently been found in right-sided (proximal) CRC and can contain ETBF and *pks+ E. coli* (88, 89). Genetically susceptible mice develop tumors upon inoculation with human colonic biofilms, but rarely with non-biofilm microbial communities, from both healthy

individuals and cancer patients (27). Taxonomy differed between biofilm-positive and biofilm-negative microbial communities (27), making it difficult to discern whether the procarcinogenic effects were biofilm dependent or due to differences in microbial composition. Nonetheless, these findings suggest that the biogeographic distribution of the microbiota and intermicrobial interactions play an understudied role in host interactions and possibly cancer.

How Does the Microbiota Alter Host Products to Influence Cancer Development?

Many microbes and their metabolites stimulate reactive oxygen species (ROS) production from host cells, leading to ROS-induced DNA damage that can promote genomic instability and mutations (90–92). In addition, bile acids are notoriously altered by the microbiota and can impact colorectal and hepatocellular carcinoma (93). Given the vast number of metabolites evoked or chemically transformed by the microbiota, carcinogenesis is undoubtedly altered by the milieu of an individual's microbiota.

How Does the External Environment Shape the Microbiota to a Carcinogenic State?

Chronic inflammation increases cancer risk and severity. A recent study demonstrates that reducing inflammation through TNF-α neutralization alters the microbiota and renders it less carcinogenic when transplanted to germ-free cancer-susceptible mice (94). In healthy individuals, the microbiota adapts over a lifetime. *B. fragilis* and other members of the microbiota continually adapt in the gut via de novo mutations, with the appearance of novel strain variants that could perhaps acquire procarcinogenic traits (11).

Do Microbial Factors Drive Specific Types of Colorectal Cancer?

One study evaluated 83 patients with a 44-patient validation cohort, in which patients were stratified by mismatch repair (MMR) status. This study found that MMR status was one of the strongest predictors of microbial community variance and that MMR-deficient patients harbor different microbes and metabolites than MMR-proficient patients (87). Larger cohorts and longitudinal studies are likely to uncover stronger links between the presence of certain microbial signatures and CRC subtypes.

SIGNIFICANCE AND THERAPEUTIC POTENTIAL

Although we continue to see an overall decrease in cancer-related deaths among men, women, and children in the United States, rates of new cancers remain stable or are increasing for some demographics (95), and the millennia-long fight against cancer persists. Two important challenges within this fight are: (*a*) modeling the complexity of the host–microbe interactions within the tumor microenvironment and (*b*) personalizing medicine to optimally treat an individual's unique disease features, including procarcinogenic microbes.

To meet these challenges, we need longitudinal clinical studies to truly demonstrate microbial causation in human carcinogenesis. Mechanistic studies and experiments with animal models build the foundation for human-based research. By understanding how and at what stage microbes impact carcinogenesis, we can step toward improved clinical intervention. By defining which microbes initiate cancer, we can identify biomarkers to predict cancer formation in susceptible individuals. By looking at microbes that promote cancer, we can estimate therapeutic efficiency.

Finally, by addressing cancer-progressing microbes, we can get a better understanding of prognosis. Furthermore, this information will allow us to target these carcinogenic microbes and microbial metabolites for elimination, in combination with strategies to enhance beneficial community function. Such knowledge can be broadly applied to other microbial-driven conditions of chronic inflammation, infection, and extraintestinal cancers. This should be our focus in the next decade to continue the fight against cancer.

SUMMARY POINTS

1. Our microbiota influence the initiation, promotion, and progression of carcinogenesis.
2. Currently, microbiota-mediated procarcinogenic effects are best exemplified by the gut microbiota in colorectal cancer (CRC).
3. Colibactin-producing polyketide synthase (*pks*+) *Escherichia coli* initiates carcinogenesis by inducing DNA damage.
4. Enterotoxigenic *Bacteroides fragilis* (ETBF) promotes carcinogenesis, both directly through *B. fragilis*–derived toxin (BFT) and indirectly through interleukin-17 (IL-17)-dominant tumor-promoting inflammation.
5. *Fusobacterium nucleatum* enhances cancer progression via its adhesins *Fusobacterium* autotransporter protein 2 (Fap2) and *Fusobacterium* adhesin A (FadA), which enhance proliferation, promote cellular invasion, and help evade antitumor immunity.
6. Defining how and when the microbiota impacts carcinogenesis will improve the timing and strategies for risk assessment and personalized cancer treatments.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to the authors whose primary literature we could not cite due to space restrictions. This work was supported by funding from National Institutes of Health (NIH) grant R01DK124617 (J.C.A.), pilot funding from NIH grant P30DK034987 (subcontract J.C.A.), a University of North Carolina Lineberger Comprehensive Cancer Center Innovation Award (J.C.A.), and SPIRE (Seeding Postdoctoral Innovators in Research and Education) [NIH IRACDA (Institutional Research and Academic Career Development Awards) program] NIH grant 5K12 GM000678-21 (R.M.B.).

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Annual Review of Medicine

Volume 72, 2021

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Errata

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