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Review

The Role of the Microbiome in Cancer and Therapy Efficacy: Focus on Lung Cancer

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Abstract. *The microbiome is extremely important for human health; more recently its role in the context of cancer became clear. Microbial effects range from enhancing cancer immunity and cancer therapy efficacy, to promoting cancer progression and inhibiting treatment efficacy. These broad implications led researchers to investigate these specific interactions, as well as how modification of the microbiome can improve cancer survival and treatment efficacy. While these interactions are better established for cancers such as gastric cancer, they are far less understood in others. As non-small cell lung cancer (NSCLC) makes up the majority of lung cancer cases, and is among the top causes of cancer deaths worldwide, understanding the mechanisms by which the microbiome may impact progression and treatment is crucial to improve patient survival and treatment response. A literature review was conducted to reveal the crosslink between human microbiome and lung cancer. This includes immune priming, induction of pro- or anti-tumor response, and the local effects of intra-tumoral microbiota. Overall, this is a complex multifactorial relationship, and there are broad implications as to how this knowledge can improve cancer treatment. Solutions include manipulation of the microbiome using probiotics, bacterial vaccines and antibiotics. Bacteria biomarkers may also be used as a diagnostic tool.*

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The microbiome, defined as the collection of genomes from all the microorganisms found in a particular environment, is an emerging and widely studied factor in human health. Its implications in cancer are manifold (1). Specific microorganisms that are found within a specific environment (*i.e.*, the microbiota) induce anti-tumor immunity through immune priming (1, 2). Dysbiosis, genotoxins, and inflammatory responses to microbiota are associated with cancer development (1). Additionally, cancer treatment efficacy can be enhanced or inhibited by intra-tumoral and gut microbiota (3-5). This knowledge leads to many questions regarding the interactions between the microbiome, cancer and cancer therapies. Most importantly, how can a better understanding of these interactions lead to improvement of current treatment efficacy? Compared to the gastrointestinal (GI) tract, the microbiota of the lung and other organs are far less understood (4, 6).

Lung cancer is the first cause of death among oncologic patients and the second most common cancer worldwide (7). Investigating microbial-cancer relationships will aid in a better understanding of the role of microbes in mechanisms underlying tumorigenesis behind this as well as other cancers and hopefully improve treatment efficacy (4). These factors are poorly understood in lung cancer (3, 8, 9). Therefore, this review aims

Table I. *Most common bacteria reported in tumor samples.*

Phylum	Genus	Species	Method	Sample type	Amount in control samples	Amount in cancer samples	Ref
Firmicutes	*Staphylococcus*	*S. epidermidis*	RT-PCR	Lung cancer tissue biopsies	N/A	25%	(19)
Firmicutes	*Streptococcus*	*S. mitis*	RT-PCR	Lung cancer tissue biopsies	N/A	21.87%	(19)
		S. pneumoniae	qPCR	Bronchoalveolar lavage (BAL)	7.30%	15.17%	(20)
	Streptococcus	Not specified	16S rRNA sequencing	Bronchial washing fluid (BWF)	N/A	12%	(21)
	Enterococcus	*E. faecalis*	qPCR	Colorectal cancer fecal samples	N/A	54%	(22)
Firmicutes	*Veillonella*	Not specified	16S rRNA sequencing	BAL fluid of LC patients (*vs.* patients with benign masses)	4%	11.4%	(23)
	Veillonella	Not specified	16S rRNA sequencing	Bronchial washing fluid (BWF)	N/A	8%	(21)
		Not specified	PCR assay	Pancreatic ductal adenocarcinoma (PDAC)	N/A	51.7%	(5)
Proteobacteria	*Escherichia*	*E. coli*	Blood culture	Lung cancer patients (with febrile neutropenia)	N/A	68.8%	(24)
	Escherichia	*E. coli*	16S rRNA sequencing	Colonic mucosa tissue samples	3%	62% (adenoma) 77% (carcinoma)	(22)
Bacteroidetes	*Porphyromonas*	Not specified	qPCR	Colorectal cancer fecal samples	N/A	45%	(22)
Fusobacter	*Fusobacterium*	Not specified	qPCR	Colorectal cancer tumor samples	81%	82% ($p=6\times10^{-5*}$)	(25)

N/A: Not available.

to provide an overview of the role of the microbiome, primarily for non-small cell lung cancer (NSCLC), reporting data that could be used in future studies to improve prevention/diagnosis, overall treatment efficacy and patient survival.

