Fusobacterium nucleatum-positive colorectal cancer (Review)

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Abstract. Colorectal cancer (CRC) is an important threat to human health and the fourth leading cause of mortality worldwide. Accumulating evidence indicates that the composition of the intestinal flora is associated with the occurrence of CRC. *Fusobacterium nucleatum* (*Fn*), one of the highly enriched bacteria in CRC tissues, invades the mucosa with adhesion factors and virulence proteins, interacts with the host immune system and promotes the occurrence and development of CRC and chemoresistance. *Fn* infection is prevalent in human colorectal carcinoma, although the infection rate varies in different regions. *Fn* may be used as a prognostic indicator of CRC. It is important to understand the multi-pathway carcinogenic mechanisms associated with CRC in order to develop novel antibacterial drugs against *Fn*. The current review summarizes the role of *Fn* and relevant research concerning CRC published in recent years, focusing on *Fn* infection in CRC, pathogenesis of *Fn*, *Fn*-positive CRC treatment, screening and prevention strategies against *Fn*-positive CRC.

Contents

1. Introduction

Colorectal cancer (CRC) was the third most common cancer type worldwide and the fourth leading cause of human

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cancer-associated mortality in 2012 (1). The incidence of CRC was highest in Europe, North America and Oceania, and lowest in South Asia, Central Asia and Africa between 1998-2002 (2). In China, according to epidemiological data in recent years, the five-year incidence was 74.6/100,000 and 58.3/100,000 in males and females in 2011, respectively. Each year, >376,000 people are diagnosed with CRC and ~191,000 patients succumb to CRC in China (3,4).

The pathogenesis of CRC is complex. In terms of environmental factors, epidemiological studies have indicated that high-fat diet, obesity and a western lifestyle increase the risk of CRC (5-7). In terms of genetic factors, the majority of CRC cases exhibit genomic instability, including microsatellite and chromosomal instability (8,9). In addition, gene mutations, including tumor suppressor gene inactivation and oncogene activation, serve an important role in the pathogenesis of CRC (8). However, there has been less focus on the role of microbial infections in the pathogenesis of CRC, despite the fact that direct evidence for an infectious cause in human cancer has been reported in the last decades (10). According to previous statistical data, 15-20% of all types of cancer are caused by infections with microbial pathogens (11).

There are >1,000 types of microorganisms in the human intestinal microbiota. A total of ~10^{14} microorganisms serve an important role in maintaining the physiological functions of the intestines, including energy metabolism, epithelial cell proliferation and apoptosis, and protection against pathogens (12). In addition to these beneficial roles, intestinal microbes can have a detrimental effect on human health. In recent years, a large number of studies have indicated that the intestinal flora is closely associated with the occurrence of CRC (13,14). Metagenomics and transcriptional analyses have revealed that compared with adjacent normal tissues, the enrichment of Bacteroidetes and Firmicutes is decreased in human CRC tissues, but the enrichment of *Fusobacterium nucleatum* (*Fn*) is significantly increased (15,16). Yamamura *et al* (17) reported that *Fn* is detected in 20, 10 and 45% of esophageal, gastric and CRC tissues, respectively. No *Fn* was detected in liver and pancreatic cancer tissues (17). *Fn* adheres to and invades the intestinal mucosa through its surface adhesion factors and virulence proteins, and ultimately promotes the occurrence and development of CRC (18). It has previously been identified that the absolute copy number of *Fn* in CRC tissues may be used as an indicator to evaluate the prognosis of patients with CRC (19). Recent studies have demonstrated that *Fn* is not only associated with the development of CRC, but also promotes

chemotherapeutic resistance (20,21). Therefore, in depth knowledge of the mechanisms underlying *Fn* carcinogenesis in CRC may increase the understanding of the intestinal flora and aid the development of effective anticancer agents against the bacterium.

