Exploring the role of respiratory microbiome in lung cancer: A systematic review

Title:

Exploring the Role of Respiratory Microbiome in Lung Cancer: a Systematic Review.

Highlights

- Respiratory microbiome is a promising and unexplored topic for cancer research
- Lung cancer is enriched in peculiar microbial communities
- Differences in microbiome composition/diversity are reported in lung cancer patients
- Lung microbiome detection and modulation in lung cancer deserves dedicated studies

Abstract

Giving the potential contribute in cancer initiation and progression, lung microbiota represents a promising topic in cancer research, although still unexplored. We performed a systematic literature search to identify clinical studies evaluating lung microbiota composition, its correlation with lung cancer patients' clinico-pathological features and prognosis. Of the identified 370 studies, 21 were eligible and included. Although studies were heterogeneous, lung cancer resulted to be enriched in peculiar microbial communities, with differences in composition and diversity according to clinico-pathological parameters. Few studies explored how lung microbiota influences cancer outcome. In light of these findings and borrowing the suggestions coming from gut microbiota, we speculate that respiratory microbiome may influence pathogenesis, progression and outcome of lung cancer. Taking advantage of the experience of chronical lung diseases, prospective studies should be designed to evaluate lung microbiota changes throughout any phase of lung cancer course, particularly with the advent of immunotherapy as pivotal treatment.

Keywords

Lung microbiota; lung cancer; prognosis.

1. Introduction

The human microbiome is defined as an ecological community of commensal, symbiotic and pathogenic organisms that share our body space. It includes bacteria, archaea, fungi, protists, and viruses located in our gut, skin, vagina, oral and nasal cavity, as well as respiratory system[1].

Recently, the microbiome has gained increasing attention in the scientific community. The remarkable improvement in genomics techniques and bioinformatics support has allowed the identification of specific microbes and/or the characterization of entire microbial communities, potentially related to many human pathologic conditions. In this field, the gut microbiome has acted as a forerunner. Indeed, many evidence are available supporting the correlation between gut microbiome alterations and development and/or progression of several acute or chronic diseases, as well as cancer[2, 3].

Since the link between *Helicobacter pylori* and gastric cancer was recognized, the existence of a crucial connection between cancer and host's microbiome, in terms of both carcinogenesis triggering (at baseline and relapse) and treatments efficacy/tolerability, came under the spotlight[4]. Alterations in specific bacterial loads, for example, have been related to the development of different tumor types, as oral squamous carcinoma, colorectal and esophageal cancer[5].

The lung microbiome (LM) forms a distinct cluster compared with the microbial communities of the oral cavity and other body areas[6]. *Proteobacteria* is the predominant phylum (60%), followed by *Firmicutes* (11%), *Bacteroidetes* (10%), *Thermi* (9%) and *Actinobacteria* (4%) in non-malignant lung tissue.

Nevertheless, the oral microbiome represents the main source of upper and lower airway communities via a well-documented physiological process known as microaspiration[7]. Several studies described microaspiration as the primary source of microbial immigration, i.e., *Prevotella* species, which participate in the immunologic homeostasis of the airways, are routinely acquired via microaspiration from the oral cavity[8-10]. Moreover, microbial migration/elimination is accomplished by a combination of mucociliary clearance, cough, and host immune defenses[7]. The concept of "gut-lung axis", as a link of immune health of gut and respiratory tract, is also intriguing. In the gut district of healthy subjects, microbial migration is "unidirectional", from mouth to anus across a variety of chemical and physical barriers. On the contrary, movement of air, mucus and microorganisms in the lungs occurs with minimal physical barriers between the larynx and the most distal sites in the alveoli ("bidirectional"). In pathological conditions, such as in gastroesophageal reflux disease (GERD), the lung environment could be contaminated by microbes derived from the upper gut tract[11]. Conversely, after pulmonary infections, several components of lung microbiota could migrate to the gut tract and cause infections[6]. Furthermore, disruptive dysbiosis of the respiratory tree and intestinal tract are linked to an increased incidence of pulmonary diseases[12, 13]. Finally, chronic lung diseases like asthma, chronic obstructive pulmonary disease, and cystic fibrosis are associated with inflammatory bowel disease[6].

