Am J Cancer Res 2024;14(8):3962-3975
www.ajcr.us /ISSN:2156-6976/ajcr0158422

Review Article

Fusobacterium nucleatum: a novel regulator of antitumor immune checkpoint blockade therapy in colorectal cancer

Mengjie Luo, Qi Li, Qingdan Gu, Chunlei Zhang

Department of Clinical Laboratory Science, Shenzhen Yantian District People's Hospital, Shenzhen 518081, Guangdong, China

Received June 12, 2024; Accepted August 22, 2024; Epub August 25, 2024; Published August 30, 2024

Abstract: Neoadjuvant immune checkpoint blockade (ICB) has achieved significant success in treating various cancers, leading to improved therapeutic responses and survival rates among patients. However, in colorectal cancer (CRC), ICB has yielded poor results in tumors that are mismatch repair proficient, microsatellite-stable, or have low levels of microsatellite instability (MSI-L), which account for up to 95% of CRC cases. The underlying mechanisms behind the lack of immune response in MSI-negative CRC to immune checkpoint inhibitors remain an open conundrum. Consequently, there is an urgent need to explore the intrinsic mechanisms and related biomarkers to enhance the intratumoral immune response and render the tumor "immune-reactive". Intestinal microbes, such as the oral microbiome member Fusobacterium nucleatum (*F. nucleatum*), have recently been thought to play a crucial role in regulating effective immunotherapeutic responses. Herein, we advocate the idea that a complex interplay involving *F. nucleatum*, the local immune system, and the tumor microenvironment (TME) significantly influences ICB responses. Several mechanisms have been proposed, including the regulation of immune cell proliferation, inhibition of T lymphocyte, natural killer (NK) cell function, and invariant natural killer T (iNKT) cell function, as well as modification of the TME. This review aims to summarize the latest potential roles and mechanisms of *F. nucleatum* in antitumor immunotherapies for CRC. Additionally, it discusses the clinical application value of *F. nucleatum* as a biomarker for CRC and explores novel strategies, such as nano-delivery systems, for modulating *F. nucleatum* to enhance the efficacy of ICB therapy.

Keywords: *Fusobacterium nucleatum*, colorectal cancer, immune checkpoint blockade therapy, biomarkers, nano-delivery systems

Introduction

Colorectal cancer (CRC) ranks as the third most commonly diagnosed malignancy worldwide, contributing significantly to 9.4% of cancer-related deaths globally [1]. Standard therapies for CRC include surgery, radiotherapy, immunotherapy, and chemotherapy. Among these, neoadjuvant immune checkpoint blockade (ICB) therapy has recently emerged as a promising treatment strategy [2]. ICB involves the administration of monoclonal antibodies, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA4), programmed cell death-1 (PD-1), and programmed death-ligand 1 (PD-L1), which have demonstrated efficacy in several clinical trials. This therapeutic approach has been widely adopted across various cancer types as a preoperative intervention and has gained significant traction in recent years. Importantly, studies have shown that ICB can enhance the immune system's ability to recognize primary tumors as antigens [3].

Although pembrolizumab and nivolumab have received approval from the Food and Drug Administration (FDA) for CRC treatment, ICB therapy has demonstrated limited efficacy in most CRC patients, with therapeutic responses often characterized by heterogeneity and short duration [4]. Merely 20% to 30% of patients undergoing ICB therapy exhibit positive regression [5]. Therefore, comprehending the factors that impact immunotherapy efficacy and providing

timely, effective treatment is crucial for the survival of CRC patients. Recent evidence suggests that, in addition to tumor genomics, host factors such as intestinal microbiota can influence susceptibility, development, and immunotherapeutic responses [6]. This effect is likely due to the regulation of innate and adaptive immunity by intestinal microbiota [7].

