

# *Fusobacterium nucleatum* and Colorectal Cancer

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**Abstract:** *Fusobacterium nucleatum* (*F.n*) is an oral anaerobic gram-negative bacillus that can colonize into the colon tissues through bloodstream infection. *F.n* have been found to be involved in both the occurrence and metastasis of colorectal cancer (CRC) through regulating immune response, virulence factor, oncogenic microRNAs, intestinal metabolites, DNA damage and other mechanisms. Therefore, *F.n* can be as an important pathogenic risk factor and a possible biomarker of CRC. Based on this, we have summarized the potential relationship between *F.n* and CRC to provide reference for the targeted therapy of CRC.

**Keywords:** *Fusobacterium nucleatum*, colorectal cancer, gut microbiome, mechanisms, biomarker

## Introduction

CRC was reported to be the third most common cancer in men and the second most common cancer in women, with a total of 1.8 million cases and 881,000 deaths worldwide in 2018.<sup>1</sup> The prognosis of CRC patients has been found to be poor with a 5-year survival rate of less than 10% after distant metastasis,<sup>2</sup> and its etiology can be very complex. A number of different environmental and genetic factors play a major role in the pathogenesis of CRC. Hereditary CRC syndromes including Lynch syndrome (hereditary nonpolyposis colorectal cancer), familial adenomatous polyposis (FAP), and multiple associated polyposis (MAP)<sup>3,4</sup> can predispose an individual to increased risk of CRC. Chronic inflammatory stimulation, schistosomiasis infection, diet and other environmental factors can also promote the occurrence of CRC.<sup>5,6</sup> Besides, a number of recent studies have found that gut microbes, including *F.n*, *Akkermansia muciniphila*, *Parvimonas micra*, *Peptostreptococcus stomatis* and *Bacteroides fragilis* could also be associated with increased incidences of CRC.<sup>7,8</sup>

*F.n* is an oral anaerobic gram-negative bacterium. A number of previous studies have indicated that the abundance of *F.n* in CRC patients was significantly higher than that in normal tissues and it was involved in the metastasis of colon cancer.<sup>9</sup> Moreover, the number of *F.n* in CRC tissues was associated with significantly shorter survival<sup>10</sup> which was usually associated with advanced disease, chemotherapy resistance, metastasis and poor prognosis.<sup>9,11–13</sup> These findings suggested that *F.n* might serve as an important risk factor for CRC, and there have been number of precedents implicating the role of microorganisms in the development of various cancers, such as HPV and cervical cancer, E-B virus and nasopharyngeal cancer, *Helicobacter pylori* and gastric cancer. Therefore, it was very important to explore the potential relationship between *F.n* and CRC. This paper has reviewed the various mechanisms through which CRC can be caused by *F.n*, so as to provide reference direction for both tumor prevention and therapy.

## Analysis of *F.n*

The various specimens used to detect *F.n* in CRC include formalin fixed paraffin-embedded (FFPE) CRC tissues, CRC frozen tissues, genomic DNA and stool of CRC patients. It has been found that the different specimens use distinct methods. For instance, the detection of *F.n* included fluorescence quantitative polymerase chain reaction (FQ-PCR) and fluorescence in situ hybridization (FISH), real-time quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR).<sup>14–16</sup> Moreover, Li et al used FQ-PCR and FISH to detect *F.n* in 101 samples of

FFPE, and the results showed that *F.n* was overexpressed in 87.1% of CRC patients.<sup>14</sup> In addition, Suehiro et al reported that ddPCR had a high sensitivity in detecting stool samples.<sup>15</sup> Leung et al also demonstrated the detection sensitivity of this technology with the result that *F.n* in 102 liquid nitrogen frozen tissue samples was significantly correlated to cancer by ddPCR,<sup>16</sup> while the accuracy of qPCR in detecting *F.n* in CRC varied from 8.6% to 82.1%.<sup>17,18</sup>

## Mechanisms Through Which *F.n* Can Promote CRC Immune Mechanism Mediated by Myeloid-Derived Suppressor Cells (MDSCs) and Natural Killer (NK) Cells