NSCLC makes up 85% of lung cancer cases, the majority of cases being either adenocarcinoma or squamous cell carcinoma (10). This makes NSCLC a major issue to be tackled in terms of improving diagnosis and treatment. Due to contact with the external environment, the lungs are heavily exposed to microorganisms (8, 11). The lungs have indeed a specific microbiota, even though they were thought to be sterile in healthy individuals according to previous knowledge. The healthy human lung microbiome predominantly consists of *Firmicutes*, mainly *Streptococcus, and Lactobacillus* (11, 12). Most commonly, lung cancer patients are infected with *Firmicutes, Bacteroidetes,* and *Proteobacteria* (13) (Table I). These include genera such as *Granulicitella, Streptococcus, Veillonella, and Mycoplasm*a (9). Additional studies have found that gram-negative bacteria such as *Haemophilus influenzae*, *Enterobacter spp.*, and *Escherichia coli* also tend to colonize in lung cancer (14). Of note, regarding gut microbiota, a lower abundance of *Firmicutes* and *Proteobacteria*, along with relatively higher levels of *Bacteroidetes* and *Fusobacteria,* have been found in lung cancer patients compared to healthy individuals (15). It appears that these phyla are reported consistently with respect to microbial changes in cancer.

Changes in the microbiome arise as both a cause and consequence of carcinogenesis, as a result of the alteration in microbial immigration, microbial elimination and microbial reproduction rates (16).

In the lungs, commensal microbial dysbiosis is associated with multiple chronic pulmonary disorders such as asthma and chronic obstructive pulmonary disease (COPD), and these disorders additionally cause complications for lung cancer progression and treatment (11). In fact, 70% of lung cancer patients are burdened by bacterial infections (11). In addition, once the balance between the host and the microbiome is disrupted, the barrier effects exerted by the microbiota disappear, leading to pathological propagation of the microbes. These microbes further promote tumor formation and progression by exploiting the immune system and inflammatory response (11, 17). Alongside the implications of the microbiome in carcinogenesis, the range of microbiota which can affect cancer treatment is extensive. These include the local effects of the intra-tumoral microbiome, and systemic effects of the microbiome of the GI tract, which can affect efficacy of cancer treatment (4, 11, 18). The multitude of ways in which microbiota influence cancer progression and drug metabolism is therefore a crucial step in the improvement of efficacy of cancer treatment.

Relevance to Cancer

Microbiome, carcinogenesis and tumor progression. Associations between microbiota and carcinogenesis are manifold (Table I). It is estimated that about 20% of cancers

are caused by infectious agents (18). Specific microbiomes and its dysbiosis can induce carcinogenesis through direct DNA damage and inflammation, or indirectly through modulation of immune response, or by chronic inflammatory responses induced by bacterial metabolites (1, 4, 18). Direct DNA damage occurs due to the metabolic imbalance and increased production of carcinogens associated with microbial dysbiosis. For example, intestinal microbiota metabolizes compounds into genotoxic forms, such as the conversion of bile acids into deoxycholic acid, which contributes to DNA adduct formation and reactive oxygen species (ROS) production (26). Chronic inflammatory response consists of the invasion and accumulation of inflammatory cells and molecules, which activate cancer-related processes such as cell proliferation, angiogenesis, and even metastasis (11). Inflammatory response is triggered due to microbial dysregulation, or stimulation, of the immune system; however, microbial effects on the immune system may also be inhibitory (8, 13). For example, ROS released by immune cells activates NFκB, which leads to increased cellular proliferation and inhibition of apoptosis, two hallmarks of cancer (13). Interestingly, activation of toll-like receptors (TLR) by microbial antigens is another mechanism by which NFκB and another promoter of proliferation and angiogenesis, STAT3, are activated and thus contribute to tumor development (13). Bacterial activation of TLR is also associated with tumor promoting IL-23 and IL-17 response, aiding in tumor progression in colon cancer models (12). TLR4 activation in other cancers such as pancreatic ductal adenocarcinoma (PDAC) is also associated with immune suppression, and tumor promotion *via* NFκB signaling (27). Therefore, the microbiome can promote carcinogenesis through modulation of the immune response. However, these effects vary greatly, and are highly dependent on the specific microbial phyla/species, location, interactions with the host or other microbiota, and whether effects are local or systemic (11, 13).

Generally, local effects exerted by microbes are carcinogenic. For example, it is well known that *H. pylori* presence in the GI tract has strong links with the development of cancer of the GI tract (predominantly gastric cancer), and promotes carcinogenesis through epithelial damage caused by inflammation, and prevention of autophagy of cancerous cells (1, 13). Similarly, the presence of infectious agents such as *M. tuberculosis* in the lungs is thought to be linked to lung cancer development *via* chronic inflammation (20). In the lungs, cancer may be initiated by chronic infection when dysbiosis leads to a more hypoxic, tumor-promoting environment (9). Lung cancer is associated with an increase in anaerobic respiration, due to the facultative anaerobic qualities of bacteria that preferentially colonize tumors (9, 28). These bacteria increase as cancer progresses, further potentiating the hypoxic and pro-inflammatory tumor environment (9). This increase in potential pathogenic bacteria is due not only to the tumor environment, but also to the impact of cancer therapy on the microbiome (28).

