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Review

The microbiome in anti-cancer therapy

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ABSTRACT

The commensal microbiome constitutes an important modulator of host physiology and risk of disease, including cancer development and progression. Lately, the microbiome has been suggested to modulate the efficacy of anti-cancer treatment. Examples include chemotherapy and total body irradiation-induced barrier function disruption, leading to microbial efflux that drives activation of anti-tumorigenic T cells; Microbiome-driven release of reactive oxygen species contributing to the efficacy of platinum salts; and microbiome-induced immune priming promoting the anti-tumor effects of alkylating chemotherapy and immune checkpoint inhibitors. Furthermore, selected commensals are able to colonize solid tumors. This 'tumor microbiome' may further impact local tumor responses to treatment and potentially be harnessed for tumor-specific targeting and therapeutic delivery. In this review, we present recent advances in understanding of the intricate role of microbiome in modulating efficacy of a number of anti-cancer treatments, and discuss how anti-cancer treatment approaches utilizing the tumor microbiome may enhance oncological treatment efficacy.

1. Introduction

1.1. The microbiome in cancer

Cancer treatment has matured over the past few decades to include a number of treatment modalities such as surgery, chemotherapy, radiotherapy, immunotherapy and hormonal therapy [1]. Newly approved therapeutic approaches have advanced the available tumor targeting arsenal. These are exemplified by anti-human papillomavirus vaccines used as a preventive treatment of cervical cancer, ipilimumab and pembrolizumab, antibodies to cytotoxic T lymphocyte antigen 4 (CTLA4) [2] and programmed cell death protein 1 (PD1) [3], respectively, used as immunotherapy treatments for metastatic melanoma. Despite these tremendous developments, many cancers still feature an unacceptable high mortality rate. A mechanistic understanding of the patient-specific processes governing treatment response, efficacy and resistance are crucial in the development of new therapeutic strategies, and in optimizing the efficacy of existing drugs based on personalized tumor and patient characteristics.

Over a century ago, William B. Coley injected heat inactivated streptococcal organisms into cancer patients leading to effective anti-tumor responses. With this work he implemented the first immunotherapy anti-cancer treatment and demonstrated a role for the immune system as a modulator of tumor growth [4]. It was later shown in the 1920s that intravesicular injection of *mycobacterium bovis* in patients with superficial bladder cancer resulted in an anti-cancer immune response leading to an increased survival rate [5], thereby demonstrating microbial immune activation leading to cancer cells clearing. The vast number of microorganisms colonizing the human body are collectively termed the microbiome and are estimated to feature equal numbers to that of human cells [6] and possess up to 100 times more genes compared to eukaryotic genes of the human body. While this term often refers to the collective genomes of these microorganisms, within this review it interchangeably refers to the collection of microorganisms colonizing the human body. The microbiome and its human host have coevolved into a "holobiont", in which complex prokaryotic-eukaryotic interactions regulate aspects of human physiology. This elaborate crosstalk is regulated by endogenous factors such as host genetics [7] and immune responses [8], and by environmental factors including nutrition [9], biogeographical localization [10] and medication profiles [11].

The most extensively studied microbiome is the gut microbiome that performs a number of vital functions such as hydrolysis of dietary compounds [12], vitamin production [13], control of pathogen colonization [14] and protection from systemic infections [15]. The gut microbiome additionally plays critical roles in immune system development, highlighted by 'sterile' germ free mice featuring an underdeveloped immune system, which is reversible upon microbial colonization [16]. Additionally, the microbiome colonizes other mucosal surfaces including lungs, vagina, gut and oral cavity, where it plays

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organ and context specific physiological functions [17].

The microbiome has also been suggested to colonize tumors [18–20]. The role of the microbiome as a contributor to carcinogenesis has been suggested in multiple models and contexts [21,22]. Recently, the gut microbiome was also shown to be an important contributor to combating cancer by modulating the efficacy of a wide range of anticancer treatments [23–26]. In this review, we focus on the effects the microbiome has on the efficacy of a number of anti-cancer treatment strategies including immunotherapy [23,24], chemotherapy [25,26] and adoptive cell transfer therapy [27], and the potential mechanisms and ramifications of these effects.