CRC is a complex and heterogeneous disease influenced by various factors, including microbial exposures [8]. Recent evidence has revealed that intestinal microbiota is strongly related to local immune responses and can affect chemotherapy outcomes of CRC patients via various mechanisms [9, 10]. Among the microbial species, *Fusobacterium nucleatum (F. nucleatum*), a Gram-negative anaerobe found in the oropharyngeal microbiome, has garnered considerable attention [11]. Importantly, with the development of sequencing techniques of bacterial 16S ribosomal and whole-genome shotgun metagenomics sequencing methods, *F. nucleatum* is abundant in CRC tissues. Studies have revealed significant differences in the abundance of *F. nucleatum* in normal tissues, adenoma tissues, and adenocarcinoma tissues of CRC patients [12, 13]. Furthermore, *F. nucleatum* has been thought to be associated with microsatellite instability (MSI), lower-level T-cell infiltrates, and shorter survival of CRC [13-15]. The potential mechanism has been identified as the role of *F. nucleatum* in inducing T-cell apoptosis and inhibiting human T-cell responses to mitogens and antigens [16-18]. *F. nucleatum* not only mediates the carcinogenesis, development, and metastasis of CRC but also regulates the response to immunotherapy, shifting the tumor environment from an anti-tumor to a pro-tumor state [19, 20]. Understanding the mechanisms underlying the interactions between *F. nucleatum* and immunotherapy response in CRC is crucial. Targeting the microbiota to improve the outcomes of immune checkpoint blockade therapy for CRC might be a viable approach. In this article, we aim to systematically illustrate the potential role and molecular mechanisms of *F. nucleatum* in determining the therapeutic responsiveness of CRC patients. We will focus on the crosstalk among *F. nucleatum*, immunotherapy, and the host, while also discussing its potential application as a biomarker for CRC screening, prediction, and diagnosis. Strategies such as nano-based delivery systems for modulating *F. nucleatum* to restore the tumor response to immune checkpoint blockade therapy will also be explored.

Role of *F. nucleatum* in CRC

F. nucleatum is a gram-negative species that commonly resides in the human oral cavity. It is a member of the *Fusobacteriaceae* family, which encompasses nine species. Despite its prevalence as part of the normal oral microbiota, *F. nucleatum* is recognized as an opportunistic pathogen due to its involvement in various human diseases [21]. Owing to its role as a coaggregation factor, *F. nucleatum* can exert coaggregation or adherence functions with diverse other bacterial species through the RadD Aid1 or CmpA adhesins, such as *Prevotella*, *Bacteroides*, *Leptotrichia*, *Selenomonas*, and *Campylobacter* species [22, 23]. Interestingly, *F. nucleatum* and its derived adhesin, Fusobacterium adhesin A (FadA), are overexpressed in fecal samples of CRC patients, indicating enteral transmission [24]. To date*, F. nucleatum* has been shown to enter into colorectal tissues mainly through three pathways, via the interaction of its Fap2 protein with D-galactose-β (1-3)-N-acetyl-D-galactosamine (Gal-GalNAc), by FadA interacting with E-cadherin, or via Lipopolysaccharide (LPS) of *F. nucleatum* binding to Toll-like receptor 4 (TLR4) [25, 26] (**Figure 1**). FadA enables *F. nucleatum* to penetrate endothelial cells through loosened junctions, allowing it to disseminate systemically [27]. It is thought that under the context of an impaired oral-intestinal barrier, such as decreased stomach acid level, the *F. nucleatum* present in the oral cavity can migrate to the gut and enter the bloodstream for opportunistic translocation [28]. Furtherly, *F. nucleatum* has been shown to own the ability to regulate Cyclooxygenase-2 (COX-2), tumor necrosis factor (TNF), interleukin (IL)-6, IL-8, and IL-1β through stimulating nuclear factor kappa B (NF-κB) pathway [29, 30]. During the process of migrating to CRC cells and adhering to biofilm, FadA plays a vital role by binding with Gal-GalNAc, which is up-regulated in the tumor context [23]. This interaction underlines the multifaceted role of *F. nucleatum* in CRC pathogenesis, from facilitating its colonization and persistence within the tumor microenvironment to potentially influencing tumor immune

