

# Colorectal cancer, radiotherapy and gut microbiota

Shu Zhang, Qiaoxuan Wang, Chengjing Zhou, Kai Chen, Hui Chang, Weiwei Xiao, Yuanhong Gao

Department of Radiation Oncology, Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center for Cancer Medicine, Guangzhou 510060, China

Correspondence to: Weiwei Xiao. Department of Radiation Oncology, Sun Yat-sen University Cancer Center, No. 651 Dong Feng East Road, Guangzhou 510060, China. Email: xiaoww@sysucc.org.cn; Yuanhong Gao. Department of Radiation Oncology, Sun Yat-sen University Cancer Center, No. 651 Dong Feng East Road, Guangzhou 510060, China. Email: gaoyh@sysucc.org.cn.

## Abstract

Colorectal cancer is closely related to inflammation and immune response. Radiotherapy, as a major treatment for colorectal cancer, plays a central role in cancer control. Inflammation caused by ionizing radiation can exert either anti- or pro-tumorigenic effects. Additionally, radiotherapy can elicit an anti-tumor response not only in radiation of target lesions but also in radiation of remote lesions. However, the immune mechanism underlying this effect has not been thoroughly elucidated yet. The combination therapeutic regimen of radiotherapy with other therapeutic methods, including chemotherapy and immunotherapy, has been applied in clinical practice. Meanwhile, radiation toxicity and radiosensitivity have long been problems that affect a patient's quality of life and morbidity. Researchers have found that the abovementioned problems are closely associated with gut microbiota. Here we discuss the impact of immune response induced by radiotherapy on tumor regression and the impact of intestinal flora on the consequent clinical efficacy.

**Keywords:** Colorectal cancer; intestinal microbiota; inflammation; immune system; radiotherapy; tumor-infiltrating lymphocytes (TILs)

Submitted Aug 28, 2018. Accepted for publication Jan 16, 2019.

doi: 10.21147/j.issn.1000-9604.2019.01.16

View this article at: <https://doi.org/10.21147/j.issn.1000-9604.2019.01.16>

## Radiation-induced inflammation and immune responses

### *Anti-tumorigenic effect*

The effects of radiation are complex, activating both tumor-promoting and tumor-suppressing immune responses. Radiotherapy triggers an antigen-specific immune response, referred to as *in situ* vaccination, to prevent tumor progression (1). Irradiated tumor cells may undergo a process essential for effective immune response initiation called immunogenic cell death (ICD), which requires effective tumor antigen exposure and the resulting activation of antigen-presenting cells (APCs). Radiation-damaged tumor cells will release damage-associated molecular patterns (DAMPs), whose corresponding ligands are pattern recognition receptors (PRRs) expressed on

APCs (1,2). DAMPs can be further divided into 3 groups: those expressed on the tumor cell surface, those actively secreted, and those passively secreted. ICD is characterized by the exposure of calreticulin on the cell surface, active secretion of adenosine triphosphate (ATP) and passive release of high-mobility group B1 (HMGB1) by stressed or dying tumor cells (2,3). With APC activation of ATP, cell surface costimulatory ligands CD80 and CD86 expressed on APCs will be upregulated, and a series of anti-cancer events, including effector T-cell expansion and regulatory T cell (Treg) reduction, will be elicited (1). Extracellular ATP functions as a stimulation signal for APCs. This radiation-induced ATP-APC anti-tumor immune response is strongly associated with autophagy-dependent extracellular ATP accumulation (3). Additionally, autophagy is related to the release of HMGB1, which will elevate the autophagy level, in a bidirectional interplay (4). Because

not more than 20% of radiation-induced cell death relies on apoptosis (5), as an important cell death pathway, autophagy and its association with radiotherapy are now increasingly recognized by researchers.

Ionizing radiation elevates chemokines involved in T-cell recruitment, converting the tumor microenvironment (TME) into “inflamed” tissue, which is more prone to effective T-cell attack. Radiation induces local vascular endothelial inflammation to increase T-cell trafficking in the tumor area and maximize effector T-cell function (1). Effective T-cell activation requires antigen presentation, costimulatory signals from appropriate APCs and background levels of cytokine stimulation. Treg cells express cytotoxic T-lymphocyte antigen 4 (CTLA4), which competitively inhibits costimulatory signaling molecules CD80 and CD86 expressed on APCs with CD28 expressed on T cells (2). Theoretically, CTLA-4 blockade during radiotherapy may enhance the *in situ* vaccination effect of radiotherapy. Radiotherapy induces not only effector T-cell expansion but also Treg cell upregulation, limiting the positive immune system against cancer cells. The effects of radiation on Treg cells have not been well characterized and may be dose-dependent. Some experiments have shown that Treg cells demonstrate an attenuated suppressive phenotype after radiotherapy and that radiotherapy can suppress the proliferation of Treg cells, specifically at a dose of 0.94 Gy (1). Another T-cell activation pathway is the OX40-OX40L signaling pathway. OX40 and its ligand OX40L belong to the tumor necrosis factor receptor and tumor necrosis factor superfamily (TNFR/TNF). OX40 is transiently expressed on activated T cells, and OX40L is mainly expressed on APCs; both of them actively regulate the function of T cells (including CD4+ T cells, CD8+ T cells, NKT cells and memory T cells) and their crosstalk with APCs (6,7). Blocking OX40-OX40L signaling helps to suppress immunity, which may be applied to clinical practice as therapy for autoimmune diseases. Regarding cancer treatment, experiments have shown that the agonist OX40-specific antibody or soluble OX40L-immunoglobulin fusion protein, that is ligation of OX40, enhances both CD4+ and CD8+ T-cell immunity to tumor cells, leading to more effective tumor elimination (6). Combined with the above, amplifying T-cell activation signaling might work synergistically with immune checkpoint blockade in immune activation post radiotherapy.