1.2. The microbiome and cancer immunity

Immune system – cancer interactions play important roles in cancer prevention, development and progression, and are reviewed in depth elsewhere [28,29]. The microbiome has been recently suggested to bear an important immune modulatory capacity, thereby impacting cancer immunity. The microbiome can affect cancer at all of its developmental stages, including elimination, equilibrium, and escape [29]. During elimination, foreign epitopes (*e.g.* BAGE-1, Cyclin-A1 and LY6K) produced by cancer cells induce an immune response during which, T cells eliminate cancer cells [28,29]. The microbiome may modulate this T cell mediated tumoricidal activity, as administration of antibiotics severely reduced the efficacy of adoptive T cell transfer in several mouse models of cancer [30,31]. As tumors grow, they implement mechanisms associated with angiogenesis while also creating local inflammation surrounding the tumor site [32], characterized by accumulation of tumor-infiltrating myeloid cells [33–35]. The tumoricidal potency of these tumor-infiltrating myeloid cells has been suggested to depend on signals perceived from the microbiome [25]. Cancer cells that survive the elimination phase begin to propagate in a manner that creates a dynamic equilibrium between tumor growth and the reactive immune response. When cancer cells become unrecognizable to immune surveillance by means of cancer cell genetic mutagenesis or by accumulation of epigenetic modifications [28], the cancer enters the “escape” phase. This phase involves the recruitment of Treg and myeloid suppressive cells to tumor sites, where they contribute to local immune suppression. The microbiome at this stage may modulate Treg induction [36] and the activity of tumor-infiltrating myeloid cells [25].

1.3. The tumor microbiome

Microorganism colonization of tumors has been reported as early as the 1950s [37], with multiple subsequent reports further demonstrating a tumor microenvironment colonization by microorganisms [18–20,38,39]. In rodent models, systemically administered bacteria have been shown to localize and replicate within tumor tissues [40]. Microorganism presence in tumor tissue can be directly related to tumorigenesis, as exemplified by *H. pylori* in gastric cancer [41], or may represent a coincidental infection. Tumor growth leads to development of new vasculature that is characterized by irregular organization and leakiness, which is thought to permit bacterial entry into the tumor microenvironment. The irregularity of vasculature leads to an insufficient blood supply to tumor cells [42] and ineffective chemotherapy drug delivery and response in these regions [43]. Insufficient blood supply combined with an increased oxygen demand from rapidly proliferating tumor cells, forms pockets of necrosis and hypoxia within the tumor niche [44]. These conditions, together with tumor microenvironment immune suppression, may favor local bacterial replication [45].

Tumor colonization by microorganisms can be a result of coinciding infections (*e.g.* wound infection during surgery [46]) or bacterial translocation from the gut lumen upon disruption of the gastrointestinal epithelial barrier [47].

Some indications suggest that an intricate interaction exists between the ‘tumor microbiome’ and local tumor immunity, as exemplified by *Fusobacterium nucleatum*, an oral microbiome commensal [48] linked to a number of pathogenic conditions [49,50] including colon adenocarcinoma [51]. In colon adenocarcinoma, *F. nucleatum* inhibits NK cell cytotoxicity and T cell activity, thereby limiting their ability to kill tumor cells [52].

2. The microbiome in anti-cancer therapy

The microbiome has been suggested to play an intricate role in modulating the efficacy of a number of anti-cancer therapeutic approaches [23–26]. In the sections below, we will discuss the role played by the microbiome in these anti-cancer therapies and discuss the suggested mechanisms driving these effects.

2.1. Platinum-based chemotherapy agents

Platinum-based cytotoxic compounds have been utilized for many years as chemotherapy agents, with the first such compound being FDA-approved for cancer treatment as early as 1978 [53]. Anti tumoral activity by these agents is mediated by disruption of genomic DNA through DNA adduct formation, binding guanine and forming intra-strand crosslinks, leading to a cytotoxic effect resulting in cancer cell apoptosis [54]. One such agent, oxaliplatin, has the ability to induce immunogenic cell death driving T cell immunity [54]. Iida et al. [25] investigated the possibility that commensal microbiome may modulate the anti-cancer effects of this platinum-based compound. Mice harboring subcutaneous tumors treated with oxaliplatin featured tumor regression and improved survival. Conversely, mice treated with antibiotics or germ-free mice showed reduced tumor regression and impaired survival. The effects of oxaliplatin on tumor progression were brought about by reactive oxygen species (ROS) production in tumor-infiltrating myeloid cells leading to tumor cell DNA damage and subsequent tumor regression (Fig. 1A). ROS production in tumor-infiltrating myeloid cells was found to be dependent on *Cybb* expression of ROS generating NADPH oxidase 2 (NOX2). Accordingly, ROS production by myeloid cells was impaired in $Cybb^{-/-}$ mice. Interestingly, ROS production was also found to be impaired in antibiotics treated wild type mice, but could be rescued by administration of LPS. Mice lacking components of the TLR pathway ($Tlr4^{-/-}$ and $Myd88^{-/-}$ mice) did not respond to treatment with oxaliplatin, indicating that TLR agonists from members of the commensal microbiome may promote ROS generation by innate immune cells within the tumor microenvironment leading to tumor cell death.

