REVIEW

Fusobacterium nucleatum and colorectal cancer: A review

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Abstract

Fusobacterium nucleatum (*F. nucleatum*) is a Gram-negative obligate anaerobe bacterium in the oral cavity and plays a role in several oral diseases, including periodontitis and gingivitis. Recently, several studies have reported that the level of *F. nucleatum* is significantly elevated in human colorectal adenomas and carcinomas compared to that in adjacent normal tissue. Several researchers have also demonstrated that *F. nucleatum* is obviously associated with colorectal cancer and promotes the development of colorectal neoplasms. In this review, we have summarized the recent reports on *F. nucleatum* and its role in colorectal cancer and have highlighted the methods of detecting *F. nucleatum* in colorectal cancer, the underlying mechanisms of pathogenesis, immunity status, and colorectal cancer prevention strategies that target *F. nucleatum*.

Key words: *Fusobacterium nucleatum*; Carcinoma; Colon and rectal carcinoma; Host immunity; Gut microbiome

Core tip: *Fusobacterium nucleatum* (*F. nucleatum*) promotes the progress of colorectal adenomas involving in multiple potential mechanisms. *F. nucleatum* positivity in colorectal cancer (CRC) is different in different research groups. Some potential biomarkers may be regarded as a criterion for judging CRC prognosis. Some chemoprevention and immunotherapy strategies on *F. nucleatum*-positive colorectal cancer need to be further explored in the future.

INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent malignant neoplasm and the fourth most frequent cause of cancer death in the world, and the five-year survival rate is nearly 65%[1]. For a long time, the mortality rate of CRC has declined in areas where medical resources are abundant, while the mortality rate has risen in areas with poor medical conditions[2]. CRC is a complex disease that is influenced by both genetic and environmental factors such as dietary habits and lifestyle. Recently, increasing evidence has indicated an association between the intestinal microbiota and CRC[3-5].

More than 100 trillion (10^14) microorganisms reside in the intestinal tract and play an extremely important role in human health. These microbes maintain intestinal homeostasis by regulating various biological activities such as mucosal barrier, immune and metabolic functions[6,7]. Once the intestinal balance is damaged, it may cause numerous intestinal diseases including inflammatory bowel diseases (IBD) and colorectal neoplasms[8-10]. There is accumulating evidence to suggest that the gut microbiota is associated with colorectal neoplasms[11-18]. Several studies have validated that the levels of *Bacteroides*, *Prevotella, Escherichia coli*, *Bacteroides fragilis* (ETBF), *Streptococcus gallolyticus*, *Enterococcus faecalis*, and *Streptococcus bovis* are significantly higher in CRC tissue compared to those in adjacent normal tissue[4,11-16,18]. ETBF has been confirmed to selectively stimulate *STATA3* in the colon, induce inflammation infiltrates of T helper type 17 and promote the development of CRC[19]. *Enterococcus faecalis* has been reported to facilitate tumorigenesis through activating the DNA damage pathways[20]. Furthermore, the abundance of both *Fusobacterium nucleatum* (*F. nucleatum*) and *C. difficile* was found to be significantly higher in CRCs compared to the healthy control group[21]. Additional studies have also confirmed that *F. nucleatum* associates with some Gram-negative bacteria, including *Streptococcus*, *Campylobacter spp.* and *Leptotrichia, and* synergistically promotes the occurrence of CRC[22,23].

