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Immuno-oncology-microbiome axis of gastrointestinal malignancy

Lin Q *et al.* IOM axis

Abstract

Research on the relationship between the microbiome and cancer has been controversial for centuries. Recent works have discovered that the intratumor microbiome is an important component of the tumor microenvironment (TME). Intratumor bacteria, the most studied intratumor microbiome, are mainly localized in tumor cells and immune cells. As the largest bacterial reservoir in human body, the gut microbiome may be one of the sources of the intratumor microbiome in gastrointestinal malignancies. An increasing number of studies have shown that the gut and intratumor microbiome play an important role in regulating the immune tone of tumors. Moreover, it has been recently proposed that the gut and intratumor microbiome can influence tumor progression by modulating host metabolism and the immune and immune tone of the TME, which is defined as the immuno-oncology-microbiome (IOM) axis. The proposal of the IOM axis provides a new target for the tumor microbiome and tumor immunity. This review aims to reveal the mechanism and progress of the gut and intratumor microbiome in gastrointestinal malignancies such as esophageal cancer, gastric cancer, liver cancer, colorectal cancer and pancreatic cancer by exploring the IOM axis. Providing new insights into the research related to gastrointestinal malignancies.

Key Words: Gut microbiome; Intratumor microbiome; Gastrointestinal malignancy; Tumor microenvironment; Immunity; Therapy

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Core Tip: The gut and intratumor microbiome can influence tumor progression by modulating host metabolism and the immune and immune tone of the tumor microenvironment, which is defined as the immuno-oncology-microbiome (IOM) axis. The proposed the IOM axis provides a new target for tumor microbiome and tumor immunity. Current studies have shown that immunotherapy with fecal microbiota

transplantation or microbial metabolism have certain effects. This review aims to explore the mechanism of the IOM axis of gastrointestinal malignancies, to reveal the mechanism and progress of gut and intratumor microbiome in gastrointestinal malignancies. Providing new insights into the research related to gastrointestinal malignancies.

INTRODUCTION

According to statistics, gastrointestinal tumors were one of the leading causes of death in the United States in 2020^[1]. Improving the survival rate of patients with gastrointestinal tumors has always been an urgent task. In recent years, with the deepening of research on tumor immunity, various immune checkpoint inhibitors (ICIs) have shown great effects in clinical practice^[2,3]. Among gastrointestinal tumors, there appears to be heterogeneity in the effect of immunotherapy across different tumor types^[4,5]. Pembrolizumab improves the prognosis of patients with esophageal cancer (EC)^[4]. However, the effect of niraparib plus nivolumab in pancreatic cancer was not satisfactory^[5]. Recent studies have described that the gut microbiome can reprogram tumor microenvironment (TME) immunity by participating in innate and/or adaptive immunity^[6]. Regulation of the microbiome can enhance the effectiveness of immunotherapy^[7]. Of all tumors, gastrointestinal malignancies have received the most attention due to their large number of microbial residues in the gut^[8]. Using microorganisms as one of the targets of immunotherapeutic effects seems to be an effective measure.

The human microbiome inhabits every surface and cavity of the body^[9], including bacteria, archaea, eukaryotes, and viruses that colonize humans^[10]. The microbiome affects overall immune function through many different mechanisms, resulting in a broad response from resistance to immune activation^[10]. The gut microbiome has long been recognized as playing a major role in human health and disease^[6], influencing host metabolism and shaping the immune system and disease conditions, including cancer^[11]. The gut microbiota plays a key role in shaping the immune system^[12]. The

human gut microbiota¹³ can influence the development and progression of gastrointestinal tumors by disrupting DNA, activating oncogenic signaling pathways, producing protumor metabolites, and suppressing antitumor immune responses^[13,14]. In recent years, with increasing research on the relationship between tumors and the microbiome, tumor tissues that were previously considered sterile are rich in microorganisms. After statistical analysis, the bacterial composition in tumor tissue is approximately 0.68%, equivalent to approximately 10^5 to 10^6 bacteria per 1 cm³ of tumor tissue^[15,16]. Although the abundance of microorganisms is relatively lower for tumor genomes, this intratumor microbiome is a potentially important player in tumor progression^[15]. The intratumor microbiome is mainly intracellular and present in cancer cells and immune cells^[17]. The gut and intratumor microbiome can influence tumor progression by modulating host metabolism and the immune and immune tone of the TME, and these immune-mediated interactions and collective feedback loops have been defined as the the immuno-oncology-microbiome (IOM) axis^[15]. The proposal of the IOM axis provides a new target for the tumor microbiome and tumor immunity. This review aims to reveal the mechanism and progress of the gut and intratumor microbiome and gastrointestinal malignancies by exploring the mechanism of the IOM axis in gastrointestinal malignancies. Providing new insights into the research related to gastrointestinal malignancies.

COLORECTAL CANCER

Colorectal cancer (CRC) is one of the most common cancers in humans, with a leading incidence rate^[18]. The initiation of CRC is a heterogeneous process that is influenced by the environment, microbial exposure, and host immunity^[9]. Interactions between CRC and the microbiome have been revealed in numerous studies, with increasing evidence highlighting the critical role of the TME in the initiation and progression of carcinogenesis. In this microenvironment, multiple relationships between tumor development, immune responses, and the microbiome have been identified. All stages of CRC are accompanied by an immune response^[19]. The regulation of the tumor

immune response by the microbiome plays an important role in the pathogenesis of CRC (Figure 1).