Therapies may not only impact cancer but may also alter the microbiome and further impact tumor progression. Chemotherapeutic treatments such as 5-fluorouracil (5-FU) and cyclophosphamide (CTX) alter the microbiome so that there is an increase in pathogenic *Firmicutes* such as *Staphylococcus*, and *Proteobacteria* such as *E. coli* and *Pseudomonas*, with a simultaneous decrease in commensal *Bacteroides* (28). These changes are consistent with findings on how the microbiome changes in the context of cancer (5, 13, 29). These phyla are considered pro-inflammatory due to the immune response they evoke, meaning that dysbiosis caused by tumor progression, inflammatory response, and cancer therapy results in a cycle that continuously perpetuates itself (28).

Microbiome and immune response. Alongside its relevance to carcinogenesis and tumor progression, the microbiome can also affect the host immune response in various ways (Figure 1). These interactions can either enhance or inhibit anti-tumor immunity and progression. Microbiota, specifically those of the GI tract, have a strong influence on the systemic cancer immune set point of the host (4, 30). This consists of the balance of factors such as the local tumor environment, the host, and environmental factors, which influence both the strength and timing of the host immune response against cancer (4). The microbiome supports or counteracts tumor progression *via* systemic and local effects (11, 13). For this reason, having a diverse microbiome is associated with better overall survival in many types of cancers (4, 31).

Microbiota influence the immune response either by inhibitory or activating effects (1). The gut-lung axis is a bi-directional system that connects the microbiomes of the GI tract and the lung, and changes in one tissue affect the other (30, 32). Translocation of gut microbiota and their products across the epithelial barrier and into the bloodstream is a key regulator of the gut-lung axis (32). This translocation occurs as cytokines or immune cells carry bacterial products into the circulation (32). In addition, translocation stimulates TLR response and subsequent T cell expansion in distant tissues (30). *Bifidobacterium*, a key commensal bacterium of the gut, stimulates Th17 cells and neutrophil responses in melanoma mouse models, which can be seen in other cancer types as well (32, 33). These immune responses are associated with reduced tumor growth (32, 33). Translocation of bacteria from the GI tract, which can be a result of chemotherapy and radiation therapy, can enhance tumor-specific responses through, for example, TLRs, or through induction of memory responses (4, 32). The latter was observed for relations between *Enterococcus hirae* and

Table II. *Potentiating or inhibitory effects of microbiota on cancer therapy for NSCLC*

Treatment	Bacteria	Enhancing or Inhibiting?	Effects	Ref
Cisplatin	*Lactobacillus* *Bifidobacterium*	Enhancing	Decrease in oncogenic VEGF and Ras levels.	(31)
Gemcitabine	*Mycoplasma* *Gammaproteobacteria* (*E. coli)*	Inhibiting	Bacterial CDA metabolizes nucleoside analogues and reduces efficacy.	(5, 18)
Ipilimumab	*B. fragilis*	Enhancing	Aid in tumor specific cytotoxic T cell expansion to promote tumor specific response.	(4)
Anti-PD-1	*B. fragilis* *A. muciniphila*	Enhancing	Aid in tumor specific cytotoxic T cell expansion to promote tumor specific response.	(30)

small-cell lung cancer (SCLC) and ovarian cancer (4). This is due to the possibility of bacterial antigens matching tumor antigens, and thus aiding in the immune response against the tumor (4). Antigen matching is necessary for an effective innate anti-tumor response (34). Bacterial antigens are picked up by antigen-presenting cells (APCs), activating NK cells and a tumor specific T cell response (34). This response is often defective in cancer patients, meaning T cells are directly activated by tumor-associated antigens, thereby weakening the tumor specific response (34). Commensal gut bacteria such as those belonging to *Bifidobacterium*, *Bacteroides,* and *Clostridium* genera aid in CD8 T cell expansion in the gut and distal organs as well (33). Conversely, microbiome dysbiosis is also associated with inhibitory effects on the anti-cancer immune response, such as by activating inhibitory effects on NK cells *via* immune suppressive T-regulatory cells (1). Also, *B. fragilis* is implicated in regulating inflammatory response by activating T-regulatory cells and suppressing pro-inflammatory molecule IL-17 *via* its polysaccharide A antigen, thereby controlling the inflammatory response and intestinal homeostasis (35). *Lactobacillus* is a gut commensal bacterium that has inhibitory effects on TNF production (36). This is beneficial because high levels of TNF can paradoxically promote tumor progression (37).

