The Interplay Between Human Microbiota and Lung Cancer: Mechanisms and Implications

Review Article

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Abstract—Our microbiome is shaped by multiple factors such as genetics and the immune system, diet, exposure to antimicrobials and other drugs, environmental factors or exchange/interaction with other microbiomes. Multiple studies have suggested that deregulation of the human microbiome contribute to the development of cancer. In this review, we try to elucidate which alterations in the microbiota are related with carcinogenesis; specially emphasizing in its role in lung cancer. We have analyzed a substantial body of studies, which conclude that the microbiota is a critical biomarker for tumorigenesis and cancer therapy response. It also significantly influences clinical characterization, prediction of response to specific treatments—particularly immunotherapy —and plays a key role in cancer prognosis. We ultimately concluded that the gut-lung microbiome axis is crucial for carcinogenesis. Dysbiosis is associated with prognosis. More prospective studies are needed to assess the true role of the microbiome in prognosis and response of lung cancer.

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Keywords—Microbiome, Lung cancer, Immunotherapy, Target therapies

Resumen—La Interacción Entre el Microbiota Humana y el Cáncer de Pulmón: Mecanismos e Implicaciones
El microbioma humano se refiere a la población total de microorganismos (bacterias, hongos, virus, arqueas) que colonizan el intestino, la cavidad oral, la nasofaringe, el tracto respiratorio y la piel, incluidos sus metabolitos y funciones dentro del cuerpo. Nuestro microbioma está moldeado por múltiples factores, incluidos los factores intrínsecos, como la genética y el sistema inmunológico, así como factores extrínsecos, como la dieta, la exposición a antimicrobianos y otros fármacos, factores ambientales o el intercambio/interacción con otros microbiomas. Múltiples estudios han sugerido que la desregulación del microbioma humano puede contribuir al desarrollo de diversas enfermedades complejas, incluido el cáncer. En esta revisión, intentamos dilucidar qué alteraciones en la microbiota están relacionadas con la carcinogénesis, haciendo especial énfasis en el eje intestino-pulmón y su papel en el cáncer de pulmón. Con este fin, hemos analizado un cuerpo sustancial de estudios, incluidos ensayos clínicos y metaanálisis, todos los cuales concluyen que la microbiota ha emergido como un biomarcador crítico y regulador en la tumorogénesis y la respuesta a la terapia contra el cáncer. También influye significativamente en la caracterización clínica, la predicción de la respuesta a tratamientos específicos, especialmente la inmunoterapia, y juega un papel clave en el pronóstico del cáncer. Finalmente, concluimos que el eje microbioma intestino-pulmón es crucial no solo en la carcinogénesis, sino también en el desarrollo de diversas enfermedades respiratorias inflamatorias. Además, la disbiosis está asociada con el pronóstico y sirve como predictor de respuesta a diferentes tratamientos, incluidos la quimioterapia, la radioterapia y la inmunoterapia. Sin embargo, se necesitan más estudios prospectivos dentro de ensayos clínicos para evaluar el verdadero papel del microbioma pulmonar e intestinal en el pronóstico y la predicción de la respuesta a los diferentes subtipos de cáncer de pulmón.

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Palabras clave—Microbioma, Cáncer de pulmón, Inmunoterapia, Terapias dirigidas

INTRODUCTION

The The human microbiome is the total population of microorganisms (bacteria, fungi, virus, archaea) that colonize the gut, oral cavity, nasopharynx, respiratory tract and skin, among others. Most of the information accumulated so far focuses on the so far focuses on the so-called bacteriome (bacterial microbiome). Our microbiome is shaped by multiple factors, including intrinsic factors, such as genetics and the immune system, as well as extrinsic factors such as diet, exposure to antimicrobials and other drugs, environmental factors or exchange/interaction with other microbiomes.

Microbiota refers to the community of living microorganisms residing in a specific ecological niche, such as the human intestine, while microbiome is the collective term for the microorganisms, their genes, and their metabolites within that given ecological niche.

Mucosal surfaces exposed to the external environment are colonized by a vast number of microbes consisting of bacteria, viruses, fungi and archaea, which are collectively referred to as the commensal 20 microbiota. While oral cavity, skin, respiratory tract, urogenital tract and gastrointestinal tract are major body sites that each harbor unique microbiota, the primary habitat of commensal bacteria is the gastrointestinal tract (Figure 1). Numerous studies in the past two decades, have explored the interactions between the host and microbiome, and have demonstrated the critical role of microbiome in regulating diverse physiological and pathological processes of the host.

In the last years the role of microbiome has become one of the principal areas of intensive interest. There is an increasing number of studies that relate microbiome and disease, or its influence in several body reactions. Multiple studies have suggested that deregulation of the human microbiome may contribute to the development of various complex diseases, including cancer. In particular, it has been studied the relationship between gut microbiota and several diseases such as IBD, liver disease, diabetes, obesity, cardiovascular and immune-related disease, neurologic and psychiatric diseases, anxiety, depression. The current challenges focus on deepening the understanding of the composition and interactions of the microbiome, as well as deciphering the biochemical mechanisms involved in pathological processes in order to develop strategies capable of preserving and able to preserve and improve the microbiota, to demonstrate whether the observed changes in the microbiome are the cause or the consequence of these diseases and, ultimately, to improve the health of individuals.

Cancer is one of the leading causes of mortality and morbidity worldwide. It is a multifactorial and complex disease influenced by both genetic and environmental factors. Risk factors such as smoking, alcohol consumption, as well as exposure to sunlight and toxic substances, chronic inflammation is a major risk factor in carcinogenesis. In recent decades, the carcinogenic role of red meat Has also been analyzed, as nutrition is gaining an essential role in the genesis and progression of diseases, and cancer is no exception.

Various studies have related alterations in the microbiota with the development of carcinogenesis. Advanced age and dietary patterns not only contribute to cancer susceptibility but also lead to changes in the microbiota. Commensal microbiota has emerged as a crucial biomarker and regulator of both tumorigenesis and the response to cancer therapy. The influence of specific bacteria in establishing a pro-inflammatory environment has been emphasized. However, no single species is solely responsible for inflammation. The mechanisms by which microbes influence tumorigenesis in the intestine—a particularly rich and immunologically complex microbial environment in the human body—have not been fully elucidated.

In this review, we will examine the relationship between alterations in the microbiome and lung cancer (LC), as well as its influence on clinical characterization, prediction of response to certain treatments, especially immunotherapy (IO), and its role in cancer prognosis.

HUMAN MICROBIOME

Gut microbiota

Gut Microbiome (GM) is defined as the set of microorganisms that colonize our digestive tract. A human being hosts 10^{13} bacteria in his/her gastro-intestinal tract, as much as human cells in his body. Is in the human gut where we can found the biggest population of bacteria in the organism, thus being the principal responsible of the microbiota functions in the human body. *Bacteroidetes* and *Firmicutes* followed by *Proteobacteria, Fusobacteria, Tenericutes, Actinobacteria* and *Verrucomicrobia* are the most dominant phyla, making up to 90% of the total microbial population in humans. The fungi, protists, archaea, and viruses are also present in the intestinal microbiota, but they have not been as extensively studied at the moment.