F. nucleatum, a common Gram-negative anaerobic bacterium, is one of the most prevalent species in the oral cavity, and several studies have demonstrated that *F. nucleatum* is associated with oral inflammation diseases, such as periodontitis and gingivitis[24-26]. It has also been associated with pancreatic cancer, oral cancer, and premature and term stillbirths[27-30]. In addition, *F. nucleatum* is closely connected with liver abscess[9,31], appendicitis and infections of the head and neck, including mastoiditis, tonsillitis and maxillary sinusitis[32-35]. Increasing evidence has indicated that the levels of *F. nucleatum* are significantly elevated in tumor tissues and stool specimens of CRC patients relative to those in normal controls[36-42]. Researchers have reported that *F. nucleatum* may contribute to the development of CRC and that it is considered to be a potential risk factor for CRC progression[17,43]. Investigators have demonstrated that a higher abundance of *F. nucleatum* in CRC is associated with a shorter survival time[44]. Several researchers have also shown that a high-abundance of *F. nucleatum* induces a series of specific tumor molecular events, including CpG island methylator phenotype (CIMP), microsatellite instability (MSI), and genetic mutations in *BRAF*, *CHD7, CHD8* and *TP53*[44,45]. However, *F. nucleatum* was previously regarded as a passenger bacterium in human intestinal tract[46,47]. Recently, it has been considered to be a potential initiator of CRC susceptibility[37,45]. Kostic *et al*[48] have confirmed that *F. nucleatum* promotes colorectal tumorigenesis in *Apc^{min/+}* mice. Rubinstein *et al*[43] have reported that *F. nucleatum* stimulates tumor cell growth in CRC by activating β-catenin signaling and inducing oncogenic gene expression *via* the FadA adhesion virulence factor. Together, these studies show that *F. nucleatum* plays an important role in the initiation of CRC and promoting tumor cell growth in CRC*,* supporting that *F. nucleatum* is a cause of CRC rather than a consequence. In this review, we have summarized the recent reports on *F. nucleatum* and its role in CRC and have highlighted the methods of detecting *F. nucleatum* in CRC, the underlying mechanisms of pathogenesis, immunity status, and colorectal prevention strategies that target *F. nucleatum*.

F. nucleatum invades human epithelial cells, activates β-catenin signaling, induces oncogenic gene expression and promotes growth of CRC cells through the FadA adhesion virulence factor.

METHODS FOR DETECTING *F. NUCLEATUM* IN CRC

To detect *F. nucleatum* in CRC, investigators have used several different methods, including fluorescent quantitative polymerase chain reaction (FQ-PCR), fluorescence in situ hybridization (FISH), quantitative real-time polymerase chain reaction (qPCR), and droplet digital polymerase chain reaction (ddPCR). Furthermore, sample collection methods also vary among studies, some of which are derived from formalin-fixed paraffin-embedded (FFPE) CRC tissues, CRC frozen tissues, genomic DNA, and feces collected from CRC patients.

As shown in Table 1, the detection method and the detection rate of *F. nucleatum* in CRC differ among studies. In one Chinese study, the *F. nucleatum* abundance was measured in frozen tissues from 101 CRC patients by FQ-PCR, and FISH analysis was conducted on 22 CRC FFPE tissues with the highest abundance of *F. nucleatum* to confirm the FQ-PCR results, and the positive rate of *F. nucleatum* was detected to be 87.13% (88/101)[40]. Analyzing 598 CRC patients in 2 American nationwide prospective cohort studies, researchers detected the abundance of *F. nucleatum* in FFPE tissue samples obtained from CRC patients by qPCR and found that the positive percentage of *F. nucleatum*

Table 1 Positive detection rates of *Fusobacterium nucleatum* in colorectal cancer reported by different research groups

Author (publish date)	Total cases (*n*)	Positive cases (*n*)	Positive percentage	Detection method	Detection samples
Li *et al*[40] (3/2016)	101	88	87.13%	FISH and FQ-PCR	Frozen tissue and FFPE tissue
Mima *et al*[38] (8/2015)	598	76	13%	qPCR	FFPE tissue
Nosho *et al*[49] (1/2016)	511	44	8.6%	qPCR	FFPE tissue
Tahara *et al*[45] (1/2014)	149	111	74%	qPCR	Genomic DNA
Ito *et al*[39] (2/2015)	511	286	56%	qPCR	FFPE tissue
Suehiro *et al*[50] (3/2017)	158	85	54%	ddPCR	Feces

qPCR: Quantitative real-time polymerase chain reaction; FQ-PCR: Fluorescent quantitative polymerase chain reaction; ddPCR: Droplet digital polymerase chain reaction; FISH: Fluorescence in situ hybridization; FFPE: Formalin-fixed paraffin-embedded.