While systemic effects of the gut microbiome may promote tumor immunity, local effects appear to be the opposite. Tumor-associated inflammation actually enhances cancer cell proliferation and contributes to tumor progression in other ways, such as by immune suppression (2). The lung microbiome of healthy mice (and humans) is primarily made up of *Firmicutes* (*Staphylococcus, Streptococcus* and *Lactobacillus)*, whereas lung tumor samples tend to have increased levels of *Proteobacteria* (11). In both mouse and human lung adenocarcinoma models, γδ T cells are highly present (11). In a lung cancer mouse model, tumor-associated, IL-17-producing γδ T cells enhanced tumor progression (11). In response to local dysbiosis, IL-17 release stimulated pro-inflammatory responses, thereby leading to a cycle of tumor cell expansion and further local immune response led by IL-1β, IL-23, and neutrophil infiltration (11). Importantly, IL-17 was not released from T cells in the spleen or lymph nodes, suggesting that commensal bacteria in the lungs were responsible for this tumor-potentiating response (11). It is evident that tumor cells utilize microbiota to stimulate an inflammatory response that can continue to feed the tumor microenvironment. While Th17 cells in the gut may produce systemic anti-tumor effects, local Th17 cells seem to aid tumor progression *via* induction of an inflammatory response. Additionally, local, tumor-specific T cells are likely to express PD-1 on their surface, leading to immune suppression and impaired tumor immunity (11). In the GI tract, *Fusobacterium* promotes carcinogenesis through ROS production and local inflammation (27). Since *Fusobacterium* infection of the pancreas is linked to carcinogenesis, the mechanisms by which it does so may be similar.

Microbiome and metastasis. Pro-inflammatory molecules released in response to chronic infection aid in the process of metastasis in addition to that of carcinogenesis (11, 18). For example, NFκB is activated by PAMPs, and can promote tumor cell invasion on top of tumor cell proliferation (11). Additionally, evidence that *Fusobacterium nucleatum* FadA protein binds and inhibits the tumor suppressive function of β-catenin in colorectal cancer models suggests that microbiota regulate cell proliferation and metastatic transformational abilities (1). This phenomenon is also seen with *B. fragilis* in colorectal cancer (38). Mice with colorectal cancer given *F. nucleatum* had more metastasis compared to controls, and increased expression of genes related to cell motility, such as E-cadherin (39). The antibiotic chloroquine reversed these effects (39). Interestingly, *F. nucleatum* can travel from the primary tumor site along with colorectal cancer cell metastases and may contribute to tumorigenesis at distant sites (40). Tumor growth was inhibited with antibiotics treatment (40).