Microbes have been studied for decades; the current aim is to relate them with human disease. In the recent years, there has been an increasing interest in discovering the human GM, its relationship with, not only infectious disease, but with chronic pathology such as obesity, diabetes, neurodegenerative diseases or even cancer. Nevertheless, the most blossoming topic is the microbe's role in novel therapeutics.

The use of massive sequencing techniques, especially those of the second generation (Next Generation Sequencing) have represented a great technological advance that has allowed us to gain a deeper understanding of the microbiome

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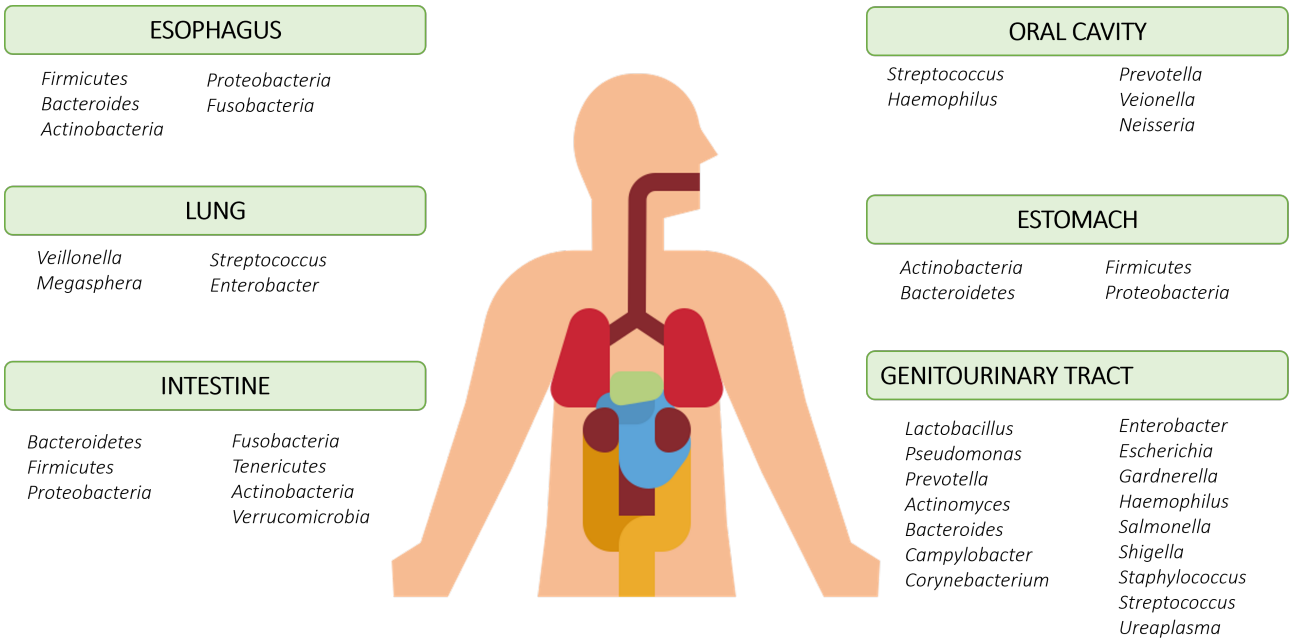

Figure 1: Principal body microbiota.

and its functions. However, the flood of multi-omics data derived from the study of the microbiome (the gut microbiome contains some 3 million genes, which is roughly equivalent to genes, which is approximately 150 times the size of the human genome) requires progress in the field of systems biology in order to make progress in the integration of data from different sources, as well as in the design of metabolic models on which to metabolic models on which to analyze these data in a biologically relevant framework.

Gut microbiome is not any more a guest but a partner. The current feeling is that human gut bacteria develop in communication with human cells and promoting immune interactions. It plays a crucial role in maintaining healthy metabolism and homeostasis of the human body. GM has been related with several functions including support and protection against pathogens, participating in digestion and metabolism, including modulation of insuline resistance and affecting its secretion. However, in the last years, there has been an increasing number of studies which attribute crucial functions to GM like controlling epithelial cell proliferation and differentiation, and, which is going to be the point of interest in this review; enhancing the immune system (IS).

The impact of maternal microbiota and antibiotic therapy during pregnancy on the baby's immune system has also been studied. Maternal MB produces compounds that affect the fetal or newborn immune system. For instance, short-chain fatty acids (SCFAs) produced by bacterial fermentation of dietary fibers, such as acetate ingested by the mother, enhance the fetal lung's ability to generate regulatory T cells (Tregs) and thereby reduce the severity of dust mite-induced allergy. The effect of SCFAs on T reg generation has been demonstrated through G protein-coupled receptors (Gpr41 and Gpr43) and direct inhibition of histone deacetylases (HDAC). In conclusion, alterations in maternal microbiota during pregnancy can impact and disrupt the development of the newborn. Similarly, colonization of the intestine by microbiota during the neonatal period induces significant transcriptional changes in the adult intestine.

The intestinal mucosa consists of a single layer of epithelial cells composed of intestinal cells and intraepithelial lymphocytes. This unique structure promotes the interaction of the MB with the IS. Paneth cells and goblet cells are located among the intestinal epithelial cells, secreting antimicrobial peptides and mucus, respectively, which additionally protect the epithelium. The lamina propria is a connective tissue layer that lays beneath the mucosal layer and contains Peyer's patches and various immune cells, such as antigen-presenting cells (APCs), innate lymphoid cells, T and B cells. This intestine-associated lymphoid tissue is representative of the largest component of the IS and plays a critical role in local and systemic immune responses. Here, the intestinal microbiota regulates the immune response through two main mechanisms: activating the innate immune response via Toll-like receptors (TLRs) and/or activating free fatty acid receptors (FFAR) through microbial metabolites such as SCFAs, including acetate, propionate, and butyrate. SCFAs, particularly butyrate, have demonstrated to enhance immunity by promoting the production of immunoglobulin A (IgA) by plasma cells. IgA not only functions by blocking bacterial adherence to epithelial cells but also directly affects bacterial pathogenicity or virulence.