accounted for 13% (76/598) of the CRC samples. This detection rate was significantly lower than that reported in the Chinese study (87.13%)[38]. In one Japanese study, the experimental specimens were obtained from CRC FFPE tissues from 511 Japanese patients, and the abundance of *F. nucleatum* was detected by qPCR. *F. nucleatum* was detected in 8.6% (44/511) of the CRC tissue samples, which was similar, albeit slightly lower, to that reported in the USA (13%)[49]. In another study, the richness of *F. nucleatum* was evaluated by qPCR, and the samples were prepared from genomic DNA extracted from 149 primary CRC tissue samples; *F. nucleatum* was detected in 74% (111/149) of the CRC tissue samples[45]. In a recent study, the samples consisted of FFPE tissues from 511 CRC patients, and *F. nucleatum* was detected in 56% (286/511) of the CRC patients by qPCR[39]. In another study, *F. nucleatum* was detected in the stool samples collected from CRC patients, and the sensitivity and specificity were found to be 72.1% (75/104) and 91.0%, respectively, while the high-abundance of *F. nucleatum* in patients exhibited a false positive rate of 7.0%[42]. In another study, the levels of *F. nucleatum* were measured in fecal specimens from Japanese CRC patients by ddPCR, and *F. nucleatum* was found to be present in 54% (85/158) of the specimens[50]. Furthermore, some researchers used a qPCR assay to detect *F. nucleatum* in FFPE tissue from CRC patients and revealed that *F. nucleatum* was present in 2.5% (4/157) of rectal cancers and 11% (19/178) of cecum cancers, with a significant linear trend along all subsites[51]. The percentage of *F. nucleatum*-enriched CRC gradually increases from rectum to cecum[51], suggesting that the rate at which *F. nucleatum* is present may also differ among intestinal sites.

Common specimens for detecting *F. nucleatum* in CRC include frozen tissues, FFPE tissues, genomic DNA and feces. The use of both frozen tissue and FFPE tissue specimens are limited by surgery or colonoscopy. Specimens derived from the feces of CRC patients are easy to obtain, but they often result in high false positive detection rates. As mentioned above, qPCR, ddPCR, FQ-PCR and FISH are applied to detect the levels of *F. nucleatum.* While the qPCR assay is the most popular technique to measure the abundance of *F. nucleatum* in CRC tissues, it is difficult to detect *F. nucleatum* in the feces[52]; in addition, a higher false positive rate is seen in the high abundance group of *F. nucleatum*[42]. It has been reported that ddPCR improved the sensitivity of *F. nucleatum* detection in the feces compared to qPCR, and ddPCR was demonstrated to be 1000 times more sensitive than qPCR[53]. In addition, ddPCR resulted in a higher detection rate of low concentrations of microorganisms compared with qPCR[54]. FQ-PCR is a convenient and rapid method for detecting pathogens and displays a higher sensitivity and specificity than qPCR[55]. In addition, it is difficult to contaminate FQ-PCR during experimental operation compared with qPCR[55].

