

# A new strategy for treating colorectal cancer: Regulating the influence of intestinal flora and oncolytic virus on interferon

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**Colorectal cancer (CRC) has the third highest incidence and the second highest mortality in the world, which seriously affects human health, while current treatments methods for CRC, including systemic therapy, preoperative radiotherapy, and surgical local excision, still have poor survival rates for patients with metastatic disease, making it critical to develop new strategies for treating CRC. In this article, we found that the gut microbiota can modulate the signaling pathways of cancer cells through direct contact with tumor cells, generate inflammatory responses and oxidative stress through interactions between the innate and adaptive immune systems, and produce diverse metabolic combinations to trigger specific immune responses and promote the initiation of systemic type I interferon (IFN-I) and anti-viral immunity. In addition, oncolytic virus-mediated immunotherapy for regulating oncolytic virus can directly lyse tumor cells, induce the immune activity of the body, interact with interferon, inhibit the anti-viral effect of IFN-I, and enhance the anti-tumor effect of IFN-II. Interferon plays an important role in the anti-tumor process. We put forward that exploring the effects of intestinal flora and oncolytic virus on interferon to treat CRC is a promising therapeutic option.**

## INTRODUCTION

Colorectal cancer (CRC) ranks third in incidence and second in mortality worldwide. There are expected to be 2.2 million new cases of CRC worldwide and 1.1 million deaths due to CRC by 2030, while China is expected to have more than 191,000 deaths and 376,000 new cases per year.<sup>1</sup> About half of all humans will develop benign colorectal tumors, and about 3% of these patients will progress to CRC. The treatment of CRC includes systemic therapy and preoperative radiotherapy, endoscopic and surgical local excision surgery, extensive local and metastatic CRC surgery, local ablation therapy for metastatic CRC, specifically targeted therapy, and immunotherapy. However, the survival of patients with metastatic CRC disease remains poor. Therefore, investigating new strategies for treating CRC is of great interest.

In addition to genetic factors, colonic inflammation is also a significant cause of CRC, regulated by various factors such as microbiota, immune cells, and cytokines. The relationship between gut microbiota and CRC has become one of the main directions for research

and treatment of CRC. Microflora can regulate the activation of diverse immune cells through the interaction between signaling, such as nuclear factor kappa B (NF- $\kappa$ B), inflammatory body, and type I interferon (IFN-I), and then affect inflammation and CRC. Therefore, studying the microflora mechanism helps formulate effective CRC prevention and treatment strategies. Immunotherapy is one of the crucial options for CRC treatment. This strategy harnesses the patient's immune system against tumor cells and effectively improves the specificity of the treatment plan for cancer cells.<sup>1</sup> The colonization of intestinal flora at the tumor site can reinforce the anti-tumor effect of immunotherapy. Therefore, *in vivo*, using specific bacteria or their engineered progeny strains may play an influential therapeutic role in enhancing anti-tumor immunotherapy and may serve as a powerful complement to therapeutic approaches such as surgery and radiotherapy. Meanwhile, the oncolytic virus (OV) uses a natural or modified virus as a vector to activate anti-tumor immune reaction and induce cell apoptosis, which is a new tumor treatment method. Interferon (IFN) is an effective immunostimulatory that is important for immune reaction. It is the first line of defense against pathogens and can even act as an effective mediator to strengthen the immune system. Changes in IFN signaling can support tumor resistance to immune checkpoint blockade (ICB). Gut microbiota and OVs can interfere with the IFN signaling pathway to treat CRC. Therefore, it is necessary to explore the effect of intestinal flora and OVs on IFN to better examine the mechanisms of immunotherapy resistance in CRC.

## INTERACTION BETWEEN IFN AND CRC

IFN-I (IFN- $\alpha$ , IFN- $\beta$ ) and IFN-II- $\gamma$  jointly regulate the immune response in tumors,<sup>2</sup> and IFN signaling plays a dual role in promoting tumor development and immune evasion. IFN- $\alpha$  and IFN- $\beta$  are effective anti-viral cytokines. At the same time, IFN-II also showed its anti-tumor effect through various mechanisms.

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### IFN promotes the development of CRC

IFNs have many different pathways of action in CRC. It has been reported that IFN-I upregulates the expression of the tumor suppressor protein p53.<sup>3</sup> IFN- $\beta$  was found to inhibit the transformation of wild-type mouse embryonic fibroblasts *in vitro*. In addition, ribonuclease L (RNase L), an endoribonuclease regulated by IFN-I, can function as a tumor suppressor in human cells.<sup>4</sup> Therefore, IFN-I provides cells with greater potential to control the transformation process. Endogenous IFN-I and IFN- $\gamma$  do not function in a completely overlapping manner, but they play a role in different host cell populations. In the study of tumor transplantation and monochloroacetic acid (MCA)-induced carcinogenicity, it was found that mice that received plasmids encoding autoantigens and IFN- $\gamma$  developed fewer tumors and produced weaker regulatory T (Treg) cell responses than mice that received plasmids encoding only autoantigens expressed by tumors. These results suggest that the early production of IFN- $\gamma$  blocks the development and immunosuppression of Treg cells.<sup>2</sup> Furthermore, dysregulation of IFN- $\gamma$  production and IFN- $\gamma$ -mediated signaling has been shown to promote spontaneous oncogenicity in the colon of mice expressing SOCS1 only in T and B cells.<sup>5</sup> IFN- $\gamma$  can inhibit tumor immune response. For instance, IFN- $\gamma$  can promote the production of B7-H1 (programmed death-ligand 1 [PD-L1]), arginase (Arg), indoleamine 2,3-dioxygenase (IDO), and other immunosuppressive molecules in the tumor microenvironment (TME).<sup>6,7</sup> IDO contributes to immune tolerance of TME, is the pathogenic driver of CRC progress, and is related to the poor prognosis of CRC. IFN- $\gamma$  is an effective inducer of IDO expression. IFN- $\gamma$  strongly stimulates the expression of programmed cell death ligand 1 (PD-L1) in the TME, thereby hampering anti-tumor immunity and ICB therapy. The immunogenic effect of IFN- $\gamma$  may inevitably be accompanied by the enhancement of the immune escape mechanism in TME, and the tumor immune escape effect can be overcome by specific combination therapy. For example, breaking the restriction of single targeted drugs can improve the combined efficacy of ICB, one of which is to interfere with the aromatic hydrocarbon receptor pathway in tumors expressing IDO.<sup>8</sup> Many cells produce IFN- $\gamma$ , such as natural killer (NK) cells,<sup>9</sup> helper T (Th) 17 cells, antigen-presenting cells (APCs), and innate lymphoid cells (ILCs). IFN- $\gamma$  binds to IFN- $\gamma$  receptors (IFNGRs) and stimulates Janus kinase (JAK)-signal transduction and transcription (STAT) signaling, which activates the transcriptional program of IFN-stimulated genes (ISGs) and regulates immune responses. In contrast, cytokine signaling (SOCS) suppressors, mainly SOCS3 and SOCS1, are negative regulators of IFN- $\gamma$  signaling.<sup>10</sup> In conclusion, IFN- $\gamma$  can lead to anti-tumor immunosuppression *in vivo* by activating SOCS family proteins and inducing the expression of immunosuppressive molecules in various ways, limiting the treatment of CRC.

IFN-I and IFN-II participate in many mechanisms for regulating immune response in cancer, thus balancing immune escape and surveillance.<sup>11</sup> The action pathways of these IFNs provide therapeutic ideas in the clinical practice of CRC and explain the mechanism of IFN promoting the development of CRC, which consists of three stages: first, elimination, which is based on the recognition of tumor cells and their killing by the innate or adaptive immune system; second, persistence,

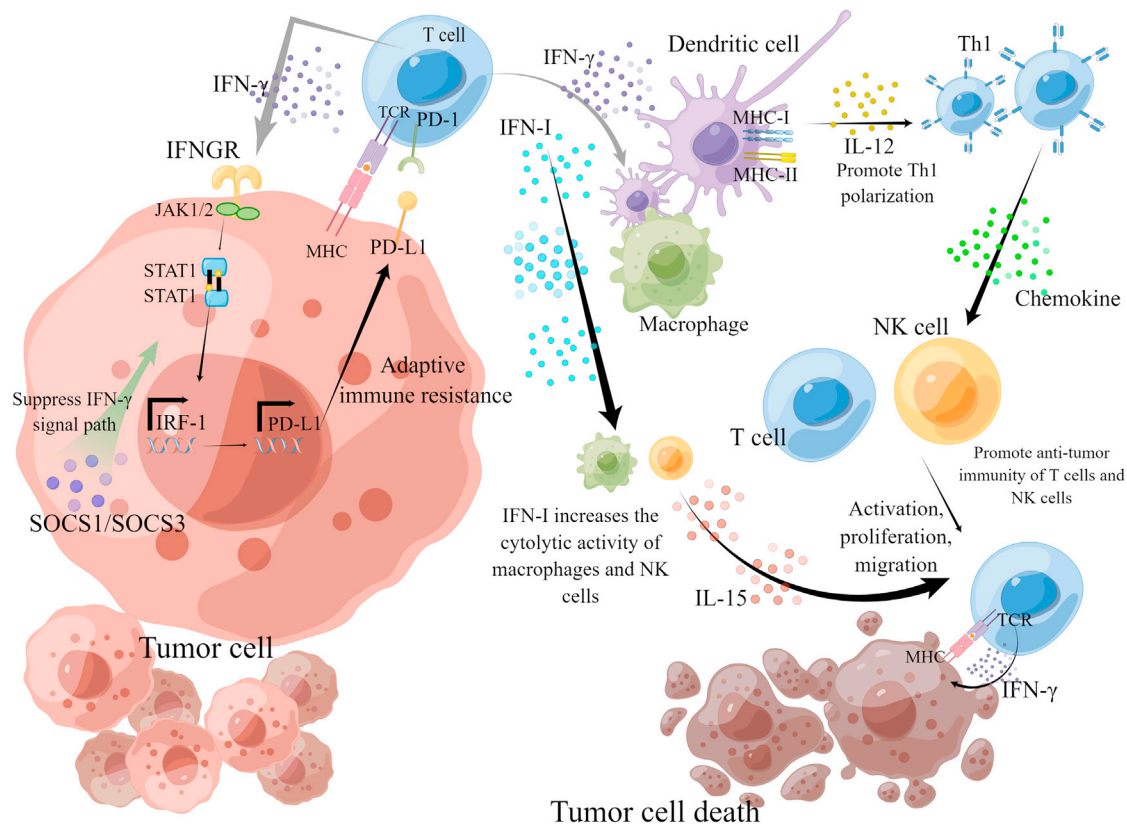
based on the failure of the elimination step, resulting in a balance between the growing cancer cells and the stress of the immune system; third, escape, when the growth of cancer cells can overcome the protective effect of the immune system, and escape begins due to immune inhibition or generation of resistant cancer cell clones.

