Microbiome dysbiosis in lung cancer: from composition to therapy

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The correlations between microbiota dysbiosis and cancer have gained extensive attention and been widely explored. As a leading cancer diagnosis worldwide, lung cancer poses a great threat to human health. The healthy human lungs are consistently exposed to external environment and harbor a specific pattern of microbiota, sharing many key pathological and physiological characteristics with the intestinal tract. Although previous findings uncovered the critical roles of microbiota in tumorigenesis and response to anticancer therapy, most of them were focused on the intestinal microbiota rather than lung microbiota. Notably, the considerable functions of microbiota in maintaining lung homeostasis should not be neglected as the microbiome dysbiosis may promote tumor development and progression through production of cytokines and toxins and multiple other pathways. Despite the fact that increasing studies have revealed the effect of microbiome on the induction of lung cancer and different disease status, the underlying mechanisms and potential therapeutic strategies remained unclear. Herein, we summarized the recent progresses about microbiome in lung cancer and further discussed the role of microbial communities in promoting lung cancer progression and the current status of therapeutic approaches targeting microbiome to alleviate and even cure lung cancer.

npj Precision Oncology (2020) 4:33; https://doi.org/10.1038/s41698-020-00138-z

INTRODUCTION

The human body coexists with a complex array of commensal microbiome that colonizes the host microenvironment forming a dynamic micro-ecological system developed during evolution. The commensal microbiome shares a symbiotic relationship with the host during the long-term coexistence development which eventually forms a dynamic microecosystem. Currently, the human microbiome has received extensive attention and been demonstrated to play a critical role in various aspects of human health and disease status via immunity, metabolism and inflammation.

Lung cancer poses a great threat to global public health and is ranked as the most common cancer (11.6% of all cancers) with over 2.09 million diagnosis and 1.7 million deaths worldwide in 20182,3. It is generally divided into two histological-pathological types including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Treatments for lung cancer include surgery, chemotherapy, radiotherapy, targeted therapy and emerging immunotherapy. And the best recommendations depend on patient’s TNM stage and unique health situations5. However, most patients are diagnosed at an advanced stage, with high mortality and poor benefit from limited treatment options6. On the other hand, multiorgan metastatic and relapse in pre-treatment and post-treatment are critical causes of death without effective therapy. There are growing emergency and social demand in exploring the carcinogenesis and new therapeutics for this deadly disease. Lung cancer has been widely considered to be a complicated disease caused by interactions between host and environmental factors7. Among diverse environmental risk factors, microbes present a vital part in maintaining microecological balance and regulating host immune responses to multi-treatments. Although the healthy lung tissues were long considered as a sterile environment, it was found recently that there were certain microbial species existed in the lung tissues impacting the balance between health and pathogenesis in the lung microenvironment with the advancements of high-throughput next generation sequencing (NGS) technologies8,9. Increasing studies have profiled the microbiome in respiratory samples from healthy adult lungs10,11. It has discovered the most common phyla including Bacteroides, Firmicutes, and Proteobacteria and genera like Streptococcus, Pseudomonas, Veillonella, and Prevotella12. Nowadays researches in the lung microbiome and important discoveries in the microbiome’s association with lung diseases are growing rapidly. It is believed that improved understanding of this association will provide novel insights into the pathogenesis of lung diseases. In this review, we summarized and evaluated the current development of the interplay and the underlying mechanisms between microbiota and lung cancer. Furthermore, we also discussed the prospects about the carcinogenesis and therapeutic applications of microbiome on lung cancer.

LUNG MICROBIOME AND GUT MICROBIOME

Microbiome was defined by the bacteria, fungi, virus, protozoa and their related genes and genomes, as well as metabolites13. At present, more and more efforts have been focused on the most compelling commensal microbial ecosystem interacting with the human body—especially the gut microbiome which is considered as a forgotten organ mediating host homeostasis via complex mechanisms14. Emerging gut microbiota research are fully facilitated by the rapid development and application of high-throughput molecular technologies15, bioinformatics and metagenomics16,17. It was reported that gut microbiome was closely

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correlated with various chronic diseases such as gastroenteric cancers within which carcinogenic *H. pylori* was colonized frequently, as well as multiple pathologic processes of remote organs in the host. Conversely, lungs were regarded as a pair of sterile organs until new studies assisted with high-throughput sequencing technologies challenged the old false dogma. It is now generally recognized that microbial disturbances influence a variety of lung diseases. The lung microbiota is composed of bacteria, fungi, and viruses, which derived from the inhalation of mucus, nasopharynx, oropharynx, and environmental bacteria, fungi, and virus, which derived from the inhalation of variety of lung diseases. The lung microbiota is composed of genus *Propionibacterium*, *Streptococcus*, *Haemophilus*, and *Veillonella*, but they do not cause infection of human lungs. It is not surprising when considering multiple unknown interactions in other tissues. All these interactions among microbiome, immunity and metabolism in these microbial niches affect multiple lung pathogenesis of COPD, asthma, cystic fibrosis and lung cancer. Recently, increasing interests have been raised about the field including commensal lung microbiome communities and the possible mechanisms maintaining microecological effects on human respiratory system. Based on previous investigations, several controversial hypotheses have been proposed including “Microbiota-Brain-Gut axis”, “Microbiota-Gut-Liver axis” and “Microbiota-Gut-Skin axis”.