UNDERLYING MECHANISMS OF *F.NUCLEATUM* PATHOGENESIS IN CRC

A previous study has shown that lymph node metastases are present in 52 out of 88 (59.1%) cases with a high-abundance of *F. nucleatum* and in 0 out of 13 (0%) subjects with a low-abundance of *F. nucleatum*, which indicates that a high abundance of *F. nucleatum* is associated with CRC progression and metastasis[40]. It has been suggested that high levels of *F. nucleatum* may be associated with poor outcomes of CRC. Some researchers have also reported that the load of *F. nucleatum* DNA in CRC tissue is correlated with higher colorectal cancer-specific mortality[44] and that *F. nucleatum* DNA may serve as a potential poor prognostic biomarker[44]. *Fusobacterium* was shown to be enriched in the mucosa-adherent microbiota and have the ability to adhere to and invade human epithelial and endothelial cells[27,52,56]. Recently, several researchers have suggested that *F. nucleatum* is a pathogenic bacterium rather than a bacterium that promotes colorectal carcinogenesis[43,57]. Several studies have shown that its virulence factors are closely linked with colorectal lesions. It has been demonstrated that *F. nucleatum* invades human epithelial cells, activates β-catenin signaling, induces oncogenic gene expression and promotes growth of CRC cells *via* the FadA adhesion virulence factor[43]. A second virulence factor, an autotransporter protein, Fap2, has been shown to potentiate the progress of CRC *via* inhibiting immune cell activity[58].
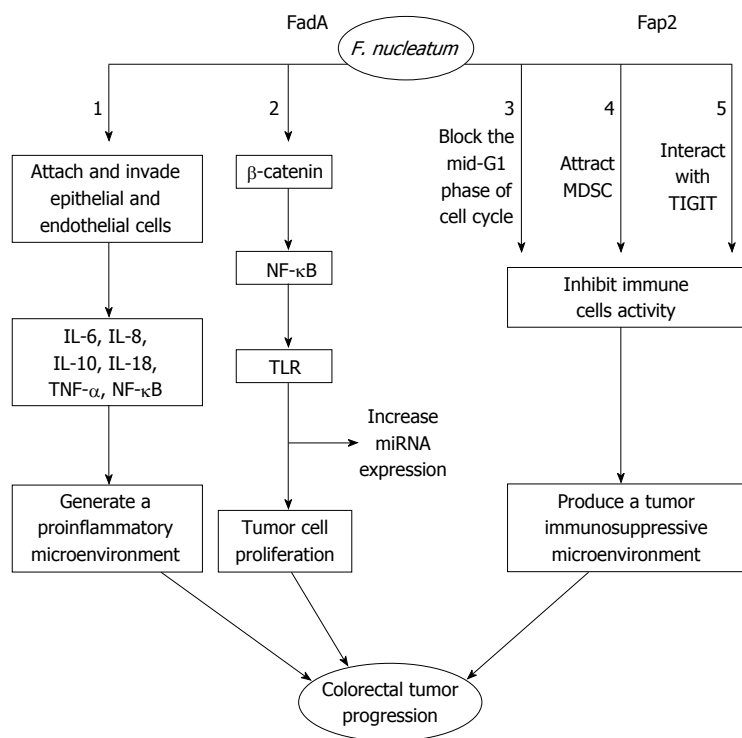
As shown in Figure 1, *F. nucleatum* attaches and

Figure 1 Underlying mechanism of *Fusobacterium nucleatum* pathogenesis in colorectal cancer. (1) In pathway 1, the FadA in *Fusobacterium nucleatum* (*F. nucleatum*) adheres to and invades human epithelial cells and endothelial cells, and inflammatory cytokine (IL-6, IL-8, IL-10, IL-18, TNF-α and NF-κB) levels increase in a proinflammatory microenvironment that accelerates the progression of colorectal tumors; (2) In pathway 2, FadA interacts with E-cadherin on the epithelial cell, activates β-catenin signaling, increases NF-κB inflammatory gene expression and promotes tumor cells proliferation. However, *F. nucleatum*-infected cells increase the expression of miRNA by activating Toll-like receptor and further promote the release of miRNA; (3) In pathway 3 and 4, *F. nucleatum* dampens human T-cell activation in a tumor immunosuppressive microenvironment that supports tumor cell growth by blocking the mid-G1 phase of cell cycle and attracting myeloid-derived suppressor cells; and (4) In pathway 5, the interaction between Fap2 of *f. nucleatum* and the human inhibitory receptor TIGIT induce human lymphocytes cell death and generate a tumor immunosuppressive microenvironment that promotes colorectal tumor progression. MDSC: Myeloid-derived suppressor cell; TLR: Toll-like receptor.

