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Perspective

The role of *Fusobacterium nucleatum* in colorectal cancer: from carcinogenesis to clinical management

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Abstract

Colorectal cancer (CRC) is a common malignant tumor that affects people worldwide. Metagenomic analyses have shown an enrichment of *Fusobacterium nucleatum* (*F. nucleatum*) in colorectal carcinoma tissue; many studies have indicated that *F. nucleatum* is closely related to the colorectal carcinogenesis. In this review, we provide the latest information to reveal the related molecular mechanisms. The known virulence factors of *F. nucleatum* promote adhesion to intestinal epithelial cells via FadA and Fap2. Besides, Fap2 also binds to immune cells causing immunosuppression. Furthermore, *F. nucleatum* recruits tumor-infiltrating immune cells, thus yielding a pro-inflammatory microenvironment, which promotes colorectal neoplasia progression. *F. nucleatum* was also found to potentiate CRC development through toll-like receptor 2 (TLR2)/toll-like receptor 4 (TLR4) signaling and microRNA (miRNA)-21 expression. In addition, *F. nucleatum* increases CRC recurrence along with chemoresistance by mediating a molecular network of miRNA-18a*, miRNA-4802, and autophagy components. Moreover, viable *F. nucleatum* was detected in mouse xenografts of human primary colorectal adenocarcinomas through successive passages. These findings indicated that an increased number of *F. nucleatum* in the tissues is a biomarker for the diagnosis and prognosis of CRC, and the underlying molecular mechanism can probably provide a potential intervention treatment strategy for patients with *F. nucleatum*-associated CRC.

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Introduction

Colorectal carcinoma (CRC) is one of the most common malignant tumors of the digestive tract, which is deemed to be a major public health concern currently.[1] Overall, colorectal cancer ranks fourth for incidence (9.2% of the total cancer cases), but second in terms of mortality (9.2% of the total cancer deaths).[2] The mechanism of CRC malignancy has not been completely established. The occurrence of CRC is a multi-factor and multi-step process caused by the synergy of environment, diet, and lifestyle along with genetic factors, while inflammation has been identified as an important risk factor.[3,4]

The digestive tracts of humans have over 10^{14} bacteria, eukaryotes, and viruses, which form the so-called gut microbiota. These microorganisms play a significant role in normal human physiological activities, including digestion, metabolism, epithelial homeostasis, and gut lymphoid tissues development. In addition, antigens and metabolic products of the gut microbiota assist in cytokine production against potential pathogens.[5] Dysbiosis in gut microbiota, such as changes in their population or composition, distributes to different locations in the intestine, which could cause specific diseases, for example, cardiometabolic disorders, inflammatory bowel diseases, neuropsychiatric diseases, and cancer.[6] Numerous evidence proved that gut microbiota influenced tumor development through the virulence factors of the pathogenic bacteria. Recent papers and reviews have suggested a close correlation between certain bacterial strains and CRC.[7–9]

Fusobacterium nucleatum (*F. nucleatum*) is an anaerobic, gram-negative bacillus, present in species-specific reservoirs in the human mouth, gastrointestinal tract, and other body parts. It has been regarded as an opportunistic pathogen, found in the anaerobic samples of patients with oral, intestinal, and other infections. 16S ribosomal ribonucleic acid (rRNA) gene sequence analysis and application of metagenomic sequencing technology indicated the close relation of *F. nucleatum* with CRC. It was abundant in CRC patients compared to the control group.[10] Moreover, the presence of *F. nucleatum* had a close relationship with the poor prognosis of CRC patients and probably promoted chemoresistance.[11,12]

Investigators have reported several CRC tumorigenesis molecular events that are related to the enrichment of *F. nucleatum*, for instance, microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and oncogenic mutations in tumor protein p53 (*TP53*), BRAF, chromodomain-helicase-DNA-binding protein (CHD)7, and CHD8.[11,13] Firstly, *F. nucleatum* has been proven to promote CRC tumorigenesis in the $Apc^{min/+}$ mice model.[14] Subsequently, it was proved to promote CRC cell growth by activating E-cadherin/β-catenin signaling via FadA adhesin,[15] and the function of more virulence factors of *F. nucleatum* associated with CRC was discovered. It was confirmed that *F. nucleatum* protected the tumor cell by restraining the immune cell activities by its Fap2 protein. However, the detailed mechanism of the effect of *F. nucleatum* abundance on CRC development and the related pathobiological implication are not completely understood. In order to facilitate future studies on *F. nucleatum* and to provide new therapeutics for CRC disease, this review will highlight the following issues in detail: (1) *F. nucleatum* interaction with microbiota in human body; (2) diseases related with *F. nucleatum*; (3) the roles of *F. nucleatum* virulence factors; (4) regulation of *F. nucleatum* in CRC clinical outcomes; (5) relationship of *F. nucleatum* with the subtypes of CRC; and (6) potential management of *F. nucleatum*-associated CRC.