2.2. Alkylating agents

One of the most frequently used chemotherapeutic agents for the treatment of lymphomas and solid tumors is Cyclophosphamide (CTX), an alkylating agent that also induces immunomodulatory effects and immunogenic cancer cell death [55]. Cyclophosphamide affects the tumor immunosuppressive environment by inducing a reduction in Tregs [56] and by increasing the number of T_H1 and T_H17 cells that feature an effect on tumor outgrowth [57]. A study by Viaud et al. [26] investigated the potential role played by commensal microbial communities as participants in the anti-tumor immunological response brought about by CTX treatment. Mice treated with CTX were found to develop an impaired gut epithelial barrier, similar to mucositis occurring in patients undergoing treatment with CTX and other alkylating agents. Dysbiosis (deviation from the bacterial ecosystem equilibrium), detected in the small intestine 7 days following drug administration, was associated with mesenteric lymph node and splenic translocation of Gram-positive commensal bacteria, including *Lactobacillus johonsonii* and *Enterococcus hirae* [26]. In the spleen, CTX induced the conversion of naïve CD4+ T cells to pathogenic T_H17 cells. Germ

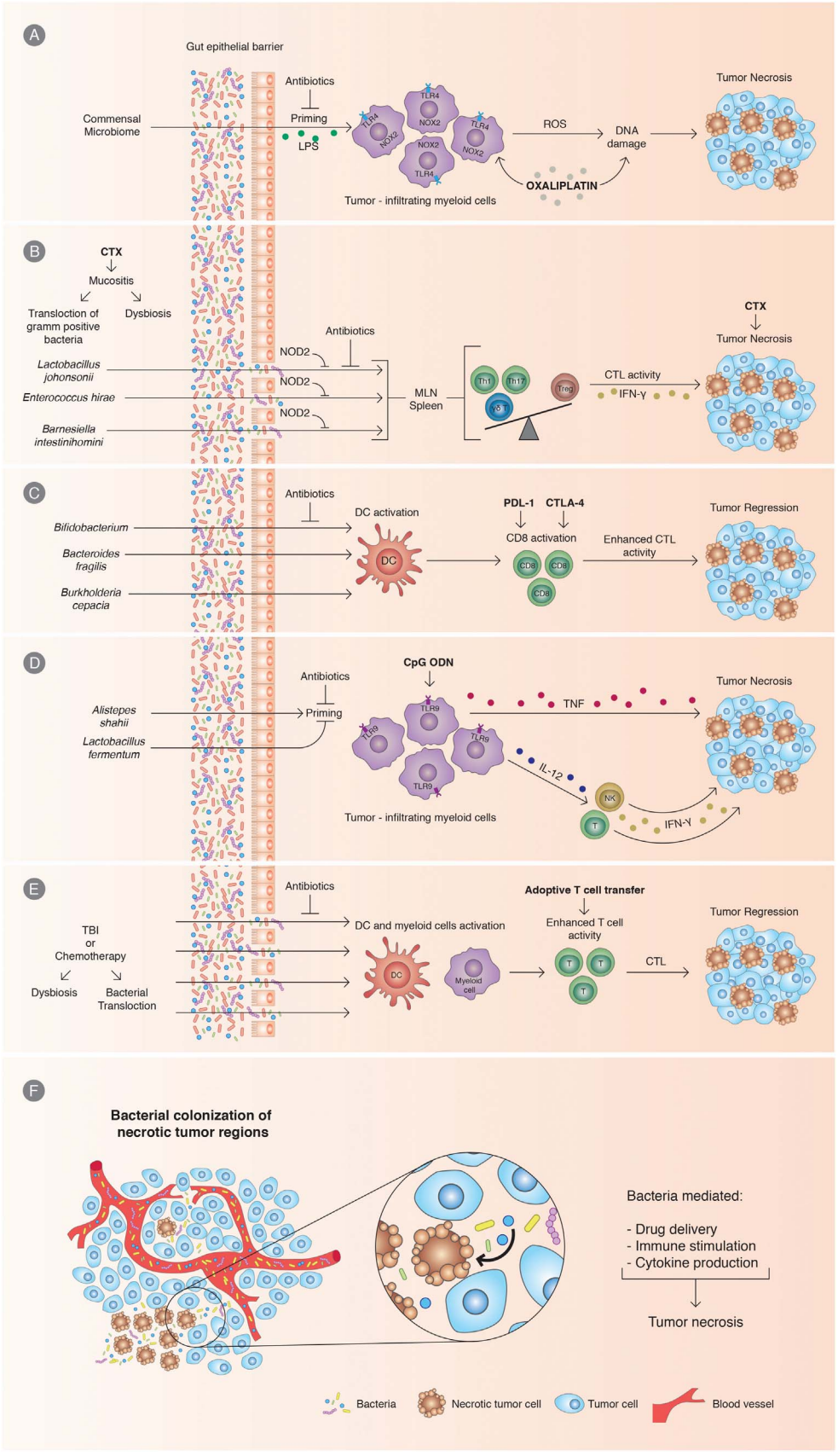

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Fig 1. Mechanisms of microbiome modulation of anti-cancer treatment.A. The gut microbiome primes tumor-infiltrating myeloid cells in a TLR4-dependent manner for enhanced production of ROS upon oxiplantin treatment leading to tumor regression. Broad spectrum antibiotics impair the response to immunotherapy [25]. B. CTX impairs gut epithelial barrier function leading to bacterial translocation into the blood