LM composition and function is dysregulated (increase in alpha diversity) by environmental exposures and tobacco smoking[14]. As for gut microbiome, the alteration of lung ecosystem may predispose to several respiratory diseases[15]. In particular, qualitative and/or quantitative changes in LM have been related to the development and/or exacerbations of asthma, chronic obstructive pulmonary disease, cystic fibrosis and pulmonary interstitiopathies[16, 17].

Regarding lung cancer, previous respiratory diseases, such as pneumonia and tuberculosis, have been demonstrated to represent independent (regardless of smoking status) risk factors, sharing the inflammatory response as the most probable causal link[18]. Moreover, the rationale supporting a pathogenetic correlation between microbial dysregulation and carcinogenesis is further strengthen by available data that have associated recurrent exposure to certain antibiotics to cancer risk in specific organ sites[19]. Of interest, a consistent amount of recently emerged evidence found that an abnormal gut microbiome composition (as the one induced by antibiotics use) might impair the systemic response to antitumor immunotherapy, thus limiting immune checkpoint inhibitors efficacy in advanced cancer patients[20-22].

Nevertheless, several crucial points need further clarification. First, to establish the real impact of LM (composition and diversity) in driving lung cancer susceptibility, development and progression. Second, although the existence of a bidirectional communication within the gut-lung axis is recognized, understanding the underlying mechanisms mediating this complex cross-talk is crucial to maintain and ideally favorably modulate this interaction. In this light, only preliminary analyses are available characterizing and comparing fecal and sputum microbiome of lung cancer patients with healthy control subjects[23]. Finally, methodological/bioinformatics issues are arising, such as cohorts heterogeneity, sampling material and technique (brush, bronchoalveolar lavage or sputum), nucleic acid extraction, analytical methods and data interpretation[24].

Herein, we conducted a systematic review including the available clinical studies that investigated lung microbiota composition and diversity, and its correlation with lung cancer patients' clinico-pathological features and prognosis. In particular, borrowing the model of gut microbiome, we wondered if lung microbiome could modulate the likelihood to respond to immune checkpoint inhibitors. Overall, we aimed to provide preliminary insights about the potential role of lung microbiome in lung cancer.

2. Methods

This systematic review was realized to answer the question: "Is there any role for the respiratory microbiota in lung cancer?". The study was planned, conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[25]. The substantial heterogeneity and the recognized risk of bias identified across and within studies did not allow for pool estimates or meta-analysis of the investigated variables.

2.1 Search strategy

We have performed a systematic literature search using PubMed (Medline), Embase, Web of Science, and the proceedings of major international meetings (American Society of Clinical Oncology, ASCO; European Society of Medical Oncology, ESMO; and International Association for the Study of Lung Cancer, IASLC) to identify relevant studies published between January 01th, 2000 and April 30th, 2020. The following keywords were used: airway [all fields] OR lung [all fields] OR pulmonary [all fields] OR respiratory [all fields] AND microbiota [all fields] OR microbiome [all fields] AND lung cancer [all fields] OR non-small-cell lung cancer [all fields]. An additional search was carried out through the references of the included studies.

2.2 Study selection and eligibility criteria

Observational studies, clinical trials and case reports evaluating lung microbiome quantity and composition in lung cancer were included. Relevant international meetings proceedings, although not published in full, were also analyzed. Only English language articles were considered eligible. On the contrary, studies with insufficient lung microbioma composition data or outcome information, performed only in preclinical models, not including cancer patients, or focused on microbiome other than lung, were excluded.

2.3 Data extraction and synthesis

Study characteristics (first author, year of publication, number of patients/samples, study aim, sample type and analytical method, endpoints assessed and main results) were extracted from the included articles and summarized in **Table 1**. Data extraction was initially performed by two authors (F.P. and L.B.) and then independently reviewed by an additional author (S.P.).

3. Results

3.1 Literature search

Of the 370 studies found in the search, 323 were excluded after removing the duplicates and carefully reading the title and abstract, because not relevant for the question addressed in this systematic review and/or focused only on gut microbiota/microbiome. After reading the full text of the remaining 47 articles, 26 were additionally excluded because missing relevant lung microbioma composition information, or having insufficient or not available data. Moreover, one study was excluded because it was based only on culture methods, which offer a lower resolution of microbiome composition not comparable to high-throughput approaches, and other two because performed only in preclinical models. Overall, 21 studies satisfied the required criteria and were selected for the present systematic analysis (the workflow of the literature search is shown in **Figure 1**).