F. nucleatum interaction with microbiota in human body

Fusobacterium species are found in the mouth and mucosal sites of human and various animals. The universality of its presence in the healthy tissues indicates that Fusobacteria is a natural constituent of the microbiota. In the human oral cavity, *F. nucleatum* plays an important role and is closely associated with other microbiota. Firstly, *F. nucleatum* has an elongated rod shape, which provides structural support to connect with primary colonizers, like the *Streptococcus* species to the secondary colonizers, including *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.[16] The *F. nucleatum* can form highly ordered corncob-like structures when co-cultured with *Streptococcus sanguinis* (*S. sanguinis*), where up to ten *S. sanguinis* can bind to a single *F.*

nucleatum cell.[17] Thus, it is clear that *F. nucleatum* interacts with other microorganisms by providing structural support, which is essential for polymicrobial biofilms. In addition, numerous evidence has demonstrated that *F. nucleatum* regulates biofilm organization and interacts with the host cells by producing various adhesins. One of the well-studied fusobacterial adhesins is the RadD protein, which is capable of binding with SpaP, an adhesin of *Streptococcus* mutants.[18] As a result, co-aggregation of these two bacteria and even advanced biofilm organization are facilitated by the binding of RadD protein and SpaP protein. In addition to bacteria, RadD is also found to be able to bind with the yeast *Candida albicans*, which is detected in the mouth and gastrointestinal tract of healthy adults.[19] Besides physical interactions, *F. nucleatum* also associates with the other microbiota through cross-feeding and metabolic interactions. It has been well studied that, in culture, *F. nucleatum* can use ornithine from *Streptococcus gordonii*, which is ArcD-excretion dependent.[20] Other metabolic pathways, including amino acid fermentation and glycolysis of *F. nucleatum*, may be affected by related microbiota in a species-specific manner, according to broader metaproteomic investigations.[21] Recent studies employing the multiplex visualization method combinatorial labeling and spectral imaging-fluorescence *in situ* hybridization (CLASI-FISH) have demonstrated a much more complex correlation between *F. nucleatum* and the other microbiota than expected.[22,23] It raises more questions, which remain to be answered in future research.

Diseases related to *F. nucleatum*

Up to date, a wide spectrum of human diseases have been recognized to be associated with *F. nucleatum*, including oral infections, adverse pregnancy outcomes (APO), gastrointestinal disorders, cardiovascular diseases, and Alzheimer's disease.[24] The *F. nucleatum* is considered to be a normal oral commensal that generally exists in the oral cavity. However, it turns out to be an invasive pathogen under unhealthy conditions and can be detected in several extra-oral sites. Here, we introduce the major diseases that are closely linked with *F. nucleatum* and the occurrence location, followed by a detailed explanation of the pathogenesis process.

Oral infections

F. nucleatum is responsible for different forms of periodontal and endodontic diseases, including gingivitis, chronic or aggressive periodontitis, and pulp necrosis.[25–27] The abundance of *F. nucleatum* increases with the severity of disease and inflammation level of patients. Compared to healthy samples, the quantity of *F. nucleatum* in the periodontal site and saliva increased profoundly in patients with gingivitis and periodontitis. Supported by animal studies, oral infections, such as periodontitis, endodontic infections, and gingivitis have been recognized to be polymicrobial infections. It has been proven to have synergy effects in virulence with other species such as *Tannerella forsythia*, *P. gingivalis*, and *Streptococci*.[28–30] Besides, recent studies also indicated a relation between *F. nucleatum* oral infection, and oral squamous cell carcinoma as well as esophageal cancer.[31,32] Some environmental factors, such as smoking and uncontrolled type-2 diabetes, promote the increase in *F. nucleatum* abundance.[33,34]