In fact, the human body is a dynamically balanced integrity and microorganisms in various body sites can interact with each other directly including mucosal dispersion, respiratory and digestive activities, or indirectly via inflammatory substances, cytokine, and metabolites in systematic circulation as shown in Fig. 1, which exhibited the possible microbial communication between the oral cavities and lungs, as well as gut. The alterations of local lung microbiome communities mainly depend on three aspects which can be summarized as microbial migration, elimination and growth rates under the condition of health and disease. Some researchers have brought forward that oral microbiome may be the primary sources of lung microbiome from a long-term and validated observation (through swallowing, mucosal dispersion, and micro-aerosols or secretions generated in oral cavities). The respiratory tract and gut could communicate with each other via biological processes including micro-aspiration and inhalation. Bacterial metabolites from the intestinal tract can regulate the differentiation tendency of naive T cells, effector T cells, Tregs, or Th17 release, which further induces systematic inflammation and immunity response. Furthermore, they could be transported into host bloodstream to regulate the systemic immune activity and alter the microbial communities located in respiratory tract. Although some theoretical models had been put forward, no specific studies were conducted to approach the relationship and communicating manners in terms of the species diversity and the relative abundance of microbiota at different sites within respiratory and gastrointestinal tract.

The temperature and pH environment in gastrointestinal tract are relatively constant and migration of microbes is unidirectional and always altered by complicated physical and chemical conditions. By contrast, the lung is frequently exchanging gas with outside environment to maintain abundant reserves of oxygen and microbiota. Furthermore, there is no physical barrier and gradient diversity of pressure and temperature in upper respiratory tract which provide bidirectional conditions for lung-resident microbial migration and dynamic changes. On the other hand, Lozupone et al. reported that the progressive maturation pattern of diversity, stability, and resilience of the lung microbiota from birth to adult mice was consistent with both human lung and gut microbiota during the first 3 years. Previous studies revealed that early-life formation of microbiota and immunologic environment in human airways and gastrointestinal tract may be derived from the skin and external environment. Despite distinct differences in micro-anatomic features, composition and population dynamics in gut and lung microbiota, these two organs share a similar homeostasis and certain physiological characteristics such as microbiota maturation process, mucosal immune system, co-evolution and communication with immune cells and continuous exposure to outside environment. Intriguingly, increasing clinical studies indicated that multiple lung diseases were more likely to develop in patients with gastrointestinal disorders. These significant discoveries lead us to reconsider whether the microbiome interaction network really exists and modulates host susceptibility to either internal or external pathogenic factors. Gut microbiome have been confirmed contributing to the chronic obstructive pulmonary diseases, the progression of asthma and the worsening acute lung injury. More and more studies have also implicated the connections and modulatory effects of specific microbial metabolites in gut and lung via circulation. For example, a significant reduction of the microbial metabolites including the fatty acids, acetate, butyrate and propionate, as well as isocids in the feces from patients with bronchial asthma was observed when compared with healthy controls. *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* have been reported to suppress the inflammatory responses in pediatric allergic asthma through inducing anti-inflammatory cytokine IL-10 and inhibiting the secretion of pro-inflammatory cytokines like IL-12. Previous studies also found that alterations in colonic luminal, serum, and hippocampal metabolomic profiles in mice treated with ketogenic diet were significantly correlated with seizure protection, supporting the distant modulation of intestinal microbiome in other organs. Bingula et al. reported that the gut bacteria and their fragments were taken up into DCs (dendritic cells) and macrophages through phagocytosis and then migrate into gut or lungs.

![Dynamic connections of microbiome inhabiting different human body sites](image)

**Fig. 1** Dynamic connections of microbiome inhabiting different human body sites. Oral, lung, and gut microbiome could communicate with each other via direct manner including mucosal dispersion, respiratory and digestive activities, and indirect manner via inflammatory substances, cytokine, and metabolites in systematic circulation. Bacteria and its metabolites from intestinal tract modulate the differentiation tendency of naive T cells and Th17 release, modulating the systematic inflammation and immunity.
to regulate immune response. Tsay et al. demonstrated that gut flora can induce lung inflammatory reaction against bacterial pneumonia and enhance neutrophils infiltration through TLR4 in mice. Taken together, the complex and interventional ecosystems regulate various pathological processes and maintain the physiological equilibrium of gut and lung. Thus, a new hypothesis was put forward as “Microbiota-Gut-Lung axis” based on the diverse and complex gut-lung microbiome networks established based on numerous long-term epidemiological observations. However, the mechanisms underlie the “Microbiota-Gut-Lung axis” remains elusive and more robust evidences are still required to kindle the lamp.

