Fusobacterium and Colorectal Cancer

Ziwei Zhou 1, Jiewen Chen 2, Herui Yao * and Hai Hu *

1 Department of Oncology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China, 2 Breast Tumor Center, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China

Colorectal cancer (CRC) is the third most common cancer worldwide and its pathogenesis has been extensively explored over the past decades. Recently, microorganisms in the gastrointestinal tract have emerged as potential etiological agents. In particular, a direct proportional association between Fusobacterium and CRC has been described. Since then, the functional impact of Fusobacterium in CRC development has been studied using various mouse models. Although some epidemiologic studies did not establish an obvious relationship between Fusobacterium and CRC, numerous pathogenic mechanisms leading to the disease have been described. For instance, Fusobacterium can activate the E-cadherin/β-catenin signaling pathway and is associated with particular epigenetic phenotype, such as microsatellite instability (MSI) and hypermethylation, via its strong adhesive and invasive abilities resulting in malignant transformation of epithelial cells. Also, Fusobacterium could alter the tumor microenvironment (TME) significantly by myeloid-derived suppressor cells (MDSCs), tumor associated macrophages (TAMs), and tumor associated neutrophils (TANs) recruitment and local immune suppression. Herein, we provide an in-depth review of the relationship between Fusobacterium and colorectal cancer. In light of the emergence of microbiome-based therapeutics, potential therapies and preventive strategies for colorectal cancer related to Fusobacterium are also discussed.

Keywords: Fusobacterium, colorectal cancer, tumor microenvironment, epigenetic changes, tumor immunity

INTRODUCTION

Commensal bacteria in the colon might play a significant role in the maintenance of health (1–4). Intestinal microbiota promotes the maturation of human immune system and maintenance of natural barrier integrity (5). Bacterial dysbiosis in the gut has been associated with numerous human diseases, including obesity (6, 7), intestinal diseases (8, 9), cardiovascular diseases (10), autism (11), malignancies (12–14) and others. Garrett et al. identified that colitis could be transferred from T-bet−/− x Rag2−/− ulcerative colitis (TRUC) mice to wild type mice with particular intestinal bacteria (Proteus mirabilis and Klebsiella pneumoniae) transplantation (15). The metabolic phenotypes and adiposity status could also be modulated by cultured gut microbiota from human and coordinate diets in mice (6). This finding denotes the importance of gut microbiota symbiosis and the dysfunctional proportion could exacerbate human diseases, since both normal and pathogenic flora are important in the regulation of homeostasis (16). Particularly, colonic dysbiosis has been associated with CRC (17) (12, 18) (19) (20). Among the multitudinous
genera, *Fusobacterium* stood out as being oftentimes increased in CRC (14, 21) (Table 1). As in the case of *Helicobacter Pylori* correlation with gastric cancer, *Fusobacterium* may be an essential microbial carcinogen that fuels the initiation and development of CRC (21, 22). *Fusobacterium* is a genus of gram-negative anaerobic bacteria. It may act as a main anchor of biofilms that can induce periodontitis (25) and other infections (26). *Fusobacterium* was considered as part of the normal flora of the oropharynx formerly, but lately its pathogenic role especially as a driver of periodontitis (27) and its association with intestinal diseases has been demonstrated. Although it is still unclear whether *Fusobacterium* is the passenger or driver of CRC, many studies have concluded that *Fusobacterium* is a novel risk factor for CRC development and progression, as well as a determinant affecting patient survival outcomes (13, 28, 29). Kostic et al. (14) illustrated that in colorectal adenoma, an early event in CRC development, *Fusobacterium* is found to be enriched in comparison with surrounding normal tissue suggesting an essential role of *Fusobacterium* in the early onset of CRC. Moreover, a recent retrospective study with 13,096 adult patients suggested that those presented with *Fusobacterium nucleatum* (one of the species of *Fusobacterium*) have increased risk of CRC (30). Additionally, significantly larger proportions of *Fusobacterium* has been detected from feces of adenoma and CRC patients in comparison to healthy controls, which further confirms Kostic's finding. Distal metastasis of colonic cancer was also found to be colonized with *Fusobacterium* and other assembled microbes. Investigators have also shown that tumor proliferation and cancer growth could be reduced via decreasing the load of *Fusobacterium* by antibiotic treatment (metronidazole) (31). Moreover, *Fusobacterium* is associated with certain epigenetic phenotypes of CRC—high degrees of microsatellite instability (MSI) and CpG island methylation phenotype (CIMP) (32, 33), which could provide promising opportunities to develop diagnostic tools or treatment biomarkers for CRC.