MDSCs are considered as myeloid cells that can exist in the bone marrow, spleen or tumor microenvironment, and act as precursors of tumor-associated macrophages (M2 type macrophages), granulocytes and dendritic cells (DC). MDSCs are a kind of immunosuppressive cells reported to be involved in immune suppression which promoted angiogenesis and carcinogenesis.<sup>19</sup> Studies have also suggested that the occurrence of CRC could be related to the gut microbiota.<sup>20</sup> For example, enrichment of *F.n* in CRC could selectively expand immunosuppressive myeloid cells to form immunosuppressive tumor microenvironment (TME) which can effectively inhibit T cell proliferation and induce T cell apoptosis in CRC, with the higher levels of infiltrating T cell subsets (ie, CD3<sup>+</sup>, CD8<sup>+</sup>, CD45RO<sup>+</sup>, and FOXP3<sup>+</sup> cells) being associated with better prognosis.<sup>21</sup> In addition, MDSCs can also respond to the different signals and differentiate into M1 and M2 phenotypes during the occurrence and development of the tumors. The macrophages of these two phenotypes showed an inverse relationship to the tumor development. It was observed that M1 phenotype hindered the formation and development of tumors, while M2 phenotype promoted the tumor formation and migration. Moreover, inhibition of M2 polarization or promotion of M1 polarization was found to play positive roles in mediating anti-tumor effects.<sup>22</sup> In addition, previous studies have also found that M2-type macrophages were the main phenotype of macrophages in human CRC, and *F.n* infection promoted M2-type macrophage polarization as well as the tumor growth in the tumor microenvironment.<sup>23,24</sup> A similar phenomenon was also found in different preclinical experiments. The numbers of M2-type macrophages in the intestinal microenvironment of CRC mice was reported to increase significantly 7–8 times after *F.n* was fed.<sup>20</sup> In addition, *F.n* infection was found to promote the polarization of M2-type macrophages and the progression of CRC in a TLR4-dependent manner by activating the IL-6/p-STAT3/c-MYC and the TLR4/NF-κB/S100A9 signal pathways.<sup>20</sup> Meanwhile, Park et al reported that *F.n*-high MSI-H CRCs were significantly associated with a high density of CD68<sup>+</sup> tumor-infiltrating macrophages and promoter CpG island hypermethylation of the *CDKN2A(p16)* gene. Interestingly, MSI also promoted the infiltration of tumor-infiltrating macrophages, which played important roles in regulating both tumor invasion and metastasis<sup>25</sup>. The proliferation of CD103<sup>+</sup> DCs in the tumors of mice fed with *F.n* promoted the expansion of Foxp3<sup>+</sup> regulatory T cells, a CD4<sup>+</sup> T cells subsets that effectively inhibited cytotoxic and effector T cells, thereby substantially attenuating anti-tumor immunity.<sup>26,27</sup> Similarly, other studies have indicated that tumor-associated neutrophils (TANs) were also involved in the tumor development, metastasis and angiogenesis.<sup>28</sup>

NK cells can actively participate in the early defense mechanism by releasing the various chemokines and cytokines to kill infectious pathogens and tumor cells. High levels of *F.n* in the gastrointestinal tract could significantly reduce NK cell activity.<sup>29</sup> T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) is a receptor that can effectively inhibit NK cells and other immune cells and could be activated by poliovirus receptor (PVR) and nectin-2 molecules. These ligands primarily initiate the inhibitory signal through affecting the cytoplasmic domain and the immunoreceptor tyrosine-based inhibition motif (ITIM) after binding to TIGIT.<sup>30</sup> Fap2 protein has been found to be involved in the binding of *F.n* to cancer cells and can interact directly with TIGIT which is mainly expressed on NK, Treg, CD8<sup>+</sup> and CD4<sup>+</sup> T cells.<sup>31</sup> The binding of Fap2 to TIGIT can thereafter inhibit the activity of NK cells against tumor cells which can lead to the growth and progression of CRC.