**LUNG MICROBIOME AND HOST METABOLISM**

Dysregulation of host metabolism by microbiome alterations have been intensively studied in the intestinal tract. The carcinogen acetaldehyde and deoxycholic acid produced by microbiome were reported to be involved in esophagus and liver carcinogenesis. Visconti et al. reported that the blood and fecal metabolites were analyzed together with the characterization of gut microbiome by metagenomic shotgun sequencing. Their findings supported a correlation between active pathways of gut bacteria and metabolites found in feces and blood. Metabolism is essential for maintaining human body homeostasis through numerous pathologic and physiologic processes. There are also emerging studies approaching lung microbiome associated with host metabolism. Griggs et al. discovered that specific metabolic profiles correlated with bacterial organisms were associated with glycerophospholipid and lineolate pathways, which play an important role in the pathogenesis of pneumonia in HIV-infected individuals through 16s rRNA sequencing in the bronchoalveolar lavage fluid. Bei et al. also found that primary metabolites secreted by *Pseudomonas aeruginosa* using substrates produced by *Rothia mucilaginosa* might contribute to its pathogenesis in the progression of cystic fibrosis. One of the high-profile metabolites is short-chain fatty acids (SCFA) which is produced by large amounts of commensal microbes and acts as a crucial signaling molecule in host cells. And many studies have focused on the role of SCFA in host gut and immunity, while the functions of SCFA in respiratory system, epithelium and immunity remain unclear. It has been shown by Gauguet et al. that mice lacking SCFA in the gut are prone to suffer from more bacterial load like *Staphylococcus aureus* which could be modulated by pulmonary Th17 immunity. Previous study by Caet al. demonstrated that dietary supplementation with short-chain fatty acids (SCFAs) can ameliorate this enhanced asthma susceptibility by modulating the activity of T cells and DCs in mice. Besides, some researches have demonstrated that modulation of gut microbiome in preclinical model can alter host immune response and susceptibility to pulmonary infection factors. Furthermore, SCFAs has been reported to regulate differentiation of bone marrow cell and maintain host immunological homeostasis. Under certain circumstances, SCFAs can modulate the composition of gut microbiome and induce myelopoiesis resulting in an anti-inflammatory milieu in the airways. Therefore, the concept of “gut-bone marrow-lung axis” has been brought forward, which represents a mechanistic explanation on how the gut microbiota derived SCFAs modulate the host immune system against exogenous pathogenic factors. However, currently most of the studies are focused more on the possible association between the intestinal-pulmonary axis and lung inflammatory diseases with few studies involving lung cancer. Although recent investigations in microbial metabolism associated with cancer occurrence and progression are shedding novel insight, limited evidences are available to establish solid connections concerning distal metabolic regulations between gut and lung and the mechanism still awaits further investigation.

**LUNG MICROBIOME AND HOST IMMUNITY**

The microbiome regulates host immune activity directly or indirectly by mediating host susceptibility to various pathogenic factors and therapeutic outcomes. The dynamic interaction between microbiome and immune system enables host to recognize and prevent bacterial or fungal invasions and infections. In preclinical studies germ-free (GF) mice lacking intestinal microbiome displayed severe immune dysplasia with incomplete mucous layer, immunoglobulin secretion disorder, and decreased size and number of lymph nodes. The special subgroup CD4+ Th17 cells play an important role in microbial interactions, mucosal immunity functions and host response to inflammatory diseases of intestinal tract, lungs and skin. Mantis et al. reported that IgA mainly regulated bacterial virulence in the gut by blocking bacterial adherence to mucosal epithelial cells. The microbiome with low-density and less-stable microbiome was susceptible to lose balance of commensal bacteria and be invaded by exogenous pathogens. A recent study revealed that high density of commensal microbiota might promote the clinical outcome of vaccine for infants. Gut microbiota can stimulate Th17 response and modulate the generation of IL-17, which is involved in the elimination of certain pathogens. In addition, IL-17 pathway is also involved in the pathogenesis of several pulmonary pathologies including asthma, sarcoidosis, obliterative bronchiolitis, and bone marrow transplant-related pneumonitis. Gollwitzer et al. reported that bacteria resident in the lung regulated the expression of certain innate immunity genes including IL-5, IL-10, and IFN, and the expression level of PD-L1 on CD11bC DCs and FoxP3+ CD25+ Treg cells were higher in the lungs of SPF (specific pathogen-free) neonates than GF (germ free) mice. Steed et al. reported that a microbiially associated metabolite, desaminotyrosine (DAT), protects host from influenza via enhancing type I IFN stimulation and reducing lung cancer immunopathology. Similarly, Takeshi Ichinohe et al. found that commensal microbiota could regulate immunity in respiratory mucosa through inflammasomes and provided immune activation signals at steady state after influenza virus infection. The most recent study revealed that the fermentable fiber inulin could alter gut microbiota structure and the associated metabolites like short-chain fatty acids which eventually improve the response of mice to influenza virus infection by dampening damage induced by neutrophils and enhancing anti-viral CD8+ T cell responses. And enrichment of lung microbiome with oral taxa was found to be associated with Th17 inflammation, in which TLR4 responses were impacted by lung microbiome composition. Moreover, commensal microbiota was shown to drive proliferation and activation of Vg6+ Vd1+ T cells in lung cancer. Nevertheless, there is no consistent definition of a healthy or beneficial lung microbiota, partly due to limited understanding in approaching the association between lung-resident microbiome and host immunity.

**CONTRIBUTION OF MICROBIOME TO CANCER**

Cancer is generally thought to be a multifactorial pathological process, where normal cells begin to proliferate in an unprogrammed manner resulting in inhibition of apoptosis, autophagy, inflammations and DNA damage. There are increasing commensal and pathogenic microorganisms defined in the human body with reported carcinogenic properties and most of them are significantly correlated with carcinogenesis epidemiologically. Here, we made a summary of previous studies in microbiome correlated with lung cancers have been listed in the Table. The results demonstrated the close relationship between microbial communities and respiratory tract. The initiations of surface boundary tumors are often associated with the host mucosal immune barrier destruction. When the mucosal surface is damaged, the microenvironment of the original tissue and commensal microbiome will be reconstructed if the injury cannot be.
Table 1. Summary of lung cancer microbiome.