invades human epithelial and endothelial cells[27,56]. This attachment and invasion depends on the *F. nucleatum* FadA adhesion protein[59,60]. The FadA protein exists in two main forms. The first form is the intact pre-FadA consisting of 129 amino that is anchored to the membrane, and the second form is the secreted mature FadA (mFadA) consisting of 111 amino acids that are secreted outside of *F. nucleatum*[61]. When mFadA combines with pre-FadA, the pre-FadA-mFadA is internalized, and FadAc is activated[61]. The internalization of the pre-FadA and mFadA complex ensures that *F. nucleatum* binds to and invades host epithelial cells[61]. The host endothelial receptor for FadA is the vascular endothelial cadherin (CDH5), which is a member of the cadherin family[59]. The CDH5 receptor is required for *F. nucleatum* to adhere to and invade endothelial cells[59]. *F. nucleatum* invasion induces the production of cytokines such as interleukin-8 (IL-8), which is regulated by the p38 MAPK signaling pathway but independent of Toll-like receptor (TLR), NOD-1, NOD-2 and Nuclear Factor-kappaB (NF-κB) signaling[62]. *F. nucleatum* promotes the expression of several inflammatory genes such as NF-κB and cytokines, including IL-6, IL-8 and IL-18[43]. *F. nucleatum* also promotes the release of

inflammatory cytokines particularly IL-8, IL-10 and tumor necrosis factor-α (TNF-α) in a proinflammatory microenvironment that accelerates colorectal tumor progression[37,62,63]. Another receptor of FadA is the cell-adhesion molecule E-cadherin expressed on non-CRC and CRC cells[43]. E-cadherin is a strong tumor suppressor that inhibits tumor growth and development[64].

FadA binding to wnt7b E-cadherin on CRC cells promotes *F. nucleatum* adhesion and invasion of host epithelial cells, activates β-catenin signaling that leads to increased expression of *Wnt* genes, oncogenes, transcription factors, and inflammatory genes, and promotes tumor cells proliferation[43]. FadAc, but not mFadA, binds specifically to the E-cadherin-5, the cytoplasmic or the transmembrane domains of E-cadherin, and results in E-cadherin phosphorylation and internalization[43,65]. As a result, a series of events, which include a decrease in β-catenin phosphorylation, an accumulation of β-catenin in the cytoplasm, and translocation toward the nucleus, leads to the activation of β-catenin-regulated transcription (CRT)[43]. CRT increases the expression of *wnt* signaling genes such as *wnt7a*, *wnt7b* and *wnt9a*, the oncogenes *myc* and cyclin D1, transcription factors such as the lymphoid enhancer factor (LEF-1), NF-κB such as NF-κB2, T cell factor

such as TCF1, TCF3 and TCF4, and proinflammatory cytokines including IL-6, IL-8 and IL-18[43]. On the other hand, *F. nucleatum* infected cells increase the expression of microRNA-21 (miR21) by activating TLR4 signaling to MYD88, which leads to the activation of NF-κB[41]. Subsequently, hyperactive NF-κB attaches to the promoter of miR21 and induces the oncogenic cascade in CRC[41]. Moreover, *F. nucleatum* reduces CD3+ T-cell density in CRC tissue[38]. A previous study has shown that FDC364, sonic extract of *F. nucleatum*, inhibits human T-cell responses to antigens and mitogens[66]. By blocking the mid-G1 phase of cell cycle, the *F. nucleatum* inhibitory protein suppresses human T-cell activity[67]. This effect may promote an immunosuppressive micro-environment that allows tumor cell growth[67]. By releasing short-chain fatty acids (acetate, propionate, and butyrate) and short-peptides (formylmethionyl-leucyl-phenylalanine), *F. nucleatum* also selectively attracts myeloid-derived suppressor cells (MDSCs)[48,68]. MDSCs, a group of heterogeneous cells, show strong T-cell suppressive activity in the immune response[69]. MDSCs and their effectors are key components of the neoplasm and promote tumor progression[48,70]. *F. nucleatum*-associated tumors increase the myeloid-lineage infiltrating cells, including CD11b+, tumor-associated macrophages (TAMs), M2-like TAMs, tumor-associated neutrophils, conventional myeloid dendritic cells (DCs) and CD103+ regulatory DCs[48]. These cells play an important role in dampening antitumor immunity and promoting tumor progression[69,71-73]. Collectively, these studies have shown that *F. nucleatum* produces a tumor immunosuppressive microenvironment and promotes CRC progression. Fap2, a galactose-sensitive adhesion protein, plays an important role in coaggregation and cell adhesion[74]. In *F. nucleatum*, the virulence factor Fap2 protein suppresses immune cell activities through interacting with TIGIT[58]. The interaction between Fap2 and TIGIT protects tumors containing *F. nucleatum* from host immune cell attack[58]. TIGIT is an inhibitory receptor in humans that is expressed on T cells and natural killer (NK) cells[75]. The Fap2 has also been reported to induce human lymphocyte cell death[57]. In addition, Fap2 mediates *F. nucleatum* enrichment by interacting with Gal-GalNAc overexpressed in colorectal tumors[76]. Gal-GalNAc is a host polysaccharide overexpressed in CRC[76]. In summary, *F. nucleatum* produces a tumor immunosuppressive microenvironment that promotes CRC progression.