### Regulate IFN to treat CRC

IFNs have emerged as central orchestrators of tumor-immune system interactions. Studies have shown that hosts that do not respond to IFN show increased tumor incidence, and tumors produced in IFN-unresponsive environments are highly antigenic. IFN-I has broad immunomodulatory effects, and many cell types may be physiologically relevant targets for their anti-tumor activity. For example, IFN-I has been found to activate dendritic cells (DCs), increase the cytolytic activity of macrophages and NK cells, induce interleukin (IL)-15 production, increase T cells' survival, and produce stromal-derived vascular inhibitory molecules.<sup>12</sup> It is also clear that IFN- $\gamma$ , but not IFN- $\alpha$  and IFN- $\beta$ , controls tumor cell immunogenicity, possibly as a result of selective production of IFN- $\gamma$  in the TME. Tumors need to respond to IFN- $\gamma$  to be immunogenic. The ability of IFN- $\gamma$  to promote tumor rejection is mediated at least in part by up-regulating major histocompatibility complex (MHC)-like pathways for antigen processing and presentation in tumor cells.<sup>2</sup> This suggests that the effect of IFN- $\gamma$  on tumor cells may coordinate the progression of the immune response to growing tumors, allowing early recognition or clearance of cancer cells by the innate immune system to be transformed into an immune attack by the adaptive immune system.

Studies have shown that IFN- $\gamma$  stimulation leads to nuclear translocation and phosphorylation of STAT1, resulting in STAT1-TET2 binding, and the regulation of this mechanism may enhance the body's anti-tumor immunity. Multiple IFN- $\gamma$ -responsive genes, including PD-L1, CXCL9 (MIG), CXCL10 (IP-10), and CXCL11 (I-TAC), were found to result in gene silencing of DNA methylation. At the same time, DNA demethylation involving Tet2 can increase the level of 5hmC on the promoters of IFN- $\gamma$ -responsive genes, thereby improving the immune response.<sup>13</sup> Therefore, modulation of the IFN- $\gamma$  epigenetic signaling pathway can affect tumor immunotherapy. IFN- $\gamma$  increases the production of MHC-I and MHC-II in cancer cells, increases the production of IL-12 in APCs, promotes Th1 polarization, and promotes tumor translocation of immune cells through the secretion of Th1-type chemokines. In addition, IFN- $\gamma$  has direct anti-cancer effects on cell replication<sup>14</sup> and induces apoptosis<sup>15</sup> and necrosis<sup>16</sup> in cancer cells. Notably, IFN- $\gamma$  can result in cancer cell apoptosis, necrosis, or ferroptosis.<sup>17</sup> Clinical studies have found that the loss of IFN- $\gamma$  gene expression is frequently observed in CRC patients, thus enhancing the IFN- $\gamma$  signaling cascade pathway is a reasonable and novel approach to treat CRC patients.<sup>18</sup>

Currently, researchers are using these cytokines to arm OV.<sup>19</sup> The rationale for using IFN-I as transgenes in OVs is that, in addition to their anti-viral function, they also have anti-tumor potential due to their role in the maturation of DCs and cytotoxic T cells. An oncolytic adenovirus armed with IFN- $\alpha$  produced modest increases in anti-tumor efficacy in a mouse model of pancreatic cancer when



**Figure 1. The main action pathway of IFN in inhibiting tumor cells**

IFN-I can activate DCs, increase the cytolytic activity of macrophages and NK cells, induce the production of IL-15, activate T cells, and increase the survival of T cells. The IFN- $\gamma$  signal cascade pathway is the main anti-tumor pathway of IFN-II. IFN- $\gamma$  binds to IFN- $\gamma$  receptors (IFNGRs) on tumor cells and stimulates Janus kinase (JAK) signal transduction and transcription (STAT) activator signaling pathway, which stimulates phosphorylation and nuclear translocation of STAT1, thus activating the IFN-stimulated gene transcription program and regulating the immune response. IFN- $\gamma$  enhances the production of MHC-I and MHC-II in cancer cells and IL-12 in APC, promotes Th1 polarization, and promotes tumor transport of immune cells through the secretion of Th1 chemokines, thus achieving the anti-tumor purpose.

combined with radiation. Similarly, after arming OV vesicular stomatitis virus (VSV)<sup>20</sup> and measles with IFN- $\beta$ ,<sup>21</sup> it was observed that their anti-tumor efficacy was improved to some extent.

Therefore, it can play a role in treating CRC by regulating IFNs to play a variety of mechanisms. It is clear from many studies that IFN-I can promote many immune functions, but further work using appropriate *in vivo* tumor models is needed to reveal which immune functions are physiologically relevant to anti-tumor immunity. IFN- $\gamma$  regulates various biological programs that may be *a priori* involved in suppressing tumor growth, so additional experimental studies are needed to determine the full induction of IFN- $\gamma$  on tumors that contributes to tumor growth suppression and is useful for the treatment of CRC (Figure 1).

## REGULATE INTESTINAL FLORA AND IFN TO TREAT CRC

As an essential participant in the intestinal environment, intestinal microorganisms are nearly connected with the occurrence and development of CRC. Some scholars have pointed out that intestinal microflora preferentially colonizes the tumor site and promotes

immunotherapy through the signal of stimulators of IFN genes (STING) in the tumor site. Living bacteria will migrate on their initiative and continuously generate secondary metabolites, thus triggering the STING pathway in DCs.<sup>22</sup> Therefore, intestinal flora and IFN are closely associated with CRC.

### The basic mechanism of intestinal flora

Under normal conditions, intestinal bacteria maintain a stable flora, which is vital in nutrient intake, regulating intestinal immunity, and maintaining the intestinal mucosal barrier. If the intestinal balance is broken, it will lead to the secretion of inflammatory cytokines, inflammatory mediators, proteases, and oxygen free radicals, which will not only aggravate the mechanical barrier damage of intestinal mucosa but also cause bacterial translocation and the activation of immune regulation of digestive tract, triggering the occurrence, progress, and even deterioration of CRC.<sup>23,24</sup>

### Direct contact with tumor cells

The human gut microbiota contains 1,013–1,014 microorganisms, of which the genes in gut bacteria are over 100 times that of the human

**Table 1. Summary of the mechanisms of different intestinal microflora in the initiation, development, and metastasis of CRC**

Enteric microorganisms	Mechanism of action	Authors	Year
<i>F. nucleatum</i>	through FadA, it selectively binds to E-cadherin on the surface of CRC and activates the intracellular $\beta$ -catenin signaling pathway	Hashemi Goradel et al. <sup>30</sup>	2019
<i>E. faecalis</i>	metalloproteinase is produced, directly destroying the intestinal epithelial barrier and inducing inflammation	de Almeida et al. <sup>33</sup>	2018
<i>E. coli</i>	gene toxin <i>E. coli</i> was produced by the NRPS-PKS hybrid gene cluster	Vizcaino et al. <sup>34</sup>	2015
ETBF	produces BFT, which has proteolytic activity, can damage intestinal mucosa, induces cancer-promoting signal cascade, and triggers medullary cell-dependent colon tumors	Chung et al. <sup>35,36</sup>	2018
<i>P. anaerobius</i>	its surface protein PCWBR2 directly combines with IEC receptor integrin $\alpha$ 2/ $\beta$ 1 to activate the carcinogenic PI3K-Akt signal pathway, thus promoting the proliferation of tumor cells	Belkaid et al. <sup>37</sup>	2014
SE	secretion of AvrA activates the STAT3 signaling pathway in colon cancer cells	Duijster et al. <sup>38</sup>	2021
CJ	CDT production leads to DNA double-strand break	Lara-Tejero et al. <sup>39</sup>	2000

genome.<sup>25</sup> In the healthy human body, intestinal flora can shape the intestinal epithelium,<sup>26</sup> collect the energy needed by the body,<sup>27</sup> produce an immune response, etc,<sup>28</sup> which plays a huge role.<sup>29</sup> Many symbiotic bacteria live in the intestine, including a few tumor-promoting species, such as *Fusobacterium nucleatum*, *Enterococcus faecalis*, *Escherichia coli*, enterotoxigenic *Bacteroides fragilis* (ETBF), *Peptostreptococcus* (*Peptostreptococcus anaerobius*), *Salmonella enteritidis* (SE), *Campylobacter jejuni* (CJ).<sup>30</sup> Pathogenic bacteria play a role by directly contacting tumor cells through their virulence factors. *F. nucleatum* selectively binds to E-cadherin through *Fusobacterium* adhesin A (FadA), thereby triggering the intracellular  $\beta$ -catenin signaling pathway, inducing carcinogenesis and inflammation.<sup>31</sup> *F. nucleatum* can also induce the sharp decrease of m6A modification in tumor cells and patient-derived xenotransplantation (PDX) tissues by downregulating m6A methyltransferase like 3 (METTL3), which helps generate CRC invasion.<sup>32</sup> *E. faecalis* has been proved to produce metalloproteinases, which can directly destroy the intestinal epithelial barrier and cause inflammation.<sup>33</sup> *E. coli* produced the gene toxin *E. coli* through the hybrid gene cluster<sup>34</sup> of polyketide synthetase (PKS) and non-ribosomal peptide synthetase (NRPS). ETBF is similar to *E. coli* and produces metalloproteinase toxin BFT (bacteroides fragilis toxin), which can damage intestinal mucosa, induce cancer-promoting signal cascade, and trigger myeloid-dependent colon tumors because of its proteolytic activity.<sup>35,36</sup> *P. anaerobius* combines with in-

testinal epithelial cell (IEC) receptor integrin  $\alpha$ 2/ $\beta$ 1 through the putative cell wall-binding repeat 2 (PCWBR2), which enhances the carcinogenic phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) signaling, thus promoting the proliferation of cancer cells.<sup>37</sup> AvrA secreted by SE can activate STAT3 signaling in CRC cells.<sup>38</sup> CJ generates a gene toxin, cytolethal distending toxin (CDT), which results in DNA breaks<sup>39</sup> and promotes the occurrence of CRC. Tumor-related flora can directly regulate the signal pathway of tumor cells, and various flora can activate the  $\beta$ -catenin signal pathway related to tumor dryness and proliferation, thus achieving the goal of promoting tumor (Table 1).