Tumor-infiltrating lymphocytes (TILs) are beneficial to the survival of human CRC^[8,19]. One of the features of CRC is a strong imbalance of T cells^[9,20]. One of the features of CRC is a strong imbalance of T cells^[21]. Tosolini *et al*^[22] analyzed T helper (Th) cell subsets in CRC and found that the expression of immune markers was different in adjacent mucosa and tumor tissue, suggesting that specific Th cell subsets were recruited at the tumor site. Experiments by Cremonesi *et al*^[20] showed that infiltration of different T-cell subsets in CRC correlated with the expression of well-defined chemokine genes such as chemokine (C-C motif) ligand (CCL)3, CCL4, and CCL20. Exposure of tumor cells to gut bacteria induced upregulation of most chemokine genes by flow cytometry^[20]. Upregulation of chemokines leads to higher T-cell recruitment into tumor xenografts. Therefore, whether CRC cells are exposed to intestinal bacteria and the degree of exposure may be one of the factors that affect the abundance of TILs. However, *Fusobacterium nucleatum* (*F. nucleatum*)^[23-25], which is abundantly enriched within CRC tumors, can interact with the immune cell inhibitory receptor T-cell immunoglobulin (Ig) and ITIM domain expressed by TILs through the adhesin Fap2, inhibiting the activity of tumor-infiltrating T cells and protecting tumor cells from immune cell attack^[26]. The effect of *F. nucleatum* on T cells may not be limited to this. *In vitro* experiments have shown that *F. nucleatum* inhibitory protein can block human T cells in the G1 phase of the cell cycle to prevent their proliferation^[27]. *F. nucleatum* can also use the trimeric autotransporter adhesin CbpF on its surface, inhibiting T-cell function by activating the inhibitory receptor carcinoembryonic antigen cell adhesion molecule 1^[28].

Th17 cells were found to be elevated to promote tumorigenesis^[29]. Microbial metabolites penetrate tumors, activate tumor-associated bone marrow cells, and mediate interleukin (IL)-23 secretion. In turn, the IL-23-driven Th17 response can promote tumor growth. Enterotoxigenic *Bacteroides fragilis*, on the other hand, increases IL-17 expression by triggering a Th17-type inflammatory response, which shifts the

colonic epithelium from an inflammatory to an oncogenic state^[30]. The mechanism of bacterial induction of carcinogenesis can be explained by the hypothesis of the “bacterial driver-passenger model”, in which “driver bacteria” of CRC promote tumorigenesis by inducing a sustained Th17 type inflammatory response, which is subsequently replaced by opportunistic “passenger bacteria” within the tumor, disrupting local innate immunity and ultimately leading to cancer progression^[30]. Group 3 innate lymphoid cells (ILC3s) are the innate counterpart of Th17 cells, which modulate adaptive Th17 cell responses and act with Th17 cells against extracellular microorganisms^[31]. ILCs are lymphocytes that do not express multiple antigen receptor types expressed on T and B cells^[31] and play a key role in regulating host-microorganism interactions on the intestinal mucosal barrier surface^[21,32]. ILC3s are abundant in mucosal sites and are involved in the innate immune response to extracellular bacteria and the suppression of gut commensal bacteria^[31], and intestinal T cells control microbiota composition and intestinal immune response^[33]. One study found that CRC is characterized by a significant decrease in ILC3s, accompanied by an increase in Th17 cells, suggesting that the progression of CRC is associated with impaired dialog between gut innate and adaptive immunity^[21]. Whereas ILC3s and effector T cells interact *via* major histocompatibility complex class II (MHCII), this experiment found that deletion of ILC3-specific MHCII in mice lead to increased CRC invasiveness and susceptibility. Thus, the disruption of the interaction between MHCII⁺ ILC3s, effector T cells, and microbiota may be a mechanism to increase CRC invasiveness. In addition, gut commensal fungi, such as *Candida albicans*, promoted CRC tumorigenesis in animal experiments^[34]. Commensal fungi activate glycolysis and IL-7 production in macrophages, while IL-7 induces IL-22 production in ILC3s, leading to tumor progression. However, other studies suggest that ILC3s in the TME may have both pro- and antitumor functions, depending on the cytokine types in the microenvironment^[35,36].

Macrophages play a vital role in the maintenance of the innate immune response^[37]. Tumor-associated macrophages (TAMs) are the main component of immune cells in the

TME. On the one hand, M1 TAMs are induced by cytokines such as tumor necrosis factor (TNF)- α , secrete IL-6 and IL-23, participate in the polarized Th1 response, and exert antitumor immunity^[37,38]. *Akkermansia muciniphila* (*A. muciniphila*) is a gut probiotic. Compared with the control group, the levels of M1-like TAMs were increased in *A. Muciniphila*-treated *Apc*^{Min/+} mice, and M1-like TAM-related cytokines, such as IL-23, TNF- α , and IL-27, were significantly induced in CRC^[39]. It was also shown that *A. Muciniphila* promotes the enrichment of M1-like macrophages in a nucleotide-binding and oligomerization domain-like receptor thermal protein domain associated protein 3 (NLRP3)-dependent manner *in vivo* and *in vitro*, acting as a suppressor of CRC proliferation. NLRP3 activation and macrophage phenotypic polarization may be induced by toll-like receptor (TLR) 2. On the other hand, M2 is the main phenotype of TAMs. M2 TAMs are induced by IL-4, IL-10, and IL-13, secrete anti-inflammatory cytokines such as IL-10 and IL-1 β , and participate in the polarized Th2 response, while activated Th2 cells produce lymphocytes producing IL-4 and IL-13, enhancing the expression of epidermal growth factor in TAMs and promoting the occurrence and development of tumors^[37,38]. Gut dysbacteriosis results in increased expression of cathepsin K (CTSK) in colon cancer cells, and CTSK binds to TLR4 to stimulate M2 polarization of TAMs *via* the mechanistic target of rapamycin-dependent pathway^[40]. At the same time, CTSK can stimulate M2 TAMs to secrete cytokines, including IL-10 and IL-17, which in turn mediate CRC cell invasion and metastasis through the nuclear factor kappa B (NF- κ B) pathway. In addition to dysbacteriosis, *F. nucleatum* can also promote macrophage infiltration through CCL20 activation while inducing polarization of M2 macrophages to promote CRC metastasis^[41].