Prolonged exposure of tumor-infiltrating lymphocytes (TILs), mainly referring to CD8+ T cells, to cancer cells

can lead to complete or partial loss of their function, producing a state referred to as T-cell exhaustion, which is partly blamed for radio-resistance. Several pathways modulate CD8+ T-cell exhaustion, among which the PD-1-PD-L1 axis has been best studied (2). Upregulation of PD-1 on T cells in the TME and PD-L1 on tumor cells results in radio-resistance. Radiation primes tumor antigen presentation and elevates major histocompatibility complex (MHC) expression on tumor cells. It was reported that blockade of the PD-1-PD-L1 axis may contribute to radio-immune therapy because its combination with radiotherapy is effective both at the primary tumor site and in generating an abscopal effect (8). Tumor-associated macrophages (TAMs) mostly show the M2 phenotype, which expresses anti-inflammatory cytokines and contributes to biological processes, including angiogenesis, tumor cell growth and metastasis. Low-dose radiotherapy can reprogram TAMs to the M1 phenotype, which expresses pro-inflammatory cytokines and MHC-I/II, enhances tumor antigen presentation and renders tumor cells more susceptible to T-cell attack (9). Provided that various immunosuppressive effects can be sufficiently overcome, radiotherapy may have the potential to prime the anti-tumor immune response more effectively.

### *Pro-tumorigenic effect*

Radiotherapy-induced chronic inflammation is the main driver of fibrosis, in which constant immune responses occur alongside tissue remodeling and repairing processes in the tumor stroma. Tumor cells coevolve with their stroma, and tumor-associated stroma plays a vital role in tumor progression. Cancer-associated fibroblasts (CAFs) constitute most of the cells within the tumor stroma. CAFs are a heterogeneous cell population whose function varies according to tumor type and stage and may act either as tumor-promoting or tumor-inhibiting in the TME. However, CAFs are increasingly recognized as the promoters of tumor progression. Neoadjuvant chemoradiotherapy, the standard treatment for locally advanced rectal cancer, produces CAFs in cancer-associated stroma in rectal cancer, indicating that neoadjuvant treatment has an impact on CAFs (10). Furthermore, the increased CAF ratio indicated poor recurrence-free survival, yet was inversely correlated with the Ki67 labeling index. CAFs are perpetually activated and no longer revert to the normal phenotype in the TME (11). It is hypothesized that CAFs activated by chemoradiotherapy may be induced into a

resting phenotype in cancer cells that will survive genotoxic assaults (10).

CAFs are involved in tumor initiation and tumor progression, including tumor metastasis and tumor angiogenesis (12). In the early stage of tumor progression, the architecture of the tissue surrounding the tumor becomes highly distorted by aberrant accumulation of extracellular matrix (ECM) components. The hallmark event of this process is the breakdown of ECM (13). CAFs are the main source of ECM degradation molecules. CAFs secrete lysyloxidase (LOX) and uPA/uPAR, both of which are involved in ECM collagen crosslinking, thus increasing the bioavailability of growth factors, which are conducive for tumor growth and are typically sequestered by ECM (13). CAFs also secrete matrix remodeling enzymes (MMPs), the proteases more typically known for their ability to degrade ECM. MMPs may help tumor cells cross tissue boundaries and escape from the local tumor site to distant organs (12). Transforming growth factor beta (TGF $\beta$ ), which, in turn, is also an activator of CAFs, and C-X-C motif chemokine 12 (CXCL12) are involved in tumor angiogenesis. CAF-secreted MMP1 cleaves and activates protease-activated receptor-1 (PAR1) expressed on the cancer cell surface to stimulate cancer cell invasion and migration through PAR1-dependent Ca<sup>2+</sup> signaling (12,14). Simultaneously, the cancer cell-produced molecule Cyr61/CCN1 stimulates MMP-1 production by adjacent CAFs, creating a positive feedback loop potentiating tumor progression (14). Heparanase is an enzyme involved in bioactivities such as ECM breakdown and the promotion of radiation-induced tumor cell invasion. It is also modulated, although indirectly, by MMPs as the inhibition of MMP2 has been shown to abrogate radiotherapy-induced tumor invasion and progression (2). As mentioned above, CAFs express significantly higher levels of CXCL12 than stromal fibroblasts in noncancerous stroma. CXCL12 binds to its ligand CXCR4, which is expressed on tumor cells, to promote tumor cell proliferation, recruit epithelial progenitor cells into tumor masses and improve tumor cell growth and tumor angiogenesis (15).

