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REVIEW

Worldwide Research Trends on Lung Cancer and Microbiota: A Bibliometric and Visualized Analysis

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Background: The microbiota is intricately linked to lung cancer's development, progression, diagnosis, and treatment, garnering significant academic interest. This study employs bibliometric methods to trace trends and advancements, emphasizing the extraction and analysis of clinical research data, and outlines current challenges and future research directions.

Methods: We selected the literature in the Web of Science Core Collection database. To provide an overview, annual publications, countries/regions, and keywords were evaluated through a bibliometric analysis, and extracted and analyzed key clinical information.

Results: A total of 579 publications were reviewed, with 177 clinical studies chosen for detailed analysis. These publications, spanning from 1997 to 2025, showed a consistent yearly increase in the number of articles, despite some fluctuations. *Science* and *PLOS One* were the most frequently cited journals in this area. Key contributors to this field include Yan Zhang, Jun Chen, Zoltan Lohinai, and Edit Dulka. China was the leading producer of publications, indicating its close monitoring of the field, though it did not emphasize international collaborations, resulting in less influential publications. Major topics in this field included lung cancer, gut microbiota, microbiome, immunotherapy, inflammation, and biomarkers. Future research will likely focus on biomarkers, machine learning, immunotherapy and mechanisms. Clinical studies have identified common microbiota, such as *Prevotella, Bacteroidetes, Streptococcus, Veillonella*, and *Bacillus*, as potential microbial biomarkers.

Conclusion: This field has a bright research prospect, and there is a huge unmet clinical need. Future studies need to expand the sample size and further explore microbiota associated with the mechanisms, diagnosis, treatment and prognosis of lung cancer. Solving problems such as small sample size, heterogeneity of the population, sequencing bias, and precise diagnosis, conducting cross-regional and multicenter research, and identifying specific microbiota that have an important impact on cancer treatment are future challenges.

Keywords: lung cancer, microbiota, bibliometric

Introduction

Lung cancer is one of the most common cancers and the most common cause of cancer-related deaths.[1] The latest cancer statistics report that more than 350 people die from lung cancer per day, and the rate of death from lung cancer far exceeds that of other cancers.[2] This is related to the fact that lung cancer patients have insidious symptoms in the early stage, and most of them are diagnosed in the advanced stage when they are detected. Further, the poor prognosis of lung cancer patients is associated with the complicated pathological mechanisms, and limited treatment effects.[3,4]

Microbiota live in symbiosis with their hosts and can cause disease in some cases. The development of sequencing technology has expanded the boundaries of human understanding of lung cancer, and the role of microbiota in the development, progression, diagnosis, and treatment of lung cancer has been further revealed. During lung carcinogenesis,

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the changes in the diversity, quantity, and composition of microbiota may directly cause DNA damage, establish an inflammatory niche, elicit metabolic and immune disorders, generate virulence factors, interfere with the cell cycle, and induce tumor formation and progression,[3–6] and microbiota could alter the efficacy of anticancer treatments, such as radiotherapy, chemotherapy, and immunotherapy, thereby affecting the development of lung cancer.[4,7,8] Compared with host genetics, the microbiota could be more easily changed through several usage strategies,[9,10] the modulation of microbiota has also emerged as a frontier in anticancer therapy research. Besides, many studies showed that microbiota biomarkers have high predictive values in lung cancer,[11] and hold promise to overcome the limitations of early diagnosis and personalized treatment of cancer,[3,8,12] it is beneficial to distinguish lung cancer stages and achieve accurate screening and treatment of lung cancer.[13]

In this study, we provided a comprehensive overview of research trends and frontiers in this field with the help of bibliometric methods. Notably, we focused on the extraction and analysis of information from clinical research evidence in this area and provide an overview of current practical challenges and future directions for research in this field.

Materials and Methods

Data Retrieval and Analysis

We used the Web of Science Core Collection (WOSCC) as the data source on April 9, 2025.[14,15] The search conditions were as follows: (1) language: English, (2) literature type: article or review, and (3) search formula: TS = (lung OR pulmonary) AND TS = (microorganism OR microbiota OR microbe OR microbiome) AND TS = (neoplasia OR neoplasm OR tumor OR cancer OR malignancy OR carcinoma OR malignant). To avoid biased selection caused by a continuously updated database, all data were derived on the same day. We obtained 2285 publications. To ensure the accuracy of the screening results, two investigators independently screened the original data to exclude publications not topic-related, and any inconsistencies were resolved by consultation with a third investigator. Detailed screening and analysis processes are illustrated in Figure 1. Finally, 579 publications were included in our study, including 438 articles and 141 reviews.

We gathered information regarding the following aspects: year of publications, countries/regions, institutions, authors, number of publications and co-citations, journals, keywords, references, and key clinical information. All extracted data were cleaned and standardized prior to analysis, such as merging singular and plural forms of keywords, unifying country names, and removing useless words.

Data Analysis

Microsoft Office Excel (2019) was used to analyze annual publication trends and draw charts. CiteSpace (version 6.4) was used to analyze and visualize knowledge areas and emerging trends, including core journals, burst detection of keywords/co-cited references, and timeline of co-cited references.[16] The set parameters were as follows: (1) time slicing, 1997–2025, choosing 1 year as a per time slice; (2) selection criteria, keeping the default settings; and (3) pruning, pathfinder and pruning sliced networks.

The analysis and visualization of institutions, authors, and keywords were implemented using VOSviewer (version 1.6.19). To better present the data, Scimago Graphica (version 1.0.24) and Gephi (version 0.9.7) were also used for the visual analysis of countries, institutions, and authors. The journal impact factor (IF) was retrieved from the Journal Citation Reports (JCR) of 2023.

Extracted Key Information from Clinical Studies

We further screened clinical studies from the included 579 studies, excluded reviews, case reports, animal and cell experiments, and only included clinical research articles. Then, we extracted the following key information, including year of publication, country, analysis method, limited Time for antibiotic use before sampling, sample type, sample size, pathological type, clinical stage, treatment, main finding, Major change microbiota in lung cancer, and limitation, all of these were extracted to Microsoft Office Excel (2019). To ensure the accuracy of the screening and