| Categories | Phylum/Genus | Sample source | Major findings |
|------------|--------------|---------------|---------------|
| Bacteria   | Staphylococcus, Streptococcus, Lactobacillus, Pasteurellaceae, Herbaspirillum, Sphingomonadaceae, Aggregatibacter, and Lactobacillus etc | Paired mouse lung cancer and normal tissues | Commensal microbiota induced γδ T cells promote inflammation and lung cancer development⁸² |
|            | Capnocytophaga and Veillonella | Human saliva | Capnocytophaga and Veillonella were significantly enriched in the saliva from lung cancer patients⁸³ |
|            | Streptococcus, Veillonella (smake testing); Veillonella, Prevotella, and Streptococcus (in vitro) | Human air brushes | The enrichment of the lower airway microbiota with oral commensals was relevant to the upregulation of lung cancer pathogenesis ERK and PI3K signaling pathways⁸⁴ |
|            | Veillonell, Megaspheara, Actinomyces, Arthrobacter, Capnocytophag, Rothia, Streptococcus, and Veillonella | Human BALF | In different metastatic states of lung cancer, differential genera between squamous cell carcinoma and adenoscarcinoma were different. And in different histologic types of lung cancer, distant metastasis-related genera were not the same¹² |
|            | Bifidobacterium, Aecalibacterium, Bacillus, Streptococcus infantis, Veillonella etc | Human feces | 13 selected gut microbial signatures can be established for the potential prediction of the ref. ⁹⁴ |
|            | G. adiacens, Enterococcuspp, Streptococcus intermedius, Escherichia coli, Streptococcus vinidans, Acinetobacter junii, and Streptococcus sp. | Human sputum | G. adiacens and associated correlated microbes were significantly correlated with lung cancer status and stage¹⁵ |
|            | Proteobacteria | Human BALF | A predominance of proteobacteria existed both in cancerous lungs and other airway disorders¹¹ |
|            | Acidovorax | Paired human lung cancer and tumor tissues | Mutations in TP53 were correlated with the presence of Acidovorax in the lung microenvironment²⁴ |
|            | Proteobacteria, Firmicutes, and Bacteroidetes | Paired human lung cancer and tumor tissues | A significantly lower abundance of Proteobacteria (Acinetobacter and Acidovorax) and higher prevalence of Firmicutes (Streptococcus) and Bacteroidetes (Prevotella) in lung cancer patients compared to emphysema-only patients⁸⁹ |
|            | Thermus and Legionella | Paired human lung cancer and tumor tissues | Thermus is more abundant in tissue from advanced stage patients and Legionella is higher in patients who develop metastases⁹⁵ |
|            | Veillonella and Megasphaera | Human BALF | Veillonella and Megasphaera were relatively more abundant in lung cancer patients⁹⁶ |
|            | Granulicatella, Abiotrophia and Streptococcus | Human oral and sputum samples | Granulicatella, Abiotrophia, and Streptococcus were significantly enriched in lung cancer patients attributed to household coal burning exposures compared to healthy controls³⁵ |
|            | Streptococcus and Neisseria | Paired human lung cancer and tumor tissues | The abundance of genus Streptococcus and Neisseria displayed an increasing trend from healthy to noncancerous to cancerous site²⁸ |
|            | Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae | Paired human lung cancer and tumor tissues | Greater abundance of Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae were associated with reduced RFS or DFS⁹⁰ |
| Virus      | HPV | NHIRD | There was a significant increase in lung cancer risk among Taiwanese women who were exposed to HPV infection⁹⁴ |

BALF bronchoalveolar lavage fluid, RFS recurrence free survival, DFS disease free survival, HPV (human papillomavirus, NHIRD (National Health Insurance Research Database).
signaling pathways are involved in the process of carcinogenesis associated with microbiome interventions. In addition, destruction of boundary surface between host and microbes could also activate pattern recognition receptors and their signaling cascades which leads to imbalance in the symbiotic microenvironment. The existence of gut microbes was also involved in other aspects of cancer initiation and progression such as angiogenesis, invasion, tumor immune microenvironment and apoptosis which can be promising research hotspots for in-depth investigation.

Prior studies have uncovered some correlations between microbiome and lung inflammatory and organizational structure in several respiratory diseases including COPD (chronic pulmonary disease), IPF (idiopathic pulmonary fibrosis), asthma, CF (cystic fibrosis) and non-CF Bronchiectasis, as shown in the Table 2.