stream. Translocation of certain gram positive bacteria (*e.g. Lactobacillus johonsonii, Enterococcus hirae, Barnesiella intestinihominis*) to the spleen and MLN increases the ratio of Th1 & Th17 to Tregs, which leads to immune-mediated tumor regression [26,58]. C. Efficacy of PDL1 and CTLA-4 antibodies treatments depends on maturation of DC cells, driven by defined commensal microbes (*e.g. Bifidobacterium* species, *Bacteroides fragilis* and *Burkholderia cepacia*) [23,24]. D. Certain members of gut microbiome (*e.g. Alistipes shahii*) prime tumor-infiltrating myeloid cells for enhanced production of TNF and IL-12 upon CpG ODN treatment, thereby leading to tumor regression, while a number of *Lactobacillus* species, (*e.g. Lactobacillus fermentum*) impair the response to immunotherapy [25]. E. TBI or chemotherapy-induced lymphodepletion damages gut epithelial barrier leading to bacterial translocation into the blood stream, which in turn promotes DC and myeloid cells activation, resulting in enhanced activity of adoptively transferred T cells [30,31]. F. Genetically engineered bacteria accumulate in necrotic regions of tumors where they may perform an array of functions including drug delivery, cytokine production, and immune stimulation [92,96,97].

free or antibiotic-treated tumor-bearing mice featured a reduced CTX-induced conversion to T_H17 cells, coupled with impaired tumor regression. In contrast, oral supplementation of *Lactobacillus johonsonii* and *Enterococcus hirae* into antibiotics treated mice enhanced CTX-mediated T_H17 cell conversion (Fig. 1B). Interestingly, adoptive transfer of pathogenic TH17 cells into tumor-bearing mice treated with antibiotics restored the antitumor efficacy of CTX [26].