F. NUCLEATUM AND IMMUNITY STATUS IN CRC

Some researchers have demonstrated that *F. nucleatum* modulates the tumor immune microenvironment while promoting CRC development[48]. Recently, it has been confirmed that biomarkers such as immune antibodies, miRNA, TAMs, and tumor-infiltrating T-cell subsets play a significant role in *F. nucleatum*-associated

CRC[44,48,77,78].

Several studies have shown that *F. nucleatum* infection causes high levels of serum *F. nucleatum*-IgA antibodies in CRC patients[77]. Researchers have confirmed that serum anti-*F. nucleatum*-IgA combined with CA19-9 and CEA has a higher sensitivity than CA19-9 and CEA alone in screening early CRC[77]. This study suggests that serum *F. nucleatum*-IgA antibodies may be regarded as a potential diagnosing biomarker for early CRC[77]. In addition, some researchers have found that the levels of the *fadA* gene in colon tissue from CRC patients are > 10-100 times higher in comparison with normal subjects[43]. This study also reveals a gradual increase in *fadA* gene copies in normal individuals compared to CRC patients[43]. The *fadA* gene has become a potential ideal diagnostic marker to identify individuals with CRC risk[43]. The *miR-21* gene has been demonstrated to promote tumor cell growth and migration *via* inhibiting sec23a protein expression[79]. The data also indicated that *F. nucleatum* induces a high level of miR-21 expression in advanced CRC[41]. The amount of miR-21 in CRC tissues has been shown to be associated with poor clinical outcomes[41]. Studies have reported that non-coding RNAs (lncRNAs) play a crucial role in the diagnosis and prognosis of CRC[80]. One study has found that low levels of NR_034119 and NR_029373 are associated with a short survival rate of CRC[80]. These researchers suggested that several lncRNAs (NR_034119, NR_029373, NR_026817, and BANCR) are potential diagnostic biomarkers for CRC and that NR_034119 and NR_029373 are potential prognostic indicators for CRC[80]. Another study reported that the level of lncRNA PANDAR was higher in CRC cells and tissues relative to adjacent normal tissues[81], and high levels of PANDAR expression were associated with short overall survival[81]. The authors suggested that the amount of PANDAR expression may be a prognostic indicator for CRC.

A previous study reported that *F. nucleatum*-positive tumors increased TAM infiltration[48]. TAMs play an important role in innate immunity, and subpopulations of regulatory T-lymphocytes (Tregs) are a component of the acquired immunity. A recent study has found that intense infiltration of TAMs in colorectal tumor tissue is associated with shorter disease-free survival and overall survival of CRC[78]. Infiltration of TAMs CD68+/iNOS− in colorectal tumor stroma is confirmed to be related to the poor prognosis of CRC[78]. Some researchers have reported that tumor-infiltrating T-cell subpopulations distinctly regulate the prognosis of CRC[82]. For instance, in tumor-infiltrating T-cell subsets, CD45RO+-cell density, but not that of FOXP3+-cell, is significantly associated with a long survival of CRC patients[82]. CD45RO+-cell is considered to be a potential good prognostic biomarker for CRC[82]. The FOXP3+ transcription factor, which plays an important role in regulating the immune system, is regarded as an immunosuppressive factor. Some scholars have reported that infiltration of FOXP3+ in colorectal tumor