Radiation increases the expression of TGF $\beta$ 1, which is a specific isoform that induces CAFs activation. Radiation-related cell injury induces inflammatory cell recruitment, and the infiltrated macrophages are the main source of pro-fibrotic mediators, which include TGF $\beta$ , the typical activator of CAFs (16). Within the primary tumor site, it was shown that TGF $\beta$  secreted by CAFs induces tumor cells to undergo the epithelial-mesenchymal transition

(EMT), thereby promoting tumor cell motility, invasion and metastasis. Additionally, at metastatic sites, activated fibroblasts are likely to play a role because they have also been found at distant metastatic sites. Studies have shown that systemic signaling cascades, as a consequent response to cancer, aid in the construction of the tumor-supportive microenvironment, including CAFs, at metastatic sites. Because the microenvironment preparation for micro-metastases is the rate-limiting step in tumor colonization, researchers have questioned whether tumor cells might bring their stroma with them (13). Both CXCL12-CXCR4 signaling and TGF $\beta$  signaling are responsible for the maintenance of the tumor-promoting ability of CAFs. Studies have shown that TGF $\beta$  and CXCL12-CXCR4 signaling converge to stimulate each other in CAFs to maintain myofibroblasts activation and its tumor-promoting ability (17). TGF $\beta$  signaling is involved in multiple biological processes, resulting in its heterogeneous nature of being tumor stimulatory or suppressive. Likewise, the tumor type, stage and other intrinsic or extrinsic factors determine CAF function as tumor stimulatory or suppressive.

In addition to CD8<sup>+</sup> cytotoxic T cells, CD4<sup>+</sup> T helper cells are also key regulators in tumor inflammatory processes. Different phenotypes of CD4<sup>+</sup> T cells play different roles in tumor progression but act as tumor suppressors in general. Treg cells are a subgroup of T cells that modulate the immune system to a suppressive state and inhibit the proliferation of effector T cells; thus, they mainly function as pro-tumor cells. Because the inflammatory microenvironment in the gut is closely correlated with colorectal cancer, Treg cells were thought to be exceptionally tumor suppressive in colorectal cancer. However, a study has shown that Treg cells contribute to colorectal cancer progression in multiple ways (18). Th1 cells stimulate interferon  $\gamma$  (IFN $\gamma$ ), the factor that upregulates PD-L1, which provides off signals to CD8<sup>+</sup> T cells (19). However, Th17 cells can function as either pro-tumor or anti-tumor immune cells depending on the tumor microenvironment. Their major tumor-promoting effects are linked to angiogenesis and the recruitment of pro-tumor neutrophils. By contrast, Th17-produced IL-17 can synergize with IFN $\gamma$  to induce the secretion of CXCL9 and CXCL10 by tumor cells, which, in turn, help recruit cytotoxic T cells (19). Taken together, the complex inflammatory reactions launched by the immune system to an irradiated tumor and the surrounding stroma are neither wholly immuno-stimulatory nor immuno-suppressive. The

immune system plays a dual role in the bioprocesses of tumor progression and remission.

## Tumor-infiltrating immune cells

### *Tumor-infiltrating lymphocytes*

The immune system plays an essential role in tumor defense, whose fundamental role is to maintain tissue homeostasis by continuous immune surveillance and to initiate inflammatory reactions that involve the activation of immune cells. TILs are mononuclear immune cells that infiltrate tumor tissues, representing the activation of the immunological response. Immune cells that infiltrate tumors comprise cells involved in innate and adaptive immunity, including tumor-associated macrophages, dendritic cells, and T cells. These infiltrating immune cells shape the tumor microenvironment through the cell factors they produce to an either tumor-promoting or tumor-preventing microenvironment. Cellular cross-talk also plays a vital role in microenvironment shaping, as demonstrated by T-cell regulated macrophage polarization to either a pro-tumor M1 or an anti-tumor M2 phenotype (20). TILs have been extensively studied in colorectal cancer and breast cancer, in which the abundance of TILs is a strong indicator of a patient's prognosis. Cytotoxic treatments such as chemotherapy and radiotherapy may sometimes act to initiate this immune cell-infiltrating system to reject cancer cells. Considering breast cancer as an example, TILs are usually more frequent in aggressive subtypes of breast cancer, namely, triple-negative breast cancer (TNBC) and HER2+ breast cancer. Evaluation of TILs on hematoxylin and eosin stain (H&E) slides may be a helpful parameter to assess a series of indexes, including pathological complete remission (pCR), response to treatment and the patient's prognosis. Studies have demonstrated that a higher level of TILs results in increased pCR rates in TNBC and HER2+ breast cancer, as well as better event-free survival (EFS) regardless of pCR, with a 3% decrease in the rate of an event for every 1% increase in TILs (21). Recommendations for a feasible and reproducible assessment of TILs on H&E sections of breast cancer have been published by the International TILs Working Group 2014 (20). The first step is the selection of the tumor area for evaluation, whose boundaries will be the indicator of TILs for assessment. Immune infiltrates outside the tumor borders at some distance from the tumor cluster should not be included in stromal TIL evaluation but can be recorded as

a separate element, because these infiltrates may be indicative of a reactive immune response (20). TILs can be subdivided into two groups, intratumoral TILs and stromal TILs. Intratumoral TILs are defined as lymphocytes located in tumor nests, typically present in lower numbers and less reproducibly measurable. Studies have proven that scoring intratumoral TILs adds no more information to what is provided by stromal TILs; and thus, TIL assessments are recommended to mainly focus on stromal TILs (20). Other types of cancers are relatively less studied in the field of TILs. Assessing TILs on H&E biopsies may guide clinical treatment.

### *Tumor-infiltrating immune cells and immunotherapy*

Researchers have long observed dense immune infiltration in mismatch repair-deficient tumors, hypothesizing that mismatch repair-deficient tumors activate the immune response. Mismatch repair-deficient tumors harbor thousands of somatic mutations, inducing substantial neoantigen. Immune checkpoint ligands are upregulated on activated T cells after priming. It is reasonable to assume that the microenvironment of mismatch repair-deficient tumors strongly expresses T-cell inhibitory ligands such as PD-L1, CTLA-4 and IDO1, which counterbalance the active immune response by the dense infiltrating immune cells. A study has shown that mismatch repair-deficient colorectal cancer is more responsive to PD-1 blockade than mismatch repair-proficient tumors (22). Patients with mismatch repair-deficient tumors other than colorectal cancer may also benefit more from anti-PD-1 therapy; therefore, the assessment of tumor genomes may help guide immune treatment.