Likewise, Daillere et al. [58] showed in sarcoma bearing mice treated with broad spectrum antibiotics, that oral administration with *Enterococcus hirae* led to a restoration of CTX anti-tumor efficacy, by inducing differentiation of Th17 and pTh17 (pathogenic T helper 17) cells and promoting tumor-specific Th1 and CTL activity (Fig. 1B). A potential involvement of NOD1 and NOD2 signaling in these processes was suggested by mice deficient in both Nod1 and Nod2 ($Nod1^{-/-}Nod2^{-/-}$) featuring increased bacterial translocation into secondary lymphoid organs following initiation of CTX treatment, leading to increased CTX treatment efficacy [58]. Analysis of mucosal (small intestinal) and stool microbiome revealed that CTX-treated $Nod1^{-/-}Nod2^{-/-}$ mice featured a dysbiotic community structure, characterized, in the large intestine, by an overrepresentation of *Porphyromonadaceae* family members of the genus *Barnesiella*, with both small intestinal *Enterococcus hirae* and large intestinal *Barnesiella intestinihominis* featuring an immune modulating capacity in augmenting CTX efficacy (Fig. 1B). When administered to non-antibiotics treated mice, both strains were able to increase CTX efficacy and modulate systemic and tumor immunity [58], including the induction of effector CD8+ T cell tumor accumulation. Interestingly, in the tumor microenvironment, *Enterococcus hirae* decreased the Treg/CTL ratio and *Barnesiella intestinihominis* led to an increase in IFNγ-producing γδ tumor infiltrating lymphocytes. Importantly, intestinal epithelial cell Nod2 ablation was associated with an increased *Enterococcus hirae* translocation to secondary lymphoid organs [58]. Tumor bearing mice lacking NOD2 ($Nod2^{-/-}$), but not those lacking NOD1, displayed increased CTX efficacy with reduced tumor growth correlating with lower numbers of Tregs infiltrating the tumor microenvironment, accompanied by a higher number of IFNγ-producing γδ tumor infiltrating lymphocytes. Likewise, tumor bearing wild type mice that were administered *Enterococcus hirae* and *Barnesiella intestinihominis* featured a reduced Treg/CTL ratio in the tumor microenvironment, accompanied by a larger number of IFNγ-producing γδ tumor infiltrating lymphocytes. Thus, in the context of CTX treatment, it appears that NOD2 limits the translocation of immune modulating commensal bacteria that, on the one hand, protects against microbial-induced intestinal epithelial cell death, but on the other, limits the efficacy of CTX induced tumor responses.

2.3. Checkpoint inhibitors

Antibodies that block immune inhibitory pathways by targeting suppressive receptors that serve as negative regulators of T cell activation [59], represent a new and exciting anti-cancer treatment strategy. Application of checkpoint inhibitors leads to tumor specific T cell activation resulting in tumor cell recognition and immune-mediated destruction. Currently, FDA approved checkpoint blockade immunotherapies target the cytotoxic T lymphocyte-associated protein 4 (CTLA4) and the programmed death 1 (PD1) located on T cell surfaces [2,3]. These therapies, however, are associated with variable efficacies and are useful in a minority of patients, with treatment success influenced by factors including, among others, host genetic background [60] and lymphocyte count [61].

Recent works suggested a potential involvement of the gut microbiome in influencing the efficacy of checkpoint inhibitor treatment strategies for both CTLA4- and PD1-targeting checkpoint inhibitors [23,24]. Sivan et al. [24] used subcutaneously injected melanoma cells in genetically identical C57BL/6 mice derived from two different facilities harboring different gut microbiome configurations, and demonstrated differential tumor growth rate and intra-tumoral CD8+ T cell infiltration to be influenced by the gut microbiome in these settings [24]. Likewise, the efficacy of PDL1 antibody treatment in tumor-harboring mice in each facility, manifesting as a reduction in tumor size and an increase in circulating tumor specific CD8+ T cells, followed a microbiome-dependent pattern. Furthermore, *Bifidobacterium* species were identified as being associated with improved anti-tumor responses (Fig. 1C). In fact, when fecal microbiome transplantation (FMT) from mice residing in one facility and featuring a better anti-PD1 reactivity was performed into mice from another 'less responsive' facility, *Bifidobacterium* species were found to increase in abundance by greater than 400 fold, while transfer of a mixture of *Bifidobacterium* species into mice of the 'less responsive' facility resulted in a reduction in tumor growth accompanied by expanded tumor-infiltrating CD8+ T cells. The proposed mechanism suggests that *Bifidobacterium*-derived signals may improve dendritic cell activation, thereby contributing to an improved tumor-specific CD8+ T cell response [24].

Vetizou et al. [23] compared the efficacy of anti-CTLA-4 antibody treatment of sarcoma induced by cellular injection in specific pathogen free (SPF), antibiotic-treated or GF mice. While anti-CTLA treatment drove a reduction in tumor growth in SPF mice, it had no effect on GF or antibiotic-treated mice, pointing towards a microbiome role in modulating treatment efficacy [23]. Administration of CTLA-4 antibodies was found to result in alterations of gut microbiome community structure, manifesting as a large intestine reduction in the relative abundance of *Bacteroidales* and *Burkholderiales*, and an increase in *Clostridiales,* and a small intestinal relative expansion of *Bacteroides* species. When antibiotics- treated SPF mice or GF mice were orally administered *Bacteroides fragilis* and *Burkholderia cepacia* prior to CTLA4 antibody treatment, they featured an induction of T_H1 responses in tumor-draining lymph nodes and maturation of DCs in the tumor microenvironment resulting in a reduction in tumor growth (Fig. 1C). Furthermore, adoptive transfer of memory *Bacteroides fragilis*-specific T_H1 cells into GF or antibiotics-treated mice resulted in partial restoration of the CTLA4 antibody treatment efficacy. To address the clinical relevance of these results, the authors characterized the gut microbiome of metastatic melanoma patients undergoing CTLA4 treatment. They identified patients with microbiome consisting of a large proportion of *Bacteroides* species, while the number of patients with this microbial composition increased following treatment. FMT from these patients into tumor-bearing, CTLA4-treated GF mice resulted in a significant reduction in tumor size [23].