2. *Fn* infection in CRC

Fn is an opportunistic anaerobic bacterium in the mouth and is one of the most common gram-negative bacteria in extraoral infections (22). *Fn* serves an important role in periodontitis, appendicitis, gingivitis and invasive infections of the head, neck, lungs, liver, heart and brain (23-25). It has been reported that *Fn* is widely present in pregnancy complications, including premature birth and stillbirth, as well as intrauterine infections, including neonatal sepsis (26). Previous studies have investigated the link between colonized bacteria in the mouth and extraoral infections (27,28). *Fn* in subgingival plaque may be transferred to the placenta and fetus, leading to acute inflammation and fetal death (24). Therefore, it is speculated that *Fn* is transmitted from the oral cavity through hematogenous transmission to a site that is suitable for its colonization and animal experiments support this hypothesis (29-31). In addition, adhesion factors and virulence proteins on the surface of *Fn* facilitate its colonization and invasion (18). Studies have indicated that *Fn* binds to a variety of mammalian cells and macromolecules, including epithelial, endothelial, blood and immune cells, and salivary macromolecules (32). In previous years, it has been identified that human CRC tissues are generally infected with *Fn* (15-21). At present, *Fn* may be detected in tissues by fluorescence quantitative (FQ)-PCR, fluorescence in situ hybridization (FISH), qPCR and droplet digital PCR (ddPCR) (33). Castellarin *et al* (34) compared CRC tissues in 99 cases with corresponding normal mucosa tissues using qPCR and revealed that the mean overall abundance of *Fn* was 415 times higher compared with the normal samples. Li *et al* (35) investigated 101 Chinese patients with CRC and demonstrated that the *Fn* infection rate was 87.1%, which was significantly higher compared with that in the adjacent normal tissues. In the USA, 598 CRC cases were analyzed by qPCR and the infection rate of *Fn* was identified to be higher in tumor tissues (13.0%) compared with the *Fn* infection rate in normal tissues (3.4%), which was significantly lower compared with studies conducted in China (36). A report from Japan demonstrated that the positive rate of *Fn* was 8.6%, which was similar to the rate obtained by American researchers (37). One study identified that the enrichment of *Fn* in CRC tissues increased gradually from 2.5% (4/157) in the rectum to 11% (19/178) in the cecum, indicating a relationship with the location of the lesion (38). Komiya *et al* (39) performed arbitrarily primed PCR as the strain typing method and the same *Fn* strain was detected in saliva specimens of 75% CRC-positive patients, suggesting *Fn* in CRC tissues originates in the oral cavity, and digestive tract transmission may be one mechanism underlying *Fn* diffusion. Bullman *et al* (40) investigated *Fn* enrichment in patients with CRC with liver metastasis and demonstrated that *Fn* was enriched in CRC tissues compared with normal tissues; similarly in liver metastatic CRC, the same strain of *Fn* was detected in liver and colon tissue, demonstrating that distant metastasis of *Fn* may occur along the lymphatic

vessels (40). High enrichment of *Fn* was observed in CRC tissues and precancerous lesions, including adenomas (41). Amitay and Brenner (42) demonstrated the high enrichment of *Fn* in CRC tissues and identified that there was no difference in the degree of *Fn* enrichment between adenomas and advanced adenomas. Therefore, it is inferred that *Fn* is a bacterium that grows in the tissues of neoplastic lesions and propagates in favorable conditions caused by malignant tumors, but it is not the cause of CRC. Ito *et al* (43) investigated 465 cases of precancerous lesions, including 343 serrated lesions and 122 non-serrated adenomas, and observed that *Fn* is detected in 24% of hyperplastic polyps, 35% of sessile serrated adenomas, 30% of traditional serrated adenoma and 33% of non-dental adenomas, and the *Fn*-positive rate gradually increased from the sigmoid colon to the cecum in sessile serrated adenoma. This study demonstrated that *Fn* is highly enriched in the precancerous lesion stage during the occurrence and development of CRC. Further research is required to determine whether *Fn* is involved in the occurrence of precancerous lesions. Although studies have demonstrated that the incidence of *Fn* infection varies in different countries, it is concluded that *Fn* is generally present in CRC tissues.