Immunotherapy, which aims to harness the immune system against cancer, is becoming a clinically proven effective therapy option that can lead to a durable tumor rejection response. The most common and effective approach to achieving an anti-tumor immune response is by blocking immune checkpoints and inhibitory receptors. Clinical cases such as patients who have undergone relapse after immunotherapy and those who deemed to be PD-L1-positive have earned no benefit from anti-PD-L1 therapy, necessitating the improvement of clinical applications of immunotherapy. To initiate effective and durable anti-tumor responses, combination therapy that targets every step that stall the activation process is necessary (23). Innate immunity coordinates adaptive immunity by processes such as antigen presentation. One combination therapy being

explored is to boost innate immune activation converging onto IFN signaling. The cGAS/STING pathway upregulates IFN-I, which enhances dendritic cell (DC) maturation and, hence, optimizes adaptive immunity. Additionally, cGAS/STING is a pattern recognition receptor that recognizes DAMPs released by dying cells. Thus, cytotoxic treatments such as radiotherapy will enhance STING to augment IFN-I signaling in DCs, contributing to immune-mediated regression of irradiated tumors, which can also be counted as a clue concerning the radioimmunotherapy combination (23).

That radiotherapy releases large amounts of immunogenic tumor antigens provides the basis for its combination with immunotherapy. Additional APC activation can enhance the *in situ* vaccination effect of radiotherapy (1). Inhibitory receptors upregulate sustainably on chronically stimulated T cells, turning them into a cellular state called exhausted T cells. Therefore, in addition to APC activation, blocking T-cell inhibitory signals to maintain T cells effectively activated should add benefits to tumor eradication (1). Immune checkpoint blockade using the CTLA-4 antibody ipilimumab and PD-1 inhibitor pembrolizumab and nivolumab, which have been approved by FDA, have already been applied to clinical practice. Double blockade of CTLA-4 and PD-L1 in combination with radiotherapy, in which CTLA-4 blockade predominantly inhibits Treg cells, PD-L1 blockade reverse T-cell exhaustion, and radiation enhances intratumoral T cells, produces an optimal therapeutic effect (1). Another immunosuppressive factor that will be upregulated by radiotherapy is TGF $\beta$ , which suppresses CD8 $^+$  T cells, upregulates Treg cells and incapacitates DCs. Radiotherapy combined with TGF $\beta$  inhibition contributes to more potent immune response and may result in better tumor suppression. TGF $\beta$  is also well known for its ability to promote EMT. miR-200 is a significant factor that is involved in EMT and that acts as an inhibitor of the EMT process. A study has shown that TGF $\beta$  can induce PD-L1 upregulation, while miR-200 not only directly down-regulates PD-L1 but also increases CD8 $^+$  TILs and decreases exhausted T cells, and in turn, PD-L1 inhibits miR-200 (24), indicating that the addition of PD-L1 inhibition to the radiotherapy/TGF $\beta$  blockade may further improve the anti-tumor response. Radiotherapy can elicit IFN $\gamma$ -mediated upregulation of PD-L1, leading to adaptive resistance to radiotherapy. Therefore, PD-1/PD-L1 blockade may be an important addition to radioimmunotherapy (1). When T-cell infiltration and activation are

therapeutically enhanced, ensuing IFN secretion and the resulting adaptive resistance may make PD-L1 blockade an important adjuvant for sustained anti-tumor activity. High levels of PD-L1 are often seen when immunotherapy resistance occurs, making PD-1/PD-L1 blockade an essential part of effective combination immunotherapy (23). In addition to harnessing cytotoxic T cells against cancer cells, inhibiting tumor inflammation-induced anti-tumor chemokines is another therapeutic choice. CXCL12/CXCR4 signaling pathway is involved in tumor angiogenesis. It is related to bone marrow-derived TIE-2-positive macrophages that are pro-angiogenic and will be specifically attracted to irradiated tumors by CXCL12, thereby contributing to tumor recurrence post cancer therapy (19). In this way, blocking CXCL12/CXCR4 signaling can inhibit tumor angiogenesis and metastatic seeding.

The immune system complexity varies depending on numerous intrinsic and extrinsic factors; thus immune-related therapies need to be more personalized.

## Intestinal microbiota, colorectal cancer and cancer therapy

### *Intestinal microbiota and colorectal cancer*

The human body possesses at least 10 times more intestinal microbial cells than human cells, and approximately 100 times as many genes in the gut microbiome as in human cells (25,26). An individual's enteric flora is a unique mix of bacterial species because the human intestine is colonized by microbes immediately after birth, shaped by host genetics, lifestyle, environment and exposure to antibiotics. A mature healthy gut microbiota remains relatively stable throughout adult life. Gut microbiota plays a crucial part in maintaining intestinal homeostasis, by being involved in epithelial barrier function maintenance and the processes of intestinal inflammation and immune responses (26,27). Intestinal epithelium barrier integrity is the fundamental condition for healthy functional intestine, requiring tight cellular junctions that seal apical epithelium and the mucus layer that lines the intestinal wall (26,28). It has long been recognized that colorectal cancer is closely related to intestinal chronic inflammation (26,28-30). Inflammation can be triggered by the presence of microorganisms at sites where they do not belong. Intestinal microbial dysbiosis is linked to aberrant immune responses, often accompanied by the abnormal production of inflammatory cytokines.

