УДК 579.61:616-092:616.24
https://doi.org/10.20538/1682-0363-2023-1-103-112

Microbiota: its contribution to carcinogenesis and immunity in the lungs

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ABSTRACT

Microbiota (an assembly of bacteria, protists / archaea, fungi, and viruses inhabiting a human body) is currently of great interest for science. It is determined by an association between changes in microbiota composition and malignant transformation in different organs. Lungs have long been considered sterile or free from bacteria; however, due to development of next-generation sequencing, this statement has been reconsidered. The metagenomic approach allowed to identify microorganisms at molecular level both in healthy lung tissues and in malignant ones.

The next stage of research is investigation of the effects of microbiota on homeostasis and immune stability in the lungs. The analysis of lung microbiota based on 16S rRNA gene sequencing revealed that microbiota of healthy lungs is mainly presented by bacteria of the phyla *Bacteroidetes, Firmicutes, Proteobacteria,* and *Fusobacteria*. In lung cancer, an increase in the number of bacteria of some certain genera and a decrease in microbiota diversity on the whole are noted. Dysbiosis facilitates reproduction of pathogens and development of lung diseases. It was detected that under normal conditions, microbiota maintains resistance of the lungs to bacterial colonization and plays a crucial role in providing a balanced immune response in this organ.

Keywords: metagenomics, microbiota, lungs, lung cancer,16S rRNA, immunity

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The study was funded by the state assignment for 2019–2021 (project No. 0352-2019-0011).

For citation: Buslaev V.Yu., Minina V.I., Matskova L.V. Microbiota: its contribution to carcinogenesis and immunity in the lungs. *Bulletin of Siberian Medicine.* 2023;22(1):103–112. https://doi.org/10.20538/1682-0363-2023-1-103-112.

Микробиота: вклад в канцерогенез и функционирование иммунной системы легких

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РЕЗЮМЕ

Микробиота (совокупность бактерий, простейших/архей, грибов, вирусов, обитающих в организме человека) и микробиом (их совокупный геном) являются предметом активных научных исследований. Особый интерес вызывает взаимосвязь изменений состава микробиоты и злокачественной трансформации различных органов. Легкие долгое время считались стерильным органом, однако это представление было пересмотрено благодаря развитию технологий секвенирования нового поколения. Метагеномный подход позволил идентифицировать микроорганизмы на молекулярном уровне в здоровых тканях легкого и в опухолях.

Следующим шагом стало выявление разнообразных аспектов влияния микробиоты на гомеостаз легочной системы и поддержание иммунитета. Анализ результатов исследований микробиоты легочной системы, основанных на секвенировании генов 16SpРНК, позволил установить, что микробиота здоровых легких представлена в основном бактериями, принадлежащими к типам *Bacteroidetes, Firmicutes, Proteobacteria* и *Fusobacteria*. При развитии рака легкого отмечено значительное повышение численности бактерий определенных родов и в целом снижение разнообразия микробиоты. Дисбиоз способствует активному размножению патогенов и развитию негативных состояний легочной системы. Установлено, что в норме легочная микробиота обеспечивает устойчивость к заселению легких болезнетворными микроорганизмами и играет важную роль в обеспечении сбалансированного иммунного ответа в данных органах.

Ключевые слова: метагеномика, микробиота, легкие, рак легкого,16SpРНК, иммунитет

Конфликт интересов. Авторы декларируют отсутствие конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование поддержано государственным заданием на 2019–2021 гг. (проект № 0352-2019-0011).

Для цитирования: Буслаев В.Ю., Минина В.И., Мацкова Л.В. Микробиота: вклад в канцерогенез и функционирование иммунной системы легких. *Бюллетень сибирской медицины.* 2023;22(1):103–112. https://doi.org/10.20538/1682-0363-2023-1-103-112.

INTRODUCTION

Lung cancer (LC) is currently recognized as one of the most widespread causes of cancer mortality in both men and women. Less than 20% of LC patients live longer than 5 years after the diagnosis was established [1]. At present, it is understood that lungs are not sterile and free from bacteria. The results of recent surveys show that lung microbiota can affect homeostasis of human respiratory system and play a role in LC development or in formation of metastases in the lungs as a consequence of primary cancer in other organs. Lung microbiota dysbiosis affects the risk of developing cancer at several levels, for example, by causing chronic inflammation or activation of oncogenes. Studying the effects of human respiratory microbiota on LC development and therapy effectiveness may be crucial in assessing the risk of pathology and developing a strategy for its treatment.