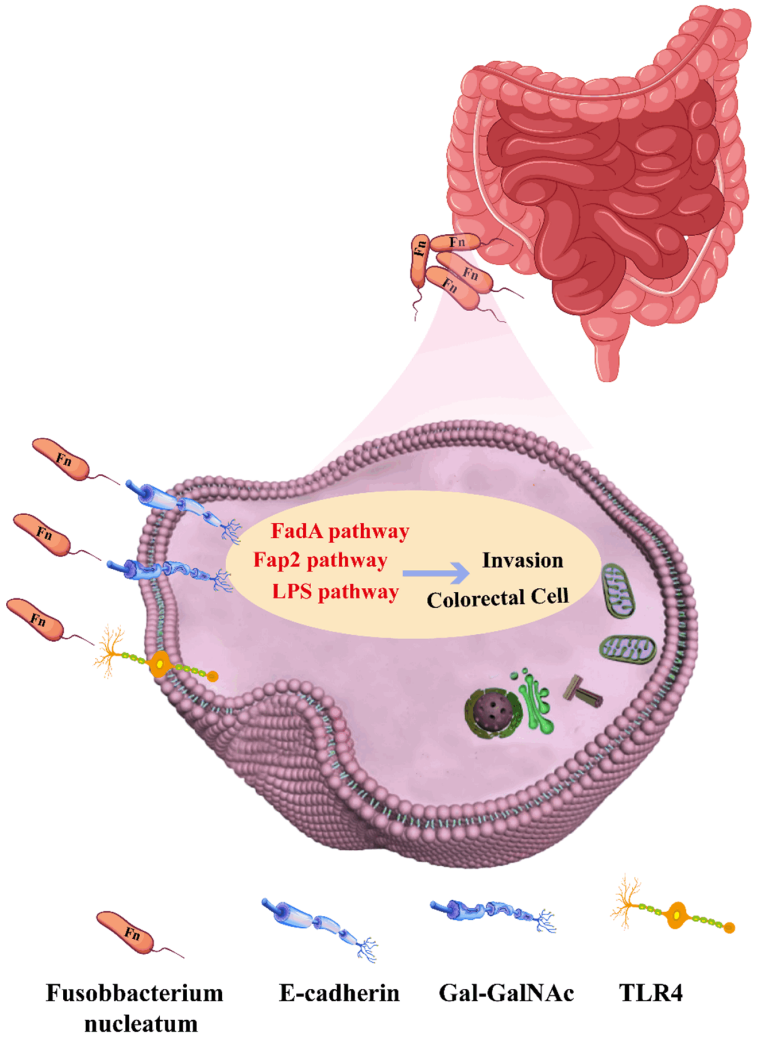

Figure 1. The three main pathways of *F. nucleatum* entering into colorectal cancer (CRC) tissues, via the interaction of its Fap2 protein with D-galactose-β (1-3)-N-acetyl-D-galactosamine (Gal-GalNAc), by FadA interacting with E-cadherin, or via Lipopolysaccharide (LPS) of *F. nucleatum* binding to Toll-like receptor 4 (TLR4), enabling attachment and invasion of CRC cells.

responses. Additionally, it contributes to the activation of the Wnt/β-catenin pathway via binding with E-cadherin, which is associated with inflammatory signaling and oncogenesis. Annexin A1 is weighed 35-40 kDa and binds phospholipids in a calcium-binding manner [31]. This molecule has been proposed to be a crucial component of FadA, enabling it to fulfill its function in diverse biological processes, including cell proliferation, differentiation, apoptosis, and migration, thereby influencing tumor growth [32, 33]. In CRC, Annexin A1 was found to be overexpressed, and its high levels were related to unfavorable prognosis, lower disease-free survival rates, and overall survival [34]. Previous research has uncovered that Annexin A1 exhibited the ability to regulate the Wnt/β-catenin signaling pathway and can activate cyclin D1 (CycD1) [23]. What is worthy of note is that Annexin A1 can induce immune suppression in the TME. This process was later confirmed to be associated with its interplay with epidermal growth factor receptor (EGFR) [34, 35]. Intriguingly, Rubinstein et al. found a complicated interplay within the multi-component complex of E-cadherin, Annexin A1, and β-catenin protein, which impacts CRC growth [36]. Furthermore, the gram-negative LPS of *F. nucleatum* can interplay with TLR4, which then triggers β-catenin and NF-κB, thereby inducing colitis-related tumor progression [37]. A high level of TLR4 has a strong association with poor prognosis and low overall survival among CRC patients [38, 39]. There has been substantial evidence suggesting that *F. nucleatum* is able to promote CRC tumorigenesis via proliferation and metabolism induction [40, 41]. Moreover, *F. nucleatum* can induce chemoresistance in CRC by upregulating baculoviral IAP repeat containing 3 (BIRC3) levels or modulating autophagy [42, 43]. The role and mechanisms of *F. nucleatum* in the initiation, progression, and development of chemoresistance to 5-fluorouracil (5-FU), oxaliplatin, and cisplatin in human CRC have been extensively discussed previously [44, 45]. This paper specifically turns attention to fusobacterial mechanisms that influence the therapeutic outcome of ICB in CRC.

Challenges in applying ICB to CRC

Over the past decades, although immunotherapies have been exerting an increasingly vital