Gastrointestinal disorders

Inflammatory bowel diseases (IBD) are found to be closely related to *F. nucleatum*. The IBD patients generally have an abundance of *F. nucleatum* in the colonic biopsies, and the *F. nucleatum* strains isolated from the inflamed tissues are more invasive than those from the normal tissues.[35] The IBD is a known CRC risk factor, which indicated CRC might be linked with similar microorganisms. The connection between *F. nucleatum* and CRC was discovered in 2012 by two individual groups, who both reported *F. nucleatum* enrichment in CRC compared to adjacent normal tissue via RNA sequencing and whole-genome shotgun sequencing.[36,37] Moreover, the association between *F. nucleatum* and appendicitis has also been reported by several groups.[38–40] However, the mechanisms of *F. nucleatum* in gastrointestinal disorders still remain to be elucidated.

Adverse pregnancy outcomes (APO)

In 2010, Han et al[41] reported for the first time that *F. nucleatum* originating from the mother's oral cavity caused stillbirth. Since then, numerous studies related to APO have been carried out, and *F. nucleatum* was proven to be associated with chorioamnionitis, preeclampsia, preterm birth, stillbirth, and early-onset neonatal sepsis. In most cases, *F. nucleatum* can be detected concurrently in the patients' amniotic fluid and cord blood, indicating its dissemination ability to placental and fetal compartments.[42] The hematogenous injection of *F. nucleatum* resulted in the specific colonization of the bacteria in the mouse fetoplacental

unit; this animal model indicated that *F. nucleatum* translocates from the patients' oral cavity to the intrauterine cavity via hematogenous transmission.[43] By far, *F. nucleatum* is the most prevalent oral species implicated in APO.

Other infections

Following the development of microbial detection technologies, *F. nucleatum* has been found to be associated with a wide range of infections, such as head, neck, brain, lung, abdomen, pelvis, urinary tract, bones, joints, and blood of human.[44,45] Different groups reported that *F. nucleatum* and *Fusobacterium necrophorum* might have a strong relationship with Lemierre's Syndrome, a well-known life-threatening upper airways infection.[45,46] Besides, *F. nucleatum* is detected in atherosclerotic plaques; thus, it is probably responsible for cardiovascular diseases (CVD).[47] It has also been detected in patients with Alzheimer's disease, rheumatoid arthritis, and other respiratory tract infections.

The question, whether *F. nucleatum* is a passenger or a driver of the related diseases has no definite answer. At least, the current evidence supports the passenger role; however, its driver role works in promoting CRC progression and negatively influencing the therapeutic efficacy. Kostic et al[14] showed that *Apc* mutation is a prerequisite genetic defect in mouse for the *F. nucleatum* to promote tumor progression. Until now, some cases have shown that improved clinical results for diseases like rheumatoid arthritis have been obtained through a concurrent periodontal treatment.[48] These findings imply that further research on the pathogenic mechanisms of *F. nucleatum* in different diseases should be considered to find new clinical treatment solutions.

The roles of *F. nucleatum* virulence factors

As described, *F. nucleatum* has been involved in inducing many diseases, not only in periodontitis but also causes other infections as a gastrointestinal opportunistic pathogen. Here, we summarized the role of *F. nucleatum* and the functions of its main virulence factors. *F. nucleatum* was recently found to participate in building oral biofilms as a mutualist, which has been tested to bridge oral microbiota, mainly by its outer membrane protein RadD (gene, *radD*) adhesin. This RadD was identified by the inactivation of Fn 1526 in *F. nucleatum* strain ATCC 23726, which is an arginine-inhibitable adhesin, to aggregate multiple species and support oral biofilm formation.[49] The *F. nucleatum* spreads to other organs by blood circulation and establishes infection,[50] and RadD was confirmed to function in the attachment and invasion to endothelial and epithelial cells.[51] Thus, RadD provides a colonized microenvironment for the first step in disease occurrence. Hence, investigation of the *F. nucleatum* secretion system is important.