stroma is associated with a poor prognosis in CRC[78]. However, several researchers also suggest that FOXP3+-cells are generally associated with a good prognosis of CRC[83]. An article recently published in Nature Medicine has shown that distinct tumor-infiltrating FOXP3+-T cell subpopulations have an opposite approach to determining CRC prognosis. The development of inflammatory FOXP3± (lo) non-Treg cells was shown to be associated with tumor invasion by intestinal bacteria, particularly *F. nucleatum*[84]. In this study, CRC patients with a high infiltration of FOXP3± (lo) T cells exhibit a significantly better prognosis, compared to those with a FOXP3± (hi) Treg cell infiltration[84]. When FOXP3± (hi) Treg cells are depleted from CRC tissues, antitumor immunity is augmented[84]. The elimination of FOXP3± (hi) Treg cells has been suggested to play a crucial role in suppressing CRC formation[84]. Recent research has also found that prudent diets such as whole grain and dietary fiber reduce the risk of *F nucleatum*-positive CRC[85].

In conclusion, anti-*F. nucleatum*-IgA, the *fadA* gene, and lncRNAs may be considered as potential diagnostic biomarkers during the early stage of *F. nucleatum*-positive CRC. The CD45RO+-cell and FOXP3± (lo) T cell biomarkers are associated with a favorable prognosis in *F. nucleatum*-positive CRC, while the miR-21, LncRNA PANDAR, and TAMs CD68+/iNOS− biomarkers are associated with a poor clinical prognosis of *F. nucleatum*-positive CRC.

PREVENTION STRATEGIES THAT TARGET *F. NUCLEATUM* IN CRC

Currently, cancer prevention strategies have been mainly focused on chemoprevention and immunotherapy. Chemoprevention, which involves the use of aspirin, cyclo-oxygenase-2 (*COX-2*) inhibitors, and selective EP2 antagonists, plays an important role in *F. nucleatum*-associated CRC. Immunotherapies, such as antibody treatment, immune-checkpoint blockade therapy and adoptive cell transfer therapies, may aid in the prevention of *F. nucleatum*-positive CRC.

Chemoprevention, including the use of aspirin, *COX-2* inhibitors, and selective EP2 antagonists, plays a significant role in the mechanisms of *F. nucleatum*-positive CRC. For instance, some researchers have reported that regular aspirin use lowers CRC incidence and mortality and reduces the risk of distant metastasis of CRC[85,86]. Regular doses of aspirin were also associated with a lower risk of CRC and low levels of CD45RO (PTPRC)+T cells, CD3+T cells or CD8+ T cells[87]. Aspirin induces neutrophils apoptosis[88] and triggers a lipoxin-driven immune-regulatory effect[89]. Aspirin directly inhibits T-cell activation and proliferation and suppresses cytokine production involved in the T cell-mediated adaptive immune response[90]. Tumor-infiltrating immune cells have been associated with a good prognosis in CRC[91,92]. The amount of *F. nucleatum* is inversely proportional to CD3+ T-cell density in colorectal carcinoma

tissue[38]. These data indicate that aspirin may support the host immune system and prevent the development of *F. nucleatum*-associated CRC.

In addition, FadA in *F. nucleatum* specifically binds to E-cadherin and activates *Wnt* signaling[43]. *F. nucleatum* increases expression of inflammatory genes and *Wnt* genes[43]. A recent study has reported that EP2 enhances the expression of NF-κB-targeted proinflammatory genes induced by TNF-α in neutrophils[93]. The levels of cytokines such as TNF-α and IL-6, COX-2, chemokine CXCL1, and *Wnt* are significantly higher in tumor lesions of EP2-abundant mice than those in EP2- deficient mice[93]. This study revealed that EP2 promotes colon tumorigenesis by means of expanding inflammation and shaping a tumor microenvironment[93]. PF-04418948, a selective EP2 antagonist, significantly inhibits the formation of colon tumors[93]. This suggests that selective EP2 antagonists may be promising drugs for the chemoprevention of *F. nucleatum*-associated CRC.