influence on cancer therapy, the inevitable impact brought by tumor-induced immunosuppression in facilitating tumor progression has also been observed, as the immune system plays a dual role in tumor regulation [46]. During the procession of carcinogenesis, new antigens are generated and recognized by the immune system, leading to the killing of tumor cells, thus hindering tumor progression. The immunogenicity of tumor cells directly determines the efficacy of the immune response; otherwise, the immune response will be impaired. Significantly, cancer cells can reshape an immunosuppressive context, thus developing immune escape, which contributes to tumor progression. Therefore, strategies to mediate the immune system to render it "immune-reactive" and fight cancer cells are urgently needed. To address this, targeting immune checkpoint molecules, including PD-1 or CTLA-4, has been the most important method tested to date since it was approved as a second-line treatment strategy for CRC with dMMR/MSI-H by the FDA in 2017, following a phase III multicenter trial [4]. In recent years, ICB has played a crucial role in the therapy of various malignant tumors due to its ability to activate the antitumor immune response by inhibiting the interaction between T cell inhibitory receptors and their ligands [47]. More importantly, patients who benefit from ICB therapy typically show a long-lasting response compared with chemotherapy [48]. For CRC, the Keytruda (pembrolizumab, anti-PD-1 monoclonal antibody) has demonstrated remarkable responses in patients with dMMR/MSI-H [49, 50]. Unfortunately, according to the Keynote-016 study, in which three cohorts including the MSI-H/dMMR CRC cohort, MSI-H/dMMR non-CRC cohort, and MSI-H/pMMR CRC cohort were investigated for the therapeutic effect of Pembrolizumab, and it was found that the ICB therapy is not satisfactory for patients with pMMR [51]. Since CRC patients with pMMR escape this immune surveillance mechanism and are resistant to ICB therapy, they occupy the vast majority at approximately 85%, demonstrating the reality that the application of ICB therapy is very limited for CRC [52].

Considering the limited application of ICB therapy among CRC patients, efforts to identify underlying mechanisms and markers that affect and predict immunotherapeutic response are ongoing. Treatment responsiveness to ICB therapy can be attributed to both the cancer cells and tumor microenvironment (TME). The human immune response is strongly associated with tumor genomics due to the reshaping of the immune microenvironment by genomic mutations-derived neoantigens [53]. Currently, a lack of antigenic mutation burden (TMB), exclusion of T cells, recruiting of immune-suppressive cells, and being not responsive to interferon (IFN) have been proposed as potential mechanisms [54, 55]. Mutated peptides derived from carcinogenesis can be recognized by and bind to MHC molecules and be presented to T cells, leading to the activation of $CD8^+$ and $CD4^+$ T lymphocytes to execute anti-tumor function. Tumor-infiltrating $CD8^+$ T cells have been thought to play the most important role in the process of generating IFN-γ [56]. Hence strategies targeting $CD8^+$ T cell function, including modulating IFN-γ and augmenting major histocompatibility complex-class I (MHC-I) presentation in dendritic cells (DCs), have been proposed [57]. Th1 cells are also involved in ICB therapy, regulated by signaling factors like TGF-β, transcription factor p73 (tumor protein p73), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs), which affect the expression of IFN-γ [58-60]. IFN-γ signaling serves an important role in both anti-tumor immunity and immune escape. By stimulating MHC-I and MHC-II expression in tumor cells and antigen-presenting cells (APCs), it promotes T-cell and NK cell function to kill tumor cells [61]. However, by boosting PD-L1 expression, it can hamper anti-tumor immunity and ICB therapy [62, 63].

Mechanisms underlying the influence of *F. nucleatum* on ICB therapy

Ineffective immunotherapy response results from intrinsic and extrinsic factors, with multiple mechanisms involved. Extrinsic factors, such as the gut microbiome, are thought to make a vital inflammatory contribution to carcinogenesis and immunotherapy response [64]. Multiple biological processes such as cell proliferation, apoptosis, progression, migration, immunotherapeutic sensitivity, and therapeutic resistance of tumors, are subject to the influence of the cross-talk between tumor cell and their specific microbiota [65]. The role of gut microbiota in modulating myeloid-derived cell functions in the tumor microenvironment has also been demonstrated [66]. On the one hand,

gut bacteria can stimulate the innate immune system. On the other hand, they can regulate lymphocyte subpopulations in secondary immune organs and influence local mucosal immunity [67]. In fecal transfer experiments that compared tumor growth in mice with different commensal microbiota, differences in spontaneous antitumor immunity were observed. The results demonstrated that several gut microorganisms were tightly associated with anti-tumor responses via affecting the T-cell that infiltrates into the tumor [68]. The most widely used immune checkpoint inhibitors, such as anti-PD-1, play an important role in immunotherapy research. Intriguingly, it has been recently reported that the intestinal microbiota can influence the efficacy of PD-L1-mediated antitumor immunotherapy [69]. Though as a member of human normal oral and gut microbiota, *F. nucleatum* has also been recognized as an opportunistic pathogen. Mima et al. reported that the amount of *F. nucleatum* was higher in carcinoma tissue than in paired adjacent non-tumor tissue based on quantitative PCR [70]. Further investigation found that CRC patients with higher *F. nucleatum* DNA had significantly shorter survival, suggesting it might be a prognostic biomarker [15]. Studies have also found that *F. nucleatum* is correlated with therapeutic response, chemoresistance, and, more importantly, the modulating of immune checkpoint therapy for CRC [71, 72]. There are also accumulating findings indicating that *F. nucleatum* in CRC tissue is positively related to high levels of MSI-high and the CpG island methylator phenotype (CIMP) [73, 74]. Therefore, a complex correlation between *F. nucleatum* and ICB therapy exists in CRC, and the associated mechanisms are summarized in **Figure 2**. In this context, to provide a better understanding of potential mechanisms for effectively targeting *F. nucleatum* in colorectal therapy, we would like to review the role of *F. nucleatum* in therapeutic response to ICB therapy.