A novel adhesin called FadA was identified to regulate the host cell adhesion.[51–53] Témoin et al[53] investigated the structure of FadA in 2011 using a *fadA*-deleted mutant of *F. nucleatum* 12230-US1. They reported that FadA has two forms, pre-FadA and mFadA, while the pre-FadA-mFadA complex (FadAc), other than the mFadA, is essential for its function. The discovery of *F. nucleatum* has uncovered that it might be a causative factor in the tumorigenesis of CRC. Research groups have reported that *F. nucleatum* was overabundant in the CRC tissues.[15,36] In 2013, Rubinstein et al[15] demonstrated that vascular endothelial cadherin (E-cadherin) is a receptor for FadA, their binding activates β-catenin signaling and triggers inflammatory responses to increase the gene expression of the transcription factors nuclear factor-κB (NF-κB) and the Wnt pathway components and to promote CRC cell proliferation. Therefore, virulent factors of *F. nucleatum* were believed to be involved in CRC development. The Fap2, located on the surface of *F. nucleatum* ATCC23726, has been recognized as a galactose-binding lectin.[54] The human colorectal adenocarcinomas expressed higher levels of Gal-GalNAc. The role of Fap2 was confirmed to mediate *F. nucleatum* recruitment to CRC cells; a mouse model infected with a *fap2*-mutant and O-glycanase confirmed this function.[55] Gur et al[56] found that *F. nucleatum* protected CRC cells from natural killer (NK) cell cytotoxicity, tumor-infiltrating lymphocytes, and T cell attack through interaction with T cell immunoglobulin and ITIM domain (TIGIT) inhibitory receptor mediated by Fap2. These results remind us that autotransporters (Type V secreted effectors) of *F. nucleatum* are worth investigating.

In 2017, a *Fusobacterium* phospholipase class A1 autotransporter (strain ATCC 25586), named FplA, was characterized by the biochemical toolbox. The FplA was found to have an affinity for phosphatidylinositol 4,5-bisphosphate [PI (4,5) P_2] preferentially, while having a stronger affinity for phosphatidylinositol 3,5-bisphosphate [PI (3,5) P_2] and phosphatidylinositol 3,4,5-trisphosphate [PI (3,4,5) P_3] lipids. This suggested that FplA has a role in shaping the *F. nucleatum* intracellular niche; however, its specific function in CRC has not been fully studied yet.[57]

It is clear that *F. nucleatum* virulent factors are related to biofilm formation, tumor cell attachment, and invasion. Besides, more autotransporters of *F. nucleatum*

remain to be confirmed. In the latest report, Liu et al[58] identified autotransporter proteins of outer membrane vesicles (OMVs) of gut mucosa-derived *F. nucleatum* by proteomics technology. The FadA, membrane occupation and recognition nexus protein 2 (MORN2), and YadA-like domains were identified through an *in silico* analysis. The results further displayed that adhesins of *F. nucleatum* have roles in bacterial colonization and dissemination, and its antigenic domains and epitopes lead to immune responses. Overall, studies on the virulence factors of *F. nucleatum* provide new insights to understand the pathogenic mechanism of *F. nucleatum*-associated CRC.