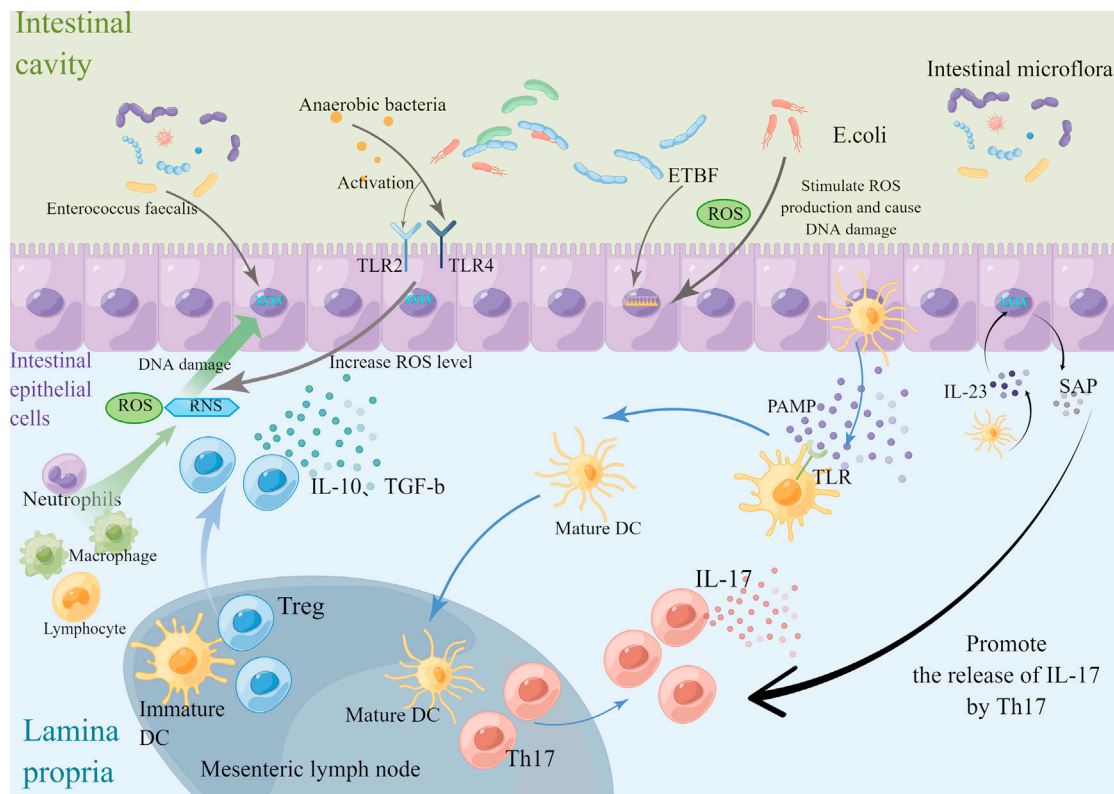
### Produce inflammatory reaction

Microbe and host immune systems interact in regulating the development of CRC. As a significant part of the TME, various immune cell groups are of great significance in inhibiting or promoting the growth of CRC. Intestinal microflora closely interacts with the immune system. Immune reactions stimulated by bacteria can cause persistent low-grade inflammation, leading to tumors. On the contrary, inflammation cannot induce CRC without compounds derived from microorganisms or bacteria.<sup>40</sup> The formation of inflammatory cells and their related mediators may cause intestinal epithelial damage, which is beneficial to the change of the microenvironment of CRC.

The innate immune system is crucial in mediating inflammatory responses in CRC patients.<sup>41</sup> It has been confirmed that microbial nucleic acids are among the main targets of innate immune recognition. Microbial antigens and potential pathogens will also be perceived by pattern recognition receptors (PRRs) encoded by host strains, which recognize specific pathogen-associated molecular patterns (PAMPs). In the innate immune response, Toll-like receptors (TLRs) are specific PRRs that can recognize pathogens and thus enable the innate immune response to proceed smoothly.<sup>42</sup> Dense microbial communities in the intestine can produce a variety of molecules identified by TLRs,<sup>43</sup> resulting in the excitation of the NF- $\kappa$ B signal and the transcription of multiple cytokines.<sup>41</sup> Microorganisms can generate molecular patterns that activate innate immunity. However, symbiotic bacteria also seem to change DNA repair pathways and induce microbe-associated molecular patterns (DAMPs) that trigger innate epithelial immunity.<sup>44</sup>

In addition, symbiotic microorganisms can modulate the adaptive immune system in various ways, which include stimulating and directing the differentiation of T cell groups in the gut, especially Treg cells and Th17 cells.<sup>45</sup> Microorganisms strictly control Treg cells in the intestine, and Treg groups exert immunosuppressive functions on various immune cell groups through transforming growth factor (TGF)- $\beta$  and IL-10.<sup>46,47</sup> Th17 cells may play a role in tumor initiation by changing signaling pathways, such as STAT3, presenting different sites between intact epithelial cells and developed tumor cells.<sup>48</sup> In contrast to non-pathogenic Th17 cells induced by TGF- $\beta$  and IL-6, Th17 cells can acquire a pathogenic pro-inflammatory phenotype in response to serum amyloid protein (SAP) or IL-23<sup>49,50</sup> and promote the release of IL-17 (Figure 2). In addition, the microbiota controls the efficacy of tumor immunotherapy by modulating cancer cell-killing functions of cytotoxic





**Figure 2. The mechanism of inflammatory reaction and oxidative stress reaction of intestinal flora**

In inflammatory reactions, symbiotic microorganisms can regulate the adaptive immune system in various ways. Symbiotic microorganisms produce adaptive immune responses by stimulating the differentiation of Th17 cells and Treg cells, among which Treg cells exert immunosuppressive function on various immune cells through IL-10 and TGF- $\beta$ . Th17 cells acquire pathogenic inflammatory phenotype with IL-23 and SAP, and they promote the release of IL-17. In the oxidative stress reaction, inflammatory cells, including neutrophils, macrophages, and lymphocytes, produce a large number of ROS and RNS under the stimulation of inflammation, which leads to DNA damage. Anaerobic bacteria activated TLR2/TLR4 on intestinal epithelial cells (IECs) and increased the level of ROS in cells. *E. coli* and ETBF also stimulate ROS production and cause DNA damage. Inflammation and oxidative stress promote each other, which further leads to the occurrence of CRC.

T cells.<sup>51–54</sup> In particular, fungi account for a large proportion of intestinal microflora, and it has been proved that fungi can induce the production of inflammasomes. Fungal recognition receptors enable downstream signals through kinase SYK and the common adaptive protein CARD9. In addition, the lack of caspase recruitment domain 9 (CARD9) or C lectin receptors (CLRs) Dectin-1 and Dectin-3 has been shown to exacerbate DSS-induced colitis in mice.

With the change of intestinal flora on TME and the deepening understanding of the immune microenvironment of CRC, microsatellite classification (dMMR/MSI-H and pMMR/MSS) has become a key biomarker for the diagnosis and treatment of CRC patients, so it has important clinical value.<sup>55</sup> Microsatellite instability (MSI) results from nucleotide insertion or deletion accumulation in the genome. MSI can be divided into MSI-high (MSI-H), MSI-low (MSI-L), and microsatellite stability (MSS).<sup>56</sup> The composition and distribution of immune cells and cytokines in TMEs of CRC patients with different MSI types of tumor-infiltrating lymphocytes are different. MSI-H tumor significantly increased the recruitment of tumor-infiltrating lymphocytes, including activated cytotoxic T lymphocytes

(CTLs), Th1 cells, and CD4<sup>+</sup>T cells, as well as NK cells and macrophages. In addition, tumor necrosis factor (TNF), IL-1, IL-6, and IFN- $\gamma$  in TME, and other related cytokines,<sup>57</sup> are added to MSI-H tumor. These cytokines regulate the activation or inhibition state of TME immunity, and different cytokines have different mechanisms and sources. Please refer to Table 2 for details.

Among them, it has been demonstrated that increased IFN expression correlates with better prognosis and induces the secretion of chemokines and adaptive immune responses with a microenvironment enriched in IFN-I compared with other CRCs. At the same time, various inflammatory mediators infiltrate to form an inflammatory TME, and continuous inflammatory stimulation leads to the exhaustion of T lymphocytes, thereby upregulating inhibitory receptors, such as PD-1.<sup>58</sup> The binding of these immunosuppressive receptors to corresponding ligands in the TME regulates anti-tumor immune responses. Activated T cells can express PD-1 on their membranes after antigen recognition and can also produce IFNs that can induce the expression of PD-L1 in various tissues. The binding between PD-1 and its ligand limits T cell activity and extreme immune responses. This immune mechanism