### THE BIOLOGICAL FEATURES OF *FUSOBACTERIUM*

#### A Heterogeneous Genus of Bacteria

*Fusobacterium* is a cylindrical shaped, gram-negative, non-spore-forming, strictly anaerobic genus. Although *Fusobacterium* is part of the normal microbiome, recent findings indicated that increased *Fusobacterium* levels have been detected in various inflammatory (39–41) and cancer samples (33). There are 14 species in *Fusobacterium*, such as *F. necrophorum* (inhabitant of the alimentary tract and being responsible for Lemiierre's syndrome) and *F. varium* (found in the ulcerative colitis). Among them, *F. nucleatum* is one of the key pathogens which plays a role in oral plaque formation, due to its adhesive ability, serving as a bridge organism during colonization and biofilm formation (42). Although several studies suggest that *Fusobacterium* strains might vary in their virulence potential, it is has been speculated that some *Fusobacterium* strains can acquire genes through horizontal transfer and obtain increased virulence potential from different species and strains (43–45). Regardless of the mechanism in which *Fusobacterium* attains its virulence, evidence points to the positive correlation of this *Fusobacterium* toward CRC malignancy.

### Adhesion and Invasion of *Fusobacterium* into Host Tissue Cells

*Fusobacterium* is an invasive organism. *Fusobacterium* invades host with the aid of a surface adhesion molecule called FadA, which is abundantly expressed on *Fusobacterium* (46). Rubinstein and his colleagues suggested that FadA, a membrane protein, encoded by *Fusobacterium* binds to E-cadherin on the epithelial cell surface and leads to E-cadherin phosphorylation and internalization. This, in turn, activates β-catenin signaling pathways (22) and consequently leads to inflammation and tumorigenesis gene transcription (such as NF-κB, Myc ad Cyclin D1, lymphoid enhance factor/T cell factor). Furthermore, they detected higher FadA expression levels in CRC tissue compared with normal tissue (27). Additionally, there are 14 strains of *Fusobacterium*, and each strain is associated with different phenotypes of CRC—high degrees of microsatellite instability (MSI) and CpG island methylation phenotype (CIMP) (32, 33), which could provide promising opportunities to develop diagnostic tools or treatment biomarkers for CRC.

#### TABLE 1 | Studies with positive detection of *Fusobacterium* in colorectal diseases.

| Authors          | Diseases | Methods | Samples         |
|------------------|----------|---------|-----------------|
| McCoy et al.     | Adenoma  | qPCR, FISH | Human tissues   |
| Wong et al.      | Adenoma  | qPCR    | Feces           |
| Ito et al.       | Adenoma  | qPCR    | FFPE tissues    |
| Kostic et al.    | CRC      | RNA-seq, qPCR, WGS | Human tissues |
| Mima et al.      | CRC      | qPCR    | FFPE tissues    |
| Kostic et al.    | Adenoma  | qPCR, 16S rDNA, Sequence, WAS, FISH | Human tissues, Feces |
| Tahara et al.    | CRC      | qPCR    | Human tissues   |
| Wang et al.      | CRC      | ELISA, WB, qPCR | blood samples and feces |
| Mehta et al.     | CRC      | qPCR    | FFPE tissues    |

**qPCR**, quantitative real-time polymerase chain reaction; **FISH**, Fluorescent quantitative polymerase chain reaction; **RNA seq**, RNA sequencing; **WAS**, Whole-genome sequencing; **ELISA**, Enzyme-linked immunosorbent assay; **WB**, Western blotting; **FFPE**, Formalin-fixed paraffin-embedded.