3. Carcinogenic mechanisms of *Fn*

A number of studies have been conducted to study the carcinogenicity of *Fn* (44,45). There are three biomolecules that are located on the surface of *Fn* including lipopolysaccharides (LPS), adhesin A (FadA) and fusobacterium autotransporter protein 2 (Fap2), which are involved in and promote the occurrence of colorectal cancer (Fig. 1) (18,31,46). Chronic inflammation may promote the occurrence and development of tumors; therefore, the role of cytokines in the pathogenesis of CRC has drawn much attention (47). Tumors may form following chronic inflammation or exhibit characteristics of chronic inflammatory infiltration during progression (48,49). In an intestinal tumorigenesis APC$^{min/+}$ mouse model, *Fn* increases the tumor multiplicity and selectively, and recruits tumor-infiltrating myeloid cells (50). Recruiting tumor infiltrating immune cells produces a proinflammatory microenvironment, which is conducive to the occurrence of CRC (51,52). The degree of enrichment of bone marrow-derived suppressor cells (MDSCs) is significantly increased in *Fn*-fed mice compared with the control group (50). MDSCs have two major subpopulations, granulocytes and monocytes, both of which function as T-cell inhibitory phenotypes (52-54). Therefore, it has been speculated that *Fn* promotes the occurrence and development of colon tumors by downregulating the T-cell-mediated adaptive immune antitumor effects. A study by Mima *et al* (36) supported this hypothesis, as it reported that the presence of *Fn* is negatively correlated with the density of CD^{3+} T cells in CRC tissues. *Fn* promotes the release of proinflammatory cytokines, including interleukin (IL)-8, IL-10 and tumor necrosis