The best-known inflammatory disorder of the intestine is inflammatory bowel disease (IBD), and patients with IBD harbor a significantly higher risk of developing colitis-associated colorectal cancer. The gut microbiome can strengthen the host defense against harmful enteric pathogens and control intestinal inflammation. By contrast, changes in the composition of the intestinal microbiota may disrupt intestinal homeostasis and lead to colitis and even tumorigenesis (26). *Fusobacterium varium*-produced butyric acid is cytotoxic to colorectal epithelium, which will lead to ulcerative colitis and impairment of intestinal barrier function (31). The observed fact of no tumor formation in germ-free models of colorectal cancer mice suggests that colorectal cancer may initiate only with the inflammatory responses induced by microbiota-derived stimuli (26,29). Hosts recognize the gut microbiome through various pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), especially TLR2 and TLR4, which are involved in intestinal homeostasis maintenance by triggering immune responses to eliminate harmful pathogens and preserve host epithelium integrity (28,32). However, uncontrolled TLR activation will lead to exaggerated inflammation and excessive intestinal epithelial cell proliferation, resulting in mucosal damage and microbiota translocation and rendering the host susceptible to colitis and colorectal cancer (26). Nevertheless, defects in TLR signaling, such as MYD88-deficient mice that cannot signal through IL-1 family receptors and most TLRs, are also pathogenic. MYD88-deficient mice show significant defects in intestinal barrier repair and are susceptible to colitis and colorectal cancer because they have insufficient levels of chemokines (e.g., IL-6, TNF and CXCL-1) that are indispensable in intestinal homeostasis (26,33).

It is believed that progression from normal epithelium to colorectal adenocarcinoma requires a series of genetic alterations. Intestinal microorganism dysbiosis may play a role in this process; conversely, it may be shaped in the gradual transition processes of intestinal epithelium. Gastric cancer is a typical example of bacteria-induced carcinogenesis in which infection of *Helicobacter pylori* is closely associated with the initiation and development of gastric cancer. By contrast, thus far, no pathogenic bacteria that are specifically related to colorectal cancer have been determined. Instead of infection of some specific bacterial pathogen, it seems that the pro-tumor effect of microbiota is caused by dysbiosis in the gut microbiome where multiple species of microbiota contribute to colorectal

cancer (32,34). The bacterial driver-passenger model developed in recent years may explain the current cognition on microbial carcinogenesis of colorectal cancer. Colonization of pathogenic bacteria, which function as driver bacteria, leads to chronic inflammation at the tumor site by inducing excessive cell proliferation and DNA damage to intestinal epithelial cells (IECs). The consequent barrier dysfunction will provide a preferred niche for opportunistic pathogens (passenger bacteria), which gradually outcompete the driver bacteria and cause tumor formation (35). Studies have revealed that the gut microbial composition is significantly different from that of normal human microbiota. *Bacteroidetes* and *Firmicutes* are the two dominant phyla in human intestinal microbiota that account for more than 90% of the microbiota (36). Studies have shown that *Bacteroidetes* and *Firmicutes* are reduced in colorectal cancer tissues (37), while *Fusobacterium* is enriched (38-40). Additionally, diversity of microbiota in cancerous tissue is lower than that in normal tissues (40). Recently, based on the phenomenon that all right-sided patients, who have relatively worse prognosis than left-sided colorectal cancer patients, can be detected the existence of bacterial biofilm, the driver-passenger model may be extended. Instead of some relatively specific pathogenic bacteria, microbial biofilm consisting of a high density of invasive bacteria may act as the “driver” at the early stage of colorectal cancer formation (41).

Gut microbiome disorder is not only associated with colorectal problems but also with systemic carcinogenesis. Experiments have shown that the composition of commensal microorganisms in patients with liver cirrhosis is different from that in healthy controls, suggesting that intestinal dysbiosis may be an indicator for monitoring patient health (42). Moreover, the intestinal bacteria *Helicobacter hepaticus* enhances intestinal adenoma multiplicity and even significantly promotes mammary carcinoma, indicating that the inflammatory status of the gut may have systemic effects (26). Genetic mutations that weaken colonic epithelial barrier repair or strengthen inflammatory responses improve tumor formation by facilitating extensive mucosal damage or by creating an inflammatory environment that favors excessive tissue repair and tumorigenesis.

### ***Intestinal microbiota and chemotherapy***

The abundance of *Fusobacterium* was found to be higher in colorectal cancer tissues than in normal tissues. More of

*Fusobacterium* in the intestine is associated with a high degree of microsatellite instability (MSI) (38,40), and the abundance of *Fusobacterium* is inversely correlated with a patient's prognosis (43). A recent study on chemo-resistant colon cancer reported that *Fusobacterium* is enriched in patients relapsed after chemotherapy of 5-FU, and its enrichment is an independent indicator of tumor cell aggressiveness (39). *Fusobacterium* promotes the resistance of 5-FU by activating the autophagy pathway through TLR4-MYD88 innate immune signaling-dependent miR-18a\*/miR-4802 loss (39). Apart from the primary tumor site, *Fusobacterium* is also enriched in distant metastatic tumors, indicating the relative stability between paired primary and distant metastatic tumor sites (44). Another study revealed that bacteria-expressed cytidine deaminase (CDDL), which is mainly found in *Gammaproteobacteria*, can metabolize gemcitabine to its inactive form, leading to gemcitabine resistance. Patients with pancreatic ductal adenocarcinoma (PDAC) showed significantly more abundant bacteria (mainly *Gammaproteobacteria*) in PDAC samples than in healthy pancreas controls. Instead of potentiating other signaling pathways to induce chemo-resistance, this study showed that the drug concentrations can be lowered in the presence of intratumoral bacteria, resulting in paradoxically lower drug concentrations at the target tumor site than in other organs (45).