3.2 Characteristics of the included studies

Trial characteristics of the studies included are listed in **Table 1**. The 21 identified studies analyzed the respiratory microbiota and its composition using different sample types: bronchoalveolar lavage (BAL) or bronchoalveolar lavage fluid (BALF) in seven[26-32], lung tissue (fresh frozen or formalin-fixed paraffin-embedded - FFPE samples) in seven[12, 14, 33-37], sputum in five[23, 27, 38-40], brushing in two[41, 42], buccal sample in five[30, 39, 40, 42, 43]. Of note, only one study compared fecal and sputum samples of lung cancer patients[23]. Regarding the analytical method for evaluating microbiota composition, 19 out of 21 analyses applied the 16S rRNA sequencing alone or associated with other techniques[14, 23, 26, 27, 29-36, 38-44]. Traditional sequencing of rRNA genes is widely applied in the clinic for the diagnosis of infectious diseases. The 16S rRNA (component of the small subunit 30S of a prokaryotic ribosome) sequencing, in particular, can distinguish among different types of bacteria at the genus level[45]. The remaining two studies applied other sequencing techniques[28, 37]. One study compared cancer and non-cancer cases (healthy and a heterogenous group of disease cases) performing a characterization of both microbiome (with 16S rRNA sequencing) and host bronchial cells (with RNA sequencing)[42].

All the studies investigated the microbiota composition in lung cancer (n = 946). Microbiota composition of lung cancer was compared with healthy controls in thirteen studies (n = 272)[23, 26-28, 30, 32, 34, 38-43], with normal/non-malignant lung tissue in eight studies (n = 440)[14, 31, 33, 34, 36, 37, 41, 44] and other non-cancerous lung diseases in four studies (n = 83)[28, 29, 35, 42]. One study analyzed also 52 patients affected by other cancer types[32]. Two studies used the data from TCGA consortium as validation cohort, representing the largest screenings of lung microbiome performed so far[26, 34]. Another study included a validation set[28]. The correlation between lung microbiota composition and patient's characteristics (e.g., smoking habits, gender, age at diagnosis) and/or disease features (e.g., stage, histology, number of metastatic sites) was specifically assessed in fifteen out of twenty-one studies[14, 26-30, 32-36, 38-40, 43]. The effect of lung microbiota on oncogenic pathways activation/inhibition was explored in four studies[33, 34, 37, 42]. The influence of lung

microbiota on risk of developing lung cancer was suggested in one study[39]. Of note, only two studies were available evaluating the potential impact of lung microbiota composition on clinical outcome[26, 36].

3.3 Synthesis of main results

3.3.1 Microbiota composition in lung cancer compared with heathy subjects/normal tissue and other lung diseases

According to the taxonomic analyses, at the phylum level, the airway microbiota of lung cancer patients was particularly enriched in *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes* and *Verrucomicrobia*[26, 28, 35]. Among them, *Firmicutes* was the most abundant[35]. At the genus level, *Prevotella* (belonging to *Bacteroidetes*) was among the dominant lung microbiota components, together with *Bifidobacterium* (*Actinobacteria*), *Acinetobacter (Proteobacteria)* and *Ruminococcus* (*Firmicutes*)[28, 35]. Although considering the intrinsic limitation of 16S rRNA sequencing methods in providing a reliable identification at the lower taxonomic rank of species, those species most represented in lung cancer were *Prevotella intermedia, Prevotella melaninogenica* and *Propionibacterium acnes*[28].