Both studies suggested a role for commensal microbiome species in modulating checkpoint inhibitor treatment response. Interestingly, these two studies utilizing the same mouse tumor model identified different bacterial strains that improve therapeutic response. This may

be the result of the checkpoint blocker used, mouse housing facility particularities affecting the host microbiome or potentially other factors alluding to differential experimental conditions.

2.4. Cancer innate immune modulation

CpG motifs present in bacterial DNA are recognized through pattern recognition receptors (PRRs) to induce an immune stimulatory effect [62]. Synthetic oligonucleotides containing unmethylated CG dinucleotides (CpG ODNs) have a similar response to bacterial DNA in their ability to stimulate the innate immune system [63]. In a study by Iida et al. [25], factors affecting the efficacy of CpG ODN were investigated in mice subcutaneously transplanted with three cancer cell lines (EL4 lymphoma, MC38 colon carcinoma and B16 melanoma). Mice treated with antibiotics, as well as germ free mice, featured an impaired response to immunotherapy. This effect was due to decreased production of TNF-α and IL-12 by tumor-infiltrating myeloid cells and reduced secretion of INF-γ by tumour infiltrating NK and T cells. Interestingly, correlating the gut microbiome community composition to the extent of TNF production suggested that a number of bacterial species, such as *Alistipes shahii*, were important in priming tumor myeloid cells in contributing to an improved efficacy of immunotherapy (Fig. 1D). Conversely, a number of *Lactobacillus* species, such as *Lactobacillus fermentum*, were suggested to impair the response to immunotherapy. Furthermore, gavage administration of bacterial LPS rescued TNF-α expression and tumor necrosis in antibiotic treated mice [25].

2.5. Adoptive cell therapy

Cancer cells express antigenic proteins that can rarely be found in healthy tissues (tumor-associated antigen, TAAs) or ones that are specific to cancerous tissues (tumor-specific antigens, TSA) [64]. The discovery of TAAs and TSAs has allowed for the development of adoptive cell transfer (ACT) therapies [64], utilizing TAA & TSA-responsive tumor-infiltrating lymphocytes (TILs) isolated from a tumor biopsy, expanded in-vitro and then re-infused into the patient. Advantages of this approach include the use of both CD4+ and CD8+ TILs [65–67], and the ability to differentiate [68], *in-vitro* activate [69] and *ex vivo* sort [70] transfused T cells for functional optimization. Furthermore, following patient infusion these cells are capable of massive expansion [71,72], can traffic to every site in the body, thus allowing for the potential clearance of tumors even in the central nervous system [73]. With these advantages noted, ACT mediates an immune response only in a minority of patients [27,74–76]. Importantly, lymphodepletion by total body irradiation (TBI) and/or chemotherapeutic drugs prior to ACT increases objective response rates in patients of up to 72% [27] compared to 30% response rates in patients treated with ACT therapy alone [27,74–76].

Some of these chemotherapeutic and TBI-mediated positive effects on ACT responsiveness may be mediated by translocation of gut commensal bacteria following treatment-induced barrier function disruption (Fig. 1E). Indeed, administration of broad spectrum antibiotics severely reduces LPS plasma levels and the beneficial effects of lymphodepletion on ACT in several mouse models of cancer [30,31]. Conversely, LPS administration to irradiated animals enhances the function of infused T cells, leading to long-term cure of mice bearing large tumors [30]. Microbial translocation augments the antitumor activity of adoptively transferred CD8T cells *via* TLR4 signaling in irradiated mice [31]. Microbial translocation also enhances maturation of DC evidenced by increased expression of the costimulatory molecules CD80, CD86, and CD70, as well as MHC class II [77–80], leading to activated DCs potentiating the function of transferred T cells. These host DC effects are reversible upon antibiotic treatment [30]. Furthermore, bacterial translocation activates the innate immune system and heightens the levels of pro-inflammatory cytokines in irradiated mice [31,81,82,77]. Collectively, these studies suggest that lymphodepletion-induced microbial translocation enhances *in vivo* function and persistence of infused T cells, thereby increasing objective response rates in patients compared to those treated with ACT therapy alone. Further fundamental and clinical studies are needed to optimize these microbiome-associated immunomodulatory capabilities in enhancing ACT efficiency.