TAMs can also promote the proliferation and invasion of tumor cells by interacting with tumor cells through microbiota-derived exosomes^[37]. Microbiota-derived exosomes have the potential to activate macrophages. The extracellular vesicles released from bacteria are named outer membrane vesicles, and the coculture of outer membrane vesicles and macrophages leads to a large production of type M1 and M2 cytokines and chemokines. TLRs are an important component of the host defense

mediated by the innate immune system^[39] and are also involved in tumorigenesis^[40]. Colon epithelial cells can sense the gut microbiome through pattern recognition receptors, including TLRs^[42]. Thus, bacterial-induced chemokine gene expression is most likely initiated when TLRs trigger tumor cells^[20].

In addition to the microbiome itself, microbiota-derived metabolites are also significant factors in the regulation of TME formation^[43]. It is an important mediator of host-microbiome interactions^[44]. Short-chain fatty acids (SCFAs), beneficial metabolites of the gut microbiome, are fermented from dietary fiber, including acetic acid, propionic acid, and butyrate^[43-46]. SCFAs regulate intestinal motility and energy metabolism by secreting peptides YY and glucagon-like peptide 1 from intestinal endocrine L cells, directly inhibiting tumor cell growth and inducing host macrophage, T-cell and B-cell responses to protect against colitis-induced CRC^[45].

Of these, butyrate is the most relevant bacterial metabolite of SCFAs^[47] and has important immunomodulatory functions that can mediate the switching of pro-inflammatory cytokine expression by inhibiting histone deacetylases^[48]. The significant reduction in butyrate-producing bacteria in the gut microbiome of patients with CRC may constitute a major structural imbalance in the gut microbiome of patients with CRC^[49]. Thus, SCFAs play an important role in regulating host energy metabolism and the immune system^[50]. Studies have shown that supplementation with probiotics that produce SCFAs can inhibit the development of intestinal tumors^[51,52]. However, under certain conditions, butyrate metabolized by microorganisms may have the opposite effect. Several studies have shown that at lower concentrations, butyrate may stimulate the proliferation of colonic epithelial cells and thus promote CRC^[53,54]. This discrepancy in the effects of butyrate on CRC is known as the “butyrate paradox”^[53,54]. Therefore, increasing metabolites, such as SCFAs, has the potential to be used as an adjuvant treatment and preventive measure for CRC. However, it is necessary to further explore the action threshold of butyrate to avoid the opposite effect. Another gut bacterial metabolite, conjugated linoleic acid (CLA), is one of the most important fatty acids in the gut and is mainly produced by *Roseburia species*^[55]. The reduction in *Roseburia species*

was closely associated with the occurrence of CRC^[49]. CLA also inhibits TNF- α expression and induces the immunomodulatory cytokine transforming growth factor β 1 (TGF- β 1) to participate in and regulate the pathways of apoptosis and immune response, reducing the risk of CRC^[56]. Thus, the antiproliferative and anti-inflammatory capacity of CLA on colon cells may play an important role in CLA's protection of the host against CRC^[57].

GASTRIC CANCER

10

Gastric cancer (GC) is the fifth most common cancer in the world and the third most common cause of cancer death^[58]. The host microbiome is closely associated with the occurrence and development of GC. The gastric microbiome affects inflammation and immunity at the local mucosal level and systemically. Disorders in the close interaction between the gastric microbiome and the immune system in the TME may contribute to GC development by triggering a tumor-promoting immune response^[59] (Figure 2).

Helicobacter pylori (*H. pylori*) infection is a risk factor for GC^[60]. *H. pylori* colonizes the gastric mucosa of up to 50% of the population, manipulating host tissue to establish and maintain an immunosuppressive environment for chronic infection^[61]. *H. pylori* inhibits the effector functions of CD4⁺ T cells, dendritic cells (DCs), and macrophages by altering the cross-presentation activity of DCs to suppress antitumor CD8⁺ T-cell responses, thereby suppressing the innate and adaptive immune responses of the infected host^[61,62]. During GC formation, *H. pylori* may actively participate in GC by altering gastric mucosal immunity, particularly regulatory T (Treg)/Th17 imbalance^[61]. *H. pylori* can also produce virulence factors (*e.g.*, VacA, γ -GT, DupA) to impair immune cell activity^[63]. For example, VacA affects the inflammatory response of the host primarily by inhibiting the proliferation and effector functions of T cells^[63], but also induces the proinflammatory effects of T cells by activating nuclear factor kappa B and leading to the upregulation of IL-8^[64]. γ -GT induces T-cell cycle block by disrupting RAS signaling and inhibits T-cell proliferation^[65]. DupA increases the expression of proinflammatory cytokines (*e.g.*, IL-12 and IL-23) *via* monocytes^[66]. However, only *H.*

pylori colonization in the stomach is not sufficient to induce gastric carcinogenesis^[61]. In the past, the human stomach was thought to be the only habitat for *H. pylori* and was considered unsuitable for microbial habitation. However, recently, various studies have demonstrated that different gastric environments result in different microbial ecosystems within the stomach, and changes in specific microbial species may make the gastric microbiome more carcinogenic^[67]. Bacteria other than *H. pylori* were previously thought to be unrelated to the development of GC^[60]. Lofgren *et al*^[68] demonstrated that the presence of a complex microbiome promoted the development of *H. pylori*-induced GC. However, the specific mechanism of the intragastric microbiome interaction with *H. pylori* on immunity remains unclear.