Moreover, these metabolites can induce the differentiation of T cells into regulatory T cells (Tregs) in the lymph nodes found in the small intestine and colon, facilitating the transformation of the innate immune response into an adaptive one. The maturation of antigen-presenting cells (APCs), including dendritic cells (DCs), is induced through pathogen-associated molecular patterns (PAMPs). When these DCs

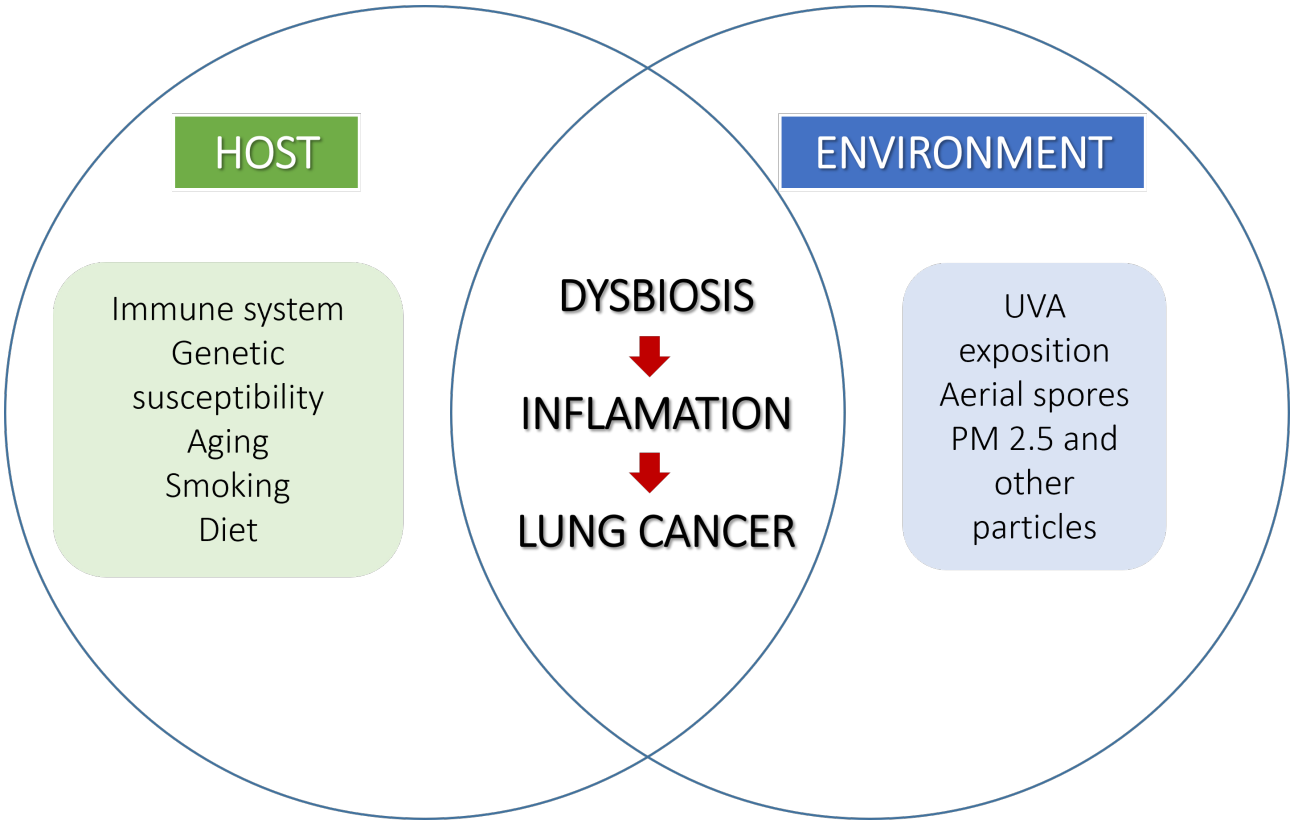

Figure 2: Triple interaction among host, enviroment and microbiome during lung cancer development and progression.

mature, they enter the mesenteric lymph nodes, where they communicate and promote the differentiation of T cells to develop into CD4+ T cells, particularly regulatory T cells (Tregs) and Th17 cells. These activated T cells play a key role in intestinal homeostasis, immunotolerance induced by Tregs, and the production of immunosuppressive cytokines (IL-10). It is worth mentioning that continuous communication occurs between intestinal microorganisms and mucosal T cells, with bacterial metabolites such as SCFAs promoting the maintenance of these cells at the intestinal level.

Eubiosis, which refers to the ecological balance, is critical for maintaining immunity. A rich and diverse microbiota can boost adaptive immune responses. Contrarily, ecological disruption, known as dysbiosis, can lead to the disruption of balance in the intestinal microbiome. This has unfavorable consequences such as reduction in diversity and a relative instability of the microbiota, along with the potential accumulation of opportunistic pathogens. This dysbiosis can also lead to a series of disturbances. For instance, the mucosal barrier is impaired, and both local and systemic immune responses are compromised. Intestinal bacteria translocate from lymph nodes and reach the systemic circulation, altering the cytokine microenvironment and promoting an inflammatory status. Th17 cells and effector T cells are activated, ultimately resulting in a profound inflammatory condition both locally and throughout the body.

Summarily, the intestinal microbiome deeply influences the immune system at both local and systemic levels. Additionally, the immune system can impact the intestinal microbiome at various levels. An imbalance in the relationship between the immune system and the microbiome could trigger a pathological process. The production of toxic metabolites, the exaggerated immune to bacterial stimuli, or sustained intestinal inflammation in the gut would be some of the key elements in the onset and development of disease.

Lung microbiota

Lung cancer (LC) is the first most common cancer and is the leading cause of cancer-related deaths in both men and women worldwide. While small cell lung cancer (SCLC) accounts for 10-15% of LC cases, up to 85% of LC are non-small cell lung cancer (NSCLC), of which the main subtypes are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Cigarette smoking is the first risk factor for LC, while other established risk factors may include second-hand smoke, air pollution, exposure to radon, asbestos, and other occupational carcinogens.

Mechanisms by which these environmental risk factors and other tumor-extrinsic factors control lung carcinogenesis remain poorly understood.

As the organ with the largest surface area in the human body and with gas exchange functions, the lung is inevitably exposed to diverse environmental microorganisms. Under these assumptions, the following doubts arise: does the lung have its own microbiota, and do the lung microbiota (LM) change when cancer is diagnosed? Moreover, how do microbiota impact lung carcinogenesis? (Figure 2).