Regulation of *F. nucleatum* in CRC clinical outcomes

In a previous study, male C57BL/6-*Apc*$^{Min/+}$ mice planted with tumor were fed with *F. nucleatum*, *E. coli*, and Berberine (BBR). The BBR is used to treat gut infections as an isoquinoline alkaloid and a pharmacological component.[59] The result showed that *F. nucleatum* enriched in the colorectal adenoma-carcinoma tissue, altered the lumen microbial structures, and also promoted colon tumorigenesis by increasing tumor-immune cytokine secretion of interleukin (IL)-17F/21/22/23/31/cluster of differentiation (CD)40L and protein expression of phospho-signal transducer and activator of transcription 3 (p-STAT3), phospho-signal transducer and activator of transcription 5 (p-STAT5), and phospho-extracellular regulated protein kinases (p-ERK)1/2.[60] Berberine intervention inhibited the ability of *F. nucleatum* to function as an opportunistic pathogen, so it might prove to be a good drug for CRC therapy. Based on the rapid development of microRNAs (miRNAs) sequencing technology, a team tested the function of miRNAs in promoting CRC progress. They identified that miR21 was highly expressed by activating toll-like receptor 4 (TLR4) binding to myeloid differentiation factor 88 (MYD88) during *F. nucleatum* invasion, then, miR21 activated the NF-κB and decreased the RAS GTPase Ras p21 protein activator 1 (RASA1) level, leading to an increase in several inflammatory factors that significantly promote the CRC cell proliferation.[4] Therefore, the *F. nucleatum* DNA or host cell miR21 were demonstrated to be biomarkers for poor outcomes (Fig. 1A). Investigation of the mechanism of *F. nucleatum*-related CRCs tumorigenesis verified that it activates β-catenin signaling by TLR4/p21-activated kinase 1 (PAK1) cascade *in vivo* in the *Apc*$^{Min/+}$ mice model, leading to intestinal tumorigenesis.[4,61,62] Some researchers concluded that CRC patients associated with the enrichment of *F. nucleatum* showed shorter survival duration and were linked with recurrence post-chemotherapy.[11] Yu et al[12] tried to unravel the mechanism of *F. nucleatum*-mediated CRC chemoresistance and confirmed that the expression of miR-18a* and miR-4802, depending on the TLR4 and MYD88 signaling pathways, respectively, were activated in *F. nucleatum*-infected CRC cells treated with 5-fluorouracil (5-FU) and oxaliplatin. During this process, *F. nucleatum* induced the expression of autophagic proteins, phosphorylated uncoordinated-51-like kinase 1 (ULK1), ULK1, and autophagy-related protein 7 (ATG7), which caused *F. nucleatum* related CRC to be resistant to 5-FU and oxaliplatin. These findings also indicated that the high amount of *F. nucleatum* is a risk factor for CRC recurrence. Simultaneously, the chemotherapeutic strategy with antibacterial drugs might be feasible for CRC patients with a high amount of *F. nucleatum* (Fig. 1B).

Additionally, some studies also reported that *F. nucleatum* also mediated CRC metastases. A high abundance of *F. nucleatum* was found predominantly in the metastatic lesions of mouse xenografts colonizing human colorectal cancer cells to promote tumor growth.[63] Importantly, *F. nucleatum* load, cancer cell proliferation, and overall tumor growth were reduced by antibiotic metronidazole treatment. These observations provide a possible treatment by antimicrobial interventions for patients with *F. nucleatum*-associated CRC (Fig. 1C).

Relationship of *F. nucleatum* with the subtypes of CRC

The subtype assignment of CRC closely correlates with the precise clinical treatment of patients since CRC is a heterogeneous disease. Guinney et al[64] built a research team aiming at precise CRC classification, which resulted in four consensus molecular subtypes (CMSs) with distinguishing features: CMS1 (microsatellite instability immune, 14%), hypermutated, microsatellite unstable, and strong immune activation; CMS2 (canonical, 37%), epithelial, marked Wnt and MYC signaling activation; CMS3 (metabolic, 13%), epithelial and evident metabolic dysregulation; and CMS4 (mesenchymal, 23%), prominent transforming growth factor-β activation, stromal invasion, and angiogenesis. The connection between gut microbial dysbiosis and CRC development was researched in different