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**Abbreviations**: MSI, microsatellite instability; MDSCs, myeloid-derived suppressive cells; TAMs, tumor associated macrophages; TANs, tumor associated neutrophils; TRUC mice, T-bet+/− × Rag2−/− ulcerative colitis mice; CRC, colorectal cancer; IBD, inflammatory bowel disease; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; F:B, Myc ad Cyclin D1, lymphoid enhance factor/T cell factor; APC, adenomatous polyposis coli; RIG-1, retinoic acid inducible gene; TregS, regulatory T cells; Gr1, granulocyte differentiation antigen 1; CD, cluster of differentiation; ROS, reactive oxygen species; MLH1, human mutL homolog 1; FIP, *Fusobacterium nucleatum* immunosuppressive protein; PCNA, proliferating cell nuclear antigen; TIGIT, T cell immunoreceptor with Ig and ITIM domains; NK cell, natural killer cell; COX2, cyclooxygenase-2; IL, interleukin; TNF, tumor necrosis factor; MMP3, matrix metalloproteinase-3; TLR, toll-like receptor; RASA1, RASp21 GTPase activating protein; RAF, a class of small G-protein; RAE, rapidly accelerated fibrosarcoma; MEK, mitogen-activated protein kinase-ERK kinase; ERK, extracellular regulated mitogen-activated protein kinase; TP53, tumor protein p53; CHD, chromdomain helicase DNA binding protein; CIMP, CpG island methylator phenotype; GI tract, gastrointestinal tract; MMR, mismatch repair; PGE2, prostaglandin E2; MYD88, myeloid differentiation primary response gene 88; ULK1, unc-51 like autophagy activating kinase 1; ATG7, autophagy-related protein 7; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; PD-1, programmed cell death 1; Th17 cells, T helper 17 cells.
with normal tissue. Consistently, the expression of oncogenes, inflammatory genes and Wnt genes are increased in CRC cells under modulation of purified FadA. Further experiments with purified FadA or F. nucleatum could only stimulated cell lines with APC or β-catenin mutations but not non-cancerous HEK-293 cells, indicated that oncogenesis promoted by Fusobacterium was secondary to these significant mutation events. As an invasive organism that survives inside host cells, Fusobacterium is also capable of releasing RNA into the host cell cytoplasm that is detected by cytosolic retinoic acid-inducible gene 1 (RIG-1), triggering activation of NF-κB and activating inflammatory genes and oncogenes (47, 48). Furthermore, FadA binds to vascular endothelial cadherin (VE-cadherin), a cell-cell junction molecule identified as the endothelial receptor for FadA. FadA binding causes VE-cadherin to relocate and increases the endothelial permeability, which then facilitates Fusobacterium and other bacteria species to penetrate into the blood stream (49). Fusobacterium also has a strong ability to induce co-aggregation and shuttle unrelated microbes [in particular Streptococcus and Campylobacter (50)] into host cell via Fap2, a large membrane protein present in Fusobacterium The shuttled microbes are known to have weak binding ability to host cells or they are non-invasive (51, 52). Being shuttled into the host cells by Fusobacterium, the toxic effects on endothelial cells become multifold.

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**Microbial–Host Interaction and Inter-microbial Interaction**

Normally, the interaction between intestinal microbiota and the host contributes to the maintenance of homeostasis and normal mucosal immunity. Abnormalities on either side will disrupt this balance and brings about disease and malignancy. It has already been established that lesions on the intestinal mucosa impairs barrier function (53). In tumor microenvironment (TME), the pH value of the tumor tissue is around 6.5-6.9, which is slightly lower than the normal physiological pH of 7.4 (51, 52). This slight change of pH can significantly affect the redefined composition of microbial community in TME (54). In APC gene deleted mouse models (CRC animal models), significant defective intestinal barrier function at tumor sites has been described and therefore concluded that changes in the local environment and a deficient barrier could provide favorable condition for Fusobacterium to reproduce and cause further mucosal injury (55). Hence, it is hypothesized that the growth of Fusobacterium, normally a commensal at low abundance, is increased with disruption of intestinal homeostasis and in turn accelerates tumorigenesis, consequently forming a vicious positive feedback cycle.

However, as only one of the estimated 10^3 microbial species in the colon (56), Fusobacterium cannot be studied in isolation and some studies have identified other bacterial species that may also contribute to colorectal carcinogenesis and have interaction with Fusobacterium. These bacteria include and Escherichia coli (E. coli) (20), Bacteroides fragilis (B. fragilis) (57), H. pylori (58), Enterococcus faecalis (Efaecalis) (59), Streptococcus bovis (S. bovis) (60) and Clostridium septicum (C. septicum) (61). Moreover, as an oral commensal and a periodontal pathogen, Fusobacterium also interacts with candida albicans within the oral cavity (62). As evidenced by other oral community members (such as members of the Porphyromonas) that has been found on colonic tumors (63), Fusobacterium may also play a role in the link between oral and intestinal microbiota. Fusobacterium has defense ability against human neutrophilic peptide-1 (64) and is one of the most important anaerobes that promote the formation of dental biofilms (24, 65). The polymicrobial nature of oral biofilms and the asaccharolytic metabolism of many of these species allows them to survive comfortably in the microenvironment of colonic lesions (66). Consequently, co-occurrence patterns of Fusobacterium with other microbiota in the oral cavity may correlate with localized intestinal dysbiosis in the setting of colorectal carcinogenesis.