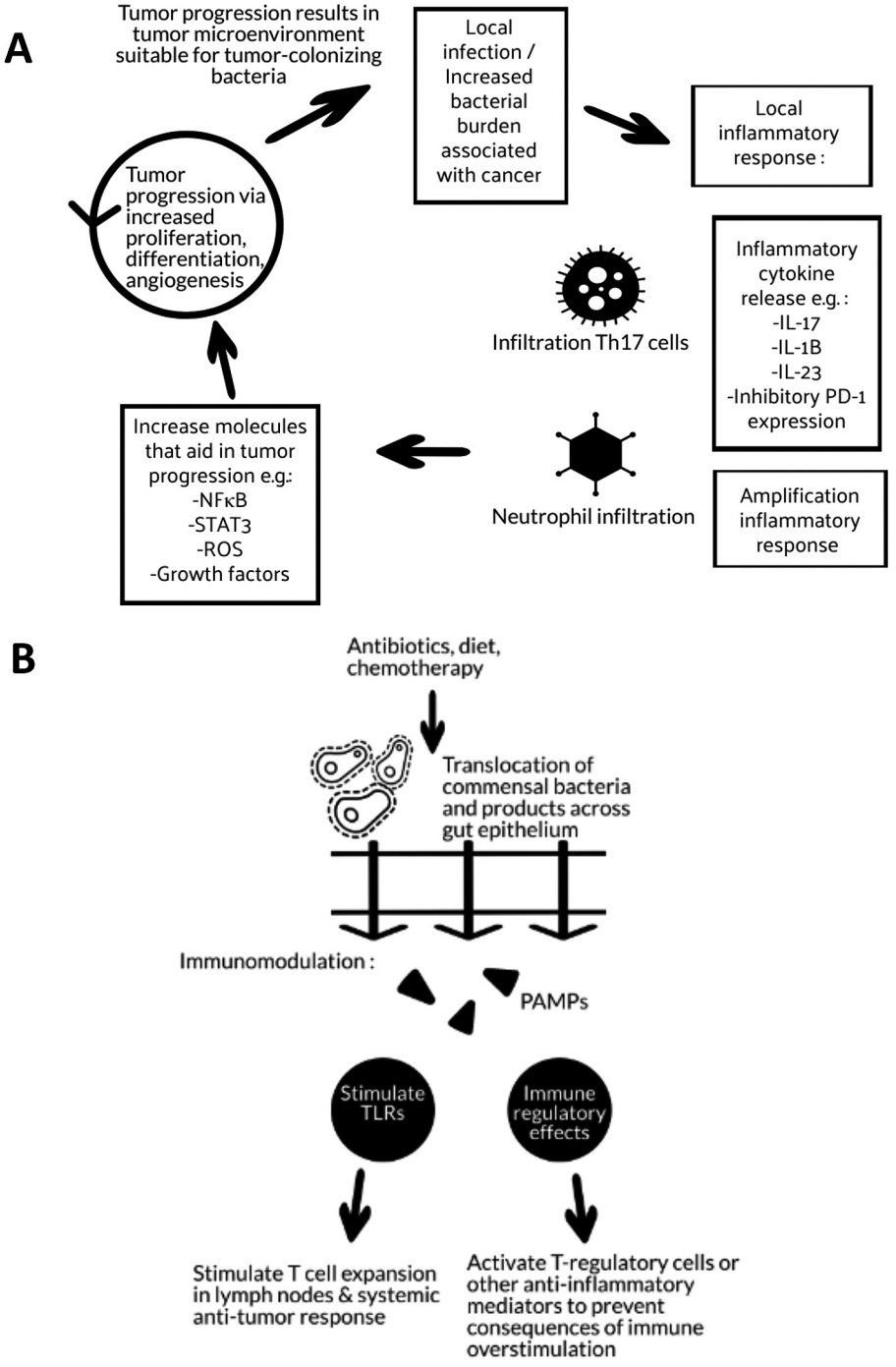

Figure 1. *The microbiome has different effects on cancer immunity and carcinogenesis depending on multiple factors including location, systemic vs. local effects, and interactions with the host. a: Locally, microbiota often potentiate cancer development. This is primarily due to the fact that local effects induce chronic inflammation. b: Systemic effects often facilitate anti-tumor immunity because they contribute to the cancer immune set point.*

Interestingly, some studies have reported associations between mycoplasma and metastasis of colon and lung cancer (18). In mycoplasma-infected cultures, lung epithelial cells developed metastatic morphologies, including the development of outgrowths, the loss of contact inhibition, and aggregation in clumps (17). Additionally, lung tumors in mycoplasma-infected mice were highly metastatic compared to uninfected mice, which had no tumor formation (17).

Varying interactions between mycoplasma and host proteins may result in tumor progression as well (18). BMP2, a common oncogenic protein in NSCLC patients involved in cell proliferation and migration, is elevated in mycoplasma-infected lung epithelial cells (17). This knowledge implies that mycoplasma species are associated with both carcinogenesis and metastasis in lung cancer. Additionally, mycoplasma-derived p37 can promote metastasis through multiple mechanisms. In lung cancer mouse models, p37-transfected cells showed an increased ability to degrade extracellular matrices and increase motility (41). This was accomplished through a p37-induced increase in matrix metallopeptidase-2 (MMP-2), and a subsequent increase in EGFR activation (41). More recently, p37-transfected hepatocellular carcinoma cells (HCC) promoted metastasis *via* interactions with EpCAM, thereby promoting an epithelial-mesenchymal transition (EMT) phenotype in circulating HCC cells (42). P37 also serves as a biomarker of poor prognosis in HCC patients (42). Additionally, mycoplasma histone deacetylase (HDAC) contributes to host cell stress tolerance and the hallmarks of cancer. Compared to controls, HDAC transfected cell lines are able to better proliferate in low serum concentrations, and have increased proliferative ability when treated with paclitaxel (PTX) and 5-FU (43). These results are due to a decrease in the host levels of BIK and p21, important apoptotic and cell cycle regulators (43).

Effects of the Microbiome on Cancer Therapy

Mycoplasma. Mycoplasma is a genus of bacteria which has varying interactions with cancer. They lack a cell wall as found with normal bacteria. Mycoplasmas are very commonly found in patient tumor tissue, suggesting a relationship between mycoplasma and carcinogenesis. Mycoplasma preferentially colonizes tumors because the tumor environment is rich in nutrients and provides a suitable environment for these small, genetically reduced bacteria (17, 18). Additionally, this preferential colonization of tumors by mycoplasma greatly contributes to the intra-tumoral environment *via* anaerobic respiration (9, 19). Mycoplasma infections have been found in up to 100% of surgically removed lung cancer tissue samples, suggesting a strong relationship between mycoplasma infection and carcinogenesis (19). Notably, mycoplasmas are found in tumor samples of various tumor types other than those of the lung. Metastasis is also increased in mycoplasma-infected cells (18).

Mycoplasma also has distinct interactions with anti-cancer drugs. For example, mycoplasma-infected cell lines showed resistance to antimetabolites (*e.g.* 5-FU) and the p53 activator nutlin due to simultaneous destabilization of p53 and inhibition of the DNA repair protein PARP1 by the Mycoplasma DnaK chaperone protein; thus, increasing the chance of malignant transformation (44). Interestingly, mycoplasma chaperone DnaK is similar to the chaperone DnaK of other bacterial species, making this a possible common mechanism of carcinogenesis and resistance with regards to the microbiome and cancer (44). Intratumoral mycoplasma also reduces the efficacy of the nucleoside analogue gemcitabine *via* its mycoplasma cytidine deaminase (CDA) (18). CDA is known as an important factor in the degradation of gemcitabine (45), a drug commonly used in the treatment of NSCLC (46). Treatment with the CDA inhibitor tetrahydrouridine (THU) and the antibiotic tetracycline can restore the sensitivity of infected tumor cells to gemcitabine (18).