When compared to healthy controls, *Actinomyces*[23], *Veillonella*[42, 43], *Capnocytophaga*[43], *Firmicutes* and *Actinobacteria*[30], *Streptococcus viridans, Granulicatella adiancens, Streptococcus intermedius* and *Mycobacterium tuberculosis*[38] emerged from distinct studies as more abundant in samples from lung cancer patients. Differences were observed also in sputum samples of never smoker lung cancer patients, being enriched in *Granulicatella, Abiotrophia* and *Streptococcus* compared to controls[40]. Of note, *Streptococcus* and *Neisseria* showed an increasing prevalence in lung cancer samples *versus Staphylococcus* and *Dialister* that gradually decreased progressively moving from healthy controls to non-cancerous to cancerous site[41]. One study has detected the presence of a specific bacterium (*Bradyrhizobium japonicum*) exclusively in lung cancer patients[28]. Regarding not-malignant lung diseases, patients affected by emphysema only presented a relative abundance in *Proteobacteria* (mainly *Acinetobacter* and *Acidovorax*) with lower presence of *Prevotella*, *Bifidobacterium*, *Acinetobacter, Ruminococcus* and *Akkermansia* compared with lung cancer patients. By contrary, lung cancer patients with or without emphysema shared a very similar phyla composition[35]. In comparison with benign lesions, lung cancer lower airway microbiota was enriched in *Veillonella, Megasphaera*[29] and *Streptococcus*, which demonstrated *in vitro* to upregulate candidate oncogenic mechanisms, as the ERK and PI3K signaling pathways[42]. The ground glass nodules, a frequent early presentation of lung adenocarcinoma, are associated with a microbiota dominated by *Mycobacterium*, *Corynebacterium* and *Negativicoccus* and are associated with an increased function of secondary metabolism genes and serine threonine protein kinase[37].

In terms of microbiota composition, fifteen studies examined both diversity within samples (alfa diversity) and between samples (beta diversity)[14, 26-32, 34-37, 39, 42, 44], whereas four studies only alfa diversity[23, 33, 38, 41]. Most of the included studies described a decreased alpha diversity in airway microbiota composition of patients affected by lung cancer *versus* healthy individuals[14, 23, 26, 28, 30, 34, 35, 41], and normal/not-malignant

tissue or conditions[14, 28, 29, 33-36, 41, 44]. Regarding beta diversity, nine studies have demonstrated different beta diversity in lung cancer patient *versus* healthy subjects and/or according to histology[14, 28-30, 32, 34, 36, 37, 42].

3.3.2 Correlation of microbiota composition with clinico-pathological features

Regarding the association between microbiota composition and smoking habit, *Acidovorax* was more abundant in former or current smokers and no correlation was found with time of cessation[34]. Other studies reported an increased proportion of *Streptococcus*[28], *Proteobacteria*[26] and ratio *Firmicutes* to *Bacteroidetes* in smokers[29].

Regarding histology, all the studies evaluating the microbiota composition between adenocarcinoma (AD) and squamous-cell carcinoma (SCC), found relevant differences, although with heterogeneous findings[14, 26-28, 30, 32-34]. The largest study described nine genera able to distinguish SCC from AD: *Acidovorax, Brevundimonas, Comamonas, Tepidimonas, Rhodoferax, Klebsiella, Leptothrix, Polaromonas*, and *Anaerococcus*. In details, *Acidovorax* was more abundant in SCC, while *Pseudomonas* in AD samples[34]. Of interest, five out of nine genera associated with SCC are significantly more represented in SCC harboring *TP53* mutation[34]. Higher quantity of *Veillonella* and *Streptococcus* and lower of *Haemophilus* were isolated in AD *versus* SCC[32]. Moreover, *Cyanobacteria* phylum was significantly more represented in AD compared to SCC and normal samples. Of note, *Cyanobacteria* is an important source of mycrocistin, a bacterial toxin involved in carcinogenesis through the reduction of CD36 (a toll-like receptor molecule) and the upregulation of PARP-1[33]. Another study evaluated the correlation between lung microbiota and histology, as well as risk of distant metastases. In particular, *Streptococcus* was significantly lower in metastatic AD *versus* not, and *Veillonella* and *Rothia* were higher in metastatic SCC as compared to localized ones[27]. Two studies have also assessed the microbiota composition in patients affected by small-cell lung cancer (SCLC). The SCLC saliva and the BALF samples were enriched in *Treponema*/*Spirochetes* and *Pseudomonas*, respectively[30]. When compared to non-small-cell lung cancer (NSCLC), *Rothia* was more abundant in SCLC[32]. Regarding the difference in alfa diversity according to lung cancer histology, one study reported higher alfa diversity in SCC than AD[34].