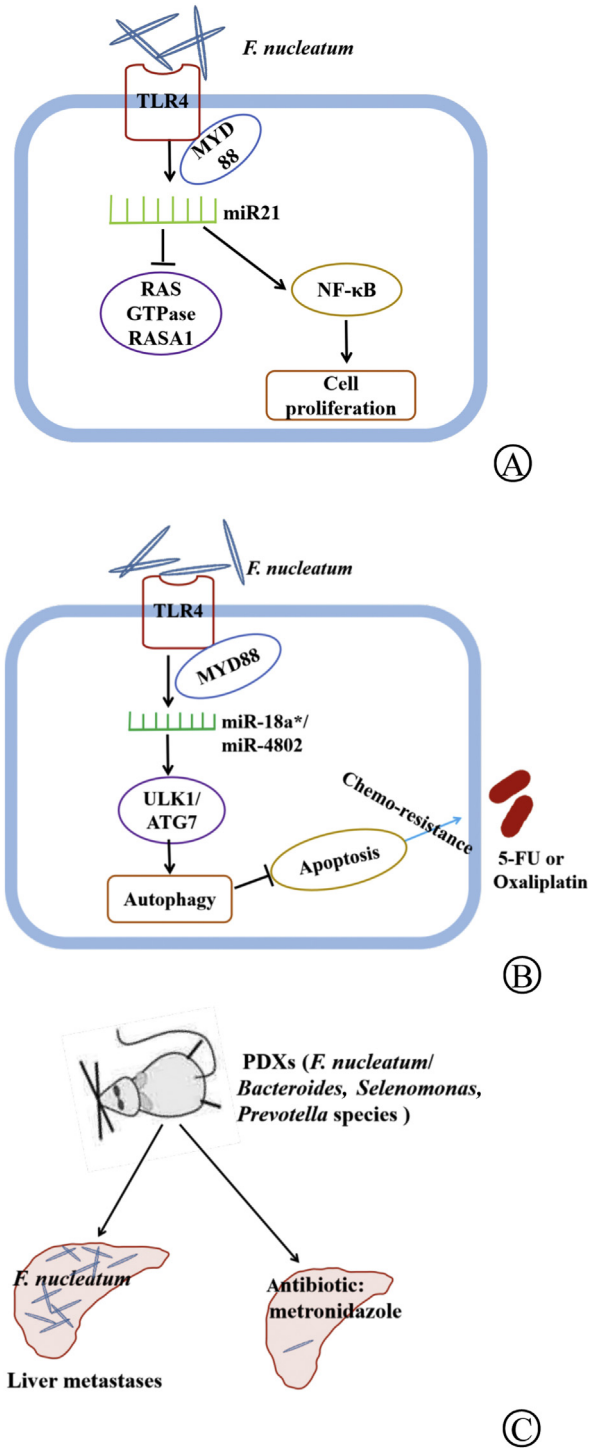

Fig. 1. *F. nucleatum* plays a role in regulating CRCs outcomes. (A) *F. nucleatum* can increase CRC cell proliferation through activating TLR4 and MYD88 signals and upregulating miR21 expression, activating the nuclear factor NF-κB, and suppressing RAS GTPase RASA1 level.[4] (B) *F. nucleatum* induces recurrence after chemotherapy. The *F. nucleatum* activates TLR4 and MYD88 signals to decrease the expression of miR-18a* and miR-4802, respectively, to further activate autophagy and induce chemoresistance.[11] (C) Mouse xenografts of human primary colorectal adenocarcinomas retain *F. nucleatum*, and antibiotic treatment reduces *F. nucleatum* load.[63] TLR4: toll-like receptor 4; MYD88: myeloid differentiation factor 88; miR: microRNA; RASA1: Ras p21 protein activator 1; NF-κB: nuclear factor-κB; ULK1: uncoordinated 51-like kinase 1; ATG7: autophagy-related protein 7; 5-FU: 5-fluorouracil; PDX: patient derived xenograft; CRC: colorectal cancer.

groups. Purcell et al[65] firstly investigated the high level of bacterial species associated with different subtypes of CRC. *Fusobacterium hwasookii* and *P. gingivalis* were identified to have the highest abundance by 16S rRNA analysis and metagenomics in CMS1. Additionally, early reports showed enrichment of *F. nucleatum* in CRC tissues compared with adjacent normal tissues.[36,37] Therefore, a detailed investigation of the connection between *F. nucleatum* and CRCs subtype is necessary.

In 2013, Kostic et al[14] found that $CD11b^+$ myeloid cells increased in $Apc^{Min/+}$ mice planted with tumor and fed with *F. nucleatum*. Tumor-associated macrophages (TAMs), M2-like TAMs, dendritic cells (DCs), regulatory T cell (Treg), and T helper cell 17 (Th17) also increased in this mice model; myeloid-derived suppressor cells (MDSC) subsets include monocytic and granulocytic cells which can suppress $CD4^+$ T cells, and activate NF-κB-driven pro-inflammatory response. These results suggested that there is a link between *F. nucleatum* abundance and immunity in CRC microenvironment. Next, Mima et al[66] conducted a study to investigate whether densities of T-cells in tumor tissue with *F. nucleatum* were associated with clinical and molecular features, including MSI, CpG island methylator phenotype, long interspersed nuclear element 1 (LINE-1) methylation, and *KRAS, BRAF, and PIK3CA* mutation status. They uncovered the following facts: first, an higher number of *F. nucleatum* in CRC tissue was significantly associated with more advanced stages of CRC; second, *F. nucleatum* in CRC was related with poor differentiation, MSI-high, MutL homolog 1 (MLH1) hypermethylation, and CIMP-high; third, higher amount of *F. nucleatum* in CRC tissues was related with lower density of $CD3^+$ T-cells, instead of $CD8^+$, $CD45RO^+$, or forkhead box P3 $(FOXP3)^+$ T-cells. It indicated that *F. nucleatum* was involved in immune response in different CRC molecular subtypes. Simultaneously, the relationship between *F. nucleatum* and the molecular features in the early stage of CRC tumorigenesis was investigated, and results showed that *F. nucleatum* was more enriched in CIMP-high premalignant lesions and positively and gradually increased from sigmoid colon to cecum in the

histological type of non-pedicular sessile serrated adenoma (SSA).[67] This research indicated that *F. nucleatum* played a role in premalignant colorectal lesions.