## Virulence Mechanism Mediated by Different Virulence Factors

Adhesin FadA has been identified as the most prominent virulence factor in *F.n*, which could effectively bind to the host cells and help the bacteria to adhere to and destroy both epithelial and endothelial cells.<sup>32</sup> A number of prior studies have also indicated that FadA-positive *F.n* was prevalent in the biopsy specimens of CRC patients.<sup>33</sup> FadA-positive *F.n* was

not detected in non-CRC patients, while patients who displayed colonization of FadA-positive *F.n* were diagnosed as CRC. Rubinstein et al found that FadA can bind to E-cadherin on both CRC and non-CRC cells which mediated the attachment and invasion of *F.n*. This interaction increased the expression of the various transcription factors, oncogenes, Wnt-regulated genes and inflammatory after activation of  $\beta$ -catenin signaling by E-cadherin which resulted in aberrant growth of CRC cells.<sup>34</sup> Similarly, Guo et al<sup>35</sup> reported that FadA enhanced the activation of E-cadherin/ $\beta$ -catenin proteins, thereby upregulating Chk2 and inducing DNA damage in CRC. It has been reported<sup>36</sup> that E-cadherin existed in 100% of the normal mucosa and in 75% of cancer specimens of CRC patients, deletion or heterogeneous expression of E-cadherin was closely associated with advanced and poor prognosis of CRC. In-depth studies revealed that<sup>37</sup> FadA exhibited a dose and time dependence on the proliferation of SW480 CRC cell.

Fap2 was a galactose sensitive hemagglutinin and adhesive protein that played an important role in *F.n* infection.<sup>38</sup> In addition to inhibiting NK cells by interacting with TIGIT, Abed et al<sup>39</sup> reported that Fap2 mediated enrichment of *F.n* in CRC by interacting with the host factor D-galactose- $\beta$ (1-3)-N-acetyl-D-galactosamine (Gal-GalNaC) expressed by tumor. Gal-galNaC has been reported to be overexpressed in human colorectal adenocarcinoma and metastatic tumors. In addition, reduction of Gal-GalNaC expression by O-canase could significantly reduce the accumulation of *F.n* in CRC. Furthermore, *F.n* could penetrate into CRC by blood-derived route in a Fap2-dependent manner. In addition, Abed et al<sup>40</sup> analyzed the Gal-GalNaC content in different kinds of tumors and found that Gal-GalNaC was substantially increased in 7 distinct kinds of adenocarcinoma and the differences were statistically significant. Lishay Parhi et al<sup>41</sup> further examined the breast tissues and found that *F.n* could also get incorporated into the breast cancer tissues through bloodstream infection and accelerate the growth and metastasis of cancerous tissue. Therefore, targeting Fap2 or Gal-GalNaC could significantly reduce the enhancement effect of *F.n* on the various cancers.

LPS could bind to its receptor TLR4. Santaolalla et al<sup>42</sup> showed that TLR4 could trigger tumor progression by activating the Wnt/ $\beta$ -catenin pathway. Similarly, Wu et al<sup>43</sup> reported that *F.n* enhanced intestinal tumorigenesis in *Apc*<sup>Min/+</sup> mice through enhancing TLR4/p-PAK1/p- $\beta$ -catenin S675 pathway, and TAK-242 could effectively inhibit intestinal tumorigenesis induced by *F.n*. These findings suggested that TLR4 can act as a potential target for the prevention and treatment of CRC associated with *F.n*.

## Expression of Oncogenic microRNAs

Multiple microRNAs play pivotal roles in the occurrence of CRC and some of them have also been used to diagnose the occurrence and progression of CRC.<sup>18,44</sup> Yang et al<sup>45</sup> reported that *F.n* up-regulated the expression of microRNA-21 by activating TLR4 signaling, and microRNA-21 reduced the level of RAS GTPase RASA1 which promoted both the proliferation and tumor development of mouse CRC cells. Meanwhile, Yu et al<sup>46</sup> found that *F.n* promoted chemotherapy resistance displayed CRC by regulating autophagy, *F.n* specifically activated TLR4 and MYD88 signal pathways to induce the genomic loss of Mir-18a\* and Mir-4802, whereas Mir-18a\* and Mir-4802 targeted the autophagy related proteins ULK1 and ATG7 respectively, which ultimately regulated chemo-resistance of CRC under both in vitro and in vivo conditions. Thus, *F.n* orchestrated the TLR4-MYD88, miR18a\* and miR4802 axis, and ULK1/ATG7 autophagy network to biologically regulate chemoresistance in CRC.