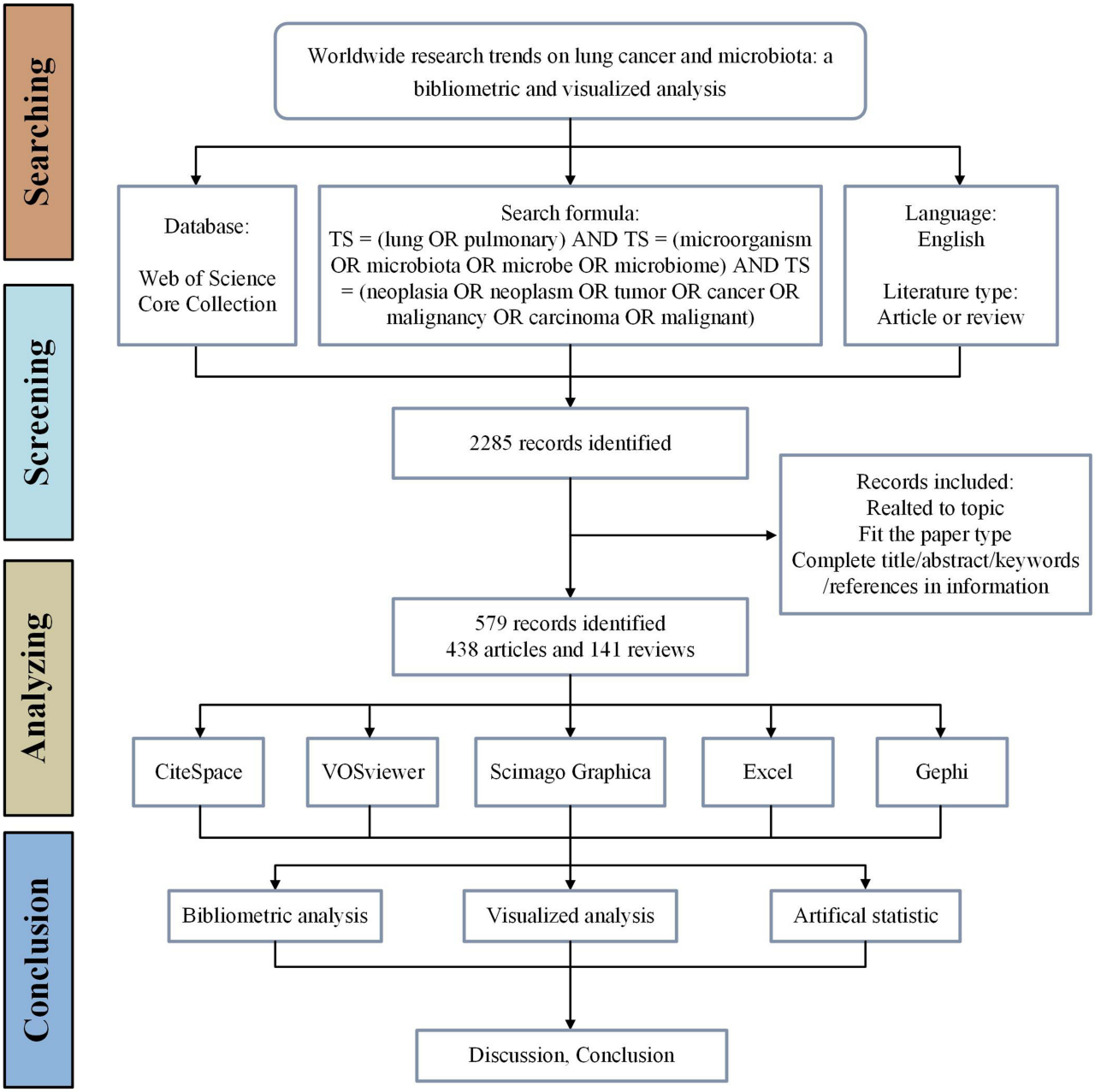

Figure 1 Process for literature screening and analyzing.

extracting results, two investigators independently screened the original data to exclude publications not topic-related, and any inconsistencies were resolved by consultation with a third investigator.

Results

Analysis of Annual Publication

The search yielded 579 publications related to this topic. As shown in Figure 2, there was an overall upward trend in the number of annual publications from 1997 to 2025. Changes in annual publications were divided into two phases. In the first stage (1997–2012), owing to low awareness of research on the microbiota in lung cancer, the increased rate of annual publications and citations was relatively slow and steady. Conversely, the upward trend with a significant increase in the number of publications in the second stage (2013–2024) showed a robust development in the field. It may be associated

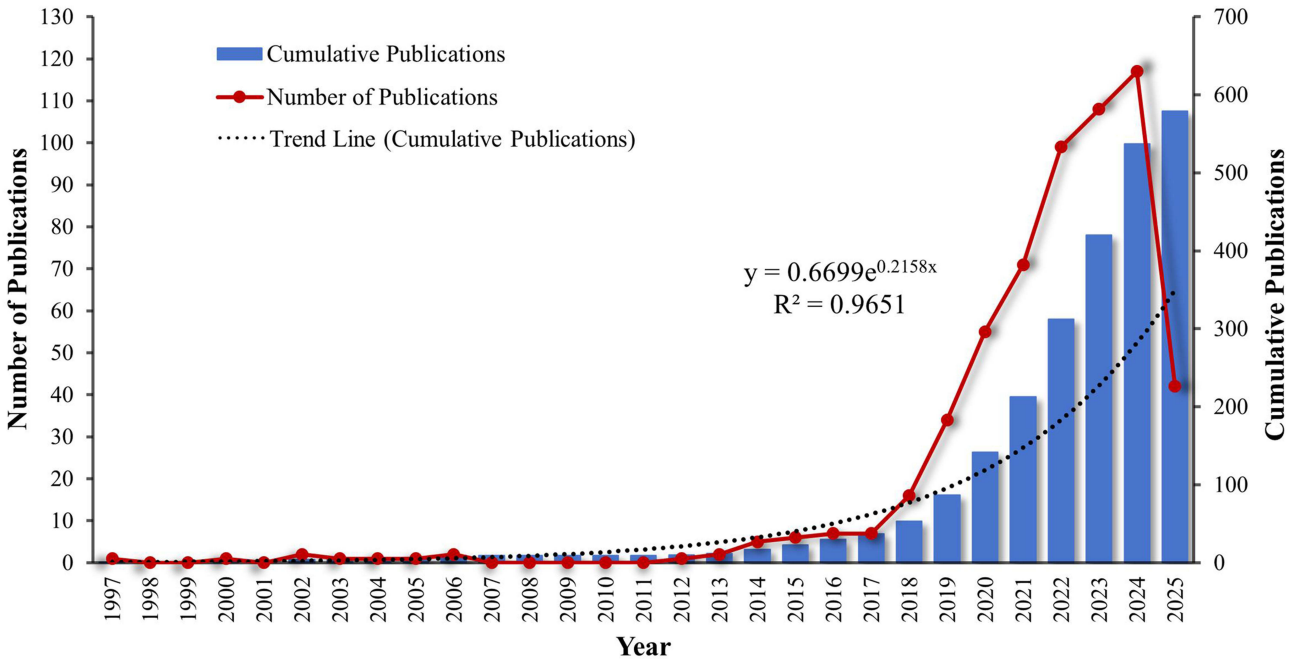

Figure 2 Total number of publications and the curve fitting of cumulative publications using exponential functions.

with the development of sequencing technology. The reason why there was a downward trend after 2025 was that we only included 42 publications that ended on April 9, 2025, and it could be clearly observed that future studies will continue to increase.

We additionally employed exponential functions to model the curves, aiming to gain a deeper understanding of the trend in cumulative publications within this field. The curve exhibited an exponential growth pattern, with an R^2 value of 0.9651 (Figure 2), suggesting that this research topic has garnered substantial interest in recent years. This trend is likely to persist in the future.

Analysis of Active Countries/Regions

The influence of microbiota on lung cancer represents a research area of significant global interest, with contributions from 55 countries/regions. As illustrated in Table 1 and Figure 3A, China emerges as the predominant contributor, with 299 publications, significantly surpassing other nations in terms of output. This suggests that China is actively engaged in monitoring developments within this field. However, despite China's substantial publication volume, its citation count and total link strength are considerably lower than those of the USA (8116 citations, and a total link strength of 46). This indicates that the USA emphasizes international academic collaborations, resulting in highly influential publications. Additionally, although Italy's publication count is relatively modest, the average citation per document is 100.31,

Table 1 Top 5 Countries/ Regions According to Publications in the Field

Rank	Country	Publications	Citations	Total Link Strengths
1	China	299	6556	46
2	USA	110	8116	85
3	Japan	41	1078	14
4	Italy	35	3511	27
5	France	25	2404	28