The microbial diversity and abundance in GC tumors were higher than those in nontumor tissues^[67,69]. The intratumoral microbiome influences GC by modulating immune responses in the TME. A recent study by Peng *et al*^[59] found that the infiltration of CD8⁺ tissue-resident memory T cells (TRM cells) in the TME of patients with GC is negatively correlated with the abundance of *Methylobacterium* in gastric tumors. The population of memory T cells in TILs, known as TRM cells, is derived from T cells that enter tissues during the primary response (such as in response to viral invasion) and plays a role in tumor immune surveillance^[70]. CD8⁺ TRM cells produce and release various cytokines, such as interferon- γ (IFN- γ), which promote the activation of other immune cells and play an important role in antitumor immunity^[71]. Intratumor *Methylobacterium* may promote the development of GC by inhibiting the immune infiltration of CD8⁺ TRM cells in the TME, leading to poor prognosis^[59]. Furthermore, the abundance of *Methylobacterium* was also found to be significantly negatively correlated with TGF- β and IL-2^[59]. TGF- β induces the expression of CD103, which plays an essential role in the permanent residence of TRM cells in epithelial tissues^[71]. This may provide a new target for the development of immunotherapy for GC.

TAMs, mainly M2 TAMs (*i.e.*, polarized M2 macrophages), are important in the progression of GC^[72]. Recent experiments have shown that *Propionibacterium acnes* (*P. acnes*) is significantly increased in GC tissues, especially in *H. pylori*-negative tissue, and

promotes M2 polarization of macrophages *via* TLR4/PI3K/Akt signaling when comparing microbial communities in GC tissues and adjacent normal tissues^[73]. M2 macrophages secrete chitinase 3-like protein 1, which binds specifically to the interleukin-13 receptor $\alpha 2$ chain of cancer cells and triggers the mitogen-activated protein kinase signaling pathway to promote regional or distal metastasis of GC^[72]. The abundance of *P. acnes* is positively correlated with M2 macrophages in GC tissues^[73]. Therefore, *P. acnes* may be one of the possible factors for GC progression beyond *H. pylori*. Recent reports have shown a sustained increase in the abundance of lactic acid bacteria (LAB) in GC patients. LAB can increase exogenous lactate production^[74]. Lactate produced by the glycolytic pathway leads to the formation of an acidic TME^[75], which is involved in cancer progression. Lactate mediates M2-like polarization of TAMs and increases vascular endothelial growth factor and arginase 1 expression in these cells, thereby facilitating immune escape^[74]. Lactate also inhibits the function and survival of T cells and natural killer (NK) cells and increases the number of myeloid-derived suppressor cells, which further inhibits the cytotoxicity of NK cells. Because LAB can produce large amounts of lactate in a short period of time^[76], it is possible that microbial lactate can shape the TME like host lactate, altering the immune response^[74].

In addition to bacteria, intratumoral fungi can also affect GC progression through the IOM axis. Yang *et al*^[77] analyzed the gastric fungal microbiome in patients with GC and healthy individuals by high-throughput sequencing and noted that the ecological dysbiosis of the gastric fungal microbiota may be related to the occurrence of GC. They also measured the mRNA levels of cytokines and chemokines in tumor and normal tissues. The results showed that the levels of pro-inflammatory cytokines and chemokines such as CXCL9, CXCL10, CXCL11, and TNF- α were significantly increased, while anti-inflammatory cytokines and chemokines such as CCL17, IL-4, IL-6, and IL-8 were significantly decreased in the GC group. It is suggested that fungi that promote the production of proinflammatory cytokines and chemokines may be involved in promoting the tumor immune response, while fungi that promote the production of anti-inflammatory cytokines and chemokines may enhance the anti-inflammatory

response in GC. IL-10 is highly expressed in various types of cancer^[78,79], and can downregulate the inflammatory cytokines IL-6 and IL-8^[77]. Therefore, the decreased levels of IL-6 and IL-8 in the GC group may be due to the increase in IL-10 levels in the local area of the tumor. IL-10 is released by TAMs, and IL-10+ TAMs infiltrate the tumor, which drives immune escape from the TME characterized by Treg-cell infiltration and CD8⁺ T-cell dysfunction^[80]. *Candida albicans* (*C. albicans*) is the most studied fungal species in GC. *C. albicans* was found to be a fungal marker of GC^[81]. *In vitro*, the mannose protein of *C. albicans* could promote tumor adhesion and liver metastasis^[82]. Similar to CRC, fungi may have a complex role in the Th17 cell family. On the one hand, Th17 cells produce IL-17 to initiate downstream immunity against *C. albicans*. On the other hand, other cytokines, such as IL-23, produced by Th17 cells can promote tumorigenesis and growth. In addition, Th-17 can promote the progression of GC through an indirect mechanism. IL-17 secreted by Th17 cells can antagonize IL-12. IL-12 induces the production of IFN- γ and promotes the infiltration of cytotoxic T cells, which plays a critical role in the Th1-type antitumor immune response^[83,84]. The promotion of cancer progression by IL-17 is also associated with neutrophil recruitment^[85]. Additionally, the complement receptor 3-related protein (CR3-RP) of *C. albicans* has antigenic and structural similarity to CR3. CR3 is involved in leukocyte adhesion to endothelial cells and the subsequent extravasation process. Thus, antibodies against *C. albicans* CR3-RP may cross-react with leukocyte CR3 and disrupt the host's antitumor defense^[85].