F. nucleatum inhibits T-cell activity

In CRC, high-level infiltrates of $CD3^+$, $CD8^+$, and $FOXP3^+$ T-cells are associated with better clinical outcomes [75]. Evidence has revealed that *F. nucleatum* is capable of inhibiting T-cell activity [76]. Another investigation reported that *F. nucleatum* can down-regulate T-cell-mediated adaptive cancer immunotherapy [29]. Further studies have shown that the modulation of T-cell-mediated immune responses by *F. nucleatum* is through the Wnt/β-catenin signaling pathway [77]. Consistent with these findings, a higher DNA abundance of *F. nucleatum* in CRC tissue is usually accompanied by lower T-cell density [70]. Moreover, *F. nucleatum* can induce T cell apoptosis and suppress T cell proliferation, which then reshapes an immunosuppressive TME [78]. Mechanistically, it has been found that the outer membrane proteins of Fap2 arrived from *F. nucleatum* can interact with TIGIT (T cell immunoglobulin and ITIM domain), thus inhibiting natural killer (NK) cell and T cell activities [79]. What's more, it is accepted that Fap2 exhibits a boosting role in inducing this apoptosis-mediated immune evading via additional fusobacterial factors. To figure out whether the effect aforementioned is brought by specific T-cell subsets, J. Borowsky et al. then revealed an inverse relationship between *F. nucleatum* and $CD3^+$ cells and $CD3^+CD4^+CD45RO^+$ cells [80].

F. nucleatum expands MDSCs in CRC

As a heterogeneous member present in the TME, MDSCs are a group of immunosuppressive myeloid cells. MDSCs in the tumor microenvironment proliferate and activate during the occurrence and development of CRC, inhibit the generation of T cells, impair their function, and hinder the immunotherapy effect of CRC [81, 82]. Compared with healthy people, the MDSCs manifest in significantly greater concentrations in CRC patients' tumor tissues [83]. To date, many studies have proposed an assistant role of MDSCs in CRC progression [78, 84]. Intriguingly, it has recently been reported that the MDSCs were enriched in the tumors of mice pretreated with *F. nucleatum* compared to the control group [29]. Consistent with this, *F. nucleatum* has been thought to recruit MDSCs and tumor-associated macrophages (TAMs) into the TME [29, 85]. Accordingly, the up-regulation of *F. nucleatum* causes microbiota disruption, which can restrain the response of cancer cells to CpG-oligonucleotide immunotherapy through decreased TNF [66]. These findings implicate that *F. nucleatum* induced an immunosuppressive TME via expanding MDSCs in CRC. Furthermore, TME plays a vital role in cancer therapy, especially for ICB therapy [86].