Some researchers proposed that a disordered microbial dysbiosis

| Table 2. Possible correlations of lung cancer microbiome with other respiratory illnesses. |
|---------------------------------|-----------------|-----------------|-----------------|
| Categories | Phylum/Genus | Respiratory illnesses | Major findings |
|-----------|--------------|-----------------|-----------------|
| Bacteria | Streptococcus pneumoniae, Haemophilus influenza, Moraxella Catarrhalis, and Pseudomonas aeruginosa | COPD | These bacteria are more colonized in COPD patients epidemiologically. Pseudomonas aeruginosa in COPD patients may indicate worse status. |
| Proteobacteria, Actinobacteria | COPD | Proteobacteria and Actinobacteria may induce a more intense inflammation in severe COPD. |
| Proteobacteria (particularly Haemophilus spp) and Bacteroidetes (particularly Prevotella spp) | COPD | More Pathogenic Proteobacteria (particularly Haemophilus spp) and less Bacteroidetes (particularly Prevotella spp) were detected in COPD patients compared to general people. |
| Veillonella and Prevotella | COPD | A significant correlation with Veillonella and Prevotella in BAL in the early COPD patients was identified. |
| Gemella, and Porphyromonas etc. | IPF | Radiographic honeycomb can alter lung microbiota of patients with IPF, which may exacerbate the anatomic disruption of IPF in a bidirectional interaction. |
| Staphylococcus aureus | IPF | Staphylococcus aureus was frequently observed culture-positivity in the BAL fluid of patients with IPF. |
| Staphylococcus sp. and Streptococcus sp. | IPF | Staphylococcus sp. and Streptococcus sp. were positively correlated with IPS progression and co-trimoxazole with antibiotic therapy can improve condition. |
| Campylobacter, Stenotrophomonas and Veillonella | IPF | There were increased Campylobacter and Stenotrophomonas and decreased Veillonella in acute exacerbation of IPF compared to stable IPF. |
| Streptococcus pneumoniae | IPF | Streptococcus pneumoniae triggers progression of pulmonary fibrosis through pneumolysin. |
| Moraxella, and Corynebacterium | Asthma | Specific bacterial genera are shared between the nasal and the bronchial mucosa which are associated with markers of systemic and bronchial inflammation. |
| Gram-negative bacteria | Asthma | A component of Gram-negative bacteria, LPS, can decrease asthma level in mice via induction of the ubiquitin-modifying enzyme A20. |
| Pseudomonas aeruginosa | CF | The oral dominant and pathogen Pseudomonas Aeruginosa can contribute to inflammation and lung structure changes. |
| Streptococcus mileri group (SMG) | CF | Streptococcus mileri group (SMG) established chronic pulmonary infections in 39% of acute pulmonary exacerbations. |
| Stenotrophomonas maltophilia or P aeruginosa | Non-CF Bronchiectasis | Host genotype (fucosyltransferase 2 secretors) is linked to increased P aeruginosa, which is consistently associated with exacerbations and poorer lung function, clinical outcomes, and mortality. |
| Proteobacteria (e.g., Haemophilus sp.) | Non-CF Bronchiectasis | Proteobacteria occupied major part in microbiome communities in sputum samples from baseline to exacerbation of Non-CF Bronchiectasis. |
| Pseudomonas sp. | Non-CF Bronchiectasis | |
| Fungus | Candida, Phialosimplex, Aspergillus, Penicillium, Cladosporium, and Eutypella | COPD | COPD patients have personalized structures and varieties in sputum microbial community during hospitalization periods. |
| Aspergillus | COPD | A. fumigatus sensitization is related to poor lung function and positive filamentous fungal culture is a common feature of COPD. |
| Aspergillus | IPF | Infection with aspergillosis contributes to chronic fibrosing pulmonary aspergillosis, which may result in chronic scarring of the lungs. |
| Alternaria alternata and Cladosporium herbarum | Asthma | A large cross-sectional study of 1132 adults with asthma found that sensitization to Alternaria alternata or Cladosporium herbarum is a significant risk factor for severe asthma in several European countries and Australia, New Zealand, and Portland. |

COPD chronic obstructive pulmonary disease IPF idiopathic pulmonary fibrosis, CF cystic fibrosis, BALF bronchoalveolar lavage fluid, LPS lipopolysaccharide.
might provoke dysregulation in host physiology and contribute to exacerbations in chronic lung diseases. Several observations reported that respiratory viruses were identified in the respiratory specimens of 39–56% of chronic obstructive pulmonary disease (COPD) patients compared to 6–19% at clinical baseline\textsuperscript{16,17}. It was also revealed that pathogenic bacteria existed in 51–70% of patients during disease exacerbations compared to 25–48% in the initial stable clinical baseline\textsuperscript{128}. Another large cohort survey reported that CXCL8/IL-8 was significantly associated with lung microbiome diversity and community structure, which can mediate host inflammatory responses during COPD exacerbations in some subjects\textsuperscript{129}. Another slowly progressive lung disease is idiopathic fibrosis (IPF) which has been confirmed to harbor a distinct microbiome from that of healthy lung status\textsuperscript{130}, and a randomized trial reported that antibiotic therapy could be beneficial to survival of IPF patients\textsuperscript{31}. Robert et al. revealed that radiographic honeycombing altered the lung microbiota of patients with IPF, which might further exacerbate the anatomic disruption of IPF in a bidirectional interaction\textsuperscript{125}. Despite growing evidence of the associations, the causal significance of an altered lung microbiota in COPD and IPF remains elusive. Furthermore, lung microbiome including bacteria or virus infection could potentially invade epithelial cells of the airways inducing host immune response or triggering the wound healing cascade in chronic pathogenic stimuli\textsuperscript{12}. Overall, these hypothesis-driven theories or epidemiological observations or cohort studies, especially whether the microbiome correlated with chronic lung diseases participate in the exacerbation or initiation of lung cancer, still awaits further investigations. Emerging studies have also raised interests about correlations between lung cancer and microbiome by high-throughput sequencing and epidemiological analysis. It was found that significantly high abundance of \textit{Granulicatella, Thermus, Legionella}, and \textit{Streptococcus} were observed in lung tumor tissues compared with control groups\textsuperscript{85,88,93}. Of note, Tsay et al. demonstrated that the enrichment of the lower airway microbiota with oral taxa (\textit{Streptococcus} and \textit{Veillonella}) was significantly correlated with the upregulation of lung carcinogenic ERK and PI3K signaling pathways. In vitro exposure of airway epithelial cells to \textit{Veillonella, Prevotella}, and \textit{Streptococcus} also led to upregulation of these same signaling pathways\textsuperscript{91}. Gomes et al. reported that lung cancer microbiota was enriched in Proteobacteria and more diverse in squamous carcinoma than adenocarcinoma, particularly in males and heavier smokers\textsuperscript{11}. Another study by Greathouse et al. revealed that mutations in TP53 were correlated with the presence of \textit{Acidovorax} in the lung microenvironment\textsuperscript{84}. The emerging technologies revealed that the niche effects of lung-resident microbiome on lung cancer should not be neglected. From a global perspective, \textit{Pseudomonas, Streptococcus, Staphylococcus, Veillonella}, and \textit{Moraxella} were frequently reported as the most relevant lung cancer-related microbiome\textsuperscript{60–12,82,84–94,133}. Notably, some lung commensal microbiota including lung cancer-related microbiome including \textit{Proteobacteria, Streptococcus, Bacteroidetes, Veillonella}, and \textit{Moraxella} have also been identified significantly correlated with lung inflammatory diseases\textsuperscript{12,26,89,91,92,95,109,110,113,125}. Yet, limited by technology development and ethics, the nasal secretions, oral saliva, sputum, and BAL fluid are often used indirectly for lung microbiome research. Most studies based on indirect samples and evidences were problematic and failed to illustrate the molecular mechanisms in this field. Despite the inevitable limitations in preclinical animal model and cell lines, emerging advances in organoid technology has allowed for innovative and meaningful investigations of 3D human lung tissues. There are increasing discussions about the feasibility of utilizing lung organoids to approach lung tissue cell–cell interaction mechanism and the potentiality of IL-17 signaling pathway during lung infection\textsuperscript{134,135}. Future research should try to use organoids to better simulate and explore the roles of microorganisms in lung cancer and the possible molecular mechanisms due to its success in investigation of microbiota associated with colorectal cancer\textsuperscript{101}.