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**THE IMPACT OF FUSOBACTERIUM IN CRC MICROENVIRONMENT**

Sporadic colorectal cancer and colitis-associated colorectal cancer are predominant classes of CRC. Sporadic colorectal cancer develops in five stages—polyyp, early adenoma, late adenoma, carcinoma and invasion; while colitis-associated cancer develops in six stages—colitis, indefinite dysplasia, low-grade dysplasia, high-grade dysplasia, carcinoma and invasion (67). In both cases, Fusobacterium was found to be an important associated factor in their development (54, 68, 69). Oncogenesis caused by Fusobacterium is closely related to the inflammatory state of the tumor. However, the crosstalk between each inflammatory component of the tumor microenvironment is very complicated. For example, the abundance of tumor infiltrating lymphocytes (TILs) correlate with improved clinical outcomes in colorectal patients (70–72), while the presence of myeloid derived suppressive cells (MDSCs) (73) and regulatory T cells (Tregs) (74) indicate immune inhibitory status and are associated with poor prognosis. Hereby, the detailed mechanism of the interaction of Fusobacterium with each TME component in inflammatory and malignancy state is discussed as follows (Figure 1).

**Fusobacterium Suppresses the Immune Response**

Kostic et al. (21) found that CD11b+ myeloid derived suppressive cells (MDSCs), including granulocytes/tumor associated neutrophils (TANs) and macrophages/tumor associated macrophages (TAMs), are more abundant in tumor tissues than normal tissue of Fusobacterium -fed APC Min mice. MDSCs are a heterogeneous group of immune cells differentiating from the myeloid lineage (21, 75). MDSCs are highly abundant in some pathological situations such as chronic infections and cancer. Due to their immunosuppressive activities and interaction with other immune cell types, tumor tissue with high infiltration of MDSCs may predict poor prognosis and drug resistance (76, 77). TANs and TAMs, the special subset of MDSCs, also contribute to the inhibition of anti-tumor immunity and result in tumor progression and metastasis, which
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FIGURE 1 | Potential mechanism of *Fusobacterium*-associated CRC. Mutant epithelial cells cause local intestinal barrier impairment, which gives *Fusobacterium* the opportunity for adherence and subsequent invasion into epithelial cells. Once FadA, a membrane protein of *Fusobacterium*, combines with E-cadherin and is internalized by epithelial cells, the β-catenin signaling pathway is activated. Phosphorylated β-catenin would enter the cell nucleus from cytoplasm and promote NF-κB genes, pro-inflammatory genes and the expression of many other oncogenes. Moreover, the microenvironment of malignancies is anoxic and acidic, which would be more suitable for *Fusobacterium* reproduction compared to other bacteria. The metabolites of aggregated *Fusobacterium*. Then recruit MDSCs, in turn suppressing anti-tumor immunity and promote CRC carcinogenesis.

has been verified in considerable experimental data from clinical and pre-clinical studies (48, 56).

In mouse models, MDSCs express both myeloid lineage differentiation antigen Gr1 (granulocyte differentiation antigen 1) and CD11b and are further classified into two subtypes—monocytic and granulocytic (78–80). *F. nucleatum* increases the production of reactive oxygen species (ROS) (81) and inflammatory cytokines such as IL-10 in CRC (34), possibly by recruiting MDSCs. ROS normally serve as second messengers to regulate many of the intracellular signaling cascades that govern multiple cellular activities (81), which causes DNA damage and other cell injuries. It is well documented that inflammatory oxidative stress can lead to p53 mutation, thus promoting oncogenesis (82). Oxidative stress also contributes to aberrant hypermethylation (83), which in turn causes tumor-suppressive gene inactivation and carcinogenesis (84, 85). Hypermethylation could also occur in promoter regions of mismatch repair protein (MLH1) gene and result in microsatellite instability (MSI) (86–88), which was recognized as an early molecular phenomenon in CRC. Moreover, MDSCs can disrupt the anti-tumor abilities of the immune system by secreting arginase-1 (89), which suppresses T cell activity and induces tumor intolerance (90, 91).