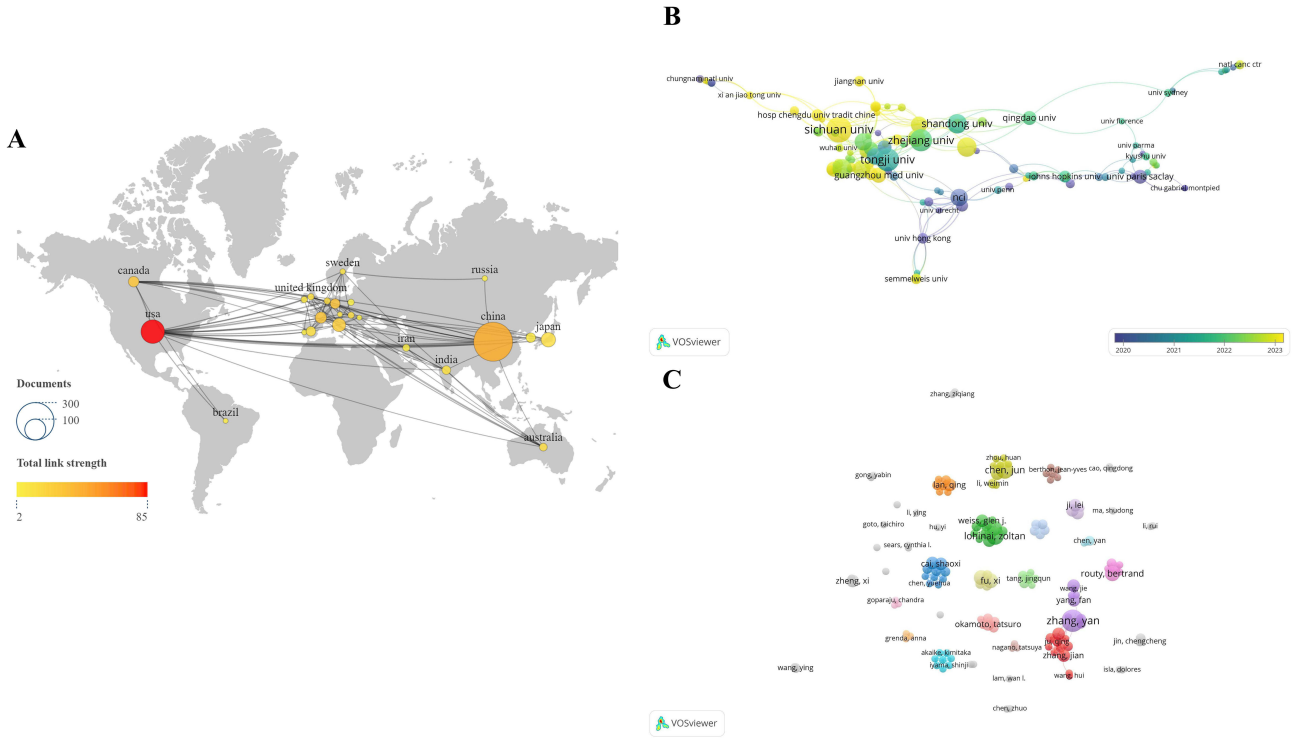

Figure 3 (**A**) Cooperation map around the world. The number of publications was represented by the size of the node, and the connection between countries/regions was represented by the color of nodes and the thickness of lines. Darker colors and thicker liners represented the closer connection between countries, which meant this country played an important role in the research field; (**B**) The visual network of active Institutes. The blue terms have been used early, followed by the green and light green terms that appeared later, whereas, the yellow terms represent newly emerging; (**C**) The cooperation network of authors.

suggesting that Italian publications are of high quality and contribute substantially to advancing this critical field. Considering all metrics, it is evident that the USA leads in this research domain.

Analysis of Active Institutions

The minimum number of documents in an organization was set to 3 in VOSviewer, and a co-authorship network consisting of the top 135 institutions was visualized (Figure 3B). The top 10 active institutions are shown in Table 2, nine of which were from China, highlighting China's focus on this field. Sichuan University leads in publications (21), followed by Tongji University (18) and Zhejiang University (17). Despite publishing only 12 papers, the National Cancer

Table 2 The Top 10 Active Institutions

Rank	Institution	Country	Publications	Citations	Total Link Strength
1	Sichuan University	China	21	281	9
2	Tongji University	China	18	738	16
3	Zhejiang University	China	17	580	17
4	Central South University	China	14	168	2
5	Shandong University	China	14	349	5
6	Southern Medical University	China	14	471	4
7	Chinese Academy of Medical Sciences & Peking Union Medical College	China	12	139	4
8	Nanjing Medical University	China	12	495	8
9	National Cancer Institute	The USA	12	1122	10
10	Zhejiang Chinese Medical University	China	12	222	15

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Institute had the most citations (1122), indicating its significant impact, followed by Tongji University (738) and Zhejiang University (580). Regarding the intensity of collaboration, Zhejiang University exhibited the highest total link strength with 17 connections, followed by Tongji University with 16 connections, and Zhejiang Chinese Medical University with 15 connections. The network analysis indicates that China has been notably active in this field in recent years. However, Chinese institutions predominantly engage in domestic collaborations, with limited international partnerships.

Analysis of Active Authors

The analysis using VOSviewer identified 4147 authors who have conducted research related to the microbiome in lung cancer. As illustrated in Table 3, Yan Zhang emerged as the most prolific author with nine publications. Jun Chen was identified as the most influential author, having accrued the highest number of citations, totaling 348, with an average of 49.71 citations per publication, across seven papers. Figure 3C depicts the collaboration network of 146 authors who have published at least three papers, nine of whom conducted research independently. Furthermore, these authors were categorized into 38 clusters, each represented by a distinct color, signifying relatively stable collaborative relationships among the authors. The largest clusters were the red cluster, predominantly consisting of authors from China, and the green cluster, primarily comprising authors from Hungary.

Analysis of Core Journals

Journal analysis has been widely used to map a research domain's disciplinary structure. Using CiteSpace, we found that 258 journals published papers and 3380 were cited. Table 4 shows that *Frontiers in Cellular and Infection Microbiology* and *International Journal of Molecular Sciences* led with 23 papers each, followed by *Cancers* and *Frontiers in Oncology* with 21 papers each. Among the top 10 most productive journals, most had relatively low IF, while 90% of the top 10 most-cited journals were Q1 with high IF. *Science* had the most citations (1591), significantly impacting the field, compared to *PLOS ONE*'s 818 citations. Furthermore, the journal map overlay depicted in Figure 4 reveals two primary citation trajectories. The majority of the journals that published the papers are associated with the fields of molecular biology, immunology, medicine, and clinical research. In contrast, the cited papers predominantly appear in journals specializing in molecular biology and genetics.

Analysis of Keywords

Keywords serve as indicators of research topics within a specific field and are essential for examining the knowledge structure, and high-frequency keywords are considered research hotspots in the field.[17] We analyzed keywords appearing at least five times, resulting in a network of 175 keywords using VOSviewer software. Figure 5A shows these keywords divided into six clusters by color: Cluster 1 (red) focuses on microbiota's role in lung disease progression through inflammation and immunity; Cluster 2 (green) relates to microbiota's role in lung cancer immunotherapy; Cluster 3 (blue) covers respiratory microbiota's role in identifying lung diseases and their mechanisms; Cluster 4 (yellow) involves

Table 3 The Top 10 Productive Authors

Rank	Author	Publications	Total Citations	Per Citations	Total Link Strength
1	Yan Zhang	9	96	10.67	17
2	Jun Chen	7	348	49.71	18
3	Zoltan Lohinai	7	131	18.71	37
4	Edit Dulka	6	110	18.33	36
5	Xi Fu	6	6	1	18
6	Qiong Ma	6	6	1	18
7	Bertrand Routy	6	252	42	15
8	Shaoxi Cai	5	104	20.8	32
9	Hangming Dong	5	104	20.8	32
10	Gabriella Galffy	5	108	21.6	30