Increasing studies have also approached to the role of intratumor tissue microbiome in cancer development and therapies. T. Geller et al. found that intratumor bacteria might contribute to gemcitabine resistance of pancreatic ductal adenocarcinoma (PDAC), in which 76% of 133 human PDACs were positive for bacteria\textsuperscript{186}. And alpha-diversity in tumor microbiome and an intra-tumoral microbiome signature were highly correlated with long-term survival in patients. They also proved that human-into-mice fecal microbiota transplantation (FMT) experiments differentially affected tumor growth, as well as tumor immune infiltration through modulating the tumor microbiome\textsuperscript{147}. To identify the characterization of the tumor microbiome, researchers from Israel conducted a comprehensive analysis of 1526 tumor tissues with adjacent normal tissues from seven cancer types (breast, lung, ovary, pancreas, melanoma, bone, and brain tumors) which revealed that the intratumor bacteria were mostly intracellular in both cancer and immune cells and supported significant correlations between intratumor bacteria or their predicted functions with tumor types and subtypes, patients’ smoking status, and the response to immunotherapy\textsuperscript{96}. In addition to bacteria, a pan-cancer comprehensive analysis based on the International Cancer Genomic Consortium (ICGC) and the Cancer Genome Atlas (TCGA) has drawn a landscape of viral associations in human cancers, which detected a high prevalence of known tumor-associated viruses\textsuperscript{138}. Yu et al. profiled the lung tissue microbiota and linked its composition and diversity to human lifestyle and clinical outcomes\textsuperscript{93}. Also, Liu et al. reported that profile of lung cancer tissue microbiota is distinct from emphysema\textsuperscript{89}. Consistently, Peters et al. demonstrated the existence of associations in lung tumor or normal tissue microbiome diversity and composition with patient recurrence-free (RFS) and disease-free survival (DFS)\textsuperscript{90}. Above all, there is still an urgent need for much more explorations approaching the potentials of intra-tumor microbiome in cancer development and therapies.

Furthermore, some pioneer preclinical studies have tried to utilize GF mice (germ-free mice, free of all microorganisms including those typically found in the gut) and SPF mice (specific pathogen-free mice, free of a specific list of testing pathogens including disease-causing or research-affecting or mice health-related pathogens, opportunistic and commensal organisms in normal, healthy mice) model to elucidate the real and precise correlations between lung microbiome and lung cancer closer\textsuperscript{82,139}. It was shown that bacteria in the lung may create a proinflammatory environment that promote lung cancer progression in the murine mouse model\textsuperscript{82}. The GF or antibiotic treated mice were significantly protected from lung cancer development induced by Kras mutation and p53 loss when compared with SPF mice. And the commensal bacteria stimulated production of Myd88-dependent IL-1β and IL-2β from myeloid cells, inducing proliferation and activation of Vγ6/Vδ1 γδ T cells that produced IL-17 and other effector molecules to promote inflammation and tumor cell proliferation. A similar result was also discovered by K. L. Greathouse et al. that a lower alpha diversity in normal lung as compared to non-tumor adjacent or tumor tissue and a separate group of taxa are identified, in which \textit{Acidovorax} is enriched in smokers with squamous cell carcinoma\textsuperscript{84}. A reasonable hypothesis is that lung microbiota homeostasis may promote the pathological processes of lung cancer. Interestingly, it was observed in spontaneous colon tumor models that the morbidity of solid tumors was significantly lower in germ-free mice compared to mice raised conventionally\textsuperscript{180}. Furthermore, high relative microbial abundance and diversity were found to be positively correlated with patient’s better responses to PD-1-based....
Fig. 2 Lung microbiome play a dual role in promoting carcinogenesis and maintaining homeostasis in different conditions. Lung microbiome can induce carcinogenesis via causing DNA damage, inflammatory response alterations, chromosome instability, abnormal signaling pathway activations and increasing mutation load through productions of bacterial toxins and multiple cytokines. On the other side, the process of colonization and maturation lung-resident microbiome community also participates in the maturation of lung and promoting host homeostasis and tolerance, as well as conferring susceptibility to lung disorders during exposure to complex external environment.