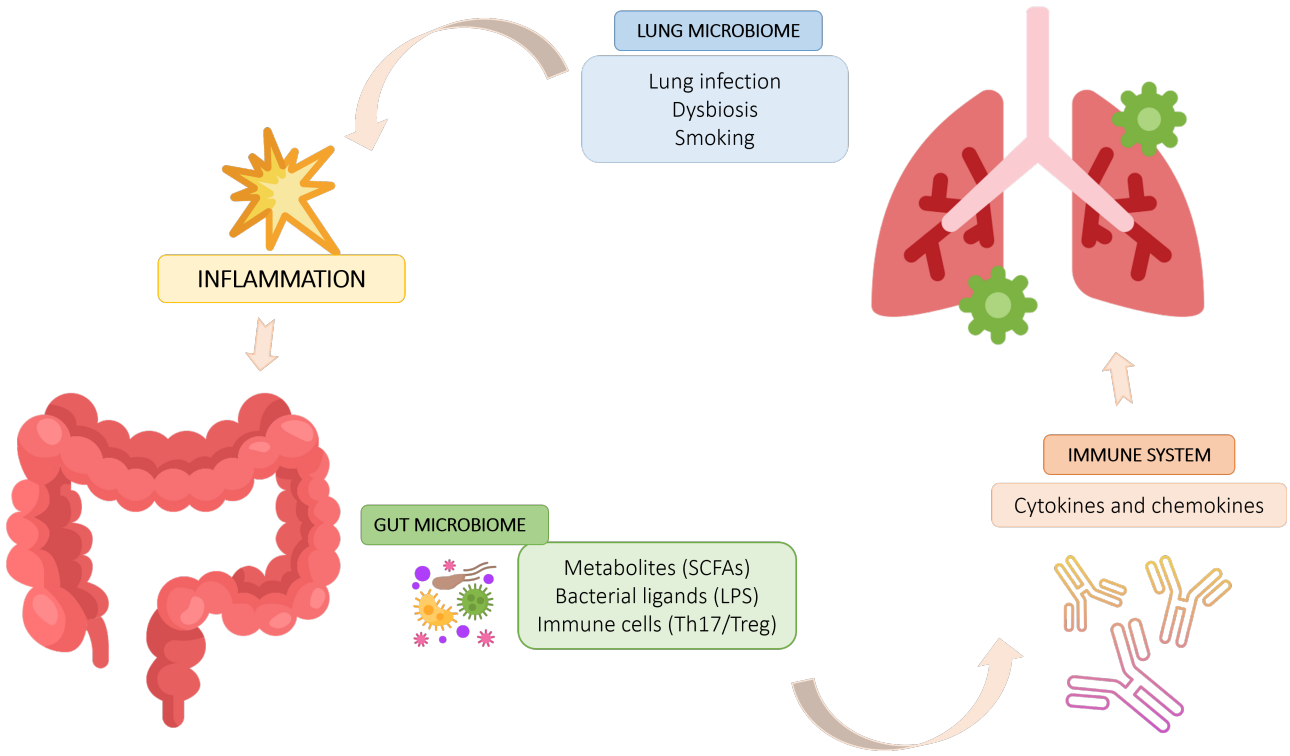

Figure 3: Gut-Lung axis.

In the last years, there has been a crescent interest in determining the role of microbiome in cancer pathogenesis, however the mechanisms by which the microbiota can affect this type of tumor have been poorly investigated. As mentioned before, even though the lung mucosal surface is large and in permanent contact with external microbes, the amount of bacterial biomass detected in healthy lungs is poor (5–8.25 log copies/mL). This could be attributed to the lung IS, a robust defense mechanism of the lung against external threats, along with the epithelium's ability to eliminate microorganisms and the inhospitable conditions for their survival within the lung mucosa. Numerous studies have attempted to link the lung microbiota with pulmonary diseases. For instance, it has been suggested that individuals with a higher prevalence of Prevotella and Veillonella (commonly found in oral microbiota) are associated with a TH17 immune response. However, further research in this area is warranted.

Most of the reviewed analysis support that LC is associated with an imbalanced local microbiome characterized by elevated total bacterial abundance, diminished alpha-diversity, and altered bacterial composition. Although the precise implications of bacterial diversity in LC remain uncertain, earlier studies indicate that increased alpha diversity is generally linked to better survival and treatment response in tumours like cervical cancer and resected pancreatic adenocarcinoma, which can be mainly attributed to the influence of the host immune response. In this same field of study, it has been suggested that the lung microbiome may contribute to lung tumorigenesis either by directly acting on tumor cells or indirectly via modulation of the tumor-associated immune response. Each tumor type was shown to have distinct microbial compositions, it was found that bacteria that can degrade chemicals in cigarette smoke were significantly enriched in LC compared with other tumor types, suggesting that high levels of these metabolites create a preferred niche for bacteria that can use these molecules for developing. Similarly, it has been described how altered microbiome in lower airway may lead to poorer outcomes in NSCLC and a decrease in overall survival. Among these bacteria, *Veionella parvula*, which is the most prevalent microorganism at this level when dysbiosis appears, leads to upregulation of several cytokines such as IL17, PI3K, thus modifying MAPK and ERK pathways. Furthermore, lower bacterial richness and diversity is correlated with lung tumors, and that alterations in relative abundance of some families (*Koribacteraceae, Bacteroidaceae, Lachnospiraceae*, and *Ruminococcaceae*) may contribute to increased or decreased disease-free survival (DFS) or progression-free survival (PFS).

Gut-lung axis

The intestine and the lungs are in constant communication, since the microorganisms can penetrate both gastrointestinal and respiratory tracts. In addition, the gut and the lungs have the same embryonic origin (Figure 3). Given these facts, in the recent years, the named gut-lung axis (GLA) has appeared to have a role both in health and disease, including gut and lung different microbiomes, but also their resultant metabolites- consisting mainly in SCFAs - and their impact on host IS.

As previously mentioned in this review, the gut microbiota has been found to regulate the immune response all over the body and especially in the lungs in allergic airway diseases or viral infections. Some studies have shown that oral antibi-

otic treatment, and its consequent alteration of intestinal microbiota, may decrease progression-free survival and overall survival in patients with advanced NSCLC, other studies appoint that therapy with antibiotics can reduce the antitumoral response of cisplatin.

It has been identified a clear cross-talk between the gut and the lungs, which is vital for maintaining homeostasis and modulating the IS. The mechanisms through which the gut impacts lung health or disease, are starting to be exposed. Multiple factors have been demonstrated to operate along the gut–lung axis, including the systemic spread of bacterial-derived components and metabolic degradation products. Among these, short-chain fatty acids (SCFAs) are the most significant immunomodulatory metabolites.

Lung cancer development is associated with changes in GM. It has been described a significant difference in microbial diversity among LC patients, mostly represented by a higher prevalence of *Enterococcus*, and others such as *Streptococcus, Prevotella, Blautia, Coprococcus, Bifidobacterium*, and *Lachnospiraceae*.

MICROBIOTA AND LUNG CANCER

For a long time, the lungs were considered sterile organs until culture-independent technologies revealed that they are inhabited by diverse microbial communities. Lung is colonized by a diverse array of microbes and the lung microbiota is profoundly involved in the development of respiratory diseases. There is little knowledge about the role of lung microbiota dysbiosis in LC.

Early reports based on culture-dependent methods linked *Chlamydia pneumoniae* and *Mycobacterium tuberculosis* with the risk of LC. Advanced analyses of the lower respiratory tract microbiome began in the past decade, and have demonstrated distinct microbiome alterations between healthy and malignant respiratory tract conditions. Several studies reported that *Veillonella, Megasphera, Streptococcus, Enterobacter*, and *Legionella* may be associated with LC development. However, the mentioned species have also been reported as part of a normal lung microbiome in healthy individuals.

LC treatment, can vary depending on the cancer type and stage, and the patient's overall health condition. The most common methods for LC treatment, including for NSCLC, include surgery, radiotherapy, chemotherapy (CT), targeted therapy, and immunotherapy (IO). PD-L1 inhibitors, also known as checkpoint inhibitors, are a class of IT drugs used in the treatment of NSCLC. Recent publications have elucidated a subtle link between the expression status of PD-L1 and/or the clinical response of the inhibitor of this protein and human gut microbiome compositional changes in cancer. Furthermore, fecal microbiome transplantation has been shown to restore the response to immune checkpoint inhibitor (ICI) therapy in patients. The tumor microenvironment, pro-inflammatory factors, and ICI play a significant role in LC development, progression, and treatment response. As of today, we lack complete knowledge of how the gut and lung microbiomes interact with the IS in tumor microenvironments. Furthermore, only a small number of studies have investigated the association between the lung tissue microbiome and clinical data of patients with LC. Ankudavicius et al, in an interesting study about lung microbiome, they demonstrated that the lung microbiome and the stool microbiome are separate bacterial milieux in patients with NSCLC founding significant associations between host microbiome and smoking habits, histology type, tumor differentiation degree, CD8+ cells in the tumor microenvironment. They also found that higher bacterial richness was associated with prolonged survival in NSCLC patients.