In general, bacteria and fungi in the stomach, which are considered unsuitable for microbial habitation, have an important role in the regulation of GC progression. They can interact with immune cells in the body or the TME, affecting the progression of GC. Moreover, the role of fungi in GC may be underestimated. However, it is not clear whether fungi exist in tumor tissue as viable or partial components.

EC

EC is one of the most invasive malignant diseases^[86]. Unlike other luminal organs of the digestive system, the esophagus does not retain food contents. It was first thought that the esophagus was aseptic, but after the study of traditional bacterial culture methods, it was found that the esophagus contained a small number of microorganisms swallowed from the oropharynx or excreted from the stomach through gastroesophageal reflux^[87,88]. With the progress of culture technology, increasing evidence shows that the normal esophagus has a unique, stable, resident microbiome^[87,88]. The distal esophageal microbiome was divided into two groups: Type I and type II^[89]. Among them, the type I microbiome, dominated by *Streptococcus species*, seems to be the main component of the normal esophageal microbiome^[89,90]. However, a study found that the microbiome of the normal esophagus is not the same as that of the esophagus with inflammation, Barrett's esophagus (BE), and EC^[87]. Type II microbiome, dominated by gram-negative bacteria, is usually associated with an abnormal esophagus^[89].

Gastroesophageal reflux disease (GERD) and BE are significant risk factors for esophageal adenocarcinoma (EAC)^[91-93]. Gram-negative bacteria that predominate in GERD and BE produce specific components, such as lipopolysaccharide (LPS)^[87,88]. LPS directly or indirectly stimulates TLR4^[94] in epithelial cells or inflammatory cells, leading to NF-κB activation^[87] promote the expression of proinflammatory cytokines and sustain the innate immune response in the esophagus^[88,94]. LPS binds plasma-derived LPS-binding protein and transmits to membrane-bound CD14 in monocytes, interacting with CD14^[95]. CD14 plays a key role in LPS-mediated signal transduction by enhancing leukocyte adhesion, activation, and cytokine production. LPS stimulation of monocytes or epithelial cells leads to the activation of TLR4 and downstream NF-κB pathways, which triggers an inflammatory response^[94]. LPS may also indirectly activate the NF-κB pathway of epithelial cells by triggering the production of inflammatory cytokines such as TNF-α, IL-1, and IL-6 by monocytes and macrophages^[94,95].

In addition, *Campylobacter* was experimentally demonstrated to be significantly increased in GERD and BE^[96]. Cytokines related to carcinogenesis, such as IL-18, were

more highly expressed in the tissues colonized by *Campylobacter*^[87]. Studies have shown that there is a very close relationship between esophageal *Campylobacter* colonization and IL-18 epithelial cell production^[97]. IL-18 is produced by gastrointestinal epithelial cells, including squamous esophagus cells, which can stimulate both congenital and adaptive responses (Th1 and Th2) and induce NK cell activity and apoptosis^[98]. Another study found that *Campylobacter* infection induced the secretion of IL-8 and TNF- α ^[99]. However, the increase in IL-8 secretion was not associated with the secretion of TNF- α stimulated by *Campylobacter*. It is not clear whether IL-8 can play a role in the initiation and maintenance of malignant transformation from GERD and BE to EAC. However, *Campylobacter species* is associated with a range of gastrointestinal diseases and may play a role in the progression of EAC similar to *H. pylori* in GC^[87,100].

Moreover, the microbiome can also directly promote the occurrence of EAC by stimulating the human systemic immune system. For example, Lopetuso *et al*^[90] identified *Leptotrichia* as the main taxon distinguishing EAC by LefSe analysis. *Leptotrichia* can promote the release of proinflammatory cytokines, such as IL-6 and IL-8. In turn, IL-6 and IL-8 can attract granulocytes and lymphocytes, thus inducing the host cellular immune response^[101]. Serum antibodies against *Leptotrichia* are very common and belong to the IgG and IgM classes^[102]. It is speculated that the immune activity caused by *Leptotrichia* may promote the occurrence of esophageal tumors^[90].

Although there are few definitive conclusions about the impact of the microbiome on EAC, the change in the microbiome in the esophagus induces GERD and BE by activating the innate immune system and makes them progress to EAC, which seems to be a very important link in the pathogenesis of EAC. Compared with EAC, the characteristics of the microbiome in esophageal squamous cell carcinoma (ESCC) are not very clear^[103]. However, there is growing evidence showing that the microbiome plays an important role in the occurrence and development of ESCC^[87]. In a study by NHANES III, an increase in serum IgG of *P. gingivalis* was associated with increased mortality from oral and gastrointestinal cancers^[104]. The results of Gao *et al*^[105] showed that *P. gingivalis* infected the cancerous and adjacent esophageal mucosa of patients

with ESCC. The titers of IgG and IgA against *P. gingivalis* in patients with ESCC were significantly higher than those in patients with esophagitis and healthy controls, which provided direct evidence for the involvement of *P. gingivalis* in the pathogenesis of ESCC. However, the specific mechanism by which *P. gingivalis* is involved in the progression of ESCC is unknown. Although the mechanism by which *P. gingivalis* affects the progression of ESCC through the IOM axis is still unclear, *P. gingivalis* can promote the infiltration of tumor-associated neutrophil 2 in tumor tissues in pancreatic cancer^[106].