Other bacteria. Apart from mycoplasma, various other bacterial species are implicated in carcinogenesis and moderating cancer treatment efficacy. Similar to mycoplasma, proteobacteria preferentially colonize tumors (28, 29). Proteobacteria are mostly anaerobic, and are therefore able to colonize the tumor, and can also further potentiate the hypoxic tumor environment (9).

Additionally, gammaproteobacteria possess CDA, similar to mycoplasma, and therefore have comparable effects on certain antimetabolites (5, 28). Gammaproteobacteria typically possess the long isoform of CDA, which is a more potent inducer of resistance to treatment as compared to the short isoform found in mycoplasma (5, 47). *E. coli*, a gammaproteobacteria, conferred gemcitabine resistance in cell cultures, and induced rapid tumor progression in mice as compared with non-infected mice, which had little to no tumor formation (5). *E. coli* also increases the cytotoxicity of the alkylating agent CB 1954 *via* its nitroreductase enzyme that produces a more potent derivative than what is expressed in non-infected cells (28). In addition to *Proteobacteria*, *Firmicutes* and *Actinobacteria* are two phyla which are commonly modified in cancer patients compared to healthy controls (13, 28). For example, *Pseudomonas*, a member of the *Firmicutes* phyla, is increased in lung adenocarcinoma patients harboring *TP53* mutations (29). The anaerobic features of these bacteria make them likely to colonize tumor tissues and again contribute to the hypoxic and inflammatory environment (9).

While specific microbial families may confer resistance to cancer therapy, others are actually necessary for some treatments to be fully effective. In a lung cancer model, mice treated with an antibiotic cocktail (ABX) that wiped out intestinal microbiota were less responsive to cisplatin, whereas mice treated with a combination of the probiotics *Lactobacillus* and *Bifidobacterium* and cisplatin had an improved overall survival rate (31). This is attributed to elevated levels of VEGF and Ras, which were overexpressed in ABX/cisplatin mice (31). Of note, overexpression of

VEGF is common in lung cancer patients, and leads to increased angiogenesis, and inhibition of apoptosis (*via* BCL-2), thereby promoting tumor development (31). Conversely, mice treated with the combination of cisplatin and a probiotic had lower levels of VEGF and Ras expression, allowing for increased expression of apoptosis-promoting BAX, and therefore reducing tumor progression in these mice (31). It is evident that *Lactobacillus* and *Bifidobacterium* probiotics may be necessary to aid in the efficacy of alkylating agents such as cisplatin, by aiding in the promotion of processes that reduce tumor growth (31).

Other evidence suggests that a healthy microbiome is necessary for effective cancer therapy. For example, cyclophosphamide (CTX) is a chemotherapeutic agent which generates better survival in lung cancer patients whose intestinal microbiome contains *E. hirae* (4). This is due to the fact that CTX causes damage to the epithelial barrier, leading to translocation and induction of an effective anti-tumor IFN-γ and IL-17 response (4). This is most likely due to the fact that *E. hirae* has antigens similar to those of the tumor of SCLC patients (4). In ABX, SPF and GF mice, the efficacy of oxaliplatin and cisplatin in lymphoma, melanoma, and colon cancer mouse models was significantly reduced (2). These platinum-based compounds form DNA cross-links and utilize ROS to facilitate DNA breakage. Genes involved in monocyte differentiation and activation are downregulated in these mice, resulting in a lack of ROS production by these immune cells (2). The study suggests that commensals may also enhance the efficacy of alkylating agents, which have similar mechanisms of action to platinum compounds (2). Overall, gut commensals are necessary for proper immune system priming and thus effective therapy efficacy.