Inversely, other authors describe an inverse relationship between different bacterial populations in the gut microbiota and the development of LC, favored by a more protective immunomodulation against lung carcinogenesis.

Lung cancer staging and microbiota

Several authors have analyzed the influence of the pulmonary microbiome on the lung cancer extension. For example, Reddy et al found that patients with involvement of multiple pulmonary lobes had a different bacterial population compared to those with single lobar involvement, and furthermore, more aggressive histologies were associated with lower microbial diversity, although broader validation is necessary to confirm these findings.

In an attempt to analyze the correlation between patient staging in LC and differences in microbiota in each case, Jiang H. et al designed a study of 115 individuals which included: healthy controls (n = 35), patients with treatment-naive NSCLC at the early stage (n = 40), and the metastatic stage (n = 40). They characterized the fecal microbiota composition and metabolism by sequencing 16S rRNA and measuring levels of SCFA through gas chromatography, respectively. They found significant alterations in the community structure of the gut microbiota in patients with NSCLC, with an increase in pathogens in Fusobacteria and Proteobacteria and a decrease in SCFA-producing bacteria, *Firmicutes* and *Actinobacteria*, particularly in the metastatic stage. The overall concentration of fecal SCFAs was significantly lower in patients with brain metastases (BM) compared to patients with early lung cancer (ELC) and healthy controls. Subgroup analysis of acetate and butyrate yielded similar results. In line with the differential expression of bacterial populations depending on the tumor's extent, Guo et al found that the increased abundance of target species, such as *Flavonifractor plautii*, was associated with advanced-stage adenocarcinoma (ADC) and a higher metastasis rate. In contrast, other authors did not find diversity significant differences across LC of different stages. In light of the previously described results, there is some evidence of different profiles in the digestive and pulmonary microbiome depending on the stage, with a greater diversity found in patients with advanced disease compared to early-stage disease when compared to healthy individuals, although further studies are necessary to confirm these findings.

Pathological lung cancer subtype and microbiota

There is very little evidence regarding differences in the pulmonary microbiota based on the histological subtype of LC developed by the patient. Although bacteria have been hypothesized as agents of carcinogenesis, little is known about the microbiota profile of the most prevalent cancer subtypes: ADC and SCC. Despite this limited evidence, several studies have linked histological subtype with differences in microbiota in lung cancer patients. An example of this is the work of Yuan et al., who found that there was a progressively increasing concentration of *Streptococcus mitis, Burkholderia oklahomensis*, and *Burkholderia latens* populations from lower concentrations in ADC in situ to higher concentrations in invasive ADC varieties.

Gomes S et al characterized the lung microbiota by analyzing the 16S ribosomal RNA gene (V3-V6) in 103 simple bronchoalveolar lavage fluid samples, identifying that the bacterial population in SCC samples was more enriched in *Proteobacteria* than in ADC cases, particularly in males and heavier smokers.

Zheng et al analyzing differences between taxa in different pathological LC subtypes, finding differences of 13 taxa between lung squamous cell carcinoma and lung ADC, 63 taxa between lung SCC and benign pulmonary diseases, and 4 taxa between lung ADC and benign pulmonary diseases reached statistical significance in all cases.

Lu H et al recruited 121 patients and 37 healthy controls with lung cancer, collecting intestinal microbiota and sputum samples. They compared the microbial profiles between the groups, identified microbial biomarkers, and generated machine learning (ML) models to distinguish healthy individuals from patients with NSCLC and patients at various stages of the disease. In this study, through analysis of gut and sputum samples, the microbiota revealed significant disturbances in both in non-small cell lung cancer, and these disturbances were associated with distant metastasis. However, only the sputum microbiota was associated with non-distant metastasis NSCLC.

Grenda et al., in a study predicting response to intestinal microbiome therapy, found that a higher percentage of Akkermansiaceae was present in patients with SCC compared to ADC. Therefore, studies conducted in this context point to different profiles of pulmonary and digestive microbiome based on histological subtype or even molecular subtype, compared to those observed in healthy subjects. A challenge in this context could be predicting the development of dysplasia or neoplastic lesions in advance based on the predominance of these populations.

Lung cancer biomarkers and microbiota

In addition to stage and histological subtype, there are studies analyzing the diversity of fecal and sputum microbiomes in patients with molecular alterations considered "driver," such as EGFR or ALK, among others. In this regard, Huang et al. analyzed the pulmonary and digestive microbiome in patients with EGFR-mutated lung ADC and found a specific differential taxonomic profile compared to other tumors without these alterations. Saifon et al. analyzed the characteristics of intestinal microbiota in two cohorts of lung cancer patients (EGFR wild-type and EGFR mutant). They found that *Proteobacteria* counts were higher in the EGFR wild-type group, whereas *Bacteroidetes* and *Firmicutes* counts were higher in the EGFR mutant group. The alpha diversity of the gastrointestinal microbiome was significantly higher in the EGFR mutant group (Shannon index: 3.82 vs. 3.25, P = 0.022). Additionally, enrichment of *Clostridia* and *Bacteroidia* was associated with a higher risk of adverse events in the EGFR wild-type cohort. They also observed that chemotherapy altered the gastrointestinal microbiota, whereas EGFR-TKIs had less effect.

Despite the growing number of publications describing new potentially treatable targets in NSCLC there is not much more data available on specific aspects of the respiratory or intestinal microbiome of these patients. Most of the limited data comes from the EGFR population, which is more common among patients with target alterations and has a longer historical trajectory, allowing for deeper insights into the pathophysiology and microenvironment of these tumors.

MICROBIOTA PATTERNS AND LUNG CANCER TREATMENTS

Several studies point to the intestinal and pulmonary microbiota as predictive markers of response to the various therapeutic options currently available for the treatment of advanced CNMP. Next, we will review the literature analyzing the different studies that describe the potential role that the microbiota may play in therapeutic prediction in the fields of CT, IT, CT-IT, radiotherapy (RT), targeted therapies in terms of efficacy, as well as toxicity.

Chemotherapy and microbiota

Most studies examining the microbiota as a predictive marker of response to CT treatments include combinations of CT-IT (chemotherapy-immunotherapy), with limited information available regarding its value in the context of chemotherapy as a standalone treatment.