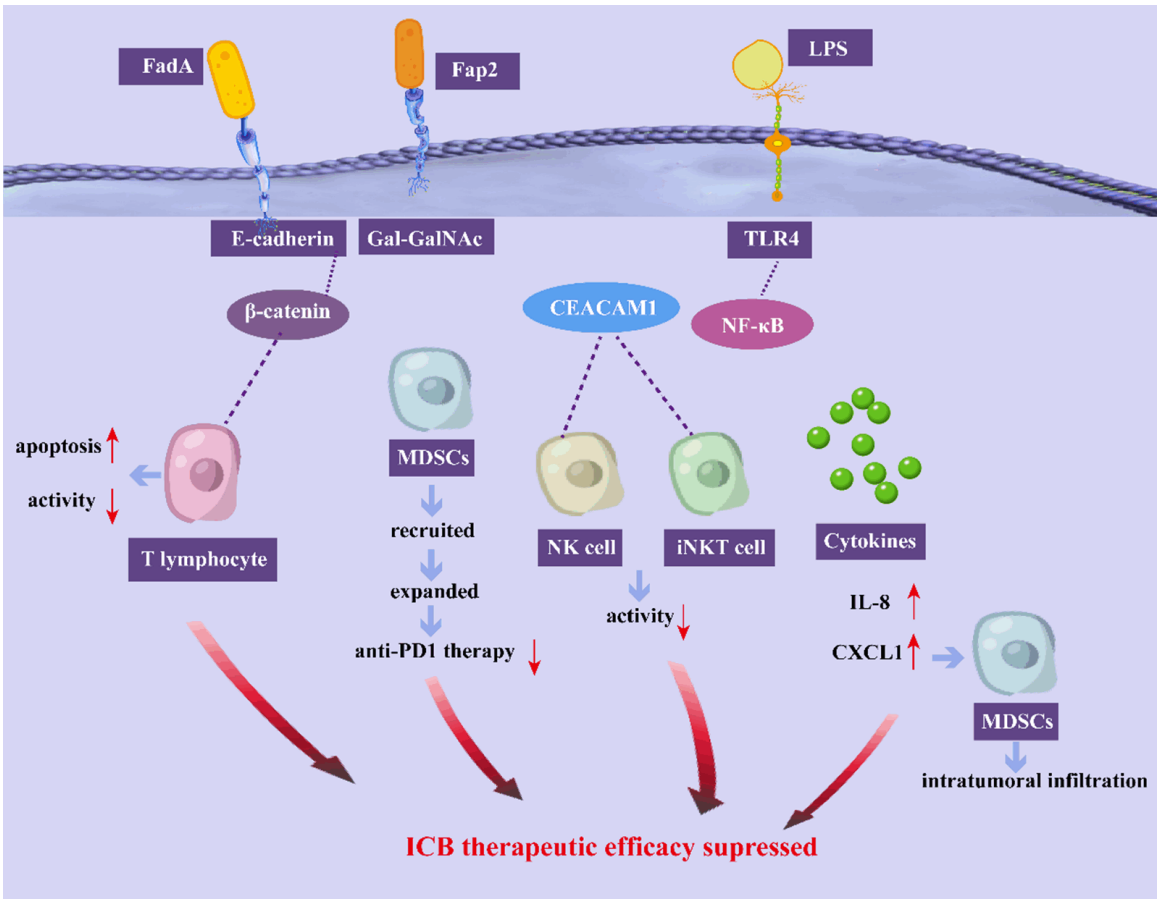

Figure 2. The potential mechanisms underlying the influence of *F. nucleatum* on ICB therapy. *F. nucleatum* can inhibit T-cell activity, downregulate T-cell-mediated adaptive cancer immunotherapy, and modulate T-cell immune responses via the Wnt/β-catenin signaling pathway. It also induces T-cell apoptosis, suppresses T-cell proliferation, and reshapes the tumor microenvironment (TME) to be immunosuppressive. *F. nucleatum* can recruit Myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), contributing to an immunosuppressive TME. *F. nucleatum* suppresses the activity of natural killer (NK) cells by activating TIGIT, reducing their ability to kill CRC cells. It also activates CEACAM1 on immune cells, which further inhibits the activity of T and NK cells. Additionally, *F. nucleatum* interferes with invariant natural killer T (iNKT) cells, impairing their function in anti-tumor immunity and contributing to immune evasion. It also upregulates pro-inflammatory cytokines like IL-8 and C-X-C motif ligand 1 (CXCL1), which recruit MDSCs and potentially disrupt PD-1/PD-L1 signaling.

Therefore, to improve the responses of CRC patients to ICB treatment, the role of *F. nucleatum* in mediating the function of MDSC in TME needs to be considered.

F. nucleatum suppress the activity of NK and invariant natural killer T (iNKT) cells

In addition to adaptive immune cells, some innate immune cells such as macrophages and NK cells, also play an important role in therapeutic response against ICB therapy. TIGIT, whose activation by fusobacteria can cause a compromised response to immunotherapy, is expressed in NK cells [79]. Therefore, related studies support that *F. nucleatum* can retrain the activity of NK cells in killing CRC cells via activating TIGIT. Previous studies have thought that the activation of CEACAM1, an inhibitory receptor on several immune cells can inhibit immune cell activities. Gur et al. have recently demonstrated that *F. nucleatum* can activate CEACAM1 to regulate anti-tumor immunotherapy by suppressing T and NK cell activities [87]. Another research has just reported that *F. nucleatum* causes immune escape of CRC by down-regulating the cytotoxicity and activity of NK cells. INKT are an innate-like T cell subset expressing an invariant T cell receptor (TCR) α-chain [88]. Gathering evidence

has suggested a strong relationship between iNKT cell functions and TME and immune surveillance [89, 90]. In CRC, iNKT cells exhibit a vital function in anti-tumor immunity by mediating cytokines levels and conventional T cells [91]. Recently, G. Lattanzi et al. confirmed that *F. nucleatum* is a contributor to iNKT cell-mediated recruitment of neutrophils and reshaping the TME, exhibiting an inhibitory property in anti-tumor immunotherapy for CRC [92].