MDSCs also interact with other immune cell types, such as an anti-tumor subtype of dendritic cells (CD103+ DCs). Mice treated with *Fusobacterium* were found to have more CD103+ DCs within tumors compared with control groups (21). These cells regulate immune responses by promoting the activation of Foxp3+ regulatory T cells, a CD4+ T cell subset that inhibits cytotoxic and effector T cells, and results in restrained antitumor immunity (92). In addition, immunohistochemical analysis indicate that a higher amount of *Fusobacterium* were associated with a lower density of tumor inhibiting CD3+ T cells in tumor tissue, thereby enabling tumor to escape immune surveillance (37). Additionally, *Fusobacterium* could suppress immune function by arresting T cells in the Mid-G1 phase of the cell cycle. Shenker and Datar discovered that *Fusobacterium* suspended cell proliferation at Mid-G1 phase by regulating different cyclin levels via an immunosuppressive protein, *F. nucleatum* immunosuppressive protein (FIP) (93). They also found that proliferating cell nuclear antigen (PCNA) was significantly decreased in expression. PCNA is a DNA clamp that acts as a processivity factor for DNA polymerase δ and accelerates cell transition through early to mid-G1. With decreased proliferative ability, T cell would fail to function or attack cancer cells thus progressing to an immunosuppressive phase.

Fap2, an outer membrane protein of *Fusobacterium*, is also involved in tumor suppression activity. Kaplan found that Fap2, together with adhesin RadD can cause lymphocyte death by direct contact with target lymphocytes. Either Fap2 or RadD alone could induce lymphocyte death, though at a much lower level. Chamutal Gur also discovered that Fap2 could bind to TIGIT, an inhibitory receptor presents on all human NK cells
and various T cells, protecting tumors from immune attack by inhibiting effective immune cell activity (94).

**Fusobacterium Promotes Development From Inflammation to Malignancy**

Recent findings support the claim that *Fusobacterium* could enhance the development from being in inflammatory state to malignancy. This finding was first reported by Kostic (21) and colleagues when APCMin/+ mice were introduced to human isolates of *Fusobacterium nucleatum*. Higher level of inflammation and more colonic tumors were found in the *F. nucleatum* group compared to control. However the colitis mice model (IL-10−/− and Rag2−/−/T-bet−/−) treated with *F. nucleatum* did not develop colonic tumors. This may suggest that *Fusobacterium* induce oncogenesis downstream of the APC pathway and the tumorigenesis does not depend on pre-existing colitis condition because the colitis mice did not develop colon tumors after *F. nucleatum* introduction. They also revealed the expression signature of inflammation: the higher expression of COX-2, IL-1 β, IL-6, IL-8, TNF, and MMP3 by human and mouse cell line while co-culturing with *Fusobacterium* suggests an NF-κB-driven pro-inflammatory response. Rubinstein et al. further indicated that the elevated expression of inflammatory genes such as NF-κB, IL-6, IL-8 and IL-18 were correlated with FadA level in CRC tissues, which is consistent with the former study. Additionally, *Fusobacterium* also binds to Toll-like receptor 4 (TLR4) of epithelial cells and activate the TLR4/MYD88/NF-κB signaling pathway. NF-κB p50/p65 separated with phosphorylated IκB and bound to DNA, followed with microRNA-21 transcription. microR-21 could inhibit RAS p21 GTPase activating protein (RASA1) and therefore activate the RAS/RAF/MEK/ERK signaling pathway (68). Thus, in the tumor-initiation period, *Fusobacterium* exerts its tumor-promoting action through the augmentation of local inflammation. Furthermore, some indicative molecules in human colon samples, such as COX-2, IL-1β, IL-6, IL-8, TNF-α, and MMP3, are suggestive of activation of NK-κB-driven inflammation (21, 55, 95). Among these expression signatures, IL-8, TNF-α and other chemokines could recruit neutrophils and macrophages, which synthesize nitric oxide (NO) and cause oxidative stress to epithelial and stromal cells. This results in DNA damage and consequently activation of p53 transcription which in turn suppresses tumorigenesis by inducing GI-S arrest, DNA repair and cell apoptosis. Moreover, p53 overexpression also leads to TP53 mutation, which is a key event during CRC development. Additionally, chronic inflammation and ROS production cause many other mutations (such as CHD7 and CHD8, members of the chromodomain helicase/ATP-dependent chromatin remodeling family (33, 96) and genomic instability), all of which would accelerate CRC development.