**Table 2. Cytokine profile associated with CRC**

Classification		Function/Effect	Source	Reference	
IFN	IFN-I	IFN- $\alpha$	inhibit virus replication, and enhance the role of MHC-II molecules. Activate DCs and increase the cytolytic activity of macrophages and NK cells, induce IL-15 production, increase T cell survival, and increase the production of stromal-derived vascular inhibitory molecules	activated macrophages, monocytes, and activated T cells	Dunn et al. and Di Franco et al. <sup>2,11</sup>
		IFN- $\beta$		Fibroblasts	Dunn et al. and Di Franco et al. <sup>2,11</sup>
	IFN-II	IFN- $\gamma$	inhibit virus replication, increase the production of MHC-I and MHC-II in cancer cells, increase the production of IL-12 in APC, promote Th1 polarization, and activate macrophages. IFN- $\gamma$ stimulation leads to nuclear translocation and phosphorylation of STAT1, leading to STAT1-TET2 binding. IFN- $\gamma$ is an effective inducer of IDO expression and a pathogenic driver of CRC progression	Th1, Tc1, NK cells	Dunn et al., Wang et al., and Du et al. <sup>2,14,17</sup>
Interleukin	IL-1F	IL-1 $\alpha$ , IL-1 $\beta$	enhance the expression of cytokines (such as IL-2 and its receptors) to stimulate T cell activation, enhance B cell proliferation and maturation, NK cytotoxicity, induce the expression of IL-1, IL-6, IL-8, TNF, GM-CSF, and PGE2 in macrophages and the expression of chemokines to play a pro-inflammatory role	monocytes, macrophages, DCs, NK, B cells, endothelial cells	Li et al., Kasprzak, Sharma and Kanneganti <sup>195-197</sup>
		IL-18	induce T cells to produce IFN- $\gamma$ and enhance NK cytotoxicity	macrophages and DCs	Li et al. and Sharma and Kanneganti <sup>195,197</sup>
		IL-36 $\alpha$ , IL-36 $\beta$ , IL-36 $\gamma$	activated monocytes, macrophages, and keratinocytes produce a variety of pro-inflammatory factors to stimulate T cells together	keratinocytes, other barrier tissues, neutrophils	Li et al., Li et al., and Xu et al. <sup>195,198,199</sup>
		IL-37	anti-inflammatory effect	monocytes, macrophages, epithelial cells, activated B cells	Li et al. <sup>195</sup>
	IL-2 family ( $\gamma$ c family)	IL-38		N terminus of apoptotic cells	Li et al. <sup>195</sup>
		IL-2	induce the proliferation of activated T cells and activated B cells, enhance NK cells' cytotoxicity, and the monocytes and macrophages' ability to kill tumor cells and bacteria	Th1	Li et al. and Heys et al. <sup>195,200</sup>
		IL-4	induce Th2 cells, stimulate the proliferation of B, T, and mast cells, upregulate B cells, macrophage MHC-II molecules and CD23 of B cells, downregulate IL-12 production to inhibit Th1 differentiation, and enhance macrophage endocytosis, mediates tumor cell proliferation, survival, and metastasis in colon cancer	Th2, Tc2, NK, NKT, $\gamma\delta$ T, and mast cells	Li et al. and Song et al. <sup>195,201</sup>
		IL-13	inhibit macrophage activation and cytokine secretion, stimulate B cell proliferation, upregulate B cell and monocyte MHC-II class molecules and CD23 expression, and mediate tumor cell proliferation, survival and metastasis of colon cancer	Th2 and mast cells	Song et al. <sup>201</sup>
		IL-15	induce the proliferation of T, NK, and activated B cells and the production of NK and CD8+ T cell factors	T, NK, monocytes, macrophages, DCs, and B cells	Li et al. and Zarogoulidis et al. <sup>195,202</sup>
		IL-21	NK differentiation, B cell activation, stimulation of T cell, Follicular helper T cells (Tfh) directed differentiation and survival	Th	Li et al. <sup>195</sup>
chemokine family	IL-3	promote growth and differentiation of hematopoietic cell precursors, mast cell growth	T, NK, and mast cells	Li et al. <sup>195</sup>	
	IL-8	mediate chemotaxis and activation of neutrophils IL-8 modulates not only stemness maintenance but also stemness promotion, such as epithelial-mesenchymal transition	monocytes, macrophages, and endothelial cells	Kasprzak and Conciatori et al. <sup>196,203</sup>	

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Table 2. Continued

Classification	Function/Effect	Source	Reference	
IL-12/IL-6 family	IL-6	promote the differentiation of myeloid stem cells, and the differentiation of B cells into plasma cells, induce the expression of acute phase proteins, enhance the proliferation of T cells, and it is vital for Th17 and Tfh-directed differentiation	Th2, monocytes, macrophages, DCs, and bone marrow stromal cells	Kasprzak and Taniguchi and Karin <sup>196,204</sup>
	IL-12	induce the proliferation of Th1, CD8+T, $\gamma\delta$ T, NK and the production of IFN- $\gamma$ and enhance NK cell and CD8+ T cell cytotoxicity	monocytes, macrophages, DCs, and B cells	Taniguchi and Karin and Engel and Neurath <sup>204,205</sup>
	IL-23	induce Th1 proliferation and IFN- $\gamma$ production, induce Th17 expansion and survival, induce macrophages to express pro-inflammatory factors (such as IL-1, IL-6, TNF), and inhibit intestinal inflammation	DCs	Taniguchi and Karin and Neurath <sup>204,206</sup>
	IL-27 (IL-30)	induce Th1 responses, enhance IFN- $\gamma$ production	DCs and monocytes	Taniguchi and Karin and Engel and Neurath <sup>204,205</sup>
	IL-35	produce immunosuppressive effects on Th1, Th2, and Th17, stimulating Treg proliferation	Treg	Taniguchi and Karin <sup>204</sup>
IL-10 family	IL-10	suppress IFN- $\gamma$ secretion and IL-2 secretion, downregulation of MHC-II molecules and cytokines such as IL-2 production, inhibition of Th1 differentiation, inhibition of T cell proliferation, and enhancement of B cell differentiation	Th, Tc, B cells, monocytes, and macrophages	Li et al. and Landskron et al. <sup>195,207</sup>
	IL-19	regulate Th1 activity	monocyte	Li et al. <sup>195</sup>
	IL-20	regulate skin inflammation	monocyte and keratinocyte	Li et al. <sup>195</sup>
	IL-22 (IL-T1F)	inhibition of Th2 and secretion of IL-4	T cells	Li et al. and Wei et al. <sup>195,208</sup>
	IL-24 (MDA-7)	induce TNF, IL-1, IL-6 expression and anti-tumor activity	Th2, monocytes, and macrophages	Li et al. <sup>195</sup>
	IL-26	enhance epithelial cell production of IL-8 and IL-10	T cells and NK cells	Li et al. and Niess et al. <sup>195,209</sup>
IL-17 family	IL-17	pro-inflammatory effect, stimulate the expression of TNF, IL-1 $\beta$ , IL-6, IL-8, G-CSF and other factors, inhibit intestinal inflammation	T cells	Hurtado et al. and Wu et al. <sup>210,211</sup>
	IL-25	induce IL-4, IL-5, IL-13 expression and Th2 related pathological changes	Th1, macrophages, and mast cell	Li et al. and Jou et al. <sup>195,212</sup>
other interleukins	IL-7	induce differentiation of lymphocytic stem cells into T and B lineage progenitor cells, and activate mature T cells	bone marrow and thymic stromal cells	Zarogoulidis et al. <sup>202</sup>
	IL-33	promote inflammatory response and induce Th2-mediated innate and adaptive immune responses	macrophages, DCs, fibroblasts, and mast cell	Jou et al. <sup>212</sup>

(Continued on next page)

Table 2. Continued

Classification	Function/Effect	Source	Reference
growth factor	stimulate the growth of monocytes, neutrophils, eosinophils, and basophils, and activate macrophages	Th, macrophages, fibroblasts, mast cells, and endothelial cells	Aliper et al. <sup>213</sup>
	promote tumor angiogenesis, and it is closely related to the invasion and metastasis of CRC	endothelial cells, smooth muscle cells, some mesenchymal and stromal cells	Maryam et al. <sup>214</sup>
TGF	associated with tumor metastasis and invasion	macrophages, brain cells, and keratinocytes	Lee et al. <sup>215</sup>
	pro-inflammatory effects include induction of monocyte and macrophage chemotaxis; anti-inflammatory effects, such as inhibition of lymphocyte proliferation	Th, B cells, macrophages, and mast cells	Lee et al. <sup>215</sup>
TNF	cause cachexia and tumor cytotoxicity, induce cytokine secretion, and activate macrophages	Th, monocytes, macrophages, DCs, mast cells, NK cells, and B cells	Kasprzak and Landskron et al. <sup>196,207</sup>
	cause tumor cytotoxicity, enhance the endocytosis of neutrophils and macrophages, and participate in the development of lymphoid organs	Th1, Tc	Maryam et al. <sup>214</sup>

controls immune tolerance to self-antigens through the negative regulation of the immune response.<sup>59</sup> Currently, MSI is considered a well-known biomarker of PD-1 blockade. Studies have shown that specific subpopulations of CRC can be better treated. Deciphering the mechanisms behind these inconsistencies may provide new insights into identifying advanced treatments and cures for CRC.

More and more evidence shows that the immune system controls the carcinogenicity of the intestine. The communication of microbial communities is connected to the immune system by diverse mechanisms, including inflammatory body induction<sup>60</sup> and TLR signaling.<sup>61</sup> Among them, NOD-like receptors (NLRs) and TLRs are associated with the incidence of CRC.<sup>62</sup> According to data, 20%–30% of CRC patients have inflammation. The inflammation mechanism is recognized as the driving factor of tumor occurrence, which is also reflected in many CRC risks of patients with IBD.<sup>43,63,64</sup> Microbes can form an inflammatory microenvironment, which in turn may affect the composition of microbes in the gut. The occurrence of CRC is related to intestinal microbes, inflammatory microenvironment, and immune regulation of the intestinal tract.<sup>24</sup>

#### Induce oxidative stress reaction

Chronic inflammation caused by gut microbiota is common in CRC. Due to the irritation of inflammation, inflammatory cells will release a lot of reactive nitrogen species (RNS) and reactive oxygen species (ROS), which will result in DNA damage and activate oncogenes or inhibit oncogenes, which is conducive to the further development of CRC. Gut microbiota can also directly release ROS, such as superoxide production induced by *E. faecalis*, which damages the DNA of epithelial cells by bystander effects.<sup>65,66</sup> *In vivo* and *in vitro* studies have shown that *E. faecalis* can produce a potent mutagen called hydroxyl radical,<sup>67</sup> which can cause point mutations, DNA breakage, and protein-DNA cross-linking, leading to chromosome instability of IECs and thus the risk of CRC<sup>68</sup> in addition to *E. faecalis* activating the epidermal growth factor receptor and promoting cell proliferation through the production of hydrogen peroxide. In contrast, anaerobic bacteria start TLR2 or TLR4 on IECs (intestinal epithelial cells) to increase ROS levels inside cells and promote cell proliferation and cholesterol synthesis.<sup>69</sup> ETBF and *E. coli* in the intestinal microenvironment are also capable of causing ROS generation in the IECs.<sup>70</sup> ETBF<sup>36</sup> causes colitis and increases the activity of the spermine oxidase (SMO) gene in the colon, stimulating ROS production and causing DNA damage.<sup>71</sup> The findings suggest that oxidative stress when acting as a metabolic regulator can promote STING-mediated immune response in DCs and emphasize the role of small ubiquitin-like modifier proteins specific protease 3 (SEN3) as a STING signaling-induced overflow in the TME with metabolic abnormalities.<sup>72</sup> In summary, intestinal flora not only have pro-tumorigenic effects on their own but they can also further contribute to colorectal carcinogenesis by producing ROS and RNS (Figure 2).