A growing body of research indicates an intricate relationship between the gut microbiome and cancer. Specific strains of bacteria found in the intestines have been shown to influence tumor development and impact the effectiveness of cancer treatments. These bacteria play a role in how CT drugs work and can affect various types of tumors, not just those in the gastrointestinal tract. Furthermore, alterations in the gut microbiome are implicated in both the body's immune response against tumors and the regulation of intestinal immunity. Thus, considering the gut microbiome as a supplementary treatment alongside conventional cancer therapies or as a predictive marker for treatment outcomes holds promise.

In addition to LC, in other tumors such as rectal cancer, a clear influence on chemotherapy response has been demonstrated with a mutational signature in ribosomal RNA of the intestinal microbiome. SBS5 was observed in non-responders ($p = 0.0021$), as well as the co-occurrence of APC and FAT4 mutations ($p < 0.05$), with an enrichment of *Hungatella, Flavonifractor*, and *Methanosphaera* in the pre-treatment biopsies of responders, while non-responders had a higher abundance of *Enhydrobacter, Paraprevotella*, and *Finegoldia*.

An example of the role of intestinal microbiota in the immunomodulation of cyclophosphamide in patients undergoing treatment with this drug diagnosed with lung and ovarian cancer is the study by Daillere R et al, where they found that the population of *Enterococcus hirae* and *Barnesiella* intestinihominis were highly present during cyclophosphamide treatment. While *E. hirae*, present in the small intestine, translocated from the small intestine to secondary lymphoid organs and increased the intratumoral CD8/Treg ratio, B. intestinihominis, present in the colon, promoted the infiltration of IFN-γ-producing $\gamma\sigma$T cells in cancer lesions.

Both populations induced an increase in the antitumor response of Th1 cells producing a higher systemic immune response in patients treated with this alkylating agent.

Radiation and microbiota

Similarly to CT and IT, the administration of RT is subject to microbiota-induced immunomodulation. Colbert et al. explored tumor and gut microbiome features affecting chemoradiation response in patients with cervical cancer and identified that an obligate L-lactate-producing lactic acid *bacterium* found in tumors, Lactobacillus iners, is associated with decreased survival in patients and induces chemotherapy and radiation resistance in cervical cancer cells.

In LC, Qui et al. analyzed a series of patients included in two phase 2 studies comparing fecal microbiota populations before and after pulmonary CTRT (concurrent). They observed that the population of *Bacteroidota* and *Proteobacteria* increased, while the abundance of *Firmicutes* decreased after CTRT in a statistically significant manner. Additionally, they found that the differential composition of bacteria as well as a specific signature based on functional study of bacterial ribosomal RNA, was related to PFS (progression-free survival).

In the context of CTRT (concurrent) in CNMP, the bacterial population was analyzed in two populations with different prognosis: the long progression-free survival (long-PFS) group (PFS11 months) vs. the short-PFS group (PFS<11 months). They found that in the long-PFS group, the *Firmicutes/Bacteroidetes* value was higher than those of the short-PFS group ($p=0.073$) and healthy individual groups ($p=0.009$). Meanwhile, the long-PFS group had significantly higher diversities in *Fungi, Archaea*, and Viruses than 16 the short-PFS group. Additionally, in this same study, they found that the antibiotic resistance genes might play a role in disease progression and provide potential information on the relationship between the use of antibiotics and the efficacy of CT-RT.

On the other hand, other authors have associated intestinal microflora profiles with a protective effect against radiation-induced pneumonitis. These authors demonstrated through fecal microbiome transplantation that pneumonitis was reduced in irradiated mice. Therefore, the gut-lung axis may represent an innovative therapeutic approach for protecting against radiation-induced lung injury. However, the protective potential of specific gut microbiota in lung cancer during radiotherapy is not yet well defined, and more studies are needed to determine the effects of microbial communities on LC radiotherapy.

Therefore, the intestinal and pulmonary microbiota can be modified by the effect of lung radiation, also presenting predictive and protective value according to previously discussed exploratory studies.

Immunotherapy and microbiota

Currently, there is a growing interest in the role of the microbiota in modulating and predicting response to immunotherapy, particularly anti-PD-1 and anti-PD-L1 therapies. Several studies have associated the gut microbiome with the host's immune system and response to immunotherapy, as well as immune-related adverse events (irAEs). For example, *Bacteroides thetaiotaomicron* and *Bacteroides fragilis* have been reported to be positively associated with the efficacy of CTLA-4 blockade Katayama and colleagues conducted a study exploring how the composition of the gut microbiome affects the effectiveness of ICIs on patients diagnosed with NSCLC undergoing ICI treatment for over 3 months correlating the microbiome composition in fecal samples with treatment response. The findings of the study revealed that responders had significantly higher levels of *Lactobacillus, Clostridium*, and *Syntrophococcus* in their gut microbiomes compared to non-responders. Conversely, non-responders exhibited higher levels of *Bilophila, Sutterella*, and *Parabacteroides*. Patients with elevated levels of *Lactobacillus, Clostridium*, and *Syntrophococcus* tended to have a longer time to treatment failure (TTF), while those with lower levels of *Bilophila* and *Sutterella* experienced a significantly prolonged TTF. In mouse models, combining oral intake of *Bifidobacterium* with anti-PD-L1 antibodytherapy led to notable improvements in melanoma control Additionally, *Bifidobacterium* has been observed to inhibit lung cancer metastasis in mouse models *Akkermansia muciniphila* was found to be more prevalent in NSCLC patients who responded positively to PD-1-based immunotherapy. In this regard, Grenda et al. also found that the presence of *Akkermansia* bacteria in the gut microbiome, particularly *Akkermansia muciniphila*, was a marker for response to IT in NSCLC patients treated with first and second-line ICI. Patients tend to respond better to treatment if this bacterium is present in the intestine, as determined by next-generation sequencing of the gut microbiome from patients treated with anti-PD-1 therapy in the first or second line.

Akkermansiaceae levels were higher in patients with disease stabilization and partial response to immunotherapy compared to patients with disease progression. Another example of such studies is the analysis of differences in the expression of 16S ribosomal RNA of the respiratory microbiota in 84 patients treated with anti-PD-L1. Although they did not find differences based on PD-L1 expression, they did observe significant changes in the microbiome of responders vs. non-responders. In the IT responder group, *Veillonella* dispar was dominant, while Haemophilus influenzae and *Neisseria perflava* were dominant in the non-responder group. Liu B et al. conducted an interesting study that carried out a functional and taxonomic analysis of the relationship between the intestinal microbiome and PFS. They found a response prediction algorithm based on the integration of previous information capable of predicting whether patients treated with immunotherapy would belong to the PFS <3m group versus >6m group. Artificial intelligence has emerged in this field as a new strategy for designing predictive and prognostic algorithms. Shoji et al. have developed a multicenter, prospective, observational study aimed at discovering the specific composition of the gut microbiome or combination of gut microbes predicting the therapeutic response to IT in 400 potentially included patients. As we have previously showed, different research has identified certain bacterial patterns that seem to be more prevalent in patients who positively respond to treatment, while other bacterial sequences are found in higher proportions among non-responders. These differences may stem from various factors, including variations between patients due to previous treatments, medications, dietary habits, geographical location, or genetic factors. Consistently, species such as *Faecalibacterium, Bacteroidales, Ruminococcaceae*, and *Clostridiales* have been recurrently associated with treatment response across multiple studies.