**FUSOBACTERIUM INDUCES EPIGENETIC CHANGES IN TUMOR CELLS**

Intriguingly, high load of *Fusobacterium* was recently reported to be associated with a specific epigenetic phenotype of CRC. *Fusobacterium* detected in colorectal cancer tissues was related to CpG island methylator phenotype (CIMP) status, high MSI and MLH1 hypermethylation (33) and up-regulating expression of microRNA-21. Interestingly, these specific molecular features of colorectal cancer occur mostly in the ascending colon (97, 98), which is also the most common colonization site of *Fusobacterium* in the GI tract. This might indicate some association between *Fusobacterium* biogeography and the colonic mucosal microenvironment.

CIMP is characterized by simultaneous hypermethylation of numerous CpG islands surrounding the promoter regions of several genes. The high level of methylation of CpG island indicates chronic inflammation and an aggravated immune response (99). Microsatellite instability is the somatic accumulation of length variations in repetitive DNA sequences (33). It has been established that defects in the DNA mismatch repair (MMR) pathway lead to disincorporation, insertions and deletions in microsatellite in this repetitive DNA sequences. MSI is frequently observed in both hereditary and sporadic CRC (100). Inflammatory state and reactive oxygen stress produced by *Fusobacterium* may contribute to epigenetic silencing of the MMR protein MLH1 and reduction of its enzymatic activity, which leads to MSI CRC (101).

microRNAs, a small non-coding RNA functioning in RNA silence and regulating post-transcriptional gene expression, rapidly emerging as promising diagnostic and therapeutic targets, may be involved in the progression of cancer as well (102). Studies suggest that *Fusobacterium* might raise the level of microRNA-21 in tumor cells via epigenetic regulation during macrophage inflammatory response (68). microRNA-21 in turn increases the levels of IL-10 and PGE2 (prostaglandin E2), which inhibit antitumor immunity mediated by T cells in the TME (103, 104), and therefore high level of microRNA-21 usually indicates worse clinical outcomes (105). Nevertheless, further studies are needed in order to pinpoint the relationship between *Fusobacterium* and microRNAs and its significance.

**POTENTIAL MANAGEMENT OF FUSOBACTERIUM-ASSOCIATED CRC**

Numerous studies have found that enrichment of *Fusobacterium* is related to worse clinical outcome in CRC patients (96). Analogous to *Helicobacter Pylori* in the setting of gastric cancer, *Fusobacterium* may be an essential pathogen that fuels the initiation and development of CRC. Research has suggested that reducing the abundance of *Fusobacterium* may help patients with intestinal diseases such as IBD to recover (106). Therefore, therapies that specifically target *Fusobacterium* or their mechanism of action could be developed for the prevention or treatment of CRC.

**Reducing Carcinogenicity of Fusobacterium**

As we discussed early, FadA, a membrane protein of *Fusobacterium*, induces adhesion and invasion, and is the
originator of epithelial injury. In FadA-E cadherin pathway, FadA gene copy number has been found to have a direct correlation with either healthy, pre-cancerous or CRC states (22), suggesting that FadA may be a promising biomarker in CRC diagnosis. Targeting FadA blockade development is also promising by inhibiting its adhesive and invasive abilities. As E-cadherin internalization via clathrin is a key process during FadA/E-cadherin/β-catenin pathway, a clathrin inhibitor (Pitstop2) could block the pro-carcinogenesis pathway (22). Similarly, Fap2, which ferries other bacteria into the host cell, is also a membrane protein of Fusobacterium. The hemagglutination and coaggregation function of Fusobacterium can be inhibited by galactose (52). This indicates that membrane blockers may suppress key pathogenic features of Fusobacterium, providing a further target in the early stages of Fusobacterium pathogenesis. However, the possibility should be taken into consideration that abundance of FadA might only reflect Fusobacterium infection status at a given time point. Since E. coli and Streptococcus gallyliticus (107) and many other microorganisms could also be significant pathogenesis factors and promote oncogenesis via different mechanisms, FadA alone may not be sufficient. Moreover, Fusobacterium is a heterogeneous genus and further gene and protein differences between low-virulence strains and high-virulence strains will need to be probed by genome and proteome analysis (108). Such approaches may allow for characterizing relevant species at a much more granular level and identifying those equipped with highly oncogenic motifs.