## Intestinal Metabolites

*F.n* could also utilize amino acids and peptides as nutrient sources in the tumor microenvironment. The various amino acid metabolites produced by *F.n*, including formylmethoxy leucine phenylalanine and short-chain fatty acids can serve as myeloid cell stimulators that can lead to myeloid cell expansion in tumors.<sup>20</sup> *F.n* also possesses basic electron transport chains that can limit their ability to inhale oxygen compared to many other strictly anaerobic bacteria in the intestinal lumen.<sup>47</sup> Thus, *F.n* might be able to persist and replicate slowly in the hypoxic tumor microenvironment. Moreover, the various adhesion molecules that can contribute to the invasiveness of *F.n* promoted bacterial aggregation and biofilm formation, thereby improving oxygen tolerance.<sup>48</sup> Overall, the different *F.n* metabolites could cause the tumor micro-environment to become more prone to the tumor growth over time by directly promoting the tumor cell proliferation, vascular growth or immune cell invasion.

## DNA Damage

Genomic instability is one of the important characteristics of CRC, such as microsatellite instability (MSI), CpG island methylated phenotype (CIMP) and KRAS, BRAF and PIK3CA gene mutations.<sup>49,50</sup> Inflammation mediated by *F.n* can affect the progression of CRC through enhancing the production of reactive oxygen species (ROS) and stimulating pro-inflammatory gene expression that can lead to abnormal DNA methylation and DNA damage.<sup>51</sup> MSI can arise due to the loss of DNA repair mechanism, which can significantly reduce the ability of cells to repair short strand DNA or tandem repeats, and the accumulation of errors can thus contribute to the gene mutation. It has been established that the mutations in various tumor suppressor genes were mutated can lead to CRC.<sup>12</sup> For example, in a study reported by Okita et al.,<sup>49</sup> *F.n* infection was associated with two distinct types of microsatellite changes in CRC. Heavy or moderate loads of *F.n* DNA were associated with MSI-H and L/E CRC respectively and were able to trigger DNA damage. Koi et al.<sup>50</sup> reported that the potential association between CIMP/MSI-H and *F.n*-infection could be explained by the role of the mismatch repair (MMR) protein complex formed between MSH2 and MSH6 (MutSa) to repair the different aberrant bases generated by excessive ROS to form 7,8-dihydro-8-oxo-guanine (8-oxoG) which was refractory to base excision repair (BER). Under these conditions, MutSa initiated repair in cooperation with DNA methyltransferases (DNMTs) and the polycomb repressive complex 4 (PRC4). DNMTs were found to effectively methylate CpG islands at the damaged sites to repress transcription of target genes and promote repair reactions. Thus, continuous generation of ROS through chronic *F.n* infection might initiate 1) CIMP-positive adenoma and carcinoma in an MSH2/MSH6-dependent manner, and/or 2) MSI-L/EMAST CRC in an MSH3-dependent manner. CpG island methylation phenotype (CIMP) has been considered to be an important precursor of the serrated adenoma pathway, represented a subset of CRC, and was characterized by significant hypermethylation of the tumor suppressor gene CpG island, leading to its inactivation and thereby can promote the tumor progression.<sup>52</sup>

## *F.n* is the Potential Marker of CRC

During CRC screening, fecal occult blood test, AFP, CEA and other indicators have been generally used for auxiliary diagnosis, and colonoscopy was mainly employed for examination, which was not only cumbersome but also highly traumatic to patients. A number of studies on *F.n* showed that it was significantly enriched in feces and tissues of CRC patients, with significant statistical differences after the comparison,<sup>53</sup> which suggested that *F.n* might serve as a useful marker for the diagnosis of CRC, but its detection efficiency in CRC was only 8.6%-87.3%,<sup>54</sup> which substantially limited its clinical applications.

## Conclusion

CRC development has been linked to a variety of gut microbes such as *F.n*, *Akkermansia muciniphila*, *Parvimonas micra*, *Peptostreptococcus stomatis* and *Bacteroides fragilis*. The potential relationship between gut microbiome dysbiosis and CRC is complex. The current studies show that *F.n*, as an important pathogenic risk factor of CRC, can actively participate in the occurrence and metastasis of CRC through affecting a variety of mechanisms, including immune regulation, virulence factor, oncogenic microRNAs, intestinal metabolites, DNA damage and other distinct mechanisms. Therefore, in-depth study and intervention of *F.n*-induced CRC pathway might provide novel direction for the targeted treatment and prevention of CRC. In addition, *F.n* can be used as a possible biomarker to detect CRC to a certain extent, but its detection efficiency is unstable, and further studies are needed to improve the detection performance of this technique.

## Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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