Targeted treatment and microbiota

Different authors have correlated the efficacy of targeted therapies in lung ADC with the respiratory and fecal microbiome. However, despite the increasing number of targets emerging in the field of CNMP, especially ADC, most studies analyzing the status of the fecal and respiratory microbiome in these patients focus on EGFR disease.

Earlier, we referenced the study by Saifon focused on the analysis of intestinal microbiota in EGFR vs. non-EGFR patients, finding differences in bacterial populations in both groups. In the context of patients harboring mutations in EGFR, Huang DH et al. found that in the airway microbiome of adenocarcinoma patients with EGFR mutations, there were differences compared to patients without these mutations.

In an Asian study with a high incidence of patients harboring EGFR mutations, it was demonstrated that those with high EGFR expression showed an abundance of *Rhizopus oryzae, Natronolimnobius innermongolicus, Staphylococcus sciuri*, etc., in bronchoalveolar lavage samples compared to those with lower expression.

To date, we have not found further studies analyzing the status of the microbiome in other targets of targeted therapies that describe differential populations or predictors of response markers in the context of dysbiosis.

Treatment toxicity and microbiota

There is a growing interest in the correlation between microbiota populations and treatment toxicity, especially with ICI (anti-PD-1, anti-PD-L1, or anti-CTLA-4). As an example of this type of study, Chau et al. found that an enrichment of *Bifidobacterium* was reported to be associated with a lower incidence of immune-related adverse events (irAEs) in lung cancer patients receiving ICIs.

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In the section on radiation and the microbiota, we have previously mentioned the relationship between profiles of intestinal microflora and a protective effect against radiation-induced pneumonitis. The same authors demonstrated through fecal microbiome transplantation that pneumonitis was reduced in irradiated mice; therefore, the gut-lung axis may be an innovative therapeutic avenue for protecting against radiation-induced lung injury. Nevertheless, the protective potential of specific gut microbiota in lung cancer during radiotherapy is not yet well defined, and more studies are needed to determine the effects of microbial communities on lung cancer radiotherapy.

It has been suggested that the composition of the microbiome might play a role in the success of IT, and by modulating ICI, might influence survival and the development of side effects. Certain species of bacteria have been identified which aid ICI response and decrease ICI toxicity, including *Akkermansia, Bifidobacterium, Faecalibacterium, Lactobacillus* and *Ruminococcaceae spp*. decrease ICI toxicity, including *Akkermansia, Bifidobacterium, Faecalibacterium, Lactobacillus*. Tan et al. described that mice undergoing antibiotic therapy fail to respond to anti-CTLA-4 therapy, but also appear to have subclinical irAEs. However, it was also described that antibody treatment itself may alter or destroy the intestinal microbiome. It is known that symbiotic microbes have the ability to induce colitis via an interleukin-1 (IL-1) mechanism.

Hayase et al described that resistance to development of irAEs was related to the *Bacteroidetes phylum*, whereas increased frequency of irAEs was associated with *Firmicutes* and they also described a phenomenon in which antibiotic administration prior to ICI therapy does result in a reduced response to therapy and it does not impact the frequency nor the severity of irAEs. In a recent study by Matson et al., a group of researchers pinpointed eight species found in individuals exhibiting reduced toxicity to ICI therapy, commonly referred to as the "beneficial bacteria." These species comprise *Bifidobacterium adolescentis, Bifidobacterium longum,*

Collinsella aerofaciens, Enterococcus faecium, Klebsiella pneumoniae, Lactobacillus sp., Parabacteroides merdae, and *Veillonella parvula*; On the other hand, absence of *Akkermansia muciniphila* has also been found in mice that are non-responsive to treatment as we previously have commented, thus have increased rates of irAEs. Gastrointestinal inmunomediated adverse events have been also described in anti-PD1/PD-L1 therapy, specifically colitis. *Bacteroides species, Klebsiella pneumoniae, Proteus mirabilis, Lachnospiraceae spp*. and *Streptococcus spp* can promote or induce colitis during ICI therapy.

McCulloch et al, analyzing patients treated with ipilimumab who had an increased representation of *Faecalibacterium* and *Firmicutes* also exhibited they observed an increased ICI sensitivity and efficacy as well as an increased risk of developing colitis or other ICI-induced toxicities.

Multiple research investigations have shown that fecal microbiota transplantation (FMT), which involves transferring the complete microbial community from one person to another, could enhance the effectiveness of ICI therapies, consequently reducing immune-related adverse events (irAEs). This procedure can be administered through oral lyophilized pills or directly via colonoscopy or gastroscopy. Initially, FMT was primarily used to address persistent *Clostridium difficile* infection. Several authors, have reported though ICI has demonstrated some usefulness in patients where anti-PD-1 therapy was no longer effective or in cases with severe CIC.

Fungi also play a significant part in shaping the balance of gut tolerance and immune reactions, but as of now, no specific fungal species have been definitively linked to the development of immune-related adverse 32 events (irAEs).

THE MICROBIOME AS A PROGNOSTIC MARKER IN LUNG CANCER

As previously noted, lung cancer (LC) remains the leading cause of cancer-related mortality worldwide. In patients diagnosed with early-stage (I - II) non-small cell lung cancer (NSCLC), the primary curative treatment is surgical resection, with or without adjuvant cytotoxic chemotherapy. However, cancer recurrence, a critical prognostic factor for mortality in lung cancer, is commonly observed: 5-year survival rates range from 92% for stage IA2 to 53% for stage IIB.

Despite recent advancements in treatment, particularly with the advent of immunotherapy, there remains critical need for biomarkers that can predict recurrence in early-stage cases. While some biomarkers, such as mutational burden and infiltrating or circulating immune cells in the tumor microenvironment, have been extensively studied, novel biomarkers derived from the human microbiota have got limited attention. Symbiotic microbiota have been identified as key biomarkers and modulators in both cancer development and therapeutic responses. These biomarkers could potentially provide valuable insights for both predicting recurrence and enhancing our understanding of the etiology of lung cáncer.

Previously in this review we have elucidated that lung microbiome is a potential modulator of lung disease, including cancer development and it's recurrence. Parallel to gut microbiome, the lower community of microorganisms residing in the human lung might be crucial in maintaining homeostasis, offering immune surveillance and protection.

It will not be preposterous to say that disbiosis at this level leads to decline in health condition, including inflammation, lung disorders, pulmonary diseases and even lung cancer.

Early epidemiological studies indicate that bacterial infections are highly prevalent among lung cancer patients, with 50-70% of them experiencing pulmonary infections that complicate the disease progression. It has been demonstrated that lower airway microbiome is linked to prognosis of NSCLC, along with pulmonary inflammation and other transcription pathways related to carcinogenesis. Among these, *Chlamydophila pneumoniae (C. pneumoniae), M. tuberculosis*, and respiratory viruses, may be related with the development and progression of lung cancer.