### ***Intestinal microbiota and radiotherapy***

Recently, researchers have noticed that gut microbiota is closely related to the response to radiotherapy and susceptibility to toxic side effects. The largest limitation of radiotherapy is the heterogeneity in radiosensitivity and radiation toxicity that correlates with tumor relapse, a patient's quality of life and mortality.

Radiotherapy toxicity is recognized as damage to healthy tissues, especially actively proliferating tissues, resulting in side effects such as hematopoietic syndrome and gastrointestinal irradiation injury (46). Patients who have received pelvic radiation often show symptoms such as diarrhea, rectal bleeding, tenesmus and fecal incontinence (47). Acute changes in the intestinal tract are mediated by the cytotoxic effect of radiation to the rapidly proliferating epithelium, and these changes are amplified by inflammation. Ionizing radiation activates the coagulation system, leading to ulceration, which exposes the underlying tissues to enteric bacteria and increases the inflammation response because the immune system struggles to contain

bacterial translocation. The ulcer may then progress to fibrosis, driven by TGF $\beta$ 1-initiated CAFs (48). In the 1960s, irradiated germ-free mice were shown to develop fewer gastrointestinal symptoms, leading to studies with antibiotics as radiation response modifiers, which had conflicting results (48). Radiation may lead to alterations in gut microbiota. A recent study assessed the fecal microbial composition of 5 mice that had undergone radiation by high-throughput sequencing of bacterial 16S rRNA gene and found that radiation induced significant alterations in the microbial composition of the intestinal tract at the genus level. Irradiation increased the level of the genera *Alistipes* and decreased the genera *Prevotella* in the large intestine (49). In another study based on 9 gynecologic cancer patients who received pelvic radiotherapy, the phyla *Firmicutes* and *Fusobacterium* were significantly decreased by 10% and increased by 3%, respectively (47). Although the above-mentioned studies covered only a small number of experimental subjects, it is reasonable to hypothesize that radiation-induced changes to the intestine are associated with changes in the gut microbiota.

It was reported that the composition of intestinal microbiota in male and female mice is different, related to different susceptibility to radiation toxicity (50). To erase the difference, fecal transplantation into sex-matched or mismatched mice was performed, and researchers found that fecal microbiota transplantation (FMT) is an effective therapy against radiation-induced death in a mouse model, and its efficiency is determined by the gender match between the donor and recipient. Furthermore, they found that FMT improved gastrointestinal tract function and intestinal epithelial integrity, as well as elevated the peripheral white blood cell counts in irradiated mice, suggesting that FMT might serve as a treatment method to alleviate radiation-induced toxicity and improve the prognosis of patients after radiotherapy (50).

Despite the radiation toxicity, radiosensitivity in relation to intestinal microbiome also needs future exploration. Relatively little is known about how the gut microbiota regulates the host response to radiotherapy. Radiotherapy exerts anti-tumor responses that are mediated by the immune response. Because the gut microbiota has been shown to affect the immune response, it can be hypothesized that the gut microbiota also plays a role in the immunogenic effect of radiotherapy (46). Detailed mechanisms underlying how the gut microbiota influences responses to radiotherapy need further study.

### *Intestinal microbiota and immunotherapy*

The gut flora educates the immune system from early life, whereas the immune system shapes the microbiota. The observed instance that Asian populations migrated to North America acquire an equal risk of colorectal cancer as local populations within one generation suggests that environmental factors play a fundamental role in altering the intestinal microbiota (26). A recent study has shown that nongenetic factors such as age, gender and seasonality have an evident impact on immune cytokine secretion and thus affect the host systematic immune system (51). Regarding functions of the immune system, distinguishing and responding to pathogenic organisms are of equal importance to recognition and tolerance of commensal microorganisms. Commensal microbes calibrate immune responses by producing molecules that mediate host-microbial interactions (52). It was observed that the gut microbiota *Fusobacterium* modulates the tumor micro-environment by upregulating tumor-permissive myeloid-derived suppressor cells (MDSCs) in colorectal cancer (53). The gut microbiota plays an important role in shaping and modulating the immune response. One major approach to adjusting the immune system to be more effective in fighting against cancer is to block immune checkpoints. Antibodies targeting CTLA-4 and PD-L1 have been successfully applied to clinical practice. Recent studies have shown that gut microbiota affects the efficacy of CTLA-4 and PD-L1 blockade immunotherapy. Tumors respond poorly to CTLA-4 blockade treatment in germ-free or broad-spectrum antibiotic-treated mice compared with specific-pathogen-free (SPF) mice, indicating that gut microbiota is required for the therapeutic effect of CTLA-4 blockade. However, CTLA-4 antibody often induces T-cell-dependent intestinal epithelial cell apoptosis, leading to mucosal lesions that are exposed to gut microorganisms (54). Anti-CTLA-4 treatment significantly alters the composition of intestinal microorganisms, while *Bacteroides fragilis* (*B. fragilis*) remains constant. Oral feeding with *B. fragilis* in germ-free mice restores the anti-cancer effect of anti-CTLA-4 treatment by inducing DC maturation and the Th1 immune response (54). CTLA-4 blockade remarkably affects the proportion of *Bacteroides* spp. in the commensal microbiome. The fecal abundance of *B. fragilis* negatively correlates with the tumor size after CTLA-4 blockade. Hence, CTLA-4 antibody modulates the abundance of *Bacteroides* spp. in the gut, ultimately affecting its own anti-tumor efficacy. In this way, measures such as fecal microbial transplantation can be taken to