Table 4 The Top 10 Productive and Influential Journals

Rank	Journal	Publications	JCR Partitions	IF (2023)	Cited-Source	Citations	JCR Partitions	IF (2023)
1	Frontiers in Cellular and Infection Microbiology	23	Q2	4.6	Science	1591	Q1	44.7
2	International Journal of Molecular Sciences	23	Q1	4.9	PLOS ONE	818	Q1	2.9
3	Cancers	21	Q1	4.5	Nature	760	Q1	50.5
4	Frontiers in Oncology	21	Q2	3.5	Cell	679	Q1	45.5
5	Frontiers In Microbiology	19	Q2	4	American Journal of Respiratory and Critical Care Medicine	561	Q1	19.3
6	Scientific Reports	15	Q1	3.8	New England Journal of Medicine	543	Q1	96.2
7	Thoracic Cancer	14	Q2	2.3	Scientific Reports	490	Q1	3.8
8	Frontiers in Immunology	12	Q1	5.7	Nature Medicine	460	Q1	58.7
9	Translational Lung Cancer Research	11	Q1	4	Frontiers In Immunology	430	Q1	5.7
10	Journal of Thoracic Disease	9	Q3	2.1	Frontiers In Microbiology	428	Q2	4

Abbreviations: JCR, Journal citation reports; IF, Impact factor.

microbial biomarkers and microbiota's role in cancer diagnosis; Cluster 5 (purple) pertains to microbial detection; and Cluster 6 (pink) is solely about machine learning. Moreover, "lung cancer" (n= 212), "gut microbiota" (n= 143), and "microbiome" (n= 122) were the top 3 high-frequency keywords that approximately accounted for 45% of the top 10, closely followed by "non-small cell lung cancer", "immunotherapy", "cancer", "inflammation", "microbiota", "dysbiosis", and "biomarker" (Figure 5B).

The burst analysis has the potential to reveal shifts in keyword usage over specific periods, thereby intuitively highlighting research hotspots in recent years and identifying emerging research directions. Among the top 25 keywords exhibiting the highest burst intensity (Figure 5C), "bronchoalveolar lavage" demonstrated the greatest burst strength, followed by "infection", "disease", "COPD", "16S rRNA", "obstructive pulmonary disease", and "dendritic cells".

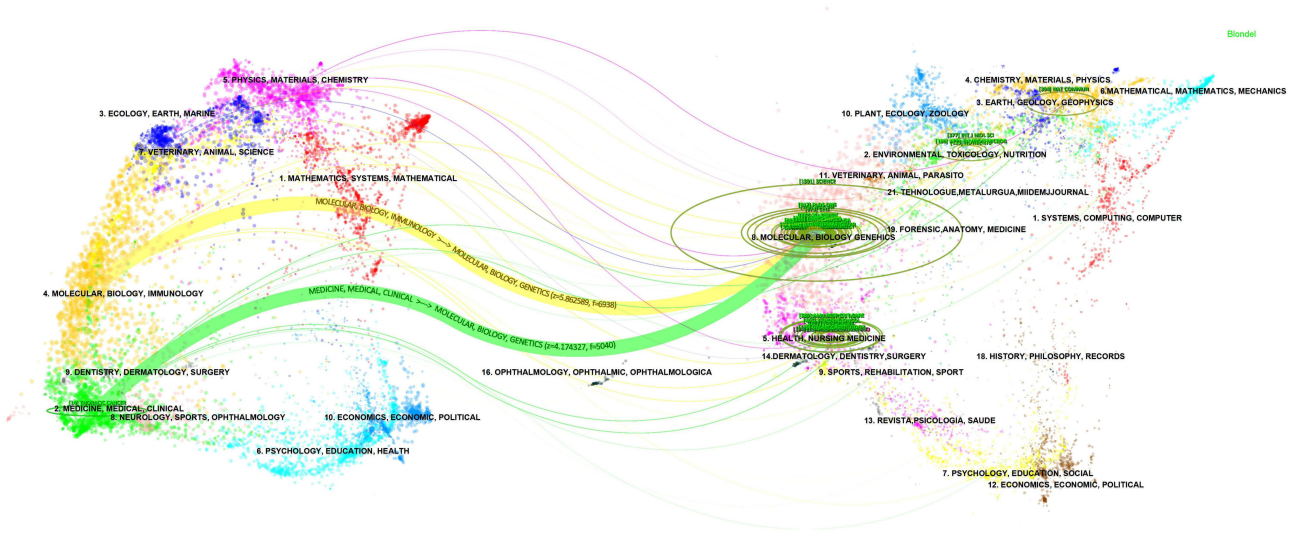

Figure 4 A biplot overlay map of journals. Left side represented the field covered by citing journals, and the right side represented the field covered by cited journals.

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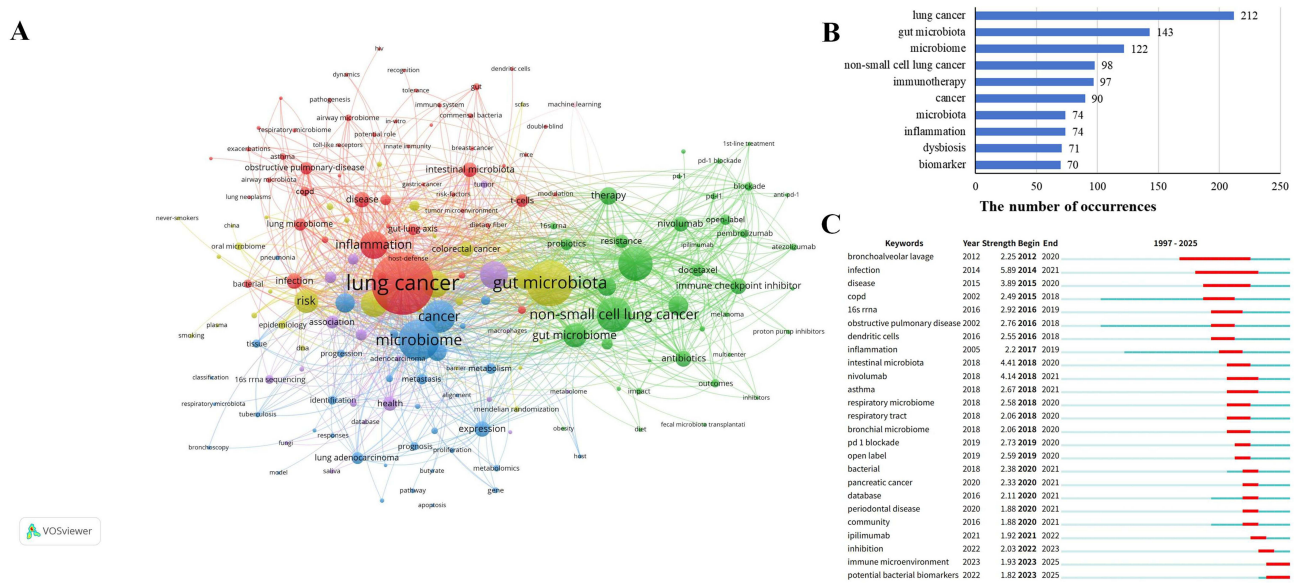

Figure 5 (**A**) Keyword cluster map according to different research fields. The size of the circle and label indicated how many publications used that term, and the thickness and length of the connected line were related to the strength of the connection and relevance between nodes; (**B**) The top 10 keywords based on occurrence frequency; (**C**) Top 25 keywords with the strongest citation bursts. The blue segment represented the period when the keyword appears, and the segment marked in red indicates a sudden increase in the cited frequency of this paper during that time.

Notably, the terms "immune microenvironment" and "potential bacterial biomarkers" displayed the most recent burst period, beginning in 2023 and continuing to the present. This suggests that these terms are likely to be significant for future research within the current field.

Analysis of Co-Cited References

Co-cited references are those in which two publications are cited simultaneously by one later paper; highly cited references can be considered the basis of research in a given domain. The minimum number of co-citations of references was set to 20, and we obtained a network consisting of 144 references. As indicated in Table 5 and Figure 6A, high-frequency co-cited references were referred successively to Routy (2018), Gopalakrishnan (2018), Lee (2016), Sivan (2015), and Yu (2016). These co-cited references were related to the effects of microorganisms on anti-PD-1 immunotherapy or the diversity of the microbiome in lung cancer, most of which originated from *Science*.