factor-α (TNF-α) (55). In addition, studies have demonstrated that *Fn* promotes the mRNA expression of TNF-α, IL-1β, IL-6, IL-8, prostaglandin-endoperoxide synthase 2 (PTGS2), nuclear factor κβ (NF-κβ) and matrix metallopeptidase 3, and the expression of these genes is induced in mouse and human cells treatment with *Fn* (56,57). The generation and release
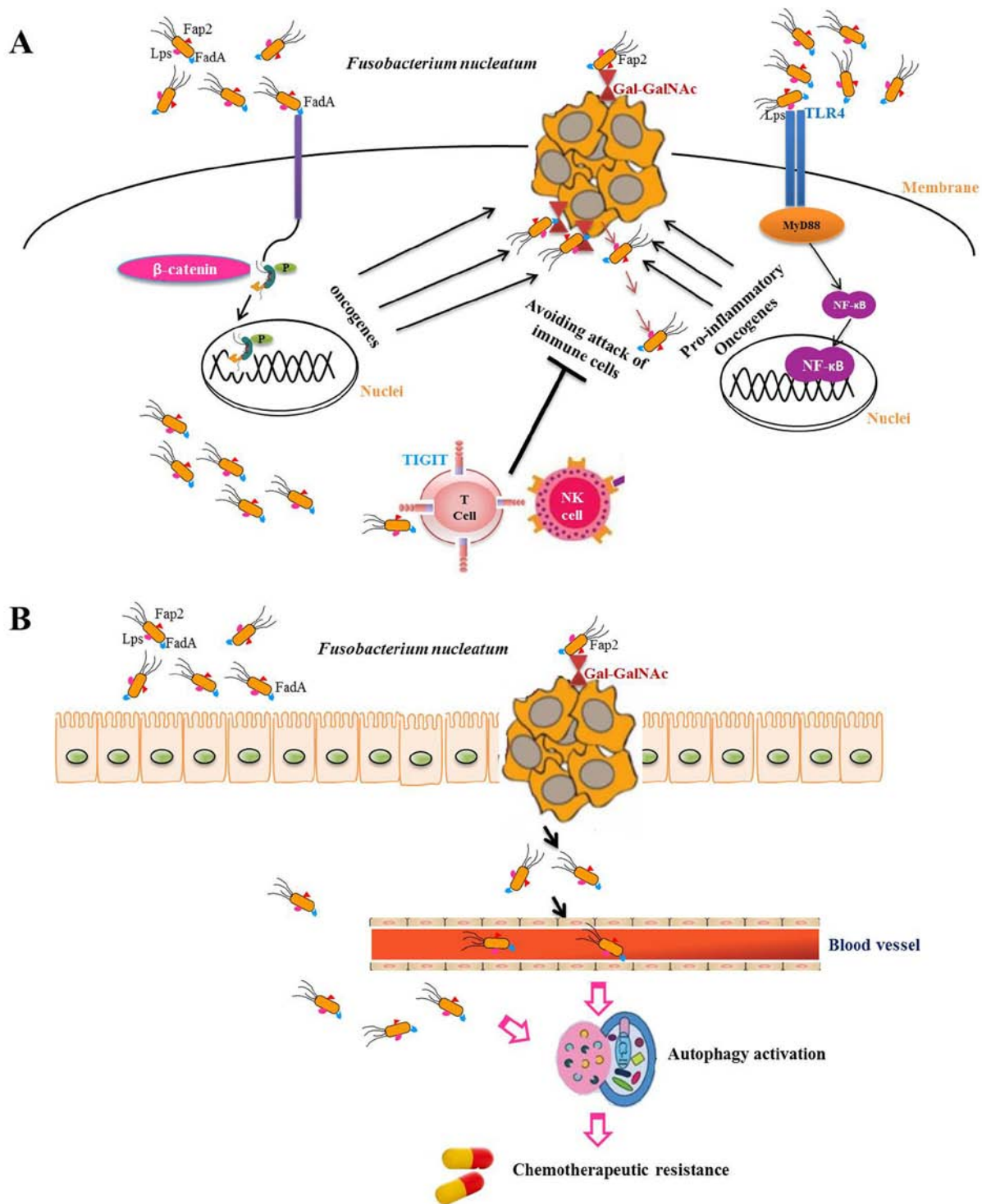
Figure 1. Potential mechanism of *Fn* in CRC. (A) Biomolecules (Lps, FadA and Fap2) are located on the surface of *Fn*. Lps breaks the intestinal barrier and facilitates the entry of *Fn* into epithelial cells. It activates MYD88 through TLR4, which further activates the NF-κβ-associated proinflammatory pathway, forming the immune microenvironment for the occurrence of CRC. FadA activates the E-catenin/β-catenin signaling pathway, which contributes to tumor development. Gal-GalNAc on tumor cells is the receptor of Fap2 and recruits *Fn* to the tumor site. In addition, FadA binds TIGT in tumor tissues and serves a role in inhibiting T cell and Nκ cell function, which are potential mechanisms of tumor immune escape. (B) Upon chemotherapy intervention, *Fn* activates the TLR4/MYD88 signaling pathway, which activates autophagy in CRC cells and contributes to chemoresistance. *Fn, Fusobacterium nucleatum;* CRC, colorectal cancer; MYD88, MYD88 innate immune signal transduction adaptor; TLR4, Toll-like receptor 4; NF-κβ, nuclear factor-κB; Gal-GalNAc, D-ga lactose-β-(1-3)-*N*-acetyl-D-galactosamine; TIGIT, T cell immunoreceptor with Ig and tyrosine-based inhibitory motif domains; NK, natural killer; LPS, lipopolysaccharides; FadA, adhesin A; Fap2, fusobacterium autotransporter protein 2; 'P' in the green circle, phosphorylation.