maximize the anti-tumor efficacy (54). Another study found that the anti-cancer efficacy of PD-L1 blockade can be elevated by the commensal *Bifidobacterium* (55). Researchers compared subcutaneous B16.SIY melanoma growth in C57BL/6 mice obtained from two different facilities, termed as TAC and JAX mice, and they found less aggressive tumors in JAX mice due to significantly higher CD8+ T-cell infiltration in JAX mice than in TAC mice. Cohousing the two mouse populations eliminates the abovementioned differences (55). Only transferring JAX fecal materials resulted in significantly slower tumor growth that was which equals to treatment with  $\alpha$ PD-L1mAb. Combination treatment with both JAX fecal transplantation and PD-L1 blockade improved tumor control (55). The response to  $\alpha$ PD-L1 mAb treatment is positively associated with the abundance of *Bifidobacterium* genus in intestinal microbiota. However, this anti-tumor effect was abolished in CD8+ T-cell-depleted mice, suggesting that the immune response induced by *Bifidobacterium* was T-cell dependent (55). Another study published more recently showed that the abundance of *Akkermansia muciniphila* (*A. muciniphila*) is positively correlated with the anti-tumor efficacy of PD-1 blockade. Oral feeding of *A. muciniphila* to non-respondent mouse models restored the efficacy of PD-1 blockade by recruiting CD4+ T-cell infiltrate into the TME (56).

### Conclusions

The gut microbiota is closely correlated with colorectal cancer initiation and progression through related inflammation and immune responses in multiple ways, and composition of microbiota is associated with tumor sensitivity to cancer therapy. However, little is known about the detailed mechanisms of how microbiota modulates tumor regression or resistance to cancer therapy. Because gut microbiota is still a brand-new frontier in colorectal cancer treatment, studies on this subject and combination cancer therapy on colorectal cancer are still greatly needed.

### Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81672987).

### Footnote

*Conflicts of Interest:* The authors have no conflicts of interests to declare.



## References

- Herrera FG, Bourhis J, Coukos G. Radiotherapy combination opportunities leveraging immunity for the next oncology practice. *CA Cancer J Clin* 2017; 67:65-85.
- Barker HE, Paget JT, Khan AA, et al. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. *Nat Rev Cancer* 2015; 15:409-25.
- Ko A, Kanehisa A, Martins I, et al. Autophagy inhibition radiosensitizes *in vitro*, yet reduces radio-responses *in vivo* due to deficient immunogenic signalling. *Cell Death Differ* 2014;21:92-9.
- Galluzzi L, Bravo-San Pedro JM, Demaria S, et al. Activating autophagy to potentiate immunogenic chemotherapy and radiation therapy. *Nat Rev Clin Oncol* 2017;14:247-58.
- Tam SY, Wu VW, Law HK. Influence of autophagy on the efficacy of radiotherapy. *Radiat Oncol* 2017; 12:57.
- Gough MJ, Crittenden MR, Sarff M, et al. Adjuvant therapy with agonistic antibodies to CD134 (OX40) increases local control after surgical or radiation therapy of cancer in mice. *J Immunother* 2010; 33:798-809.
- Croft M. Control of immunity by the TNFR-related molecule OX40 (CD134). *Annu Rev Immunol* 2010;28:57-78.
- Wang X, Schoenhals JE, Li A, et al. Suppression of type I IFN signaling in tumors mediates resistance to anti-PD-1 treatment that can be overcome by radiotherapy. *Cancer Res* 2017;77:839-50.
- Klug F, Prakash H, Huber PE, et al. Low-dose irradiation programs macrophage differentiation to an iNOS<sup>+</sup>/M1 phenotype that orchestrates effective T cell immunotherapy. *Cancer Cell* 2013;24:589-602.
- Verset L, Tommelein J, Moles Lopez X, et al. Impact of neoadjuvant therapy on cancer-associated fibroblasts in rectal cancer. *Radiother Oncol* 2015; 116:449-54.
- Li H, Fan X, Houghton J. Tumor microenvironment: The role of the tumor stroma in cancer. *J Cell Biochem* 2007;101:805-15.
- Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer* 2006;6:392-401.
- Marsh T, Pietras K, McAllister SS. Fibroblasts as architects of cancer pathogenesis. *Biochim Biophys Acta* 2013;1832:1070-8.
- Radisky ES, Radisky DC. Stromal induction of breast cancer: inflammation and invasion. *Rev Endocr Metab Disord* 2007;8:279-87.
- Orimo A, Gupta PB, Sgroi DC, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 2005;121:335-48.
- Westbury CB, Yarnold JR. Radiation fibrosis – current clinical and therapeutic perspectives. *Clin Oncol (R Coll Radiol)* 2012;24:657-72.
- Kojima Y, Acar A, Eaton EN, et al. Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. *Proc Natl Acad Sci U S A* 2010;107:20009-14.
- Timperi E, Pacella I, Schinzari V, et al. Regulatory T cells with multiple suppressive and potentially pro-tumor activities accumulate in human colorectal cancer. *OncoImmunology* 2016;5:e1175800.
- Coussens LM, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation: a magic bullet? *Science* 2013;339:286-91.
- Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol* 2015;26:259-71.
- Criscitiello C, Esposito A, Trapani D, et al. Prognostic and predictive value of tumor infiltrating lymphocytes in early breast cancer. *Cancer Treat Rev* 2016;50:205-7.
- Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015;373:12509-20.
- Minn AJ, Wherry EJ. Combination cancer therapies with immune checkpoint blockade: convergence on interferon signaling. *Cell* 2016;165:272-5.
- Chen L, Gibbons DL, Goswami S, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. *Nat Commun* 2014;5:5241.
- Kim YS, Kim J, Park SJ. High-throughput 16S rRNA