To explore the connection of *F. nucleatum* with CRC histological types, Mima et al[68] further examined the alterations in the enrichment of *F. nucleatum* along different bowel subsites (rectum, rectosigmoid junction, sigmoid colon, descending colon, splenic flexure, transverse colon, hepatic flexure, ascending colon, and cecum). The analysis demonstrated that the amount of *F. nucleatum* gradually increased along the bowel subsites from rectum to cecum in CRC.[68] Among the different ways to explore the tumorigenesis mechanisms of *F. nucleatum*, the classification of the CRC subtypes by significant clinical, pathological, and molecular features favors the treatment of *F. nucleatum*-associated CRC disease.

The impact of *F. nucleatum* on the clinical prognosis of CRC was also investigated in 2016. The data revealed that a high amount of *F. nucleatum* was related to shorter survival duration by the examination of over 1000 CRC cases. In addition, *F. nucleatum* was only related to MSI-high but not to CIMP-high or *BRAF* mutation.[11] Hence, there is a possibility for *F. nucleatum* to be used as a prognostic marker. Oh et al[69] investigated the prognostic impacts of *F. nucleatum* in CRC treated with adjuvant chemotherapy in 2019; they analyzed 593 CRC cases from surgically resected specimens of stage III or high-risk stage II CRC patients who had received curative surgery and subsequent oxaliplatin-based adjuvant chemotherapy (either 5-fluorouracil, leucovorin, and oxaliplatin [FOLFOX] or capecitabine and oxaliplatin [CAPOX]). The data showed that *F. nucleatum* played a prognostic role in a non-MSI-high/non-sigmoid/non-rectal cancer subset of stage II/III CRCs treated with oxaliplatin-based adjuvant chemotherapy. The author proposed two reasons: firstly, the genomic DNA of *F. nucleatum* extracted from formalin-fixed paraffin-embedded (FFPE) tissues might decrease due to degradation; secondly, this analysis was a retrospective rather than prospective study. Therefore, large prospective cohorts and better specimens should be examined in future research.

Potential management of *F. nucleatum*-associated CRC

In the former discussion, high abundance of *F. nucleatum* had a close relationship with the initiation and development of CRC and also mediated chemoresistance against 5-FU and platinum-based agents. There is also evidence about the enrichment of *F. nucleatum* leading to poor prognosis of CRC patients. A study has shown that reducing the abundance of *F. nucleatum* facilitated recovery of patients with intestinal diseases, like IBD.[70] Thus, targeting *F. nucleatum* or its pathogenic signaling pathway involved in tumorigenesis will probably provide new clinical strategies for the prevention and treatment of CRC. In the following part, several promising approaches will be introduced.

The first approach is to reduce the carcinogenicity of *F. nucleatum*. Adhesin of *F. nucleatum*, FadA, promotes adhesion and invasion to the host epithelial cells.[51–53] Thus, treatment targeting FadA is promising in inhibiting the adhesive and invasive abilities of *F. nucleatum*. Meanwhile, we have known that FadA is involved in the E-cadherin/β-catenin promoted tumor growth pathway; internalization of E-cadherin via clathrin is an essential step in this process,[15] where a clathrin inhibitor, Pitstop 2, could block this pro-tumorigenesis pathway. Besides, another potential target could be *F. nucleatum* outer membrane protein Fap2, which ferries other bacteria into the host cell and produces an inflammatory microenvironment.[55] These findings indicate that membrane blockers may be effective in suppressing the key pathogenic factors of *F. nucleatum*. Since *E. coli*, *Streptococcus* gallolyticus and other microbiota can also play a crucial role in the CRC tumorigenesis,[71] inhibition of FadA alone may not be sufficient. On the other hand, early studies uncovered that some *F. nucleatum* strains acquire virulent genes through horizontal transfer from other gut microbiota.[72,73] This finding suggested that administration of microecological products may produce some therapeutic effects upon CRC patients, including probiotics and prebiotics.[74] Probiotics including *Bifidobacterium longum*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* significantly reduced the abundance of *F. nucleatum* in CRC surgery patients by nearly 5-fold.[75] Thus, by regulating the whole composition of gut microbiota, the source of *F. nucleatum* virulence genes was blocked. In contrast to the other types of cancer, CRC develops in a complex microenvironment due to the change of diversity of microorganisms within the gut. A number of studies have demonstrated that drug resistance is highly related to the microenvironment in the cancerous area.[76,77] Thus, strategies to regulate the composition of gut microbiota and to lower the number of *F. nucleatum* may be promising for reducing drug resistance in CRC patients.