#### Generation of metabolites

Microbiota produce metabolites of host immunity and CRC and, in addition to direct interactions, the gut microbiota produces diverse



combinations of metabolites to generate specific immune responses that might immediately benefit or harm the host. In healthy individuals, the normal metabolism of colonic cells can maintain the balance of commensal communities and the anaerobic state in the human gut.<sup>73</sup> Diet, disease, or other injuries can cause transformation and abnormal metabolism of colonic cells, which can lead to disturbance of the host-commensal bacterial symbiosis and dysregulation of the intestinal flora.<sup>73,74</sup> Bacterial metabolites, including hydrogen sulfide, secondary bile acids, tryptophan catabolites, polyamines, and short-chain fatty acids, can synergize with other T cell-targeted immunotherapies as well.<sup>22</sup> For example, short-chain fatty acids (SCFAs) are mainly composed of propionate, acetate, and butyrate.<sup>74</sup> SCFAs, especially butyrate, can inhibit DNA damage and pathogen proliferation by significantly reducing the pH of fecal matter in the gut.<sup>75</sup> Extracellular SCFAs also interact with cell surface receptors, such as G protein-coupled receptors (GPCRs), and take part in regulating intestinal immune reactions.<sup>76</sup> Butyrate plays a vital part in the cell as a histone deacetylase inhibitor, downregulates IL-6 to attenuate intestinal inflammatory responses,<sup>77</sup> induces ROS production,<sup>78</sup> and inhibits lipopolysaccharide-induced pro-inflammatory mediators in DCs and macrophages.<sup>79</sup> In the DSS-induced colitis model, butyrate can alleviate inflammatory reactions by promoting M2 macrophage polarization.<sup>80</sup> Besides, SCFAs can accelerate the generation of Treg cells and rapidly release Th1 cytokines such as TNF and IFN- $\gamma$  to enhance immune reaction.<sup>81</sup> Metabolites produced by intestinal flora can cause a robust adaptive immune response, so bacterial metabolites have the potential to produce synergistic effects in immunotherapy.

### Intestinal flora affects IFN in the treatment of CRC

Most research focuses on how gut microbiota activate gut immunity to influence tumor immunotherapy. Therapies related to the innate immune response, including CD47 blockade, rely on the rapid immune reaction in the TME.<sup>22</sup> The IFN pathway is significant for activating cytotoxic T cells, which is the core of tumor immune monitoring and immunotherapy. Studies have shown intestinal flora can regulate the human immune mechanism by affecting IFN release.

It was shown that systemic administration of *Bifidobacterium* could cause a mass of accumulation in tumors, and non-responsive mice were endowed with anti-CD47 immunotherapy responses in an IFN-dependent manner and IFN gene stimulator (STING). After anti-CD47 treatment, local delivery of bifidobacteria effectively promoted the cross-infiltration of DCs and activated STING signaling. This study identified the gut microbiome as a priority to colonize tumor sites and promote immunotherapy through STING signaling.<sup>22</sup> At the same time, some studies have found that the modulation of gut microbiota can affect the host response to myriad types of cancer treatment, especially immunotherapy.<sup>82–84</sup> Oral bifidobacteria failed to promote CD47 blockade in mice conditionally knocked out in DCs. This finding suggests that IFN-I signaling in DCs plays an important role in treating *Bifidobacterium*-promoting CD47 blockade. IFN-I signaling can promote cross-stimulatory responses of DCs, thereby enabling adaptive immune reactions.<sup>85,86</sup> Mean-

while, it was shown that *Bifidobacterium bifidum* promotes CD47-founded immunotherapy in a T cell-dependent and IFN- $\beta$ -dependent method, and its anti-tumor function depends on STING signaling within DCs. The STING pathway adjusts the expression of IFN-I in anti-CD47-mediated anti-tumor effects. STING agonists targeting tumor tissue may be effective.<sup>22</sup> Active migration and continuous production of metabolites by live bacteria in the gut would activate the STING path in DCs. The synergetic effects of STING activators and the cyclic guanosine monophosphate-adenosine monophosphate (cGAS) product cGAMP on STING activation must be further explored.<sup>44</sup>

Modulation of the microbiome may become a new immunotherapeutic strategy.<sup>87</sup> Naive Th cells (Th0) usually mature and differentiate into Treg, Th1, Th2, or Th17 lineages. Among the classic Th1 cytokines, IFN- $\gamma$  plays an anti-tumor role in TME, while Treg or Th2-related cytokines, including IL-4, IL-5, and IL-10, can promote tumors in the TME. Depletion of the gut microbiome led to marked growth in the number of T cells secreting anti-tumor IFN- $\gamma$  and a corresponding reduction in the amount of pro-tumor IL-17a and Tc1 and IL-10-secreting immune populations. Symbiotic intestinal fungi promote activation of the inflammasome and maturation of IL-18 during colitis,<sup>44</sup> thus facilitating the secretion of IFN- $\gamma$  by CD8<sup>+</sup> T cells and the restoration of the epithelial barrier. Some fecal strains from healthy providers have been shown to promote the proliferation of IFN- $\gamma$ <sup>+</sup> CD8<sup>+</sup> T cells in the gut and to heighten ICB efficacy in mice with CRC.<sup>54</sup> In the CD4<sup>+</sup> Th cell subset, although Th1 cells are the main cells producing IFN- $\gamma$ , Th1 cells may alter function in the intestinal TME.<sup>88</sup> Aerobic glycolysis can regulate the function of CD4<sup>+</sup> T cells and affect the secretion of IFN- $\gamma$ .<sup>17</sup> An experiment<sup>89</sup> analyzed the gastrointestinal tract secretion of human BCL-G in healthy and unhealthy conditions; this *in vitro* study had shown that TNF- $\alpha$  and IFN- $\gamma$  act synergistically to increase BCL-GS/L and generate apoptosis in primary human colonic-like organs and colonic epithelial cell lines. IFN-I signaling is initiated by a nucleic acid sensor, which is the primary immune signal to detect invading pathogens. In the TME of CRC patients, aerobic glycolysis impairment often occurs, promoting further strengthening of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) binding to IFN- $\gamma$  AU-rich elements (IFN- $\gamma$ AREs), thus reducing IFN- $\gamma$  secretion.<sup>90</sup> Studies have shown that *Lactobacillus* (LAB) double-stranded RNA can activate intestinal DCs to produce TLR3-mediated IFN- $\beta$ , thereby preventing infection and colitis.<sup>91</sup> In addition, LAB can also induce IFN-I production via cGAS and RLR (RIG-I-like receptors).<sup>92</sup> The IFN-I signaling is initiated by the activation of cyclic GMP-AMP synthase-stimulator of interferon genes (cGASSTING), which is indispensable for innate resistance to RNA and DNA viruses. In-depth studies have shown that stimulatory activation of cGASSTING signaling results from membrane vesicle-mediated DNA scheduling in bacteria<sup>93</sup> and is supposed to associate the immune response with DNA sensing.<sup>94</sup> Hence, special attention must be paid to controlling the activation of the IFN-I system initiated by RNA and DNA sensors to maintain a steady state of the intestinal mucosa in response to colitis and pathogen infection.

In summary, the gut microbiome mediates the inflammatory response and oxidative stress in the intestine. At the same time, the metabolites produced by microorganisms can affect the immune status of the intestine, which might be advantageous or detrimental to treating CRC. Regulating the composition, distribution, and quantity of the intestinal microbiome and thus modulating the IFN cascade signaling to adjust the intestinal microecological and immune status for treating CRC is a direction worth further exploration.

#### USE OF OV AND IFN TAMPERING IN THE TREATMENT OF CRC

OVs are a fresh therapeutic drug that promotes anti-tumor response through the dual mechanism of selective killing of tumor cells and induction of systemic anti-tumor immunity.<sup>95,96</sup> Compared with other drugs, OVs have unique advantages, such as specific infection of cancer cells and improvement of TME. OVs can specifically infect cancer cells without affecting normal cells. On the one hand, OVs can naturally identify cancer cells by the characteristics of cancer cell biomarkers<sup>97</sup> or the properties of gene and protein expression.<sup>98</sup> On the other hand, genetic engineering has further enhanced the specificity of OVs. Virus infection can cause cancer cells to produce soluble antigens and danger signals, release IFN, and improve the TME.<sup>99–101</sup> In different subtypes of CRC, MSI leads to neoantigens that are more likely to be recognized by the immune system in the tumor, and more cytokines are accumulated here. Hence, CRC patients with MSI type have an ideal response to OV therapy.<sup>102</sup>

#### The primary mechanism of OVs

OVs selectively infect and kill cancer cells while enhancing the immune reaction of the body against the tumor.<sup>103</sup> The ideal curative effect of OVs depends on the specific recognition of cancer cells by OVs and the rapid proliferation of cancer cells. First, the virus must combine with virus-specific receptors on the cancer cell surface. Some studies have improved the tumor-specific recognition ability of the OV to enhance the tumor-killing effect.<sup>104,105</sup> Second, the rapid proliferation ability of cancer cells can promote the replication and amplification of viruses, and the genetic mutation in cancer cells can specifically improve the selective replication ability of viruses in tumor cells.<sup>106</sup>

#### Direct lysis of tumor cells

OV replication results in CRC cell apoptosis through a variety of mechanisms, such as activating apoptotic genes and related proteins, especially p53 and caspase genes. Studies have shown that VSV can activate the transcription and translation of the p53 gene in colon cancer CT608 cell line and mediate apoptosis of colon cancer cells.<sup>107</sup> Another study showed that late high expression of the p53 gene could effectively enhance cancer cell killing.<sup>108</sup> In addition, reovirus induces apoptosis by upregulating the expression of caspase and other genes in CT608 cell line.<sup>109</sup> OV can also be used as a genetic engineering vector to treat CRC. A study has shown that oncolytic adenovirus ZD55 carrying multisubstrate deoxyribonucleoside kinase of *Drosophila melanogaster* (DM-DNK) induces high and sustained expression of suicide genes in CRC cells and activates nucleoside analogues (NAs), thus achieving an anti-tumor effect.<sup>110</sup>