of these inflammatory cytokines constitutes to the immune microenvironment for the occurrence of CRC (58).

Fn adsorption and invasion into mucosa is a prerequisite for its role in carcinogenesis (59). A FadA virulence protein is expressed on the surface of *Fn*. This virulence protein is highly conserved for *Fn* (60). FadA exists in two forms: The non-secreted, intact FadA precursor (pre-FadA) consisting of 129 amino acid residues, and the secreted, mature FadA

(mFadA) consisting of 111 amino acid residues (61,62). When mFadA is mixed with pre-FadA, they form active FadAc (FadA virulence protein) composed of non-uniform filaments (62). *Fn* binds to E-cadherin on CRC and non-CRC cells via FadA virulence protein (FadA) and mediates *Fn* attachment and invasion into epithelial cells (63). It has been demonstrated that FadA regulates E-cadherin and activates β-catenin signaling, leading to increased expression of transcription factors, oncogenes, Wnt signaling pathway and inflammatory genes, as well as proliferation of CRC cells (63). The same study revealed the oncogenic mechanism of *Fn* and identified FadA as a potential diagnostic and therapeutic target for CRC. Notably, another study demonstrated that *Fn* activated the β-catenin signaling pathway via lipopolysaccharide-mediated Toll-like receptor 4 (TLR4)/p21 activated kinase 1 (PAK1) in CRC (64). TLR4 activates the β-catenin signaling pathway, forming intestinal tumors, while PAK1 is associated with CRC progression and metastasis (64,65). An improved understanding of the signaling pathways activated by *Fn* may provide new avenues for the prevention and treatment of CRC. However, E-cadherin is expressed in different types of cells, and its expression levels and cell localization patterns may be different in dysplasia and tumors (66). Therefore, FadA virulence protein cannot fully explain the selection specificity of *Fn*. Abed *et al* (67) identified that a highly expressed polysaccharide, D-galactose-β-(1-3)-*N*-acetyl-D-galactosamine (Gal-GalNAc), is present in CRC tissues. The biosynthesis of Gal-GalNAc is increased gradually from normal mucosa, adenoma to CRC tissues (67). Gal-GalNAc binds to Fap2, another protein on *Fn*, leading to high enrichment of *Fn* in CRC tissues (67). Another study on Fap2 demonstrated that *Fn* binds to the T cell immunoreceptor with Ig and tyrosine-based inhibitory motif domains (TIGIT) via Fap2 and that TIGIT is widely present on the surface of natural killer and T cells, thereby preventing immune cells from attacking CRC cells (68). *Fn* also inhibited the activity of human T cells by blocking the G1 phase of the cell cycle (69). This study suggested the presence of an *Fn*-dependent tumor immune escape mechanism. Yang *et al* (70) revealed that *Fn* activated NF-κβ signaling via the innate immune pathway [TLR4/MYD88 innate immune signal transduction adaptor (MYD88)] and miRNA-21, and increased the proliferation of CRC cells and the development of tumors. Compared with non-neoplastic colon tissues, *Fn* DNA and miRNA-21 levels are significantly increased in CRC tissues, and the levels were even higher in advanced CRC tissues. This indicated that patients with both *Fn* DNA and high miRNA-21 exhibit a higher risk of poor prognosis. This study revealed that *Fn* regulates the expression of miRNAs, but the specific mechanism of this is not yet understood.

In recent years, studies have identified that *Fn* infection is associated with CRC-specific molecular typing. Tahara *et al* (71) studied CRC samples from 149 patients and reported that *Fn* is highly enriched in the CRC group, which was correlated with CpG island methylation phenotype (CIMP; P<0.001), tumor protein p53 (TP53) wild type (P<0.015), human mutL homolog 1 methylation (P<0.003), microsatellite instability (MSI; P<0.018) and chromodomain helicase DNA binding protein 7/8 mutation (P<0.002) phenotypes. Mima *et al* (72) identified that *Fn* is associated with MSI-high, CIMP and B-Raf proto-oncogene serine/threonine kinase (BRAF) mutations. In CRC molecular typing, CIMP

and MSI are mainly identified in the right hemicolon, which may be related to the high enrichment of *Fn*, but the specific mechanism needs to be studied in more detail (72). Therefore, Mima *et al* (72) provided strong support for the pathogenic role of intestinal microbial components in CRC.

According to statistical data, 20-30% of treated patients with CRC exhibit tumor recurrence and 35% of these patients succumb to the disease within 5 years (73). Combination chemotherapy remains the main treatment for advanced CRC (74). Therefore, studies on the mechanism of CRC resistance to chemotherapy are important. A study by Yu *et al* (75) demonstrated that *Fn* abundance in recurrent CRC tissues is markedly higher compared with that in non-recurrent CRC tissues, and high-abundance *Fn* is associated with poor prognosis. Therefore, *Fn* may be used as a diagnostic marker for preventing CRC recurrence and as a prognosis predictor. In addition, a series of experiments revealed that *Fn* selectively decreases miRNA-18a*/4802 expression levels through the innate immune pathway (TLR4/MYD88) and subsequently activates cell autophagy via unc-51 like autophagy activating kinase 1-autophagy related 7 to become resistant to chemotherapeutic drugs. This drug resistance was overcome by the autophagy blocker chloroquine (75).