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Microbial biomarkers

Nowadays, the widely used and effective diagnostic tools for lung cancer are chest X-ray and CT in clinic. However, the examination by low-dose spiral CT is still unable to fully popularized due to its high cost and inconvenience. The best option for lung cancer screening is to examine groups with high-risk disease characteristics including age, gender, long-term smoking, and occupational exposure. Different from the previous methods, it will be better to explore the interaction between gut microbiota and lung cancer, and try to find the microbial alterations and specific microbes that are closely associated with lung cancer, which can provide better targets to pick out the high-risk groups for chest X-ray and CT. With development and popularization of deep sequencing, the associations between microbiota in different human body sites and various diseases have held interests from both researchers and clinicians. It has been reported that there is a significant correlation between microbiota landscape alterations and development of various cancer including lung cancer85, melanoma144 and pancreatic ductal adenocarcinoma145. As shown in Table 1, there were many long-term observations and epidemiological studies which detected significant correlations between microbiota and lung cancer based on various sample sources85,86,88,91,94. Previous studies have provided novel insights into the microbiota alteration during the development and exacerbation of various lung diseases, which help establish a non-invasive detection method106,107,110,111,118. Zheng et al. identified and established the specific gut microbial signatures for the prediction of early-stage lung cancer146. Yan et al. found that Neisseria, Streptococcus, and Porphyromonas were significantly higher in the saliva from lung cancer patients, which may serve as potential biomarkers for the disease detection/classification147. A pilot study using 16S rRNA sequencing revealed that greater abundance of Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae in lung tissue were significantly associated with a decreased risk of recurrence-free survival (RFS) and disease-free survival (DFS)148. There is no doubt that further clinical studies are necessary to establish microbial markers for predicting lung cancer in the future. Moreover, the precise role of salivary, sputum and feces microbiome in lung cancer initiation and progression is largely unknown and the potential molecular mechanisms awaits further investigation.

Radiotherapy and chemotherapy

Radiotherapy for advanced lung cancer has become a routine treatment in clinical practice, although it brings some unexpected side effects such as immune damage and radiation-induced toxicity. However, the relationship between gut microbiome and ionizing radiotherapy of cancer remains unclear as there was not too much progresses in this field. A recent study revealed that mice fecal microbiota transplantation could reduce radiation-induced damage without promoting cancer cell proliferation and migration in vivo149. Furthermore, a unique microbial signature with enhanced IL-1β, IL-6, and TNF-α expression compared with naïve microbiota, was observed in post-radiation mouse model tissue150. It is promising to identify microbes hypersensitive to radiation as predictive biotargets for improvement of curative effects. Microbiota might serve as a therapeutic strategy to reduce radiation-induced toxicity and improve the prognosis of lung cancer patients after radiotherapy149. Recent studies have implicated that gut microbiome plays a crucial role in drug metabolism, chemotherapy-induced toxicity and host response sensitivity149. The gut microbiota can directly modulate drug absorption and metabolism via microorganisms and microbial enzymes150,151. In addition, gut microbiota can also indirectly affect the rate of metabolism in oral and systemic administration via regulating gene expression, local mucosal barrier response, and the physiology of distant organs152,153. Experiments in vivo and in vitro have indicated a complex and multi-level intervention...
relationships between chemotherapeutic agents and human microbiota. Some special microbial species and metabolites have been confirmed to inhibit the antineoplastic drug gemcitabine and promote the prodrug CB1954 in blood circulation. Another subset of mainstream antineoplastic drugs is genotoxic platinum drugs, exhibiting anti-cancer effect via inhibition of DNA replication and by targeting plasma membranes and mitochondria, which leads to adverse drug reactions including intestinal toxicity, nephrotoxicity, blood–brain barrier integrity disorder and deafness. A possible explanation is that destruction of the intestinal mucosal barrier enables microorganisms or pathogens invade into the mesenteric lymph nodes and blood circulation. Furthermore, accumulating studies prove that effects of anti-neoplastic drugs with broad spectrum can be reduced, and antibiotics abuse may not only aggravate the side effects of anti-tumor drugs but also cause severe additional systemic side-effects. At present, most studies of microbiome and chemotherapy drugs remains in the stage of animal experiments, and there is only little research directly exploring the gut microbiota alterations and functions in post-chemotherapy patients with lung cancer. Additional mechanistic studies and clinical trials are still required to investigate whether modulation model of gut microbiota could become an effective clinical approach to assist treatment of lung cancer with chemotherapy drugs and minimize drug-induced toxicity.