Furthermore, COX expression in Braf[V600E] cells may prevent CD103+ DC activation and accumulation in tumors[94]. By suppressing local T-cell effector, COX-2 also promotes immune evasion and resistance to antigen-specific cancer immunity[95]. COX-2 is also considered an inhibitor of antigen-specific tumor immunotherapy[95]. This is powerful evidence that supports that COX inhibitors reduce the risk of CRC by inhibiting inflammatory pathways, and COX inhibitors may be important for immune-based therapy in CRC patients. In conclusion, aspirin, EP2 antagonists, and COX-2 inhibitors may be important tools for preventing *F. nucleatum*-associated CRC.

Immunotherapies, including antibody treatment, immune-checkpoint blockade therapy and adoptive cell transfer therapies, may be effective strategies for preventing *F. nucleatum*-positive CRC. For example, the interaction between Fap2 and TIGIT receptor protects tumors against immune cell attack and, accordingly, inhibits antitumor immunity and supports tumor cells growth[58]. Fap2 also induces lymphocyte cell death[57]. Fap2 mediates *F. nucleatum* enrichment *via* its interaction with Gal-GalNAc that is overexpressed in CRC, which may exacerbate the inhibition of antitumor immunity[76]. Therefore, anti-Fap2 antibody development may favor antitumor immune response and be a potential immunotherapy in *F. nucleatum*-positive CRC. *F. nucleatum* inhibits T-cell activity and stimulates lymphocyte cell death, which protects tumors from immune cell attack. *F. nucleatum* may have immunosuppressive function in the tumor immune microenvironment.

Recently, the approach to cancer immunotherapy involves immune-checkpoint blockade, such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed death protein 1 (PD-1). CTLA-4 and PD-1 have been reported to be involved in T cell-mediated antitumor immunity[96,97]. It was speculated that blockade of CTLA-4 and PD-1 may shape the antitumor immunity response and be an effective immunotherapy

for *F. nucleatum*-associated CRC. Other CRC treatment strategies involving *F. nucleatum*, such as *miR-21* blockade may play a significant role in *F. nucleatum*-positive CRC, as *F. nucleatum* increases expression of *miR-21* by activating TLR4 signaling to NF-κB[41]. It has been demonstrated that *miR-21* promotes tumor cells proliferation and migration by down-regulating the expression of the sec23a protein[79]. The inhibition of *miR-21* suppresses the metastasis of colorectal tumor cells by regulating programmed cell death 4[98]. In a *miR-21* knockout mouse model, expression of proinflammatory and procarcinogenic cytokines was decreased, suggesting that *miR-21* deficiency promotes the apoptosis of tumor cells by suppressing STATA3 and Bcl-2 activation[99]. It has been suggested that the *miR-21* blockade may be a potential treatment strategy for *F. nucleatum*-associated CRC. Some adoptive cell transfer therapies, such as NK cells[100], cytokine-induced killer cells[101], and tumor-infiltrating lymphocytes[102], are also being used to strengthen antitumor immunity in clinical practice. These adoptive cell transfer therapies may also be considered as an immunotherapy approach in CRC associated with *F. nucleatum*.

In sum, CRC prevention strategies that target *F. nucleatum* are mainly focused on chemoprevention, which includes the use of aspirin, *COX-2* inhibitors and selective EP2 antagonists, and immunotherapy, which includes anti-Fap2 antibody treatment, CTLA-4, PD-1, *miR-21* blockade therapies and adoptive cell transfer therapies.

CONCLUSION

In summary, the gut microbiota, especially *F. nucleatum*, has been extensively associated with CRC. *F. nucleatum* promotes the progression of CRC *via* multiple potential mechanisms. The positive detection rate of *F. nucleatum* in CRC samples varies among different studies. FadA combined with anti-*F. nucleatum*-IgA may improve the diagnosis of CRC. Several potential biomarkers, such as miR-21, LncRNA PANDAR, TAMs CD68^{+}/iNOS^{−}, FOXP3[±] (lo) T cells and CD45RO^{+} cells, may be considered as criteria for determining CRC prognosis. Furthermore, chemoprevention and immunotherapy strategies should be further explored in the future.

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