3.3.3 Correlation of microbiota composition with lung cancer risk and prognosis

Overall, only three studies speculated about the potential correlation between microbiota composition and lung cancer onset and prognosis[26, 36, 39]. The loss of airway microbiota diversity in healthy controls represented a risk factor for lung cancer development[39]. One study, deeply evaluating the prognostic implications, did not find any correlation between recurrence status or recurrence-free survival (RFS) and tumoral tissue microbiota diversity/richness. By contrary, an increased richness/diversity in normal adjacent tissue from lung cancer patients was associated with an reduced RFS and disease-free survival[36]. Finally, among SCC, a subcluster enriched in peculiar *Enterobacteriaceae* was associated with worse patients' survival[26].

4. Discussion

The human microbiota is recognized to play a relevant role in initiating and promoting carcinogenesis, as well as affecting prognosis and potentially influencing treatment outcome in a wide variety of malignancies, such as gastrointestinal tumors, cervical, nasopharyngeal and lung cancers[46]. Many data demonstrated that gut microbiome is involved in carcinogenesis and able to modulate efficacy and safety of oncological treatments, in particular immunotherapy[4]. While gut microbiota gained most of the attention, being the main *player* of the available data, the role of those microbial communities belonging to other body areas, as well as their source, interactions and local/systemic balance is still under investigation. Moving a step away from cancer, the composition and diversity of respiratory microbioma is increasingly well defined in chronic respiratory diseases, emerging as a main determinant of pulmonary health, whose changes affect their pathogenesis, exacerbations and prognosis[47].

Putting together these converging fields, exploring the potential role of lung microbiome in lung cancer represents an interesting and still unexplored area. Although studies included in this systematic review were heterogeneous (i.e., cohorts, type of sample, endpoints assessed), lung cancer resulted to be enriched in peculiar microbial communities, with relevant differences in composition and diversity compared to healthy subjects, not-malignant diseases, and according to clinico-pathological parameters.

Although the exact pathogenetic and functional underlying mechanisms are not yet clarified, the capability of human microbiota of influencing the oncogenic process through the inflammatory mechanisms and the modulation of host immune system provides the link between an increased lung cancer predisposition and previous respiratory diseases[18]. In this regard, preclinical data obtained in mice models showed dysbiosis of lung and gut microbiota with higher levels of inflammatory cytokines in lung adenocarcinoma compared to control group. Of interest, the prebiotics intervention was associated with modulation in levels of inflammation and microbiome composition[48]. Another *in vivo* study confirmed that lung adenocarcinoma-associated microbiota increase inflammation by activating lung-resident T cells and that germ-free mice or undergoing antibiotic therapy were at lower risk of developing lung cancer induced by *KRAS* mutation/*TP53* loss[28]. Moreover, the composition of the airway microbiome may trigger lung cancer pathogenesis through the induction of signaling pathways involved in carcinogenesis, as demonstrated by the upregulation of ERK and PI3K pathways induced by *Veillonella* and *Streptococcus*, significantly enriched in lung cancer *versus* controls[42].

Among the most relevant data emerging from this systematic analysis, peculiar communities were specifically described as able to potentially discriminate smokers *versus* non-smokers, lung cancer patients *versus* healthy individuals, and create peculiar “microbiota profiles” for adenocarcinoma, squamous cell carcinoma and other lung cancer subtypes. In this regard, application of respiratory microbiota components as candidate (prognostic/predictive) biomarkers throughout the entire disease course is an interesting field of research. In the only available study we identified mainly designed to evaluate the correlation between

lung microbiome and outcome, an increased richness and diversity of normal tissue was associated with a worse prognosis in lung cancer patients[36]. Similarly, other studies suggested that microbiota composition and diversity might predict an increased risk of developing lung cancer, for example in smokers, or disease progression in localized tumors[27, 39].

Besides the prognostic implications, how lung microbiota influences the activity and efficacy of oncological treatments (as radiotherapy, target therapy, chemotherapy and immunotherapy), together with the therapeutic potentialities coming from microbiome manipulation, is even more important from a clinical point-of-view. In this light, nowadays immune checkpoint inhibitors represent a pillar treatment for both locally advanced and metastatic lung cancer, thus exploring the potential predictive role of lung microbiome in different settings is not only fascinating, but also it may provide potential practice-changing information.