The poor prognosis of ESCC is also related to the presence of *F. nucleatum*^[107]. In the relationship between *F. nucleatum* and cancer, especially CRC, *F. nucleatum* is considered to selectively amplify myeloid-derived immune cells to regulate the tumor immune microenvironment^[23,25]. The immune response mediated by myeloid cells may provide the driving force for inflammation, genotoxicity, and epigenetic changes that lead to cancer^[23]. In addition, *F. nucleatum* may promote tumor invasiveness by activating chemokines, such as CCL20, in EC tissue^[87]. CCL20 plays a critical role in the migration of Treg lymphocytes. The specific receptor of CCL20 is CCR6, which is highly expressed in immune cells (*e.g.*, Treg and Th17) and epithelial tumors^[108]. Treg cells promote migration to tumor tissue in a CCR6-dependent manner in response to CCL20^[109], and the concentration of CCL20 in the tumor is positively correlated with the number of tumor-infiltrating Tregs^[108,110].

PANCREATIC CANCER

The central position of the pancreas in the abdominal cavity and the surrounding blood vessels and lymphatic vessels promote local and distant tumor spread. The TME of pancreatic cancer is characterized by a high matrix level and low immune activity^[111]. This has also become one of the reasons for the poor efficacy of ICIs in pancreatic cancer. Improving this immunosuppressive microenvironment has become an urgent problem to be solved. In recent years, with the deepening of the study of the microbiome and the application of high-throughput sequencing technology^[112], it has

been found that the gut and intratumor microbiome have an important effect on pancreatic cancer^[113-115]. This is especially true in immune-related research. The role of the IOM axis in pancreatic cancer has a dual nature. Most studies have reported the cancer-promoting effect of fungi and bacteria through the IOM axis in pancreatic ductal adenocarcinoma (PDAC).

TAMs have important potential in activating tumor-specific CD8⁺ T cells, while the important role of the microbiome has long been neglected in research on TAMs in the TME, as well as in pancreatic cancer^[116-119]. Pushalkar *et al*^[120] reported that ablation of the gut flora of mice with pancreatic cancer resulted in a decrease in immunosuppressive CD206⁺ M2-like TAMs with a concomitant increase in M1-like TAMs and increased expression of MHCII, CD86, TNF- α , IL-12, and IL-6. Moreover, like other gastrointestinal tumors, microbial ablation increased TLR expression in macrophages. Thus, the effect of the microbiome on PDAC through the IOM axis may be partly caused by acting on TLRs on macrophages. At the same time, their research also reported that the cell-free extract of the gut microbiome from pancreatic cancer mice can also promote the transformation of TAMs to an immunosuppressive phenotype. This suggests that the cellular components or metabolites of the microbiome located in the gut or tumor may play an important role in TAM-related reactions. Indole, a metabolite of the gut microbiome, such as *Lactobacillus murinus*, was found to activate the aryl hydrocarbon receptor (AhR) on TAMs. AhR activation leads to the expression of arginase 1 and IL-10 in TAMs and suppresses the expression of IFN- γ in CD8⁺ T cells. Another metabolite of the gut microbiome, trimethylamine N-oxide (TMAO), is different from indole in its production, which is completely dependent on gut bacteria^[121]. Choline is the main source of circulating TMAO. However, unlike the effect of indole, choline-supplemented mice had increased TMAO levels, accompanied by a significant reduction in PDAC burden^[122]. Flow cytometry showed that the expression of MHCI and MHCII on TAMs was significantly increased, accompanied by a significant increase in activated CD8⁺ and CD4⁺ T cells. Further research found that TMAO directly changed TAMs into a phenotype that could support the T-cell response

and reduce the burden of PDAC. However, the role of CD8⁺ T cells mediated by the microbiome in the gut/tumor seems not limited to the stimulation of gut microbiome metabolites. Riquelme *et al*^[12] found that patients with long-term survival had higher intratumor microbiome diversity than those with short-term survival. Moreover, PDAC tumor volume was reduced in mice that were gavaged with feces from long-term survival patients, and this effect could be eliminated by the depletion of CD8⁺ T cells. Although the causal relationship between the enrichment of microbial communities and tumors is still unclear, it suggests that the construction of microbial communities in the gut/tumors may be more effective than studying the role of a single strain.

The role of IL-33 and ILC2s in pancreatic cancer is highly controversial. Kras mutant PDAC cells can secrete IL-33 through the Kras-MEK-ERK pathway^[123]. Intratumor fungi and their cell-free extracts can activate dectin-1 on PDAC. The activation of dectin-1 promotes the secretion of IL-33 by PDAC cells through the Src-Syk-CARD9 pathway. This shows the important role of intratumoral fungi in mediating the secretion of PDAC cells. However, the source of IL-33 may not be limited to PDAC cells. Another study showed a different result. Sun *et al*^[124] conducted immunohistochemistry on 20 human PDAC tissues and found that pericytes and cancer-associated fibroblasts were the major cell sources of IL-33 production in PDAC tissues and promoted PDAC metastasis through the IL-33-ST2-CXCL3-CXCR2 axis, although both studies found that the expression of IL-33 in PDAC was increased. The increase in IL-33 in the TME of PDAC promotes the infiltration of TH2 cells and ILCs in PDAC through the IL-33/ST2 axis^[123]. These immune cells promote the tumorigenic process through their cytokine networks, leading to PDAC progression^[125-127]. In addition to IL-33, the role of its downstream ILC2s is also controversial. The role of bipartisan politicians of ILC2s is also reflected in PDAC. ILC2s can also promote the effective infiltration of immune cells. ILC2s can inhibit the progression of PDAC tumors through the ILC2-CD103⁺ DC-CD8⁺ T axis^[128]. The level of immunity in PDAC appears to affect the effect of ILCs.