OVs can also induce apoptosis of cancer cells by activating the apoptosis pathway of CRC cells, such as PI3K, mitogen-activated protein kinase (MAPK), and the NF- $\kappa$ B signal pathway. Studies have shown that oncolytic adenovirus induces apoptosis in CRC cell line SW620 by activating the PI3K/Akt pathway.<sup>111</sup> Another study shows that adenovirus can provide a more powerful killing effect on CRC cells by activating MAPK signal.<sup>112</sup> In addition, reovirus can activate the NF- $\kappa$ B signal pathway and induce apoptosis of CRC cells.<sup>113</sup>

What is more, the daughter viruses released after cancer cell lysis can infect surrounding cells and generate a cascade response to expand the lysis. The killing effect of lysing viruses on cancer cells increases with time.<sup>114,115</sup>

Besides direct lysis of cancer cells, OVs attack tumor vasculature and eventually lead to thrombosis and vascular atrophy, leading to ischemia and hypoxia in cancer cells and reducing subsequent viral spread within tumor tissue.<sup>116</sup> The strategy of lyssavirus attack on the tumor vascular system is to target vascular endothelial cells within the tumor while selectively enhancing tumor vascular permeability.<sup>117</sup> An experimental study showed that oncolytic adenovirus can infect, replicate, and expand in tumor-associated endothelial cells, causing inhibition of tumor angiogenesis.<sup>118,119</sup> In contrast, in clinical trials, the tumor lysing virus JX-594 was found to cause acute tumor vascular disruption, causing a significant decrease in blood perfusion at tumor sites.<sup>120,121</sup> In addition, lysing viruses can inhibit angiogenesis. Vascular epithelial growth factor (VEGF), a classical angiogenic factor, was found to be significantly reduced by cancer cells infected with lysing viruses.<sup>122,123</sup> At the same time, the secretion capacity of VEGF was also reduced in surrounding uninfected cells in the presence of relevant cytokines.<sup>124</sup>

#### Induction of organismal immune activity

Compared with directly cracking cancer cells and attacking tumor blood vessels, it is more important to induce immune activity.<sup>125</sup> OV therapy interacts with various constituent parts of the immune system in different forms and methods, improving the TME, making it favorable for fuller contact with immune cells, and activating tumor-specific cell reactions.<sup>126</sup>

OV directly acts on innate immune cells and enhances the immunogenicity of the TME. In the virus replication process, the virus's structural protein is often expressed on the surface of cancer cells. The host's antiviral immune response can recognize these antigens expressed on the surface of cancer cells and kill tumor cells.<sup>127</sup> In the process of viral oncolysis, cancer cells also produce molecules such as high-mobility group protein B1 (HMGB1), calreticulin (CRT),<sup>128,129</sup> and heat shock proteins (HSPs),<sup>130,131</sup> which activate the anti-tumor immune reaction.

OV infection can activate the TLR signaling pathway in the tumor site and cause acute inflammatory reaction,<sup>132</sup> which results in the production of IL-2, chemokines, cytokines, IFN- $\gamma$ , and granulocyte-macrophage colony-stimulating factor (GM-CSF),<sup>118,133–136</sup> which can directly kill cancer cells, induce chemotactic immune cells to infiltrate

into the cancer location, and enhance immune reaction. The infiltration of immune cells caused by virus infection and the production of cytokines secreted by immune cells improve the effect of infiltrating immune cells, resist tumor-induced immunosuppression, and benefit the activation of immune reaction. For instance, GM-CSF can significantly strengthen the anti-tumor immune effect. GM-CSF is an inducing factor to stimulate the replication of hematopoietic stem cells,<sup>137,138</sup> and it can promote the production of tumor-specific cytotoxic T lymphocytes (CTLs) as well, which can directly kill tumors.<sup>139</sup> Besides, TNF- $\alpha$  and IL-2 have also been proved to have anti-tumor effects.<sup>140,141</sup> When IL-2 is expressed by oncolytic VSV, regulatory T cells in the neovascular system and tumor tissues can be reduced,<sup>142,143</sup> and T cell-mediated immune reaction could be induced in the immune cancer stem cell model.

Besides infiltration of inflammatory cells and secretion of cytokines, the OV itself will release viral death signals. Infected cells will also release cell death signals, tumor-associated antigens (TAAs), endogenous danger signals, tumor-derived cytokines, and even immunomodulatory factors<sup>144,145</sup> to activate DCs, enhance their antigen presentation ability, activate innate immune responses, and activate DCs, neutrophils (NEs), macrophages (m $\phi$ ), NK cells, and other immune cells, enhancing the body's innate immunity.<sup>146,147</sup>

After OV infects tumor cells, it is easy to cross-present tumor antigens, activate acquired immune effects, and cause immunogenic cell death (ICD). At the same time, it leads to the release of PAMP and TAA, and finally activates DAMP and leads to the release of cytokines. First of all, OV promotes the expression of MHC-I and stimulates CD8<sup>+</sup> T cells. In addition, the infection of the OV provides inflammatory stimulation for the body, thus promoting antigen processing and the formation of the MHC-II complex and enhancing the adaptive immunity of the body.<sup>148–150</sup> In addition, OV infection can induce ICD of tumor cells, including cell death, apoptosis, and autophagy.<sup>151</sup> Immunogenic death leads to the exposure or release of PAMPs<sup>152</sup> and TAAs<sup>153</sup> as danger signals. PAMPs activate a series of signals, stimulate inflammatory corpuscles, activate different transcription factors, and finally produce pro-inflammatory cytokines and DAMP.<sup>154</sup> The release of pro-inflammatory cytokines reversed the balance between pro-inflammatory factors and anti-inflammatory factors in the TME, thus breaking the immunosuppression state of the tumor. In addition to breaking tumor-induced immunosuppression, immune cells that release cytokines can also be recruited to enhance the host's anti-tumor immunity<sup>155</sup> (Figure 3).

#### Interaction between IFN and OV

IFN has many functions, such as anti-virus, inhibiting cell proliferation, regulating immunity, and anti-tumor. Numerous cells have a complete IFN pathway, have immune clearance against OVs, and cannot carry on playing a therapy role in cancer cells.<sup>156,157</sup> Therefore, if the OV is to proliferate rapidly in the body, it is necessary to inhibit the IFN response. On the other hand, the secretion of IFN stimulates the immune response in various ways, thus the immunosuppressive response of TME can be broken, which is very beneficial to tumor

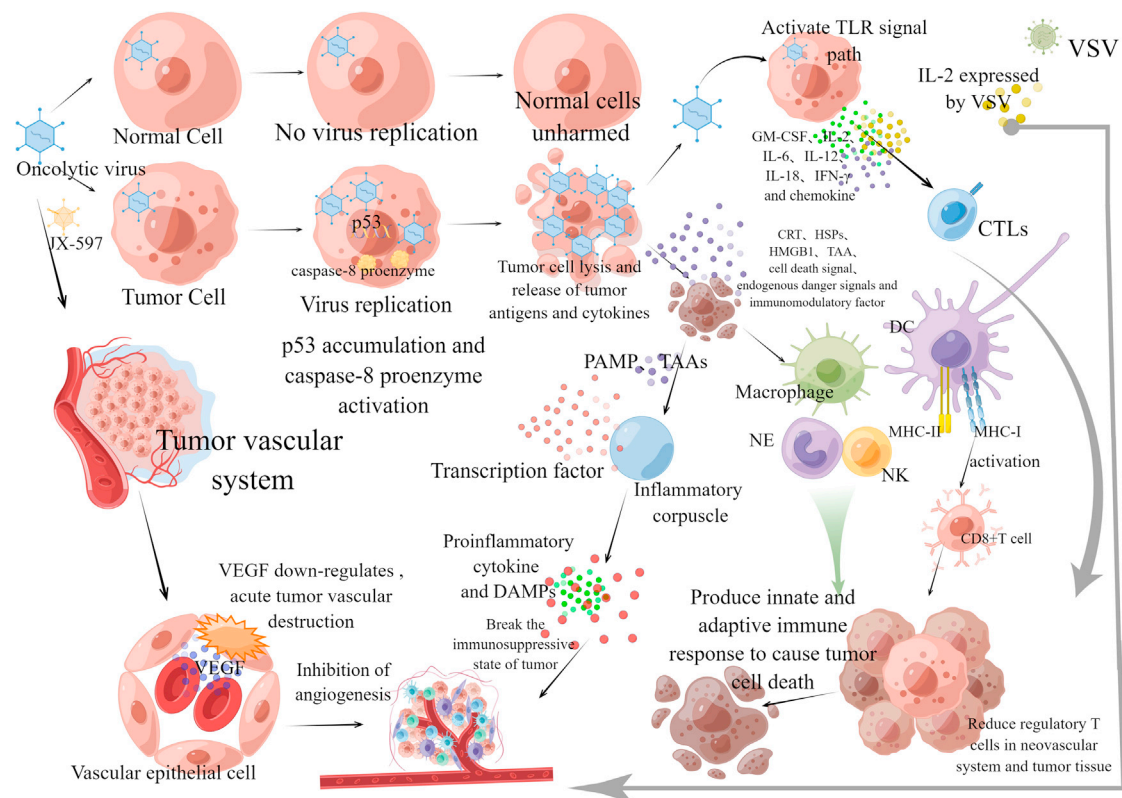
treatment.<sup>96,158,159</sup> Therefore, some OVs are designed to express IFN, to play a more powerful anti-tumor role.

#### Inhibition of the anti-viral effect of IFN-I

An important aspect of the interaction between OVs and IFN is that OVs can inhibit the anti-viral response of IFN through several important pathways, such as inhibiting the signal pathway of IFN-I, inhibiting the synthesis of IFN proteins, and producing bait proteins that combine with IFN to inhibit the anti-viral effects of IFN.