Immunotherapy efficacy is also influenced by microbiota, and this is relevant considering that immune checkpoint inhibitors (ICI) are widely used for the treatment of advanced NSCLC. Colonization of the intestinal mucosa with certain bacteria is actually necessary for an effective response to therapy. Efficacy of immunotherapy with CpG-ODN was reduced in ABX and GF mice due to the lack of tumor specific cells and effective generation of an immune response (2). Notably, SPF mice injected with *Lactobacillus* had reduced therapy efficacy due to its inhibitory effects on the TLR9-dependent immune response that is induced by CpG-ODN (2). In general, however, commensals often enhance therapy efficacy through immune priming. GF mice were fed a mix of 11 commensal bacterial strains, which included predominantly members of *Bifidobacterium, Bacteroides,* and *Clostridium* genera (48). These commensals aid in CD8 T cell expansion within the gut, thereby promoting an effective response to ICI (48); however, the T cell expansion is seen in other organs besides the intestine (35). Mice without the mix of commensals had very poor response to anti-PD1 and anti-CTLA4 therapy (48). In keeping with this, the efficacy of ipilimumab is optimal when a lymphocyte response against *B. fragilis* already exists (4). Additionally, lung and renal cancer patients colonized with *A. municiphilia* and *E.hirae* had significantly increased CD4 T cell and IL-12 response, which greatly improved response to ICI (36). This is consistent with other findings that *B. fragilis* and *A. municiphilia* promote long-term survival and better response to anti-PD-1 therapy in lung cancer patients (30). In addition, patients who were not responsive to ICI had more *Staphylococcus* present in their gut, a bacterium that is often elevated in cancer patients (9, 36). In another study, patients with abundant *Lactobacillus* and *Clostridium* in their stool tended to have a longer benefit from ICI than those with a lower abundance (49). When assessing microbiota composition in the stool samples of patients with advanced NSCLC treated with nivolumab, subjects with high microbiome diversity had significantly longer progression-free survival compared to those with low diversity (50). In the same study, responders had enrichment of *Alistipes putredinis*, *Bifidobacterium longum*, and *Prevotella copri*, whereas *Ruminococcus_unclassified* was more represented in non-responders.

Lastly, it has been demonstrated that ICI therapy can modify the composition of microbiota, and changes in the microbiota composition could potentially influence the response to immunotherapy and its toxicity (51).

Antibiotic. Antibiotics have different effects on cancer and therapy. Having a diverse and balanced microbiome can be cancer protective. As discussed previously, colonization by certain commensal microbiota can stimulate systemic anti-tumor immunity and enhance treatment efficacy (3, 4, 36, 48). Therefore, reducing certain commensals with the use of antibiotics might reduce the anti-tumor response. Antibiotic-treated mice showed reduced immune capacity, such as decreased expression of inflammatory genes, antigen presentation, and overall adaptive response (3, 30). Antibiotic use is also associated with increased risk of cancer due to the consequences of dysbiosis and the subsequent pro-inflammatory response (33). A retrospective study investigating associations between previous antibiotic use and efficacy of ICI therapy in NSCLC patients found negative correlations between antibiotic use and overall survival (52). The same trend was observed among 90 NSCLC patients treated with nivolumab, both in terms of progression-free survival and overall survival (53). In 47 NSCLC patients treated with immunotherapy and receiving antibiotics during the whole treatment period, a worse progression-free and overall survival was observed (54). Additionally, prolonged antibiotic use is strongly linked to lung cancer development (30).

With regards to therapy, antibiotics have different effects. Antibiotics may alter drug metabolism. It is suggested that

intra-tumoral bacteria play a role in resistance to cancer therapy, such as the intra-tumoral bacteria in PDAC (5). In patients with metastatic pancreatic cancer being treated with gemcitabine, antibiotics intended to reduce resistance due to bacteria, actually led to an increase in gemcitabine toxicity and adverse events (48). This is due to the fact that both gut and local tumor bacteria possessing CDA, metabolize nucleoside analogues, therefore, decreasing the toxicity of the drug, whereas the depletion of bacteria using antibiotics increased drug availability and toxicity (48). The antibiotic ciprofloxacin did, however, restore treatment efficacy in cancer cell cultures infected by CDA-producing bacteria (most likely *Proteobacteria* or *Mycoplasma*) (5).

How to Exploit the Knowledge on Microbiome to Improve Cancer Therapies

Bypassing the effects of the microbiome. Improved knowledge regarding the effects of microbiota on cancer treatment will help to determine how the manipulation of the microbiome can affect its efficacy. Antibiotics are one way in which cancer treatment efficacy can be restored (5). Importantly, this should be done with narrow spectrum antibiotics, as these target a more specific range of microbiota, and therefore reduce the chance of dysbiosis and aberrant side effects (13). Lung infection by *Mycoplasma* is highly common and results in resistance to nucleoside analog treatments (5, 17). Thus, treatment with anti-mycoplasma antibiotics could effectively restore treatment efficacy.

However, due to the many host and microbial factors that influence cancer, it is important to be cautious when administering antibiotics next to cancer therapy. For example, due to the necessity of balanced gut microbiota in promoting effective responses to therapy, antibiotic use may quicken relapse or treatment failure in patients receiving anti-PD-1 therapy (36).

Another way in which the microbiome may be modified is through fecal transplantation. This alters microbes in the intestine, as well as in the lung, due to the gut-lung axis (30). Fecal transplantation from patients responsive to anti-PD-1 therapy actually induced the same necessary T cell response in a melanoma mouse model, due to the addition of gut bacteria necessary for an effective ICI response (30).