THE MICROBIOME AND CANCER THERAPY

Antibiotics

In a retrospective clinical study, increased ² overall survival (OS) and progression-free survival (PFS) in patients with metastatic PDAC were associated with antibiotic use^[129]. Several preclinical studies have found that ablation of the gut and intratumor microbiome with antibiotics in a PDAC in situ mouse model can prevent tumor progression^[12,120,130]. In the abnormal esophagus, the use of selective antibiotics or probiotics to reverse the type II microbiome to a type I microbiome in the esophagus can reduce the risk of esophageal carcinogenesis^[94]. However, the effect caused by this antibiotic is related to the type of tumor and the type of antibiotics. Treatment of mice carrying colon cancer xenografts with the antibiotic metronidazole was found to reduce cancer cell proliferation and tumor growth^[131]. However, treatment with metronidazole in pancreatic cancer significantly increased the tumor load^[122]. In pancreatic cancer, quinolone therapy is linked to the improvement of OS. Postoperative quinolone therapy may prolong the survival time of preoperative treatment and resection of pancreatic cancer^[132]. The use of antibiotics can inhibit or kill the pathogenic microbiome in the host. However, frequent use of broad-spectrum antibiotics may interfere with the gut microbiome, leading to ecological disorders and even cancer development^[133].

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is a method that transplants the entire gut microbiota from a healthy donor into the patient's gut to correct gut microbial abnormalities and reconstruct the structure and function of normal gut microbiota^[134]. ¹ Metagenomic analysis showed that FMT significantly increased the abundance of potentially beneficial species^[135]. The microbiome remodeling induced by FMT may be related to an improved tumor immune microenvironment. Riquelme *et al*^[12] concluded that compared with short-term PDAC survivors or healthy controls, PDAC tumor-bearing mice transplanted with the fecal microbiome from long-term survivors of PDAC had antitumor immunity. The bacteria found in the tumor transferred from the intestinal tract to the pancreas, affecting the composition of the tumor microbiome and

antitumor immunity in the pancreas. Rosshart *et al*^[136] reported that FMT enhanced host resistance to mutagen/inflammation-induced colorectal tumorigenesis. The beneficial role of FMT in the treatment of diseases has been confirmed, but it has not been widely studied in gastrointestinal tumors.

Probiotics

¹⁶ Probiotics can alter the composition of the gut microbiome and have been shown to inhibit tumor development by downregulating the levels of LPS, inflammatory factors, and chemokines^[137]. Various studies have found that supplementation with probiotics that produce SCFAs can inhibit the development of tumors^[51,52]. For example, both *Lactobacillus coryniformis* MXJ32 and *Lactocaseibacillus rhamnosus* LS8 can reduce intestinal inflammation by downregulating ² the expression of inflammatory cytokines (e.g., TNF- α , IL-1 β , IL-6, IL- γ , and IL-17a) and chemokines (e.g., CXCL1, CXCL2, CXCL3, CXCL5, and CCL7) and effectively improve colitis-associated CRC^[138,139]. However, the effects of these two probiotics on the mechanism of regulating the gut microbiome and specific immune response are not clear. ²⁴ In the study of Heydari *et al*^[140], which uses an animal colon cancer model, treatment with probiotics suppressed the increase in miRNA expression and decreased the level of oncogenes, and such treatment was considered beneficial for colon cancer treatment. *Lactobacillus brevis* SBL8803-derived polyphosphate leads to apoptosis of CRC cells by activating extracellular signal-regulated kinase signal transduction. Heptelidic acid, a metabolite of the probiotic *Aspergillus oryzae*, passes through the gut mucosa to reach the pancreas and induces apoptosis in pancreatic cancer cells by activating the p38MAPK signaling pathway^[141]. Although probiotic supplements may alter the structure of the microbiome and regulate inflammation to prevent cancer^[142], probiotics may adversely affect the reconstruction of gut mucosal host-microbiome ecosystems after antibiotic treatment^[143]. Therefore, probiotic therapy may be a promising intervention method^[138], but many problems urgently require further research.

Immunotherapy

The use of ICIs has made remarkable progress in the treatment of many cancers, among which the most widely used ICIs are monoclonal antibodies targeting programmed cell death protein 1 (PD-1) and its ligand PD-L1^[144]. Although immunotherapy based on anti-PD-1 has a limited response in CRC patients, a growing body of research supports the important role of the gut microbiome in the immune system. The gut microbiome seems to influence the expression of PD-1/PD-L1 indirectly through systemic or locally mediated immune function, thus affecting the efficacy of anti-PD-1 and anti-PD-L1 therapy^[134]. The mechanisms by which the gut microbiome improves the efficacy of anti-PD-1 are as follows^[145-148]: (1) An increase in beneficial bacteria; (2) Enhancement of the function of DCs; (3) An increase in antitumor CD8⁺ T-cell activity; and (4) Promotion of T-cell tumor infiltration. Several studies have reported that oral combinations of specific symbiotic bacteria and anti-PD-1/PD-L1 antibodies almost eliminated tumor growth^[145,149]. In a mouse cancer model, oral live *Lactobacillus rhamnosus* GG enhanced the antitumor activity of anti-PD-1 immunotherapy by increasing tumor-infiltrating DCs and T cells and significantly inhibited tumor growth^[146].