**Immunotherapy**

It was previously reported that the disorder of the intestinal microbiota may affect the immunotherapeutic effect for cancer. For example, the microbiome of 249 cancer patients who underwent PD-1 immunotherapy was examined by a French research group. Among them, 69 patients were given antibiotics due to other diseases at the beginning of treatment which will disrupt the intestinal flora. Surprisingly, patients treated with antibiotics have shorter cancer-recurrence time and survival time than those who did not receive antibiotics, indicating that antibiotics consumption could greatly reduce the effectiveness of immunotherapy. A follow-up study compared the gut microbiota of the two groups of patients and isolated the probiotics Akkermansia muciniphila from the stool of the recovered patients. And this probiotic has been proved to be effective in the prevention of obesity and diabetes in previous studies. Interestingly, this study also demonstrates its contributions to cancer immunotherapy. Moreover, researchers implanted the feces of the recovered patients into germ free mice and those who received “effective” feces was responding quickly to PD-1 inhibitors. In addition, the oral probiotic Akkermansia muciniphila can also restore the same effect of immunotherapy. One possible reason is that a higher diversity of microbiome communities might be positively correlated with T cell activity, which in turn causes cancer cells to be killed more thoroughly. Inversely, patients with “bad bacteria” have more regulatory T cells which could suppress the host immune response. A recent study of Chinese patients with advanced non-small cell lung cancer who treated with the immunological checkpoint inhibitor PD-1 showed that patients with higher pre-treatment microbiota diversity presented better response to anti-PD-1 immuno-checkpoint inhibitors. Patients with favorable gut microbiome (such as those with high diversity) exhibit enhanced memory T cell and natural killer cell signatures in the periphery blood. Shi et al. revealed that systemic administration of Bifidobacterium potently stimulated STING signaling and increased cross-priming of dendritic cells after anti-CD47 treatment, which converts the non-responder mice into responder. Paulo et al. brought forward a hypothesis that gut microbiome may help prime an immune response through TLR4-signaling. Tumor-associated myeloid cells might be activated by commensal gut bacteria (via TLR4 signaling) to produce TNF and other inflammatory cytokines that mediate the tumor microenvironment and anti-tumor effect of immunotherapy. After treatment with cyclophosphamide, the translocation of specific commensal bacteria into mesenteric lymph nodes enhanced Th17 responses in the spleen and the induction of memory Th1 responses, which proved the important role of commensal microorganisms in MyD88 and TLR signaling. Intriguingly, a new research demonstrated that the commensal lung bacteria stimulated the production of Myd88-dependent IL-1β, IL-2β, and IL-17 and other effector molecules to promote inflammation and tumor cell proliferation. These studies revealed a close relationship between intestinal flora and cancer immunotherapy which provided new insights for improving the effectiveness of tumor immunotherapy. Nevertheless, further understanding of the effects and mechanisms of microbiome and immunotherapy needs more explorations.

**Probiotics, prebiotics, and microbial targeting drug**

At present, mature products targeting microbiome that have entered the commercial market include probiotics, prebiotics, and symbiotics, having showed generally safety in different clinical practice. The general effects revealed by increasing clinical data include promoting gastrointestinal homeostasis and integrity, regulating metabolism via productions of SCFAs (short-chain fatty acids) and vitamin or second bile salts, participating digestive activities and neutralization of inflammation and carcinogens. One of the major effects of administration of prebiotics and probiotics is to achieve optimal host immune homeostasis through maintaining the diversity and relative numbers of Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria and so on. On the other hand, the pharmaceutical and biotechnology companies worldwide are looking for microbial drug targets for developing and promoting chemotherapy and targeted therapy for cancer. Drug targeting microbiome have potentials to lessen the side effects resulting from chemotherapy, in which a clinical trial suggested a clinical benefit from administering neomycin concurrent with irinotecan to diminish the side effects. In another preclinical study, mice treated with the novel small-molecule inhibitors targeting bacterial β-glucuronidase were protected from irinotecan (an anticancer drug) induced diarrhea. However, the current limited investigation and knowledge about the beneficial microbiota and molecular mechanisms cannot provide the best method to dissect the host microbiome. Whether the microbial changes will cause unexpected local homeostasis disorders, inflammatory response or even precancerous lesions remains elusive. Recently, FDA issued a safety alert on the application of FMT for the risk of serious adverse events due to transmission of pathogenic organisms. Even though emerging achievements have indicated a promising application of microbiome in anti-cancer action, future studies should focus more on the causal effects of lung microbiome alterations on lung diseases and identify the healthy and beneficial lung microbiome.

**DISCUSSION AND PERSPECTIVES**

Lung cancer has been the leading cause of cancer-related deaths worldwide mainly due to its initially asymptomatic and typically diagnosis at an advanced stage. As shown in Fig. 3, the triple interaction among host, microbiome and environment maintains lung homeostasis in healthy functioning. Moreover, the microbiome potentially possesses inestimable therapeutic strategies in promoting conventional lung cancer treatments including radiotherapy, chemotherapy, surgical resection, and immunotherapy. Undoubtedly, microbiome was confirmed to be involved in various diseases initiation and development. But the
opportunities for promoting diagnosis and prognosis of lung cancers patients.

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ACKNOWLEDGEMENTS

The authors thank all the lab members in discussion and preparation of the manuscript. This study was supported by grants from the National Key R&D Program of China (2018YFC02000700), the National Natural Science Foundation (81630086, 81972172, 81971993, 81527053, 31901291), the Key Research Program (ZDRW-ZS-2017-1) of the Chinese Academy of Sciences, the Major Science and Technology Innovation Program of Shanghai Municipal Education Commission (2019-01-07-00-1-E00039), Medicine and Engineering Interdisciplinary Research Fund of Shanghai Jiao Tong University (YG2020QY06), Innovative research team of high-level local universities in Shanghai. National the Program for Young Eastern Scholar at Shanghai Institutions of Higher Learning (program QD2018106), Shanghai Pujiang Program (18PJ1406600), Innovative research team of high-level local universities in Shanghai, Shanghai Municipal Health Commission (2017BR026), Shanghai Science and Technology Committee (19XD1423200, 18140903900, 17441904300), Shanghai Municipal Human Resources and Social Security Bureau (2017114), Shanghai Education Development Foundation (175G23), Shanghai Hospital Development Center (SHDC12018122, SHDC12017X03), Fundamental Research Funds for the Central Universities (22120180510), and Shanghai Pulmonary Hospital (fkcx1801, fkcx1904).

AUTHOR CONTRIBUTIONS

N.L., Q.M., and Y.G. made substantial contributions to the design, data collection and writing of this manuscript. C.Y., L.W., J.T., Q.C., and J.L. contributed to discussion for scientific content on this manuscript. N.L., P.Z., and H.W. made substantial contributions to the conception and design of the work, critically commented on the manuscript for scientific content, were responsible for the quality of the overall manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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