4. Treatment of *Fn*-positive CRC

Fn invasion into the mucosa promotes the release of inflammatory cytokines and is involved in the formation of the immune microenvironment for the occurrence of CRC (55-58). Due to the inflammatory basis of CRC, anti-inflammatory agents may be candidates for treating or preventing CRC (76). Non-steroidal anti-inflammatory drugs (NSAIDs) are non-selective inhibitors of PTGS2 (77). PTGS2 is highly expressed in many tumor types, including CRC (78). Furthermore, *Fn* promotes the expression of PTGS2 (56). NSAIDs may serve a role in the prevention of CRC. A preliminary study on patients with familial adenomatous polyposis indicated that following 1 year of treatment with the NSAID sulindac, patients exhibit a decrease in polyps (79). A large-scale demographic observation study from 1991 reported that the use of NSAIDs reduces the risk of fatal CRC (80). Retrospective studies have demonstrated that NSAID treatment is associated with a decreased risk of recurrence of colorectal polyps and tumor (81,82). Patients who use low-dose aspirin for >5 years exhibit a decreased overall risk for developing CRC by 40-50% and NSAIDs have a positive effect on advanced CRC (83,84). Furthermore, non-steroidal antitumor drug therapy inhibits a tumor-promoting pathway by inhibiting Wnt signaling (85).

According to a previous report, prostaglandin E receptor 2 (PTGER2) increases the expression of NF-κβ-targeted proinflammatory genes in neutrophils. The expression of TNF-α and IL-6, PTGS2, C-X-C motif chemokine ligand 1, Wnt and other cytokines in tumor lesions are significantly higher in PTGER2-enriched compared with PTGER2-knockout mice (86). Therefore, NSAIDs and PTGER2 antagonists may be candidates for the prevention and treatment of *Fn*-positive CRC.

Fn is one of the most common gram-negative bacteria; therefore antibiotics against *Fn* are used to treat *Fn*-positive CRC. Bullman *et al* (40) transplanted *Fn*-infected colon cancer cell allografts into mice, maintained the animals for

several weeks and then treated them with erythromycin or metronidazole. *Fn* is resistant to erythromycin but sensitive to metronidazole according to the drug sensitivity test. The results demonstrated that compared with the erythromycin-resistant *Fn* group, the volume and number of tumors is significantly reduced in mice treated with metronidazole. This indicated that antibiotic intervention may be used as a potential treatment for *Fn*-positive patients with CRC. Therefore, the development of targeted antibiotics with a narrow spectrum may selectively eliminate pathogens while maintaining the balance of flora and avoiding the side effects caused by the use of broad spectrum antibiotics (87). It has been reported that berberine reverses the imbalance of intestinal microbiota caused by *Fn*, thereby reversing the growth of CRC *in vivo* (88).

Tumor immunotherapy is a promising area, with significant progress being made in tumor molecular biology, particularly with regards to the use of immune checkpoint inhibitors, such as programmed cell death 1 (PDCD1) inhibitors (89). The efficacy of checkpoint inhibitors depends on the patient's gut microbiota. Complex interactions between the gut microorganisms and the immune system limit the effects of PDCD1 inhibitors (90). According to the OncoKB classification system, pembrolizumab is a Food and Drug Association-approved drug for MSI-high solid tumors (level 1) and *Fn* is associated with MSI-high, CIMP and BRAF mutations (71,72,91). Therefore, PDCD1 inhibitors may exhibit anticancer effects on *Fn*-positive CRC. However, immune checkpoint inhibitors have a number of side effects, including hepatitis, diarrhea and enterocolitis, resulting from the complex interactions between host genetics, immune responses, the environment and microbes (92). Therefore, the use of immune checkpoint inhibitors has very strict indications (92). Since *Fn* binds to TIGIT, an immune cell inhibitory receptor, through Fap2 to avoid attacks of immune cells, the development of an anti-Fap2 antibody may be beneficial for the antitumor immune response (68). Nedaeinia *et al* (93) revealed that inhibition of miRNA-21 suppresses metastasis of CRC cells through modulation of programmed cell death 4. Kumar *et al* (94) studied host-pathogen protein-protein interactions (HP-PPIs) and identified that *Fn* and CRC-related proteins have 186 interactions, including 103 host proteins and 76 *Fn*-pathogenic proteins. Therefore, the development of drugs targeting HP-PPIs may be used to treat *Fn*-positive CRC. In view of the important role of TLR4 in *Fn* carcinogenesis, it may be possible to develop a drug for the treatment of *Fn*-positive tumors (65). For patients with recurrent CRC, in addition to combination chemotherapy, the benefit of autophagy blocking agents or *Fn* inhibitors requires investigation in future studies.