Studies have found that some F. nucleatum strains may acquire genes through horizontal transfer, indicating the close connection between Fusobacterium and other gut microbes (44, 45). This can be seen in the therapeutic effects of microecologic products (109), such as probiotics and prebiotics, as they reduce the overall virulence genera through regulating the whole composition of microbes and cut down the possibility for Fusobacterium to obtain virulence genes. For example, the amount of Fusobacterium was reduced in fecal samples and the microbial diversity increased following probiotic intervention in CRC patients, suggesting that microecologic products would benefit CRC patient for suppressing CRC-associated genera (110). Though limited literature has proved a direct relationship between Fusobacterium colonized in gingival sulcus and colorectal cancer tumorigenesis, there might be some connections between oral flora and gut flora, as well as connections between oral infectious diseases and intestinal diseases (such as CRC). Combined detection of fecal and oral microbes may also enhance prediction of adenomas or CRC in addition to other risk factors (111). It is possible that altering intestinal Fusobacterium abundance could be approached by targeting oral Fusobacterium, however further studies are needed for confirmation.

**Tackling Drug Resistance and Dealing With a Complicated Microenvironment**

As stated earlier, Fusobacterium has a complex influence on the tumor microenvironment. Either chemotherapy or targeted therapies for CRC face the serious dilemma of drug resistance (112, 113). Compared with other tumors, CRC uniquely occurs in an intricate microenvironment owing to the co-existence of the huge diversity of microorganisms within the gut microbiome. It is now understood that drug resistance is closely related to the tumor microenvironment (114, 115). In this sense, the microenvironment regulated by Fusobacterium might account for drug resistance in CRC. On the one hand, Fusobacterium nucleatum has been found to promote chemoresistance to colorectal cancer by modulating autophagy (116). Via binding to TLR4 on colorectal cancer cells, Fusobacterium nucleatum activated the TLR4/MyD88 innate immune signaling pathway and miRNA-18a and miRNA-4802 were downregulated sequentially, which resulted in ULK1 and ATK7 expression. Both ULK1 and ATG7 are important elements of autophagy, the downregulation of which could decrease CRC cell apoptosis induced by chemotherapeutic agents. On the other hand, Fusobacterium might contribute to anti-VEGF pathway agents, such as bevacizumab. Fusobacterium promotes inflammation and cause aberrant extracellular matrix (ECM) formation, which accelerate VEGF synthesis and secretion. Many of the Fusobacterium-associated genes, including IL-6 and IL-8, also play significant roles in alternative pro-angiogenesis pathways (117). Besides, MDSCs recruited by Fusobacterium are also associated with drug resistance (118, 119). With overactive inflammation-tumorigenesis sequences and a local immunosuppressive state, it is more likely for patients to undergo drug resistance to immune therapies. Facing treatment failure, strategies to lower the abundance of Fusobacterium may be helpful to reduce drug resistance.

**Exploring the Relationship Between Fusobacterium and Epigenetic Alterations**

Some studies implicate Fusobacterium in certain epigenetic changes of CRC, such as CpG island methylation phenotype (CIMP), MSI and microRNA expression. However, further exploration is needed to uncover mechanism of how Fusobacterium impacts on CRC epigenetic changes. Particularly, CRC tissues enriched with Fusobacterium may be associated with MSI (96). Interestingly, high-abundance of Fusobacterium suggests poor prognosis of CRC while MSI forecasts favorable prognosis. One possible explanation is that MSI is not a predominant factor in the interaction between Fusobacterium and host cells, and other factors should also be taken into consideration. Recently, MSI is regarded as a strong biomarker for PD-1 blockade (120). It would be a possible explanation that CRC cells with frameshift mutations in the absence of normal mismatch repair function produce a mutation-associated neoantigen, which may activate anti-tumor immunity and enhance the effect of PD-L1 blockade. Some studies have indicated the relationship between gut microbiota and checkpoint immunotherapy recently (121–124), but whether Fusobacterium impact on checkpoint inhibitors still remains unclear. Further studies are needed to clarify whether Fusobacterium influences mismatch repair signaling pathways and alters the patient’s immune response.
FUTURE OUTLOOK AND CAVEATS