Probiotics may also be used to enhance cancer therapy (13). Through restoration of the gut microbiota, systemic immune surveillance, and therefore tumor immunity, can be improved (4). This occurs as commensal microbiota stimulate tumor-specific T cell expansion, aiding in the immune response to cancer as well as that to cancer therapy (4). *Lactobacillus* and *Bacteroides* often decrease due to cancer or treatments such as chemotherapy, making them commonly-given probiotics in combination with cancer therapy in order to improve its efficacy (28). As described previously, mice given *Lactobacillus* and *Bifidobacterium* probiotics had improved response to cisplatin as compared to GF mice (31).

Last but not least, cancer therapy might be enhanced by bacterial vaccines. Since these vaccines are typically inactivated, or are only composed of certain bacterial components, they help to confer an anti-tumor immune response without the potential negative side effects of a chronic infection. For example, BCG is a bacterial vaccine containing components of *Staphylococcus* and *Streptococcus* (43). These bacteria are associated with inflammation and cancer, and a previous study showed that inactivated strains are effective as an adjuvant therapy in NSCLC patients (43). More recently, an increase in *Pseudomonas aeruginosa* was associated with lung cancer development and tumor progression (29). However, when a *P. aeruginosa* preparation (PAP), an inactivated preparation of the bacteria, is injected into patients with advanced NSCLC, there is an enhancement in cisplatin efficacy (34). This is because *P. aeruginosa* has very potent immune stimulating properties and therefore enhanced the tumor specific response (34). Many PAPs are associated with tumor regression in breast, liver, and gastric cancer as well (34). Thus, as an adjuvant therapy, bacterial vaccines may be used to continuously stimulate the innate anti-tumor immune response (34).

Clinical implications. Apart from the possible benefits of manipulating microbiota in order to enhance cancer treatment efficacy, knowledge of the microbiome in the context of cancer might provide biomarkers for lung cancer diagnosis and personalized treatment. In this sense, an understanding of the heterogeneity that exists between individuals can be utilized when considering personalized cancer treatments (3). Bacteria and their products may be used to diagnose cancer at earlier stages due to consistencies in microbial changes among cancer patients. Regarding lung cancer, a specific gut microbiome signature has been proposed as a potential predictor of early-stage NSCLC, based on 16S ribosomal RNA gene sequencing analysis (55). Not least, the diversity and composition of the normal lung tissue microbiome has been associated with recurrence-free and disease-free survival of resected lung cancer (56). More research is necessary to determine specific microbial biomarkers for lung cancer.

Recent studies showed controversial results on the microbiome in duodenal fluids of PDAC patients (57). However, the ability to measure and monitor cancer biomarkers in "body fluid biopsy" could greatly impact oncologic practice. Bronchoalveolar lavage fluid (BAL) is a lung fluid that can be extracted from the lungs by a bronchoscope and recent studies have suggested that BAL proteins, mRNAs, miRNAs, and lipids correlate with the pathophysiological state of the patient. Among potential biomarkers, BAL fluid of lung cancer patients often reveals significantly higher amounts of *Streptococcus*

viridans (20). In a recent study, high frequencies of *Proteobacteria* were found in the BAL of NSCLC cases, which have been further subdivided into well-defined bacterial communities associated with different histology (adenocarcinoma *versus* squamous cell carcinoma) (58). Bacteria can also be used as biomarkers to determine which treatment is the most effective for a specific patient. Indeed, as stated above, lung cancer patients colonized by *B. fragilis* and/or *A. muciniphila* respond better to anti-PD-1 therapy (20). Identification of the microbial composition of a patient may potentially improve decisions on cancer therapies in the future.

Conclusion

There is a highly sensitive balance between specific microbiota and the host. Conversely, immune priming, as well as an induction of an effective immune response happen in the event of dysbiosis. However, this response may also quickly become overstimulated and can thus damage the host and anti-tumor response. Therefore, more research is needed to better understand the complexities of these interactions. Research thus far has revealed important relations between lung/gut microbiota and lung cancer, but there is still much to be understood (59). Contradictory experimental results due to the many factors involved in the outcomes of microbiota-host interactions in the context of lung cancer and cancer therapy make it difficult to draw concrete conclusions. Moreover, in some experiments, particular strains of bacteria were linked to particular effects or conditions, but other studies suggest that the diversity of the microbiome, or the relative abundances of species, is important. Of note, a most recent comprehensive study of the tumor microbiome, analyzing 1526 tumors and their adjacent normal tissues across seven cancer types, including NSCLC, found that each cancer type has a distinct microbiome composition (60). This study described also correlations between intratumor bacteria and tumor subtypes, smoking status, and the response to immunotherapy. However, further research is necessary to better understand how manipulation of the microbiome impacts the host, cancer, and cancer therapy, with a special focus on the effects on immunotherapies as well as to prevent potential adverse effects due to modification of the microbiome.

Conflicts of Interest

The Authors have no conflicts of interest to disclose in relation to this study.

Authors' Contributions

Concept and design of the study: AH, EG, GJP; data collection and initial concept: AH; additional data and writing: AL, AG; additional writing: MT, DMD; writing and supervision: EG, GJP.

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