F. nucleatum regulates the inflammatory cytokines levels in CRC

Growing evidence has demonstrated the promoting role of pro-inflammatory cytokines in the TME, such as regulating angiogenesis and activating tumor-specific immune responses. Related mechanisms are involved in recruiting $CD8^+$ T cells, stimulating TAM polarization, and accumulating Treg cells [93, 94]. Earlier research has proposed a significantly positive role of *F. nucleatum* in generating IL-10 and TNF-a [95]. Consistently, Yin et al. also confirmed that the mice treated with *F. nucleatum* implicated higher inflammatory cytokines levels, in which IFN-γ, TNF-α, IL6, IL12, and IL17A were included [96]. Specifically, *F. nucleatum* within CRC tissues has been thought to upregulate the pro-inflammatory cytokines IL-8 and C-X-C motif ligand 1 (CXCL1), which were both thought to function in recruiting immune cells, especially for neutrophils [97, 98]. This effect has the potential for stimulating neutrophils and altering them into tumor-associated neutrophils (TANs), of which the infiltration has recently been demonstrated to predict worse outcomes of ICB therapy [99]. However, *F. nucleatum* has been evidenced to own the ability to induce the generation of these two inflammatory cytokines in HCT116 cells [97]. Therefore, it is deduced that *F. nucleatum* might produce a proinflammatory microenvironment, which was then rendered an immunosuppressive microenvironment to subsequently be not responsible for ICB therapy.

F. nucleatum affects anti-PD-1/PD-L1 signaling

As a checkpoint receptor expressing immune cells, PD-1 signaling is associated strongly with immune escape by interacting with PD-L1 [100]. However, anti-PD-1/PD-L1 signaling has exhibited dissatisfactory outcomes in CRC, except for those with MSI-high and a high TMB. What on earth influences the response of CRC cells to anti-PD-1/PD-L1 treatment is required to be identified. Numerous investigations have confirmed the relationship between *F. nucleatum* and anti-PD-1 immunotherapy in CRC. Gao et al. demonstrated that *F. nucleatum* can assist with therapeutic responses to PD-1 blockade both in mice models and CRC patients. Mechanically, it induces PD-L1 expression via recruiting $CD8^+$ TILs, stimulating STING signaling and inducing IFN-γ [101]. However, another newly published research proposed that the *F. nucleatum* can impede the action of anti-PD-1 inhibitors. By comparing the metabolomic and microbiome data from CRC patients with post-immunotherapy, the researchers found that *F. nucleatum* was related to poor response to immunotherapy. Mechanically, this study suggested that *F. nucleatum*-derived succinic acid is capable of disrupting the GMP-AMP synthase interferon-β pathway, which then dampens the therapeutic response of CRC cells to anti-PD-1 treatment [102]. Throughout these findings, there still exists controversy about the role of *F. nucleatum* in correlating with anti-PD-1/PD-L1 therapy, thus deeper explorations are appealed in futural research.

F. nucleatum as a potential biomarker and therapeutic target

Effective biomarkers are of vital importance to detect premalignant lesions and provide timely treatment, which could greatly improve the survival rate of CRC patients [103]. Consistently, biomarkers for therapeutic efficacy prediction of cancer immunotherapy are also required for clinical care, as long as for further progress of cancer immunotherapy. Up to date, PD-L1, MMR, and MSI examination are generally applied as predictive immunotherapy biomarkers for CRC. Recently, microbiota-based biomarkers have emerged as a novel hotspot due to their role and influence on immunotherapy response. Emerging evidence has supported that *F. nucleatum* significantly overexpressed feces and tumor sites of CRC patients. Wang et al. investigated CRC patients' RFS and found that high levels of *F. nucleatum* were correlated with worse RFS, compared with those with no/low *F. nucleatum* levels [104]. They highlight that the *F. nucleatum* could serve as a valuable

predictive and prognostic biomarker for unfavorable prognosis in CRC.