The nine largest clusters of the reference co-citation map were summarized in three main aspects (Figure 6B): Group 1 showed the basic research facets, including (#2 shared molecular mechanism, #7 nkt cell, #8 molecular detection), group 2 was closely related to microbial biomarker, including (#0 respiratory microbiome, #3 gut microbiota, #4 intratumoral microbiome, #5 potential bacterial biomarker). And group 3 represented treatment in this field, including (#1 immunotherapy). The modularity Q values were all 0.7747, and the total weighted mean silhouette S values were greater than 0.8, indicating that the clustering results are convincing. Figure 6C highlights the top 20 most co-cited references, indicating recent research trends: 10 clinical studies, 5 basic research articles, and 5 reviews. The thematic analysis indicates that 6 studies focus on microbial biomarkers in lung cancer, 3 on microbiome and immunotherapy interactions, 2 on microbes in tumorigenesis, 1 on global cancer epidemiology, and the rest on respiratory microbiomes and chronic lung disease. Overall, microbial biomarkers and cancer immunotherapy are key research priorities.

Analysis of Key Information from Clinical Studies

We screened 177 clinical studies for analysis, and the detailed clinical characteristics are shown in the Supplementary Table 1. Here, we conducted a comprehensive discussion of the major information.

Table 5 The Top 10 Influential Co-Cited References

Rank	First Author	Journal	Title	Year	Citations
1	Bertrand Routy	Science	Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors	2018	198
2	Vancheswaran Gopalakrishnan	Science	Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients	2018	136
3	Sang Hoon Lee	Lung Cancer	Characterization of microbiome in bronchoalveolar lavage fluid of patients with lung cancer comparing with benign mass like lesions	2016	134
4	Ayelet Sivan	Science	Commensal Bifidobacterium promotes antitumor immunity and facilitates anti–PD-L1 efficacy	2015	131
5	Guoqin Yu	Genome Biology	Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features	2016	128
6	Chengcheng Jin	Cell	Commensal Microbiota Promote Lung Cancer Development via γδ T Cells	2019	123
7	K Leigh Greathouse	Genome Biology	Interaction between the microbiome and TP53 in human lung cancer	2018	122
8	Jun-Chieh J Tsay	American Journal of Respiratory And Critical Care Medicine	Airway Microbiota Is Associated with Upregulation of the PI3K Pathway in Lung Cancer	2018	118
9	Xinmin Yan	American Journal of Cancer Research	Discovery and validation of potential bacterial biomarkers for lung cancer	2015	111
10	Marie Vétizou		Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota	2015	108

Study Groups

Forty-five studies established a healthy control group, with the largest sample size comprising saliva specimens (n = 172). Thirty-one articles categorized groups based on pathological type, 44 articles emphasized the clinical stage of lung cancer, and 41 articles concentrated on the microbiota in the context of lung cancer treatment, of which 33 were

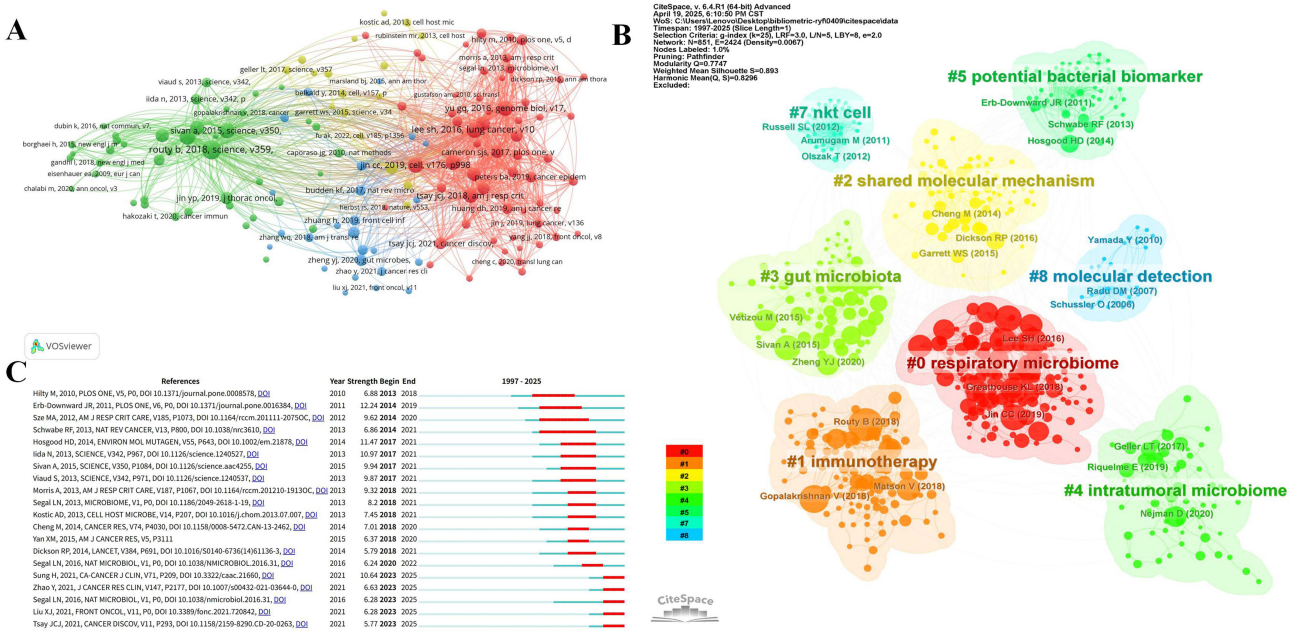

Figure 6 (**A**) The co-occurrence network of co-cited reference; (**B**) The cluster and timeline map of co- cited reference; (**C**) The top 20 references with the strongest citation bursts.

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specifically related to immunotherapy. Notably, four articles categorized groups according to tumor markers, epidermal growth factor receptor expression, and programmed cell death ligand-1 (PD-L1) expression. These groupings indicate that the current research focus is on identifying biomarkers associated with lung cancer grading, staging, gene mutations, tumor marker levels, and immunotherapy.

Specimen Types

A total of 22 different specimens were included in these studies, stool (n = 65) was the most collected specimen, followed by tissue (n = 46), bronchoalveolar lavage fluid (BALF) (n = 38), saliva (n = 15), sputum (n = 14), blood (n = 13), bronchial brushing specimen (n = 8), oral wash (n = 6), serum (n = 3), buccal (n = 3), bronchial washing fluid (BWF) (n = 3), throat swab (n = 2), oropharynx swab (n = 1), protected specimen brush (n = 1), oral swab (n = 1), exhaled breath condensate (n = 1), lung tissue flushing solutions (n = 1), pleural effusion (n = 1), nasal swabs (n = 1), nasopharyngeal swabs (n = 1), oropharyngeal swab (n = 1), tongue coating swab (n = 1). Additionally, in these 177 studies, 40 articles analyzed more than two kinds of specimens, and 10 articles analyzed three or more. Research articles analyzing the distribution differences and correlations of microbiota in multi-site specimens of lung cancer, published successively between 2020 and 2025, indicate that the inter-site microbial associations in lung cancer and the screening of predominant microbial taxa at critical sites have garnered increasing scientific attention.

Limited Time for Antibiotic Use Before Sampling

Sixty-eight studies have clearly proposed time limits for antibiotic use before sampling, including within 3 months (n = 21), 1 month (n = 16), 2 months (n = 8), 4 weeks (n = 6), 2 weeks (n = 4), 8 weeks (n = 3), 6 months (n = 3), 6 weeks (n = 1), 1 week (n = 1), 1 year (n = 1), 21 days (n = 1), 60 days (n = 1). Clearly, the absence of a standardized protocol for determining the appropriate duration of antibiotic restriction prior to sampling necessitates research that categorizes subjects based on varying antibiotic usage cycles.