Colorectal cancer is one of the most common cancers worldwide and an understanding of its relationship with the gut microbiota is beginning to emerge. Interacting with numerous microbes, the initiation and development of colorectal cancer may be fueled by pathogens or inhibited by probiotics. Researchers have demonstrated the correlation between Fusobacterium with colonic adenomas and colorectal cancer. Whether this is a cause or just an association with CRC remains unclear, although some potential mechanisms have been elucidated. While FadA-induced cell invasion and subsequent signaling pathway activation is one of the most accepted mechanisms, nevertheless, based on the evidence so far, screening or risk stratification strategies utilizing Fusobacterium should be developed. In accordance with the broad public acceptance of probiotics and prebiotics, microbiome-targeted therapies for CRC are a promising avenue for future development. Notably, fecal microbiota transplantation is now considered standard of care for recurrent Clostridium difficile infection, with promising results in other conditions reported (125, 126). However, not all of the study results are in agreement with a “causal relationship” conclusion: Dejea et al. found that no consistent bacterial genus associated with tumors by high-throughput sequencing in 30 CRC and 6 adenoma human samples (127). In another paper, Dejea and colleagues studied patchy biofilms in colonic mucosa of patients with an APC gene mutations (128). Fusobacterium was not the predominant component of the biofilms while Escherichia coli and Bacteroides fragilis were prominently associated. Moreover, although Fusobacterium was more abundant in CRC biopsies and fecal samples than normal control group (129), the elevated level was only manifested in 20–30% of CRC patients and has not been consistent. In a cohort study of 137217 adults (129) to explore the association between diets with CRC prevalence, a stronger relationship between diet and CRC was found in F. nucleatum enriched individuals than subgroups without F. nucleatum detection. This finding also suggests that Fusobacterium may play different roles in different subtypes of CRC. Additionally, the interaction between the microbiota and carcinogenesis in the colon is sophisticated. Studies have also found associations with other microorganisms triggering neoplasia as well as associated mechanisms. For instance, Enterotoxigenic Bacteroides fragilis promotes Th17 development by Tregs, limiting the availability of IL2 in the local microenvironment (130) while Escherichia coli releases colibactin, a genomic product of polyketide synthase island, which is carcinogenic and promotes CRC development (20).

CONCLUSION

In conclusion, how Fusobacterium impacts CRC warrants large-scale cohort studies and laboratory experiments. Some key studies suggest that Fusobacterium could activate the β-catenin signaling pathway to promote oncogene transcription and alter the tumor microenvironment to induce immune suppression, while others suggested that Fusobacterium is associated with epigenetic changes of malignant epithelial cells. Potential microbiome therapies in colorectal cancer are still some time away but are likely to emerge as microbiome research continues to expand into oncological research. It is our hope that this review provides new insights for further CRC and Fusobacterium research.

AUTHOR CONTRIBUTIONS

ZZ conceived and wrote the paper. HH, HY, and JC reviewed and edited the manuscript. All authors read and approved the manuscript.

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Fusobacterium nucleatum is a well-recognized periodontal pathogen. It is a Gram-negative, microaerophilic, anaerobic bacterium that is commonly found in streptococcal dental biofilm and has been implicated in various oral and systemic conditions, including periodontal disease, dental caries, and systemic conditions such as inflammatory bowel disease and colorectal cancer.

Studies have shown that Fusobacterium nucleatum is present in up to 90% of colorectal cancer tissues, and its presence is associated with a worse prognosis. The bacterium has been shown to increase proliferation of colorectal cancer cells and tumor development in mice by activating toll-like receptor 4 signaling to nuclear factor-kappaB and Up-regulating expression of microRNA-21. This suggests a role for this bacterium in colorectal cancer development.

Other studies have shown that Fusobacterium nucleatum interacts with epithelial barrier regulation, contributing to the development of colorectal cancer. It has been shown to increase proliferation of colorectal cancer cells, enhance tumor microenvironment, and promote tumor growth.

In summary, the role of Fusobacterium nucleatum in colorectal cancer is multifaceted, involving interactions with the host immune system, the tumor microenvironment, and epithelial barrier regulation. Further studies are needed to better understand the mechanisms by which this bacterium contributes to the development and progression of colorectal cancer.
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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