One strategy of OVs to inhibit the anti-viral response of the IFN cascade is to interfere with the downstream signal that produces IFN-I.<sup>120</sup> This pathway is mainly achieved by blocking the key steps of IFN response and can also be achieved by blocking the function of ISG. In addition, part of the protein shell of OVs can directly inhibit the initiation of IFN. The protein encoded by some OVs can block the key steps of the IFN-I signaling and inhibit the initiation of the IFN-I signaling pathway. For example, herpes simplex virus (HSV) encodes asparagine deaminase UL37, modifies and inactivates retinoic acid-induced gene protein (IRIG-I),<sup>160</sup> and inhibits the production of IFN. The E1A protein of oncolytic adenovirus downregulates the STAT activator protein-1 (STAT1), which decreases the signal transduction when IFN-I combines with its receptor, stops the production of immune serum globulin 3 (ISG3), and also blocks the interaction between peripheral blood signal transducer and transcription activator-1 (STAT1) and IFN regulatory factor (IRF1), thereby avoiding the induction of transcription of ISGs.<sup>161</sup> The C6 protein encoded by an oncolytic poxvirus (VV) binds to STAT2, and paramyxovirus encodes V and W proteins interacting with STAT1 and STAT2,<sup>162</sup> which inhibits the signal transduction of IFN-I. At the same time, HSV can also block cyclic GMP-AMP synthetase (cGAS) and the downstream stimuli STING,<sup>163</sup> which leads to preventing IFN-I production. Moreover, the oncolytic measles virus (MV) can bind and inactivate phosphatase PP1, thus preventing the activation and dephosphorylation of RIG-I and MDA5<sup>164</sup> and also preventing the initiation of type III IFN signaling cascade. Besides perturbing the secretion or signal of IFN-I, a subset of viruses directly intercepts ISG and its multiple features. For instance, the E1A protein of adenovirus combines with the nuclear complex to avoid histone ubiquitination, thus inhibiting chromatin opening and repressing ISG transcription.<sup>165</sup> In addition, part of the OV protein coat can block IFN-I response through various pathways, one of which is to avoid the start of the IFN-I signal cascade by finding that virus protein binds to viral RNA and RIG-I.<sup>166</sup> Some OV proteins can also directly prevent the stimulation of NF- $\kappa$ B and IRF3, thus preventing the transcription of IFN mRNA<sup>167,168</sup> and inhibiting the activation of IFN. Similarly, experiments have confirmed that herpes simplex virus<sup>169</sup> and reovirus<sup>120</sup> also have the above mechanisms, and they can also block the production of type III IFN.

Another way to inhibit the production of IFN-I is to inhibit its transformation into protein. VSV uses this strategy, and its matrix protein interacts with the nuclear pore, preventing mRNA export to the cytoplasm.<sup>170</sup> The effect of inhibition is not only aimed at IFN mRNA but also affects many genes, leading to the widespread blocking of protein production.



**Figure 3. OV is a new therapeutic drug that promotes anti-tumor response through the dual mechanism of selective tumor cell killing and inducing systemic anti-tumor immunity**

OV selectively infects and kills tumor cells, but virus replication does not occur in normal cells of the human body, so normal cells continue to survive. However, when the OV infects tumor cells, viral replication leads to the accumulation of p53 and activation of caspase-8 proenzyme, leading to tumor cell apoptosis and lysis. Besides directly cracking cancer cells, the OV also attacks tumor blood vessels and causes vascular atrophy, among which JX-594 can cause acute tumor blood vessel destruction, and, at the same time, the OV can downregulate the secretion of VEGF by tumor cells and surrounding uninfected cells and inhibit the formation of tumor vascular system. In addition, IL-2 expressed by VSV can reduce regulatory T cells in the neovascular system and tumor tissues. Compared with directly cracking cancer cells and attacking tumor blood vessels, inducing immune activity is more important. In the process of virus oncolysis, OVs replicated in tumor cells are released, as well as CRT, HSPs, HMGB1, TAA, cell death signals, endogenous risk signals, and tumor-derived cytokines. Among them, immunogenic death leads to the exposure or release of PAMPs and TAAs as danger signals. PAMPs activate a series of signals, stimulate inflammatory corpuscles, and release pro-inflammatory cytokines and DAMPs, thus breaking the immunosuppressive state of tumors. In addition, virus oncolysis can also recruit immune cells that release cytokines. OV infection can activate the TLR signaling pathway in the tumor site, resulting in the release of cytokines GM-CSF, IL-2, IL-6, IL-12, IL-18, and IFN- $\gamma$ , and chemokines, which can directly have toxic effects on tumor cells, stimulate chemotactic cells to infiltrate into the tumor site, and induce anti-tumor immunity. At the same time, cell death signals, TAA, endogenous danger signals, tumor-derived cytokines, and immunoregulatory factors released in the process of oncolysis can activate immune cells such as DCs, NK cells, macrophages, and neutrophils, and activate the innate immune response of the body. At the same time, after the OV infects tumor cells, it is easy to start adaptive immune effect, promote MHC-I expression, activate CD8+T cells, and promote the formation of MHC-II.

An animal experiment confirmed that an oncolytic VSV deleted its matrix protein in methionine 51, which inactivated the feature, thus enhancing the specificity for tumor cells with IFN pathway deficiency.<sup>171</sup>

Another way to inhibit IFN-I is to produce bait protein to bind specifically with IFN, to prevent IFN from binding with cell receptors and starting a signal cascade reaction. For example, an oncolytic pox virus (VV) encodes B18R,<sup>172</sup> a kind of soluble IFN-I receptors on infected cells, thus preventing IFN from combining with cell receptors. The protein B18R of Wyeth strain OVJx594 of oncolytic pox virus is naturally truncated,<sup>173</sup> which seriously reduces its affinity for IFN.

#### **Enhance the anti-tumor effect of IFN-II**

Another important aspect of the interaction between OVs and IFN is to promote the anti-tumor effect of IFN. It is mainly realized by promoting the activation of the IFN-II signal pathway and designing OVs into a vector that can express IFN by genetic engineering.

Some OVs can promote the body to produce IFN. For instance, HSV-1 irritates NK cells to produce IFN-II through TLR2/NF- $\kappa$ B signaling pathway and mobilizes DCs, macrophages, and other types of immune cells.<sup>174,175</sup> The accumulation of these cancer cells improves the TME in the gut and enhances the lethal effect on cancer cells. Oncolytic virus has a similar effect. It can induce a high level of IFN-II expression by



facilitating the transcription activity of IFN regulatory factor 3 (IRF-3)<sup>176</sup> and enhancing the anti-tumor effect of OV.

In addition, modification of the OV to make it a carrier for IFN expression can also enhance the killing effect on cancer cells. An *in vitro* experiment proved that a VVB18R deletion mutant that also overexpressed IFN-II only replicated in tumors, and its killing effect on cancer cells was significantly increased.<sup>177</sup> Similarly, another experiment also proved that, compared with the parent virus, the oncolytic MV encoding IFN-II is more effective in controlling the killing of cancer cells and the spread of tumors.<sup>178</sup> For adenovirus, with the adenovirus variant encoding IFN- $\gamma$ ,<sup>179</sup> it was found that the virus not only prevented cancer vascularization<sup>180</sup> but also improved cancer-killing and immune stimulation ability. In addition, the oncolytic VSV was also designed to express IFN. An experiment confirmed that the variant expressing IFN- $\gamma$  from oncolytic VSV can preferably control the tumor growth in multifarious CRC models via strengthening the anti-tumor immune response.<sup>181</sup> Similarly, research tested a IFN-II-engineered VSV variant and found that, compared with VSV, the virus can inhibit cancer and improve immune reaction by decreasing tumor infiltration of Tregs and enhancing tumor infiltration of CTLs.<sup>182</sup> It is worth noting that oncolytic VSV expressing IFN-II is presently in clinical tests on solid tumor,<sup>183</sup> which has broad development prospects (Table 3).

#### INTERACTION BETWEEN OV, INTESTINAL FLORA, AND IFN

Cancer is associated with genetic mutations that activate proto-oncogenes and cause diseases related to cell growth. In the process of gene mutation, there will be the non-genetic or genetic inactivation or activation of specific genes that inhibit or irritate tumor metastasis and proliferation. Tumor immunotherapy is prospective and has been considered an important direction for treating tumors in the past 3 to 5 years. It is considered to be an effective alternative to radiotherapy, chemotherapy, and surgery.<sup>184</sup> The OV and intestinal flora are promising in treating CRC by controlling IFN changes during immunotherapy.

OV-mediated immunological therapy is a new cancer treatment method using natural or genetically modified viruses. OVs are mainly used as gene vectors carrying specific checkpoint antibodies, which regulate immune checkpoints in the TME through the synergistic effects of cytokine and chemokine secretion, oncolysis, and so on. Genetically modified OVs can forthrightly produce checkpoint antibodies to destroy cancer cells in the TME. For instance, a novel recombinant myxoma virus (vPD-1) infects cells and produces soluble PD-1, which induces and maintains anti-tumor reactions and is more secure and more efficient than  $\alpha$ PD-1 antibodies.<sup>185</sup> NK cells and CD8<sup>+</sup> T cells require OV-mediated immune activation to enhance the efficacy of antibodies that block PD-1/PD-L1- or cytotoxic T lymphocyte-associated protein 4 (CTLA4)/B7-mediated responses.<sup>186</sup> OV encoding specific protein gene-IFN- $\beta$  can significantly enhance checkpoint-blocking therapy. Vaccines made from OVs, such as adenovirus and vaccinia virus, show high oncolytic properties and significantly induce immune

reactions against cancer patients with less lymphocyte distribution in the TME. Intestinal microbiota mediates the regulation of more than 100 trillion microorganisms in human intestines. A great deal of evidence shows that the changes in intestinal microflora are related to various cancers. Different microbiota or metabolites can contribute to changes in the immune reaction, such as the activation of Th17 cells and Tregs. Recent studies suggested that the immunocompetence and anti-tumor effect of checkpoint-blocking cures are related to different microbial species of different tumor types. Specific gut microbiota contributes to the efficacy of anti-CTLA-4 and anti-PD-1 treatment by diverse mechanisms.<sup>187</sup> *Bifidobacterium fragilis* promotes the activation of CTLs and DCs in tumor-draining lymph nodes, therefore actuating immune reactions. Interestingly, the reinforcement has nothing to do with the transfer of microorganisms to the external position of the intestine. The symbiotic derivative factors may activate DCs, while soluble systemic chemokines promote DC transfer to TME. Furthermore, during CTLA-4 blockade, the memory T cell response of mice with specific immunomodulatory species was enhanced. Preceding studies have discovered that IFN- $\gamma$  production and Th1 response are memorized in mice specific to *B. fragilis* treated with CTLA-4 monoclonal antibody (mAb),<sup>188</sup> so it may be very important to study the key mechanism of intestinal flora as an immunotherapy adjuvant to enhance checkpoint-blocking therapy. Implanting immunogenic intestinal microflora (*B. fragilis*, etc.) into selected cancer patients can prepare living annotations, restore the anti-cancer T cell responses to confront cancer cells, and promote the anti-checkpoint effect.<sup>189</sup>