Sequencing Methods

16S rRNA sequencing (n = 105) is the most widely used sequencing technology, followed by metagenomic sequencing (n = 41), metabolomic analysis (n = 13), 16S rDNA sequencing (n = 10), transcriptomic analysis (n = 10), internal transcribed spacer (ITS) (n = 6), quantitative real-time polymerase chain reaction (qPCR) (n = 5), bacterial culture (n = 5), droplet digital PCR (ddPCR) (n = 3), immunohistochemistry (IHC) (n = 2), PCR (n = 2), TP53 gene sequencing (n = 1), spatial meta-transcriptomic (n = 1), proteomic (n = 1), multiplex PCR (mPCR) (n = 1), cytology (n = 1), culturomics (n = 1), protein microarrays (n = 1), bacterial identification and antimicrobial susceptibility testing (n = 1), high-throughput next-generation sequencing (n = 1), whole metagenomics sequencing (n = 1), Onco-metagenomic next-generation sequencing (mNGS) (n = 1), reverse transcriptase-PCR (RT-PCR) (n = 1), nanopore sequencing (n = 1), metatranscriptomic analysis (n = 1), spatial meta-transcriptomic sequencing (n = 1). Fifteen studies used more than 2 sequencing types, and the use of metagenomic analysis, metabolomic analysis, and transcriptomic analysis have increased after 2022.

Different Microbiota

Through comprehensive data analysis, we identified distinct variations in microbial species across different sample types. Notably, the relative abundances of *Akkermansia, Faecalibacterium, Bacteroides*, and *Streptococcus* exhibited significant alterations in the stool samples of lung cancer patients. In tissue samples, *Proteobacteria, Bacillus, Prevotella*, and *Actinobacteria* emerged as predominant microbiotas. Sputum samples demonstrated significant changes in the abundances of *Streptococcus, Veillonella, Bacillus, Gemella*, and *Haemophilus*. In the saliva of lung cancer patients, there was a marked increase in the abundances of *Prevotella, Veillonella, Streptococcus*, and *Capnocytophaga*, while *Streptococcus, Prevotella*, and *Veillonella* were notably elevated in bronchial brushing specimens. Furthermore, the BALF samples were characterized by prominent abundances of *Streptococcus, Veillonella, Haemophilus, Bacillus*, and *Neisseria*.

Research Limitations

Among the studies reviewed, 183 explicitly identified research limitations. The most frequently cited limitation was small sample size (n = 69), followed by the use of cross-sectional study designs (n = 20). Other common limitations included the absence of validation data (n = 18), constraints associated with 16S rRNA gene sequencing (n = 18), and the failure to account for factors such as diet, habits, and other variables that could influence the type and abundance of microbiota (n = 13). Additionally, a lack of exploration into underlying mechanisms was noted (n = 10). Further prevalent limitations in current research included population heterogeneity, the conduct of single-center studies, a lack of universality in findings, inconsistencies in sample distribution, and the absence of multiple and dynamic sample collections.

Discussion

Current Overall Development Trend and Limitation

The modern microbiota era began in the late 1990s, thus research on the microbiota in lung cancer appeared relatively late. After 2013, the number of annual publications showed a clear increasing trend. This could be attributed to the evolution of sequencing technology, which promoted research on the microbiota of lung cancer. According to the number of annual publications, it could be observed that future studies will continue to increase, and there was great potential for research in this field. However, there is a general lack of extensive and close academic exchanges. Although China had the most publications, the quality of Chinese papers was not high in general with the lack of influence. The low impact of research outputs was closely related to the lack of collaboration. Thus, communication and cooperation between different countries/regions should be strengthened in the future.

Future Directions

Basic Research Direction: Mechanisms

The keyword cluster analysis indicated that terms associated with the impact of microbiota on the progression of pulmonary diseases, particularly through modulation of inflammation and immunity, constituted the largest cluster. This cluster represents the most prominent research trajectory within the field. Additionally, burst analysis identified microbial carcinogenic mechanisms as an emerging research frontier. The oncogenic mechanisms associated with microbial dysbiosis predominantly involve the modulation of host inflammatory/immune responses, the production of carcinogenic metabolites, genotoxicity, and the disruption of cellular cycles.[18,19]

Inflammatory cells and cytokines promote tumor growth by creating a supportive microenvironment.[20] Tsay et al demonstrated through multi-omics analysis that pulmonary microbial dysbiosis upregulates pathways such as PI3K-Akt, ERK-MAPK, IL-17A, IL-6/IL-8, and inflammasome signaling in KP-induced lung cancer mice.[21] Tang et al showed that gut microbiota imbalances affect lung immunity by altering the TLR4/NF-κB pathway, increasing oxidative stress and inflammation.[22] Chronic lung inflammation is a risk factor for lung cancer. Patients with both COPD and non-small cell lung cancer (NSCLC) have more Gram-negative bacteria and higher microbial stress tolerance than those with only one condition.[23] Microbial dysbiosis adversely affects immune responses by impairing pathogen clearance and the anti-tumor activity of immune cells within the pulmonary system.[24] Shi et al demonstrated that patients with low-risk lung adenocarcinoma display more active interactions between predominant intratumoral bacteria and immune cells in the tumor microenvironment compared to their high-risk counterparts.[25] Furthermore, Shen et al emphasized the pivotal role of lung microbiota in facilitating CD8(+) T cell-mediated anti-tumor immunity.[26] Additionally, the expression of PD-L1 in lung cancer tissues has been correlated with bacterial diversity and the presence of specific bacterial species. With advances in metabolomics and genomic analyses, the interplay among microbiota, metabolism, genes, and lung cancer is being elucidated. Chen et al suggested that lung cancer might arise from the interaction between L-valine and *Lachnospiraceae_UCG-006* through the aminoacyl-tRNA biosynthesis pathway.[27] Liang et al developed a microbiota-gene network showing that *Proteus* and *Bacteroides* are closely associated with gene sets that promote tumor growth and energy metabolism, possibly contributing to lymph node metastasis in lung squamous carcinoma (SCC).[28]

Furthermore, alterations in microbial populations across various body sites have an impact on the initiation, progression, and prognosis of lung cancer. Clinical studies show that stool and lung tissue microbiota are the most

extensively investigated. Due to the homology between the lung and gut,[29] research has concentrated on the gut-lung axis microbiome in patients with lung cancer. Importantly, a significant positive correlation has been observed between *Romboutsia* and *Alistipes* along the gut-lung axis in patients with NSCLC, although the causal relationship has yet to be elucidated.[30]

While some studies have shed light on microbial mechanisms in cancer, most research is still observational and lacks clear mechanistic insights. Current models and methods fall short in fully tracking the interactions between microbiota and tumors in lung cancer, leaving many cellular and molecular mediators unidentified. Additionally, the reliability of analyses is heavily dependent on microbiota sampling techniques. Future research should focus on improving experimental models, refining methodologies, and exploring mechanisms in depth to better understand how microbiota affect lung cancer progression.

Diagnosis and Disease Prediction Direction: Microbiota Biomarkers

Among the top 25 keywords exhibiting the highest burst intensity, "biomarker" has emerged as a newly prominent term in recent years, with its associated concepts forming significant clusters in cluster analyses. Research indicates that microbiota may serve as potential biomarkers for early detection, advanced metastasis, and pathological classification.[31,32] According to the analysis of clinical studies, we identified several frequently reported microbiotas. Here, we provide an initial emphasis on these specific microbiotas and synthesize their potential mechanisms of action alongside pertinent clinical findings related to the progression of lung cancer.