5. Screening and prevention of *Fn*-positive CRC

Fn can be detected in formalin-fixed paraffin-embedded CRC tissues, frozen CRC tissues and feces of patients with CRC using FQ-PCR, FISH, qPCR and ddPCR (33). For screening, it is not convenient to obtain tissue specimens, whereas feces and serum samples are more appropriate. A number of studies have identified that *Fn* infection leads to elevated anti-*Fn* antibody IgA levels in the serum of patients with CRC (95,96). Serum anti-*Fn* antibody IgA combined with carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic

antigen (CEA) is more sensitive compared with only CA19-9 and CEA in screening for early stage CRC, suggesting that serum *Fn* antibodies may be a potential marker for the diagnosis of early stage CRC (97). The fecal immunochemical test (FIT) is recommended as a non-invasive screening test for CRC. However, it is less sensitive in advanced adenomas (98). Wong *et al* (99) detected *Fn* in feces by qPCR combined with FIT, which increases the detection rate of CRC and advanced adenomas. Its sensitivity and specificity for CRC are 92.3 and 93.0%, respectively, and for advanced adenomas 38.6 and 89.0%, respectively, are reported. Huang *et al* (100) employed loop-mediated isothermal amplification technology to quickly and efficiently identify the *Fn* virulence factor FadA through the nusG gene, which increases the sensitivity by 10-fold compared with qPCR. Therefore, *Fn* may be used as a microbial marker to increase the diagnostic efficiency for CRC in the future.

Previous studies have demonstrated that a diet rich in fruits, vegetables and whole grains is associated with a lower risk of colon cancer compared with Western dietary patterns, which are dominated by red and processed meats (5-7,101). A high-fat diet and overconsumption of red meat may increase the risk of CRC (102-104). Although the mechanism of the association between diet and CRC remains unclear, it is speculated that the gut microbiota may play an intermediary role. It has been reported that diet influences the composition of gut microbiota, although long-term dietary patterns outweigh short-term changes in diet (105,106). Considering that different groups of gut microbiota have different metabolic capacities, particular dietary patterns may allow for the selection of certain microbes and inhibits others (107,108). A previous study demonstrated that certain microflora may become extinct. By providing low-fiber diets to mice, reversible changes are observed in the microbiota. However, following low-fiber diets for several successive generations, the diversity of microbiota is gradually lost and certain microbiota are undetectable (108).

Previous studies have attempted to change the enrichment of *Fn* in the intestines through diet. Specific nutrients may cause intestinal inflammation, characterized by elevated circulatory levels of IL-6, creatine protein (CRP) and TNF receptor superfamily 1B (TNFRSF1B), and promote CRC (109). Inflammatory effects of different diets may be estimated using the Empirical Dietary Inflammatory Pattern (EDIP) score, which is calculated based on the intake of 18 foods associated with levels of IL-6, CRP and TNFRSF1B in the plasma (110). Liu *et al* (111) demonstrated that higher EDIP scores are associated with increased risk of *Fn*-positive CRC, and high EDIP scores are associated with proximal *Fn*-positive CRC. The aforementioned studies indicated that the negative association between a diet rich in fruits, vegetables and whole grains and risk of CRC is more pronounced in the *Fn*-positive CRC subgroup compared with in the *Fn*-negative CRC group.

6. Conclusions

In summary, *Fn* infection is associated with the occurrence of many human diseases and *Fn* infection is prevalent in human CRC tissues. *Fn* invades the intestinal epithelial mucosa via its own adhesion factors and virulence proteins, and subsequently

interacts with the host immune system to promote the occurrence and development of CRC and chemoresistance through a variety of molecular pathways. This ultimately affects the efficacy of CRC treatment. *Fn*-positive CRC is characterized by shorter survival times and poor prognosis. *Fn* may be used as an evaluation index to predict the clinical outcomes of CRC. Eradication therapy for *Fn* may not only treat *Fn*-positive CRC but also prevent *Fn*-positive CRC recurrence. At present, there are no clinically effective drugs that target *Fn*-positive CRC. Therefore, it is expected that targeted immunotherapy drugs and biological agents with minimal side effects will be developed in the future.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

GJ designed the study, and ZY drafted the manuscript and revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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