2.6. Other microbiome effects

Some recent works suggest that in some cases the microbiome may adversely affect anticancer drug stability and half-life by modifying or degrading chemotherapeutic agents [83]. This is exemplified, in patients co-administered the anti-viral drug sorivudine and the chemotherapy 5-fluouracil, by a microbiome-induced transformation of sorivudine into (E)-5-(2-bromovinyl) uracil that, in turn, inhibits 5-fluorouracil metabolism leading to accumulation of toxic levels of the drug [84].

3. Exploiting the tumor microbiome in anti-cancer treatment

In the following section, we will highlight how increasing knowledge on the composition and function of the 'tumor microbiome' may enable harnessing tumor resident microbes as means of treatment or of tumor-specific delivery of therapeutic targets, thereby minimizing treatment-related systemic adverse effects.

3.1. Bacteria as anti-tumor treatments

Bacterial-associated anti-tumor responses involve immune stimulatory effects mediated by structural components such as LPS, peptidoglycan, flagellin and other pathogen-associated molecular patterns (PAMPs) signaling to host germline encoded pattern recognition receptors (PRRs) [85,86]. In recent years, the affinity of anaerobic bacteria towards colonizing hypoxic tumor microenvironments, including, as an example, *Clostridia* spores germinating in the hypoxic regions of solid tumors [40], has been pursued as an experimental anti-tumor therapeutic approach (Fig. 1F). Similarly, spores from attenuated *Clostridia* strains were tested in canine tumors and in a human patient suffering of advanced leimyosarcoma [87], while attenuated *Salmonella* strains were utilized as anti-cancer agents due to their tumor colonizing abilities [88]. The mechanism of microbial driven tumor reduction is suggested to involve induction anti-cancer immune responses, such as bacteremia-induced TNF-α secretion, whose vasoactive roles facilitate bacterial entry into the tumor microenvironment [89], leading to $CD8^+$ T cell activation contributing to enhanced tumor surveillance and tumor clearance [90]. Further elucidation of bacterial usage in this context merits further efficacy and safety testing in human patients.

3.2. Bacteria as anti-tumor treatment vehicles

In addition to use of bacteria as therapeutic agents, recent elegant approaches utilized bacteria as drug delivery platforms, enabling specific tumor cell targeting while reducing non-specific toxicity associated with systemically administered therapeutic agents (Fig. 1F). Over the past years, a broad range of agents has been designed for delivery by bacterial vehicles into tumors, including cytokines [91], bacterial toxins [92] and immune activating proteins [93]. Moreover, further attempts are designed to optimize the expression of agents within tumors, for example by utilization of tumor-specific bacterial promoters [94].

An interesting study by Din et al. [95] exploited a quorum-sensing inter-bacterial communication system to deliver anti-cancer treatment payloads within hypoxic tumor regions inaccessible to chemotherapy, as means of facilitating synchronized localized drug delivery. Feedback loops were engineered in attenuated *Salmonella enterica* subsp. *enterica* serovar Typhimurium. The designed molecular circuitry included a *LuxI*

promoter inducing the expression of auto-inducer acyl-homoserine lactone (AHL), which is able to diffuse and accumulate outside the cell, thereby providing a communication and synchronization signal to neighboring microbes, in which it binds and activates its receptor protein LUXR. *LuxI* was also engineered to drive the expression of ϕX174 E, a bacteriophage lysis gene triggering cell death, and therapeutic genes of choice, which in this system included the pore-forming anti-tumor toxin Haemolysin E [92], the T-cell and dendritic cell recruiting chemokine CCL21 and a fusion of Bit1 cell death domain to the iRGD tumor-penetrating peptide [96,97]. As a result, when bacterial cell population was low, AHL diffused outside the cell but did not reach a sufficient concentration to initiate synchronized activation of the molecular circuitry in the surrounding microbial community. However, as the population density increased, AHL accumulated and the molecular circuitry was activated resulting in population-synchronized AHL- *LuxI*-driven expression of the therapeutic gene(s) leading to cell lysis releasing treatment compounds into the tumor microenvironment. During lysis, the majority of cells underwent cell death, while a few survived to produce AHL and begin a new synchronized growth and drug release cycle.