Streptococcus

Streptococcus is a common gram-positive coccus widely found in the human nasopharynx and feces. It causes a variety of infections by interfering with and evading the body's immune system through adhesion, secretion of toxins and invasion of host cells.[33,34] For instance, *Streptococcus* pneumoniae has been shown to activate the PI3K/AKT and NF-κB pathways during pulmonary tumorigenesis.[35] Thirty-seven studies reported the changes of *Streptococcus* associated with lung cancer. Despite the heterogeneity observed among these findings, the majority of studies indicate that an increased abundance of *Streptococcus* is positively associated with the progression of lung cancer.[31,36–38] It is enriched in saliva, sputum, BALF, and demonstrates moderate diagnostic potential with an area under the ROC curve (AUC) of 0.693.[39] Furthermore, *Streptococcus* abundance is positively correlated with CD8(+) T cell infiltration in tumors, indicating its potential in predicting immunotherapy success.[40]

Prevotella

Genus *Prevotella* is the typical intestinal and oral microbiota that typically symbiosis with their hosts. However, under specific conditions, *Prevotella* causes lung cancer by activating inflammation-related signaling pathways, inhibiting immune cell function, and interfering with cell apoptosis, proliferation, and other pathways.[41–43] Research indicates a notable increase in *Prevotella* abundance in lung cancer patients,[44,45] correlating with the progression of NSCLC, especially in the T4 stage and metastatic groups.[46] *Prevotella* and *Alloprevotella* levels can also differentiate SCC patients from those with non-malignant tracheal tumors.[47] Although some studies, such as Zhang et al,[48] report a decreased relative abundance of *Prevotella* in NSCLC patients' saliva, there is growing evidence supporting its potential as a biomarker for lung cancer prediction.

Veillonella

Veillonella is highly colocalized in the oral and distributed in the pharynx, respiratory tract, and digestive tract. *Veillonella* infection influences the development of lung cancer by triggering the inflammatory response, affecting the function of immune cells and the production of immune factors, and impacting host cell metabolism.[21,43] Studies indicate that Veillonella is enriched in the BALF of lung cancer patients,[11] while Veillonella and Rothia in sputum may predict distant metastasis in SCC,[49] underscoring their potential as biomarkers. *Veillonella* is also abundant in stool samples of patients with less than 6 months of progression-free survival after anti-PD-1 therapy and dominates the BALF of patients with high PD-L1 expression, suggesting its abundance could be a key indicator of immunotherapy effectiveness in lung cancer.[50,51]

Haemophilus

Haemophilus belongs to the group of Gram-negative bacilli that usually colonize the human oral and respiratory tracts and cause a variety of infections. Fourteen studies had observed the changes of *Haemophilus* associated with lung cancer. Findings show *Haemophilus* is more common in lung cancer tissues than in emphysema,[52] particularly in nicotine-exposed lung cancer patients.[53] Besides, it is often found in the sputum of SCC patients and in the stool of adenocarcinoma patients.[30,54] *Haemophilus influenzae* is the main species in the bronchoalveolar lavage fluid of anti-PD-1 non-responders.[40] Despite these findings, more research is needed to understand *Haemophilus*'s role and predictive value in lung cancer.

Bacillus

Bacillus, a Gram-positive bacteria genus, is found in the lung tissues, sputum, BALF, and stool of lung cancer patients, with notable enrichment in SCC sputum.[54,55] Its levels rise with disease progression to invasive adenocarcinoma and are linked to postoperative recurrence and immunotherapy outcomes. Microbial biomarker analyses suggest that a low-diversity microbiota dominated by *Bacillus* may favor responses to immune checkpoint inhibitor (ICI) therapy.[56] PATNAIK et al noted lower *Bacillus* levels in lung tissues and BALF of stage I NSCLC patients with recurrence after surgery.[57] Thus, *Bacillus* could be a valuable biomarker for lung cancer prognosis and treatment monitoring.

In addition to the previously mentioned microbiota, potential lung cancer biomarkers like *Akkermansia, Faecalibacterium, Bacteroides*, and *Neisseria* could offer valuable insights into early diagnosis, treatment response, and prognosis. Nonetheless, individual microbial taxa frequently do not adequately account for the intricate interactions between tumors and their environments. In contrast, multiple microbial community signatures, distinguished by their comprehensive structure, stability, and functional redundancy, typically demonstrate enhanced predictive capabilities. Machine learning (cluster 6) is increasingly important in identifying these biomarkers, as it effectively manages complex microbiome data and improves predictive accuracy through continuous training. For example, Jiang et al effectively differentiated patients with early-stage lung cancer and brain metastasis from healthy controls by utilizing a fecal biomarker panel consisting of *Faecalibacterium, Bifidobacterium, Butyricicoccus, Klebsiella, Blautia*, and *Streptococcus*, achieving an AUC of 0.884.[58] Similarly, Sun et al developed a diagnostic model employing six bacterial markers that flawlessly distinguished early-stage lung adenocarcinoma from healthy individuals, with an AUC of 1.000.[59] Furthermore, Ma et al identified Actinomyces, Rothia, Streptococcus, Prevotella, Porphyromonas, and Veillonella as biomarkers for malignant pulmonary nodules, with their saliva-based LightGBM model exhibiting optimal predictive performance (AUC = 0.887).[60] Although these models demonstrate remarkable predictive capabilities, enhancing their generalizability and clinical applicability necessitates the integration of additional clinical variables, the expansion of sample sizes through external validation cohorts, and the incorporation of multi-omics data (such as metabolomic and genetic alterations) to refine the robustness of biomarkers.

Treatment Direction: Combined Microbiota and Lung Cancer Treatment

Keyword clustering analysis reveals that lung cancer therapeutics is a key research focus. The microbiota influences host responses to cancer treatments by boosting drug effectiveness, managing drug clearance and toxicity, or reducing therapeutic impact. Utilizing microbiota to improve drug efficacy is a promising strategy. For instance, Gui et al found that combining cisplatin with Lactobacillus improved survival and tumor control in mice.[61] Chen et al showed that the postbiotic JK5G reduced immune-related side effects in NSCLC patients by modulating gut microbiota, enhancing quality of life and nutrition.[62] Moreover, oral probiotics post-radiotherapy may prevent infections in lung cancer patients, and microbiota-targeted agents could enhance chemotherapy efficacy and reduce toxicity.[63,64] Notably, our data showed that the effect of microbiota in lung cancer immunotherapy had more attention, especially the combination of gut microbiota and PD-1 expression in NSCLC immunotherapy, which was the focus of this research field.

Immunotherapy is recognized as one of the most complex and rapidly advancing strategies in modern oncology. Among these, PD-1/PD-L1 immune checkpoint inhibitors have become a central focus of clinical research. Although PD-L1 expression in tumor cells is the most widely validated biomarker for predicting responses to anti-PD-1 therapy, there are considerable limitations. Specifically, a portion of NSCLC patients with high PD-L1 expression exhibit primary

resistance to anti-PD-1 treatment, with objective response rates ranging from 19% to 47% in advanced NSCLC populations.[65] Additionally, many patients experience inadequate anti-tumor immune activation.[66] This highlights the urgent need for strategies to improve therapeutic efficacy. Tomita et al have shown that therapy with Clostridium butyricum, specifically utilizing the live biotherapeutic strain C. butyricum MIYAIRI 588 (CBM588), can enhance the efficacy of immune checkpoint blockade in lung cancer patients undergoing proton pump inhibitor treatment.[67] Importantly, the combination of CBM588 with chemoimmunotherapy significantly extended overall survival (OS) in patients with advanced NSCLC. The most pronounced survival benefit was observed in the cohort with PD-L1 expression levels of less than 1%, as compared to those with PD-L1 expression levels of 1–49% and ≥ 50%.[68] While microbial modulation holds promise for improving immunotherapy outcomes, its practical application is limited. Antibiotics targeting microbiota in cancer treatment may reduce efficacy and increase cancer risks, whereas microbial modulation offers potential for lung cancer prevention. Strategies like prebiotics, probiotics, synbiotics, engineered probiotics, dietary changes, microbiota-targeted drugs, and fecal microbiota transplantation are promising for disease prevention.[9,10,69] However, the current body of evidence regarding the synergistic enhancement of ICIs by microbiota is constrained by a paucity of robust, high-quality clinical data.[70,71] This limitation highlights the critical need for this area to be a central focus of future translational research efforts.