The OV and intestinal microbiota jointly regulate the immune checkpoint in TME. Both intestinal flora and the OVs can strengthen the treatment of CRC by anti-CTLA-4 and anti-PD-1 by various mechanisms.<sup>184</sup> Intestinal microbial antigens, potential pathogens, and OVs lead to PAMPs. PAMPs activate a series of signals, stimulate inflammatory bodies, activate different transcription factors, and ultimately release pro-inflammatory cytokines and DAMPs.<sup>190</sup> At the same time, the dense microbiota and OV infection in the intestine can produce all kinds of molecules identified by TLRs, thus causing inflammatory reactions and releasing IFN, other cytokines, and chemokines. Some intestinal microorganisms, such as *Bifidobacterium*, can produce immune responses through IFN gene stimulators (STING) and IFN dependence. The OV, such as HSV,<sup>163</sup> can block the cGAS and the downstream effector STING, which results in a block of IFN secretion. At this time, OVs can inhibit the immune inflammatory reaction of intestinal bacteria by inhibiting STING and reducing IFN secretion. The OV itself has the effect of promoting the body to produce IFN. For instance, HSV-1<sup>191</sup> stimulates pDC to produce IFN-I and can also stimulate NK cells to release IFN-II<sup>174,175</sup> by the TLR2/NF- $\kappa$ B signal pathway. For intestinal flora, the IFN-I signaling in DCs is crucial to treating *Bifidobacterium*-promoting CD47 blocking. IFN-I signal can drive the cross-stimulation of the DCs and further promote adaptive immune reactions,<sup>85,86</sup> which indicates that the OV can promote the immunotherapy of intestinal bacteria against CRC by stimulating IFN secretion. To sum up, there is a close relationship between OV, intestinal flora, and IFN. We need to explore the relationship to achieve the goal of treating CRC.



**Table 3. Summary of the basic mechanism of OV regulating IFN**

Effect on IFN	Oncolytic virus	Mechanism of action	Authors	Year
Anti-viral effect of inhibiting IFN-I	herpes simplex virus	encoding asparagine deaminase UL37	Watanabe et al. <sup>192</sup>	2018
		modify and inactivate IRIG-1	Ishino et al. <sup>191</sup>	2021
		blocking the synthesis of cGAS by cyclic GMP-AMP and its downstream effector STING	Glorioso et al. <sup>163</sup>	2021
		the combination of viral protein with viral RNA and RIG-I prevents the initiation of IFN-I signal cascade	Linder et al. <sup>166</sup>	2021
		direct connection of virus protein inhibits the activation of NF- $\kappa$ B and IRF3 downstream and prevents IFN mRNA transcription	Ottolino-Perry et al. <sup>169</sup>	2015
	oncolytic adenovirus	E1A protein downregulates signal transduction and STAT1 and reduces the signal transmitted when IFN-I binds to its receptor	Delwar et al. <sup>193</sup>	2018
		prevents the formation of ISG3 and blocks the interaction between peripheral blood signal transducer and STAT1 and IRF1	Komatsu et al. <sup>161</sup>	2016
		preventing the induction of ISG transcription	Hu et al. <sup>194</sup>	2018
		the binding of the E1A protein to the nuclear complex prevents histone ubiquitination, chromatin opening, and ISG transcription inhibition	Lipatova et al. <sup>165</sup>	2021
		Paramyxovirus	encoding v and w proteins interacting with STAT1 and STAT2	Danziger et al. <sup>162</sup>
reovirus	viral proteins combine with viral RNA and RIG-I to avoid the start of the IFN-I signaling cascade. Directly inhibit the activation of NF- $\kappa$ B and IRF3 downstream, thus preventing the transcription of IFN mRNA and inhibiting the activation of IFN	Lee et al. <sup>120</sup>	2020	
oncolytic MV	inactivated PP1 was combined to prevent dephosphorylation and activation of RIG-I and MDA5	Walton et al. <sup>164</sup>	2018	
VSV	matrix proteins interact with nuclear pores, preventing mRNA from being exported to the cytoplasm	Means et al. <sup>170</sup>	2020	
	VSV $\delta$ 51 has lost its matrix protein in methionine 51	Velazquez-Salinas et al. <sup>171</sup>	2017	
oncolytic pox virus	VV encodes B18R, which prevents IFN from binding to the fine-cell receptor	Stewart et al. <sup>172</sup>	2021	
	the B18R protein of OV Jx594 is naturally truncated, reducing its affinity for IFN	Sun et al. <sup>173</sup>	2019	
Enhance the anti-tumor effect of IFN-II	herpes simplex virus	HSV-1 stimulates NK cells to release IFN-II through TLR2/NF- $\kappa$ B signaling pathway	Wang et al. <sup>174,175</sup>	2022
	oncolytic poxvirus	Recruits macrophages, DCs, and other immune cells to improve the tumor microenvironment and promote IRF-3 transcription activity	Fox et al. <sup>176</sup>	2019
	oncolytic MV	codes IFN-II to kill cancer cells and control tumor proliferation	Robinson et al. <sup>178</sup>	2017
	oncolytic adenovirus	code IFN- $\gamma$ adenoviral variants to enhance the killing and immune stimulation ability of cancer	Bah et al. <sup>179</sup>	2020
	oncolytic VSV	expresses IFN- $\gamma$ variants and enhances anti-tumor immunity	Urbiola et al. <sup>181</sup>	2018
		reduces and increases tumor infiltration, Tregs, and CTL respectively, and improves anti-tumor immunity	Ayala Breton et al. <sup>182</sup>	2015

## SUMMARY AND PROSPECTS

From the above review, we can understand that CRC is a disease of the intestinal epithelium that is heterogeneous. Although CRC is generally viewed as a disease associated with environmental and genetic factors, more and more research shows that the gut microbiota plays a significant part in accelerating cancer growth and proliferation and forming an inflammatory environment. The biofilm of intestinal bacteria, toxic metabolites, the destruction of intestinal microbiota to the steady state of the microenvironment, and the immune reaction produced are critical to the treatment of CRC. Identifying the composition of gut microbiota in healthy people is an important basis for assessing the appearance of CRC and plays a critical part in the early diagnosis of CRC. The studies have suggested that observing the variations of intestinal bacteria and fecal metabolites can prevent CRC, and a part of the intestinal flora has active anti-tumor functions in chemotherapy and immunotherapy. In addition, the OV is also very promising in treating CRC, which can be effectively integrated into tumor immunotherapy. The OV directly cleaves cancer cells, releasing danger signals, soluble antigens, and IFN- $\gamma$ , thus driving anti-tumor immune responses. Besides, some OVs can be modified to convey therapeutic genes or improve the function of tumor-related endothelial cells to strengthen the recruitment of T cells into the TME of immune rejection or rejection. These characteristics make the OV an attractive drug for optimizing the combination strategy of cancer immunotherapy.

IFN is the first recombinant cytokine used to treat cancer. IFN has multiple functions, such as anti-viral, immune regulation, and anti-tumor functions. It exerts an anti-viral effect by inhibiting the synthesis of viral RNA and DNA and activates cytotoxic T cells to enhance the immune reaction while preventing the division of cancer cells from exerting an anti-tumor effect. IFN-induced proteins are vital to cell apoptosis. These proteins have the function of promoting apoptosis, while homozygous deletion gives cell apoptosis resistance. Therefore, IFN has dual functions. In addition to its pro-apoptotic effect, IFN also has an anti-apoptotic effect. The interaction between IFN and cell receptors produces a variety of cell signal pathways, and the anti-viral effect of IFN is usually mediated by the JAK-STAT signal pathway. Therefore, more in-depth research on the IFN- $\gamma$  pathway will be carried out to deeply understand the interaction between intestinal flora and OV and IFN in CRC, which will help to make greater use of the immune system to treat cancer.

Intestinal flora and OV take part in treating CRC by influencing IFN. Intestinal microbiota promotes the stimulation of systemic IFN- $\gamma$  and anti-viral immunity, while the cancer specificity of biotherapy of OV usually depends on the defective IFN response often observed in cancer cells, so, compared with healthy cells, cancer cells increase their vulnerability to viruses. At present, OVs have been designed to better facilitate the IFN response, but, because of the over-activation of this IFN, the immune system can clear the virus faster, thus limiting direct tumor lysis. Therefore, intestinal flora takes part in controlling the direct oncolysis of OVs by promoting the production of IFN. In addition,

OVs can promote the immunotherapy of intestinal bacteria against CRC by stimulating the secretion of IFN. These findings indicate that balancing the connection between enteric microorganisms, OV, and IFN is vital in treating CRC.

In addition, to make IFN effective in clinical use, three conditions need to be fully met: first, the need for the tumor itself to produce high-level IFN; second, the tumors themselves are susceptible to IFN; third, we need to overcome the immunosuppressive activity of the tumor. The intestinal microbiota is preferentially colonized at the tumor site, and its metabolites enhance the immune reaction and promote the secretion of IFN. Meanwhile, OVs encoded by special genes can lift the sensitivity of cancer cells to IFN. Intestinal symbiotic microorganisms can adjust the polarization of T cells, induce DAMPs, and do other things to promote immune activity. Furthermore, some OVs have immunostimulatory genes, and their lysed tumor cell death has high immunogenicity, which can create a pro-inflammatory environment to better control the immunosuppressive activity of tumors. Therefore, intestinal flora combined with OVs can better meet the above three conditions to promote IFN to play an anti-tumor role. In this paper, the pathogenic mechanism and potential therapeutic methods of CRC are discussed in detail from two aspects of intestinal flora and OV, and it is proposed that the effect of intestinal flora and OV on IFN is a prospective therapeutic option for treating CRC.

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## AUTHOR CONTRIBUTIONS

J.Y. conceived the study and conceptualized and wrote the first manuscript. J.Y. prepared figures. P.Z.L. prepared tables. P.Z.L., Q.B.L., and A.Z. helped with the further conceptualization of the manuscript. X.B.K. wrote the first manuscript and revised the final manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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