Moreover, some evidence showed that the possible origin of intestinal *F. nucleatum* is from the oral cavity of CRC patients as patients who harbored *F. nucleatum*

in their tumor tissues also had oral *F. nucleatum* strains that share identical arbitrarily primed polymerase chain reaction (PCR) strain-typing patterns.[78] Although only limited reports suggested a correlation between oral infectious diseases and CRC, it is a possibility that targeting oral *F. nucleatum* could reduce the intestinal abundance of the same bacterial species, which is another promising hypothesis that remains to be verified.

Strategy to reduce *F. nucleatum* directly is also within consideration, such as the employment of antibiotics in the treatment of CRC patients. Generally, clinically isolated *F. nucleatum* strains are sensitive to several antibiotics, such as metronidazole, clindamycin, and some β-lactam antibiotics.[79] A related study had demonstrated that metronidazole treatment effectively reduced the tumor volumes in CRC patient-derived xenograft mice model.[63] However, such antibiotic agents also inhibit other species of bacteria, which may be problematic as some bacteria have been identified to have different benefits in the human body. As a result, the challenge in this approach is to develop an antibiotic which specifically targets *F. nucleatum* in the tumor tissues. *F. nucleatum* vaccination is also considered to be a possible strategy in CRC prevention and treatment. The vaccine targeting the outer membrane protein FomA expressed by *F. nucleatum* has been used in the treatment of halitosis. Through downregulation of FomA, the bacterial co-aggregation and biofilm formation activity can be blocked effectively.[80] However, to our knowledge, there is no clinical data published related to the effectiveness of *F. nucleatum* vaccine in CRC management; therefore, this approach is worth studying.

Studies on the relation between *F. nucleatum* and the subtypes of CRCs, such as MSI, CIMP, *BRAF*, *KRAS*, and *TP53* mutation status, showed that enrichment of *F. nucleatum* induced significantly differential immune microenvironment under different subtypes. Thence, immunological checkpoint blockade might be a new CRC therapy approach. Overall, a range of potential therapeutic approaches for *F. nucleatum* associated-CRC are considerable, aiming at reducing the abundance of *F. nucleatum* directly, blocking the tumorigenesis factors and pathways, upregulating the immune responses, and balancing the composition of gut microbiota.

Conclusion

Colorectal cancer is a heterogeneous disease, which occurs and progresses in a complex microenvironment partially due to the gut microbiome imbalance. Recent advances in 16S rRNA sequencing and metagenomic sequencing have revealed the diversity (structural composition) and function of bacterial species, and the roles that some of the bacterial species are playing in the human diseases. It was a mystery if *F. nucleatum* is a cause or a consequence for CRC. Researchers have discovered more enrichment of *F. nucleatum* in CRC tissues compared to noncancerous tissues. There is also an obvious connection between the existence of intestinal *F. nucleatum* and poor prognosis and drug resistance of CRC patients. Although some of the virulence factors of *F. nucleatum* have been recognized and multiple mechanisms of *F. nucleatum*-associated CRC tumorigenesis have been revealed in the early studies, *F. nucleatum* presents widely in the healthy population as a mutualist and even in other mammals. We still have not fully identified all the key virulent factors or mechanisms that transform *F. nucleatum* into invasive and cancer-promoting pathogenic bacteria. Considering the complexity of the intestinal microenvironment, the underlying mechanism that *F. nucleatum* interacts with the host cells and with the other gut microbiota still needs to be verified, and new approaches for the prevention and treatment of *F. nucleatum* associated-CRC need to be employed.

Conflicts of interest

None.

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