Antibiotics and immunotherapy

There is growing evidence that the gut microbiome can influence immunotherapy responses in patients treated with ICIs^[61,62,134]. Preclinical experiments in mice showed that the use of antibiotics could decrease the efficacy of ICIs^[144]. In a meta-analysis enrolling 2740 cancer patients, antibiotic use significantly reduced OS and PFS in patients treated with ICIs^[150]. But, In a study on pancreatic cancer, researchers discovered that by using broad-spectrum antibiotics to eliminate gut microbiota, they could trigger immunogenic reprogramming within the TME. This made treatment with ICIs more effective by increasing the expression of PD-1^[120]. Thus, whether the use of antibiotics can improve the efficacy of ICIs is controversial. At present, some meta-analyses suggest that antibiotic administration may be associated with poor prognosis

of tumor patients receiving ICIs^[151,152]. However, these studies focused on lung cancer, renal cell carcinoma, urothelial carcinoma, and melanoma^[151]. Research on gastrointestinal malignancies is still insufficient. When CRC cells were implanted into germ-free or specific pathogen-free mice, broad-spectrum antibiotics reduced their ICI efficacy^[153]. In a mouse model, *H. pylori* infection partially blocked the activity of ICIs and reduced the effect of tumor immunotherapy^[61]. However, eradication of *H. pylori* infection through antibiotic therapy did not restore the decreased response of *H. pylori*-induced cancer to immunotherapy. Therefore, the administration of antibiotics to cure *H. pylori* infection is not a good choice to improve the efficacy of cancer immunotherapy. ⁴ Han *et al*^[154] recently demonstrated that antibiotic-induced microbiome disorders enhanced the antitumor efficacy of γ - δ T cells during immunotherapy in a mouse model of hepatocellular carcinoma. γ - δ T cells can generate immune responses to a wide range of antigens. They are believed to serve as a bridge between innate and adaptive immune responses^[7]. γ - δ T cells can also infiltrate GC, pancreatic cancer, and colon cancer^[155-157]. Thus, the effect of antibiotics on ICIs in patients with gastrointestinal malignancies still needs to be further studied.

FMT and immunotherapy

¹ FMT is a potential way to improve the efficacy of anti-PD-1 therapy^[158]. Huang *et al*^[158] found that compared with colon cancer-bearing mice treated with anti-PD-1 or FMT alone, FMT combined with anti-PD-1 showed higher survival and tumor control. The enhancement ¹ of anti-PD-1 therapy induced by FMT may be mediated by changes in the microbial genome and blood metabolism. Through metagenomic analysis, FMT altered the composition of the gut microbiome. The relative abundance of *Bacteroides species* and *Parabacteroides species* increased significantly. Metabonomic analysis of mouse plasma showed that after FMT, several metabolites, including toxic ¹ acid and aspirin, may promote the response to anti-PD-1 therapy through their immunomodulatory function. Accordingly, the composition and function of the gut microbiome may be able to influence the ICI response in cancer^[7]. However, FMT did not improve the response to

immunotherapy in cancer patients infected with *H. pylori*^[62]. In the absence of ICIs, modulation of the gut microbiome with bacteria or FMT has a limited impact on the antitumor immune response or tumor growth^[134]. Therefore, FMT may serve as an important therapeutic modality to assist patients treated with ICIs to enhance systemic and antitumor immunity in cancer patients.

Microbial metabolites and immunotherapy

Intestinal epithelial cells are closely related to the immune system. Bacterial metabolites, such as SCFAs, occur in the immune response and are strongly associated with innate immunity and antibody production^[159]. The results from a cohort study showed that high levels of fecal or plasma SCFAs were associated with PD-1 treatment response and longer PFS^[160]. In mice humanized with gut microbiota from patients, butyrate promoted T-cell infiltration in the TME, thus improving the efficacy of anti-PD-1 monoclonal antibodies^[147]. Tryptophan is an essential amino acid for the human body. Indole, a metabolite of tryptophan, is a biologically active compound that plays an important role in tumor and immune regulation^[161]. Indole drives AhR on TAMs and suppresses antitumor immunity. Macrophage AhR is the central driver of TAM function in PDAC. In patients with PDAC, high expression of AhR is associated with rapid disease progression and mortality. TMAO, a metabolite of natural microorganisms, suppresses the immunostimulatory phenotype of macrophages, promotes the activity of effector T cells, and enhances antitumor immunity against PDAC^[122]. The combination of TMAO and anti-PD1 in a PDAC mouse model significantly reduced the tumor burden and improved the survival rate compared to TMAO or ICIs alone. Therefore, the immunomodulatory mechanism associated with microbial metabolites may become a new direction to improve PD-1 efficacy in cancer patients^[160].

CONCLUSION

In recent years, some progress has been made in the study of microorganisms and tumors. Although the role of the IOM axis in GC and EC needs to be further clarified, it plays an important role in the occurrence and development of gastrointestinal malignancies. Macrophages may be a key component in linking the microbiome and immunity, which has been reflected in the variety of tumors mentioned above. The microbiome may influence tumor immune responses through TLRs on macrophages. ILCs also play a vital role in the host microbiome and, together with T cells, regulate the IOM axis. The IOM axis provides a new direction for the treatment of gastrointestinal malignancies. An increasing number of studies have shown the role of the microbiome in immunotherapy. For example, specific antibiotic use may prevent tumor progression, whereas the combination of antibiotics with ICIs may reduce the efficacy of ICIs. In contrast, FMT has been found to improve the efficacy of immunotherapy. However, the effect of the microbiome on immunotherapy is still controversial, and the mechanism of action is still elusive and needs to be widely validated by more preclinical models and clinical trials.

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