As a proof of concept, Din et al. tested a combination of bacterial strains expressing all three therapeutic compounds, which were orally administered to a mouse syngeneic model of hepatic colorectal metastases, in conjunction with the chemotherapy agent 5-fluorouracil [95]. The purpose of using both the bacterial vehicle system and a systemically administered chemotherapeutic agent was to target areas of the tumor with sufficient vasculature with 5-fluorouracil, while targeting hypoxic and necrotic areas by bacterial colonization. Importantly, tumors were found to substantially shrink for 18 days followed by resumption of tumor growth. Although this approach did not constitute a long-term effect, it presents an interesting and elegant approach of targeting tumors that would likely require further optimizations in becoming therapeutically efficient in combination with other therapeutic approaches.

4. Conclusion and prospects

The microbiome involvement in augmenting anti-tumor responses to therapeutic approaches represents a new and exciting area of research with potential broad implications in cancer therapy. Many of these anti-cancer therapy-promoting effects are mediated by immune system priming and by augmenting the immune response against tumor cells. One limitation in our current ability to comprehensively understand the mechanisms driving microbiome effects on tumor therapy is related to conflicting results between studies, potentially stemming from differences in methodology, studied tumors, or in local vivarium configurations differentially impacting immune responses. For example, a study by Viaud et al. [26] demonstrated commensal bacterial translocation to play an important role in stimulating an inflammatory response leading to upregulation of IL-17 [26] driving tumor regression. In contrast, Grivennikov et al. [98] showed that bacterial translocation-induced upregulation of IL-17 leads to progression of colorectal cancer, as was suggested in a study following human patients with colorectal cancer [99]. As such, the effects of IL-17 on colorectal cancer may vary in relation to the tumor, treatment and microbiome context.

Another limitation of clinical translation of microbiome effects on tumor therapy is that most were conducted in mouse tumor models, using syngenic cancerous cell systems that have already undergone a process of immune editing before transfer into the new host. In 'real-life' cancer, in contrast, the nature of immune system interaction with cancer greatly differs during the cancer elimination, equilibrium and escape stages. Studies in humanized models and in spontaneous models of cancer development are therefore needed to address this limitation, and to determine whether the findings described in current studies are mirrored in human subjects.

With respect to antibiotic usage as means of microbiome modulation or limitation of local translocation, clinical implications in long term antibiotic use in patients suffering of immune suppression and susceptibility to treatment-related mucosal damage merit careful clinical consideration, as it may subject patients to the risk of developing systemic, life risking antibiotic resistant bacterial or fungal infection. Efforts of modulating the microbiome using more targeted approaches such as probiotics [100], prebiotic, nutritional [101], and postbiotic [102,103] interventions may enable to control pathobionts impacting cancer therapy efficacy, while not impacting the microbiome ecosystem at its entirety.

Likewise, enhanced understanding of the roles the 'tumor microbiome' plays in shaping the tumor immune, metabolic and pharmacologic microenvironment may fundamentally impact our ability to harness features of this local microbial niche towards better cancer treatment responsiveness. As such, the exploitation of bacterial tumor colonization is evolving from a passive reliance on systemically administered bacterial strains migrating to tumor hypoxic sites, to modified strains that may act as elaborate intra-tumoral drug delivery vehicles. These approaches represent interesting therapy options, particularly in combination with other anti-cancer agents that cannot reach necrotic tumor regions due to the tumor's aberrant vasculature.

Finally, the rich set of individual-specific compositional and functional datasets presented by the microbiome may enable to harness microbiome data in personalizing patient anti-cancer treatment decision-making. As such, microbiome-based patient stratification using modalities such as machine learning [101,9] may enable to tailor treatment combinations to more optimally achieve therapeutic efficacy, rather than relying on population-based data or frequently used 'trial and error' approaches. Such personalized decision making processes may enable to optimize treatment efficacy while minimizing adverse effects. In all, the expanding research focusing on elucidating the roles of the microbiome in impacting cancer treatment represents a new and exciting frontier towards future harnessing of the microbiome as a diagnostic, patient stratification, prognostic and treatment anti-cancer modality.

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