5. Future Perspectives

In order to evaluate the potential clinical application of lung microbiota detection in lung cancer, we hypothesize the idea of a prospective study in lung cancer patients treated with immunotherapy (**Figure 2**). In this observational prospective multicenter study, the microbiota composition and diversity (and its changes) will be prospectively monitored with serial nasopharyngeal swabs in a large number of locally advanced or metastatic lung cancer patients candidate to immune checkpoint inhibitors (alone or in combination). The study aims to *i)* understand microbiota changes during treatment and *ii)* correlate them with clinical outcome, ideally building a "lung microbiota signature" able to predict those patients more likely to respond or, by contrary progress, during immunotherapy course. From an exploratory point of view, analyzing lung microbiota composition at baseline and modification during treatment may clarify its potential role in predisposing disease spread through tumor and host microenvironment modulation (seed and soil hypothesis)[49].

Although the idea of conducting an exploratory study evaluating lung microbiota in immunotherapy-treated lung cancer patients is innovative and fascinating, several challenges should be faced, as emerged in reviewing the results of the studies included in this systematic analysis. The main issue was related to the relevant heterogeneity in terms of analysed cohorts and materials, sampling and analytical techniques. Regarding the type of sample, sputum emerged as a non-invasive and potentially reliable source of microbiota composition in lung cancer, although the correlation with the lower respiratory tract microbiome is not yet clarified and it is likely to be more representative of the upper tract[38]. As an example, in the study by *Wang et al.*, including both buccal and BALF samples, the microbiota composition was different according to the sampling site[30]. Moreover, in terms of results interpretation according to sample type, several analyses were performed in fresh frozen lung tissue, which is likely to represent the most appropriate source of microbiota, although more difficult to collect and manage in clinical practice[50]. In this

sense, considering the importance of sample choice for lung microbiota analysis, as well as the necessity to have easy-to-perform and ideally not-invasive procedures[51], in the proposed study a comparative analysis will be performed in parallel in a subgroup of patients to assess the concordance rate of lung microbiota composition detection between nasopharyngeal swab, bronco-alveolar lavage and sputum.

When planning the next steps, ideas should be ideally borrowed from chronical lung diseases, having a pivotal experience in airway microbiota study, definition and application[47]. Cystic fibrosis in particular may represent an ideal model, considering that lung and gut microbiome dysbiosis and their modulation through antibiotic or probiotics have been extensively studied, and microbiome-driven therapeutical interventions are currently ongoing[52]. Furthermore, preliminary data are available in autoimmune diseases. Distal airway dysbiosis was detected in untreated early rheumatoid arthritis, as well as in sarcoidosis-related lung inflammation[53]. In details, although current evidence does not prove neither a direct causal role of airway pathogens nor the existence of a peculiar lung microbiome signature, some studies have reported encouraging results about the potential utility of antibiotics in supporting the management of patients affected by sarcoidosis[54]. These data, properly integrated with the prognostic/predictive implications prospectively collected, could provide a rational for future studies focusing on respiratory microbiome modulation in lung cancer patients. Such pilot studies would increase our knowledge about some of the complex interactions between lung microbiota and lung cancer, potentially putting a new tile in the complex mosaic of cancer-immune system-host relationships.

Figure 1. Flow diagram.

Table 1. Summary of the studies included in the systematic review.

Figure 2. The Respi-Lung study: an observational prospective study evaluating the Respiratory microbiome in Lung cancer patients treated with immune checkpoint inhibitors. The study will be conducted in patients affected by locally advanced or metastatic lung cancer candidate to immunotherapy (alone or in combination), in order to monitor the microbiome composition during treatment. During this phase, a nasopharyngeal (NP) swab will be performed at baseline (at the beginning of immunotherapy), at the time of first and second radiological assessment, at disease progression and before and after any cycle of antibiotics for any cause, if done. Moreover, blood samples will be collected at the same time points to evaluate circulating immune cell subsets in comparison with respiratory microbiome characteristics and composition. A comparative analysis will be performed in parallel in a subgroup of patients to assess the concordance rate of lung microbiota composition detection between NP swab, bronco-alveolar lavage (BAL) and sputum.

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