- gene sequencing reveals alterations of mouse intestinal microbiota after radiotherapy. *Anaerobe* 2015;33:1-7.
26. Saleh M, Trinchieri G. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. *Nat Rev Immunol* 2011;11:9-20.
  27. Cui M, Xiao H, Li Y, et al. Faecal microbiota transplantation protects against radiation-induced toxicity. *EMBO Mol Med* 2017;9:448-61.
  28. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014;12:661-72.
  29. Arthur JC, Perez-Chanona E, Mühlbauer M, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 2012;338:120-3.
  30. Tilg H, Adolph TE, Gerner RR, et al. The intestinal microbiota in colorectal cancer. *Cancer Cell* 2018;33:954-64.
  31. Ohkusa T, Okayasu I, Ogihara T, et al. Induction of experimental ulcerative colitis by *Fusobacterium varium* isolated from colonic mucosa of patients with ulcerative colitis. *Gut* 2003;52:79-83.
  32. Schwabe RF, Jobin C. The microbiome and cancer. *Nat Rev Cancer* 2013;13:800-12.
  33. Salcedo R, Worschech A, Cardone M, et al. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. *J Exp Med* 2010;207:1625-36.
  34. Abreu MT, Peek RM Jr. Gastrointestinal malignancy and the microbiome. *Gastroenterology* 2014;146:1534-46.
  35. Pagès F, Mlecnik B, Marliot F, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet* 2018;10135:2128-39.
  36. Morgillo F, Dallio M, Della Corte CM, et al. Carcinogenesis as a result of multiple inflammatory and oxidative hits: a comprehensive review from tumor microenvironment to gut microbiota. *Neoplasia* 2018;20:721-33.
  37. Bhatt AP, Redinbo MR, Bultman SJ. The role of the microbiome in cancer development and therapy. *CA Cancer J Clin* 2017;67:326-44.
  38. Castellarin M, Warren RL, Freeman JD, et al. *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. *Genome Research* 2012;22:299-306.
  39. Yu T, Guo F, Yu Y, et al. *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell* 2017;170:548-63.
  40. Chen W, Liu F, Ling Z, et al. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. *PLoS One* 2012;7:e39743.
  41. Li S, Konstantinov SR, Smits R, et al. Bacterial biofilms in colorectal cancer initiation and progression. *Trends Mol Med* 2017;23:18-30.
  42. Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014;513:59-64.
  43. Mima K, Nishihara R, Qian ZR, et al. *Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. *Gut* 2016;65:1973-80.
  44. Bullman S, Pedamallu CS, Sicinska E, et al. Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. *Science* 2017;358:1443-8.
  45. Geller LT, Barzily-Rokni M, Danino T, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* 2017;357:1156-60.
  46. Roy S, Trinchieri G. Microbiota: a key orchestrator of cancer therapy. *Nat Rev Cancer* 2017;17:271-85.
  47. Nam YD, Kim HJ, Seo JG, et al. Impact of pelvic radiotherapy on gut microbiota of gynecological cancer patients revealed by massive pyrosequencing. *PLoS One* 2013;8:e82659.
  48. Ferreira MR, Muls A, Dearnaley DP, et al. Microbiota and radiation-induced bowel toxicity: lessons from inflammatory bowel disease for the radiation oncologist. *Lancet Oncol* 2014;15:139-47.
  49. Kim YS, Kim J and Park SJ. High-throughput 16S rRNA gene sequencing reveals alterations of mouse intestinal microbiota after radiotherapy. *Anaerobe* 2015;33:1-7.
  50. Cui M, Xiao H, Li Y, et al. Faecal microbiota transplantation protects against radiation-induced toxicity. *EMBO Mol Med* 2017;9:448-61.
  51. Ter Horst R, Jaeger M, Smeekens SP, et al. Host and environmental factors influencing individual human cytokine responses. *Cell* 2016;167:1111-24.
  52. Schirmer M, Smeekens SP, Vlamakis H, et al. Linking the human gut microbiome to inflammatory cytokine production capacity. *Cell* 2016;167:1125-36.

53. Kostic AD, Chun E, Robertson L, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013;14:207-15.
54. Vétizou M, Pitt JM, Daillère R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 2015;350:1079-84.

**Cite this article as:** Zhang S, Wang Q, Zhou C, Chen K, Chang H, Xiao W, Gao Y. Colorectal cancer, radiotherapy and gut microbiota. *Chin J Cancer Res* 2019;31(1):212-222. doi: 10.21147/j.issn.1000-9604.2019.01.16

55. Sivan A, Corrales L, Hubert N, et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 2015;350:1084-9.
56. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018;359:91-7.