Challenges and Limitations of Current Clinical Research

Analysis of clinical trials revealed that existing clinical studies are basically cross-sectional studies, most of which were conducted only in a single center with small sample sizes. Existing studies did not consider the effect of diet on the microbiota, failed to compare the effect of different antibiotic durations on the abundance of the microbiota and did not dynamically analyze microbial changes with multiple samples. The heterogeneity of the included populations was too large and had a significant impact on the results. In addition, it also lacked data validation sets and the current findings were not convincing. Notably, future research needs to strengthen international exchange and cooperation, carry out multi-center studies, and enlarge the sample size. To explore the laws of microbiota changes in lung cancer, find microbiota biomarkers that could accurately predict the occurrence and development of lung cancer, its pathologic typology, and its clinical classification and staging. And combine machine learning algorithms to construct a more clinically applicable prediction model for lung cancer clinical diagnosis.

In the early stage of insidious symptoms of lung cancer, although detecting specific microbiotas can help further control the optimal screening and treatment time for lung cancer, the accuracy of its detection results greatly depends on the detection method. Although 16S rRNA is the main microbial detection method, the accuracy of the results is easily affected by the extent of amplification, and it is difficult to provide absolute taxa abundance.[72,73]

The use of advanced computational tools, metagenomics, transcriptomics, proteomics, and metabolomics based on next-generation sequencing technology has increased in this domain in recent years. The integration and application of these techniques could provide a more systematic and comprehensive analysis of changes in microbiota composition and function under different conditions, which helps to obtain more accurate analysis results. In addition, the sample sizes of the current studies are relatively small, the heterogeneity of the included populations is too large, the studies lack data validation sets, and the analysis of the results does not take into account the effect of diet or other factors on the microbiota. In the research, the appropriate sequencing techniques should be utilized to explore the relationship between microbial abundance in different sample sites under multiple dynamic sequencing from a spatial and temporal perspective, or further explore the relationship between microbiota and other levels of cytokines and metabolites, to identify more accurate microbiota biomarkers and validate them in vivo.

Microbial modulation provides both opportunities and challenges to improve the efficacy of immunotherapy. Different probiotics have different abilities to survive and colonize in the gastrointestinal tract, and the gut microbiota of different ethnic and geographic populations were different.

How do we select the most appropriate probiotics, biologics, or targeted microbial drugs at each disease stage? And can antibiotics be used effectively and safely to regulate microbiota? Is it possible to keep the microbiota in proper balance over time through dietary modification? Answers to these questions would be the research challenges in this field (Figure 7).

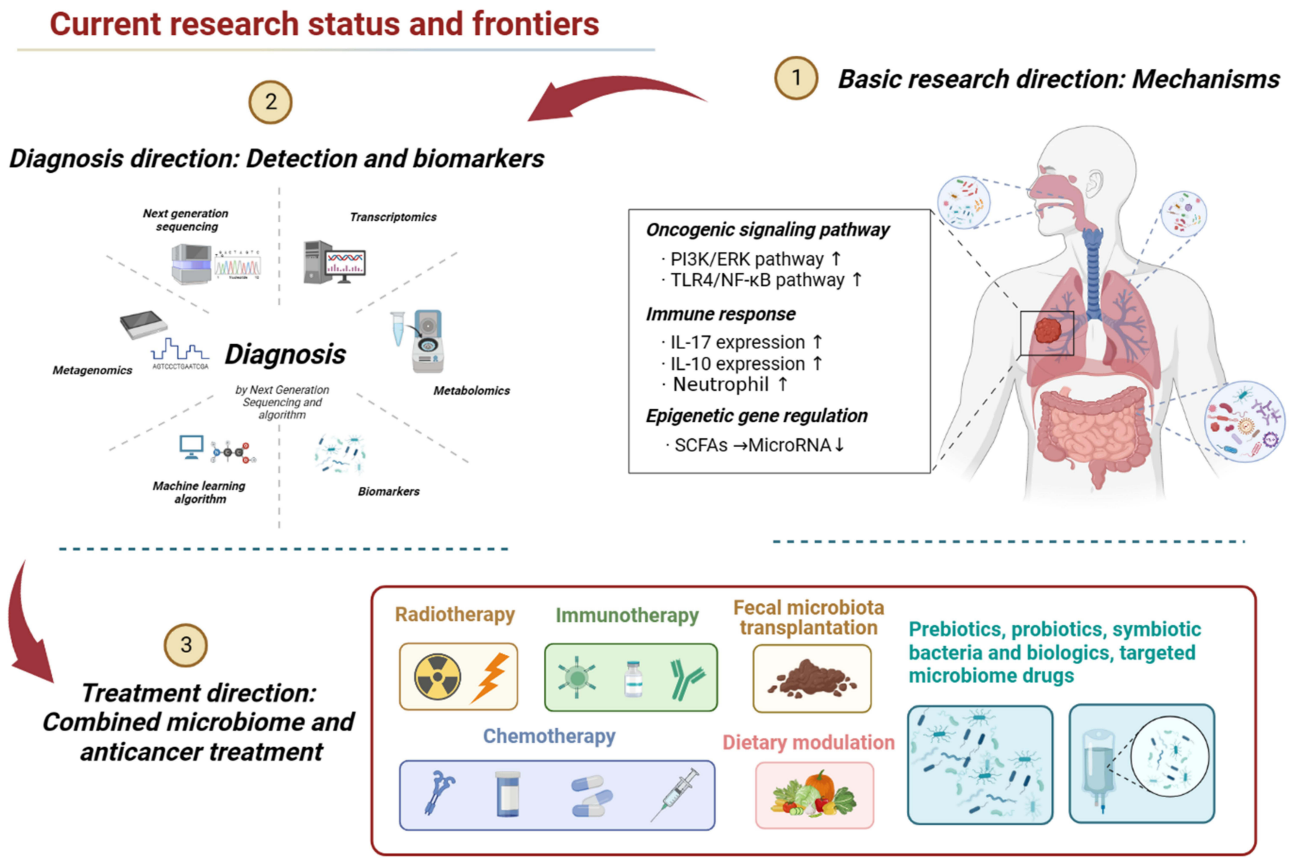

Figure 7 The current research status and frontiers for the microbiome in lung cancer.

Conclusion

The field has a bright research prospect, and there is a huge unmet clinical need. Future studies need to expand the sample size and further explore microbiota biomarkers associated with the mechanisms, diagnosis, treatment and prognosis of lung cancer. The use of microbial markers to assess the risk of lesions and the extent of disease and prognosis of lung cancer patients is conducive to changing the existing clinical diagnosis and treatment dilemmas and improving the effectiveness of clinical treatment of lung cancer. Solving problems such as small sample size, heterogeneity of the population, sequencing bias, diagnostic accuracy, and rational drug use, identifying specific microbiota that have an important impact on cancer treatment, and exploring methods to optimize microbial regulation are future challenges.

Abbreviations

WOSCC, Web of Science Core Collection; IF, impact factor; JCR, Journal Citation Reports; SCC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; COPD, chronic obstructive pulmonary disease; BALF, bronchoalveolar lavage fluid; BWF, bronchial washing fluid; PD-L1, programmed cell death ligand-1; qPCR, quantitative real-time polymerase chain reaction; ddPCR, droplet digital PCR; ITS, internal transcribed spacer; IHC, immunohistochemistry; mPCR, multiplex PCR; RT-PCR, reverse transcriptase-PCR; AUC, area under the curve; ICI, immune checkpoint inhibitor; CBM588, C. butyricum MIYAIRI 588.

Ethics Approval and Informed Consent

The Ethics approval and informed consent is not necessary for our study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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