Nanomedicine-based strategies targeting *F. nucleatum* for CRC therapy

In consideration of the inhibitory effect of *F. nucleatum* in responses to ICB therapy, eliminating this microbe from tumor tissues might be a promising strategy to enhance cancer therapy. Although deeper validation is required, the existing evidence supports that eradicating the intratumoral microorganisms can induce cancer-associated neoantigens, which might be conducive for patients receiving ICB [105]. Nowadays, some antibiotics including metronidazole and β-lactams, have been applied to eradicate *F. nucleatum* to inhibit tumor growth [106]. Noteworthy, nanotechnology is an emerging rapidly target-based modality for drug delivery to deep tumor sites owing to its high permeability and safety, providing novel strategies to enhance ICB response [107]. Therefore, nano-based medicines have the potential to specifically abolish intratumoral microorganisms [108]. Wang et al. constructed a liposomal delivery of antibiotics targeting bacteria *F. nucleatum*-infected CRC model, and the results indicated that bacteria were abolished. Moreover, the elimination of *F. nucleatum* improved ICB therapeutic outcomes by exposing microbial epitopes [109]. Qu et al. creatively constructed an ultrasonic stimulation-responsive albumin-based nanoplatform (Au@BSA-CuPpIX), which was entrapped by biomimetic mineralization [110]. This nanocomplex was found to efficiently kill F. nucleatum, at the same time, down-regulating the anti-apoptosis protein levels in tumor sites. In addition, tumor volume and metastases of xenografts and orthotopic CRC mice model were significantly reduced. To deliver antibiotics against *F. nucleatum* in tumor sites with minimal damage, Geng et al. designed a biomimetic nanomedicine (FtnDOX), which loads antibiotic MTZ into the nanovehicle through azobenzene [111]. Expectedly, this nanomedicine efficiently released antibiotics to kill *F. nucleatum and* promoted immunogenic death, enhancing tumoral response to ICB. Chen et al. devised a mimetic nanocomplex with *F. nucleatum* wrapping on the Colistin-loaded liposomes. They demonstrated that the nanomedicine effectively eliminated intratumoral *F. nucleatum* and enhanced the therapeutic efficacy of ICB. In summary, targeting *F. nucleatum* to eliminate it can turn an immunologically cold tumor into a hot state, which then enhances the immune response of tumor cells to ICB therapy. Nowadays, although antibiotics against *F. nucleatum* are the most efficient treatment, direct administration of antibiotics is facing the difficulty of compromised therapeutic efficacy and some adverse effects. To address these issues, various tumor-targeted nanocarriers for drug delivery have been proposed, which provide a novel strategy for augmenting ICB therapeutic outcomes.

Future perspectives and concluding remarks

The interplay between the host, intestinal microbes, and immunotherapy, particularly in the context of CRC, is an area of growing interest and research. Emerging preclinical and clinical findings have shed light on the involvement of *F. nucleatum* and its metabolites in carcinogenesis and therapeutic response to ICB therapy. *F. nucleatum* has been implicated in various mechanisms that influence the efficacy of ICB therapy in CRC. These include its role in regulating T-cell activity, expanding MDSCs, suppressing NK and iNKT cell functions, and upregulating inflammatory cytokines. These actions collectively contribute to the creation of an immunosuppressive TME that compromises the efficacy of ICB therapy. Moreover, the identification of *F. nucleatum* may hold promise as a biomarker for predicting responses to ICB therapy. By understanding the presence and abundance of *F. nucleatum* in CRC patients, clinicians may be better equipped to tailor treatment strategies and optimize therapeutic outcomes. Recently, nanoplatform-based strategies offer innovative approaches for accurately eliminating *F. nucleatum*. augmenting the response to ICB therapy in CRC. This field holds the potential to revolutionize the treatment paradigm for *F. nucleatum*-associated cancers and improve patient outcomes. However, while the association between *F. nucleatum* and ICB therapy in CRC is becoming increasingly evident, further in-depth preclinical and clinical exploration is urgently required to unveil the underlying cooperation and action. Comprehensive studies are required to uncover the complexities of the interaction between *F. nucleatum*, the host immune system, and immu-

notherapy agents. Despite the challenges, the development of *F. nucleatum* as a therapeutic target holds the potential to enhance the efficacy of ICB therapy in CRC. By targeting *F. nucleatum* and modulating its activity within the TME, researchers may uncover novel strategies to overcome immunosuppression and improve treatment responses. Furthermore, unraveling the complexities of the host-microbe-immunotherapy interaction contributes to a comprehensive understanding of individual tumor progression and lays the groundwork for risk assessment and precision medicine approaches in CRC management. By integrating microbiome analysis into clinical decision-making, clinicians may be able to personalize treatment regimens and optimize patient outcomes.

Disclosure of conflict of interest

None.

Address correspondence to: Chunlei Zhang, Department of Clinical Laboratory Science, Shenzhen Yantian District People's Hospital, 2010 Wutong Road, Yantian District, Shenzhen 518081, Guangdong, China. Tel: +86-13670092808; Fax: +86-0755-25215063; E-mail: czhang0755@163.com

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