Peters et al studied the differences in composition of lung tumor microbes compared to those in normal lung tissue and observed a relationship between taxa in the lung and cancer recurrence risk. They described that microbiome composition in the tumor, measured by the JSD (Jensen-Shannon Divergence), was significantly related to DFS (Disease-Free Survival, but not to RFS (Relapse-Free Survival or OS (Overall Survival). Furthermore they found that higher bacterial diversity was associated with better DFS. In contrast, normal lung microbiome was not related to RFS, DFS, or OS.

They appointed that some bacterial classes were highlighted for their association with survival; these are *Pseudomonadales* and *Actinomycetales* orders, along with the Marmoricola aurantiacus species, correlated with poorer survival rates in lung cancer tissue, particularly in terms of DFS. In the other hand, increased levels of the *Alphaproteobacteria* and *Betaproteobacteria* classes (these are *Burkholderiales* and *Neisseriales* orders, and *Mycobacterium vaccae*, were associated with improved survival rates.

Li et. Al also described that lung cancer microbiome showed significant variations in the relative abundance of some bacterial genera compared to healthy individuals. Notably, patients with lung cancer exhibited elevated levels of *Streptococcus* and *Staphylococcus*, while the presence of *Streptomyces* and *Streptococcus* was reduced in noncancerous tissues from these same patients. Furthermore, sputum collected from lung cancer patients with NSCLC is rich in *Veillonella, Neisseria*, and phage.

In a parallel investigation, Tsay et al. describe an association between a higher abundance of *Clostridia* and *Bacteroidia* in lower airway with worse survival in stage I-IIIA NSCLC patients.

They all conclude that dysregulation of the pulmonary microbiome or the gut-lung axis may lead to DNA damage, promoting genomic instability, and increasing host's susceptibility to carcinogenesis, ultimately contributing to lung cancer development. Microbiome dysbiosis results in a decrease in beneficial microorganisms, while inflammation encourages the overgrowth of pathogenic bacteria.

These studies not only focused on bacterial composition but also in their function, comprising metabolites and operational pathways. In this field they revealed that ubiquinol (also known as coenzyme Q10, which is a naturally occurring antioxidant) synthesis in normal lung tissue was protective against lung cancer recurrence, whereas glucose metabolism pathways were associated with worse survival outcomes.

In this same investigation field, Li et al elucidated that PI3K pathway activation may lead to carcinogenesis. This study also describes that patients with greater diversity and abundance of lung microbiota in unaffected lung tissues experienced shorter disease-free survival and lower relapse-free survival rates. They finally concluded, according to the rest, that lung cancer is associated with local dysbiosis, marked by increased bacterial abundance, reduced α-diversity, and altered bacterial composition.

Altogether, these recent studies, reflect the relevance of lung microbiome, not only in carcinogenesis but also in tumor recurrence and response to cancer treatment.

Regarding surgery, it has been described that preoperative composition of microorganisms in the lower respiratory tract may be linked to early recurrence of non-small cell lung cancer (NSCLC), as the microbiome of patients who experience postoperative recurrence differs from that of patients without recurrence.

Microbiome dysbiosis may influence not only tumor progression but also the efficacy of clinical therapies, particularly immunotherapy. Patients with non-small cell lung cancer (NSCLC) who receive broad spectrum antibiotics prior to immune checkpoint inhibitor therapy tend to have a poorer clinical prognosis. New strategies involving the modulation of gut microbiota in conjunction with ICIs, such as probiotics, prebiotics, FMT, and other dietary interventions, are growing as potential treatments toenhance the efficacy of anti-PD-1/PD-L1 therapy.

In conclusion microbiome may soon become a meaningful diagnostic and preventive biomarker for lung cancer. It has even been noted that sequencing of the airway microbiota may represent a novel genomic strategy for the early detection of lung cancer, providing an opportunity to predict the risk of cancer development.

FUTURE PERSPECTIVES

The study of microbiota in cancer, specifically in LC, is open to multiple applications in the fields of prevention, diagnosis, response prediction, and prognosis. In the review we have conducted, we highlight numerous studies carried out in these areas, although it is true that these results are not yet applicable to clinical practice. In addition to the markers detected in bronchoalveolar lavage or tools, another potential biological sample for searching for microbiome biomarkers due to its accessibility is saliva; however, there are few studies focusing on the analysis of bacterial populations in saliva. Valuable markers with diagnostic and prognostic potentials in LC have been discovered in saliva, including metabolic (catalase activity, triene conjugates, and Schiff bases), inflammatory (interleukin 10, C-X-C motif chemokine ligand 10), proteomic (haptoglobin, zinc--2-glycoprotein, and calprotectin), genomic (epidermal growth factor receptor), and microbial candidates (*Veillonella* and *Streptococcus*). Continuing the search for new contexts to locate microbiota-related biomarkers, microbiome driven liquid biopsy has also been studied in patients with LC, in the realm of early detection and treatment monitoring.

In addition to all the aforementioned, the future of research in LC microbiota lies in the application of artificial intelligence in designing algorithms focused on predicting response and toxicities, as we have 16 previously discussed.

Another avenue with growing interest is the study of intratumoral microbiota. Accumulating evidence indicates that intratumor microbiota might serve as an emerging biomarker for cancer diagnosis,prognosis, and even a therapeutic target across multiple cancer types, including LC. Some authors have investigated the role of intestinal microbiota in the practice of traditional Chinese medicine. They conclude that from this, they extract complementary information useful in this type of practice.

Recently it has been studied not only the microbiome but the mycobiome, which also influences lung cancer. For instance, *Blastomyces* is frequently found in lung tumor tissues. However, it remains unclear whether *Blastomyces* contributes to the lung cancer phenotype or if its presence is a result of lung cancer development. Additionally, increased levels of tumor-resident *Aspergillus sydowii (A. sydowii)* have been identified in patients with lung adenocarcinoma, where it plays a role in the progression of lung cancer. *A. sydowii* promotes tumor progression by inhibiting cytotoxic T-lymphocyte activity and the accumulation of PD-1+ CD8+ T-cells, facilitating the expansion and activation of myeloid-derived suppressor cells through IL-1.

Latest research indicates that the interaction between fungi and bacteria can provoke inflammatory responses, which vary according to the type of tumor.

Unfortunately, understanding the mechanisms through which the bacterial community interacts with the mycobiome and virome will necessitate further research.

CONCLUSIONS

There is no doubt about the increasing importance of the gut-lung microbiome axis, not only in carcinogenesis but

also in the development of various inflammatory respiratory diseases. Different populations of the lung microbiome have been identified in various histologies, stages, and molecular characteristics in the context of non-small cell lung cancer. Similarly, dysbiosis has been associated with prognosis and as a predictor of response to different treatments, including chemotherapy, radiotherapy, and immunotherapy. More prospective studies within clinical trials are needed to evaluate the real role of the lung and gut microbiome in the context of prognosis and response prediction in the different subtypes of lung cancer.

Author Contributions

Dr Zambrano attended the patient, collected the patient's data and drafted the manuscript. Dr Piñar critically read the manuscript and contributed substantially to its revision. Dr Remón attended the patient, collected the patient's data, read the manuscript and contributed substantially to its revision.

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