An assembly of microorganisms (bacteria, archea, fungi, viruses, protists) living in the human body is called microbiota, and their combined genome is called microbiome [2, 3]. Currently, the relationship between microbiota and cancer is being actively investigated. Most experimental studies are devoted to revealing the pathogenic properties of bacteria. For example, bacterial toxins can disrupt cell cycle by interfering with the synthesis of proteins responsible for DNA repair, cell division, and apoptosis. Bacteria affect the effectiveness of immunotherapy and the development of host immune responses against cancer cells [4].

Until recently, it was impossible to study bacteria in the lungs using conventional culture methods. Modern next-generation sequencing (NGS) makes it possible to effectively detect bacterial DNA [5–9]. This approach allowed researchers to identify

microorganisms at the molecular level in complex biological samples. Depending on the bacterial kingdom of interest, primers specific to conserved regions of genomes are used: 16S rRNA and 18S rRNA for bacteria and archaea, ITS1–ITS2 for fungi, V4–V9 regions of 18S rRNA for protists. Shotgun sequencing is used to identify viruses after primary extraction of viral particles [10]. A combination of conventional and novel methods of analysis, 16S rRNA gene sequencing and matrix-assisted laser desorption / ionization, and advancements in bioinformatics analysis of big repositories over the last five years made it possible to perform whole-genome sequencing of microorganisms and identify new species [11]. Interestingly, conventional culture methods are more effective in detecting the species of *Mycobacterium* genus [12].

Researchers are still discussing the effects of microbiota on lung homeostasis and its role in maintaining immunity**.** The aim of this review was to summarize the results of studies on evaluating microbiota contribution to the functioning of immunity and the development of LC published over the past 10 years.

MICROBIOTA OF HEALTHY LUNGS

Bacteria colonizing the human body belong mainly to *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria,* and *Cyanobacteria* phyla [13–16]. Currently, it was determined that the numbers of bacterial and human cells in the human body are equal. The number of bacteria inhabiting healthy human lungs is estimated from hundreds of thousands to hundreds of millions per 1 ml of lung volume [17].

A sufficient amount of information was obtained about the characteristics of lung microbiota depending on certain physiological conditions of the host organism. It was noticed that functional stability is provided by bacterial taxa that make up "healthy microbiota". Thus, under normal conditions *Proteobacteria, Firmicutes, Fusobacteria,* and *Bacteroidetes* phyla are the largest in number [18, 19]. They also include *Pseudomonas, Streptococcus, Prevotella, Veillonella, Haemophillus, Neisseria* genera, inhabiting the respiratory tract. *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria,* and *Fusobacteria*, as a rule, dominate in healthy lungs [20, 21]. However, it should be noted that when certain conditions change, some of the listed taxa may perform destructive functions. In general, in the absence of pathological conditions in the lungs, spatial differences in microbiota composition are not observed.

In most healthy people, oral commensals belonging to *Prevotella*, *Veillonella,* and *Streptococcus* genera are also found in lung microbiota, probably due to swallowing of the pharynx content, although these bacteria are not observed in all healthy individuals [22, 23]. Lung microbiota is also distinguished into pneumotypes according to quantitative and qualitative characteristics of specific taxa. The first group includes microbiome with a high bacterial count enriched with bacteria from the oral cavity, such as *Prevotella* and *Veillonella* (supraglotic predominant taxa (SPT)). The second group includes microbiome with a low content of *Prevotella* and *Veillonella* and trace amounts of bacteria from the environment, such as *Acidocella* and *Pseudomonas* (background predominant taxa (BPT)). It was shown that the SPT-pneumotype corresponds to a local Th17 immune response. Its functioning determines the immune status in normal and pathological conditions [24]. The relationship between pneumotypes and a risk of developing lung pathology is the focus of many studies. Besides, there are studies that confirmed that microbiota of healthy lungs differs from that of the oral cavity and other parts of the respiratory system and consists mainly of *Proteobacteria* (up to 60%).

LUNG MICROBIOTA IN LUNG CANCER

Malignant transformation in LC promotes structural changes in the microbiota composition. In LC patients, *Actinomyces* and *Peptostreptococcus* genera are most often detected in the lower respiratory tract. Pathogenesis is also associated with the activity of oral cavity bacteria (*Streptococcus* and *Wechsler*), which are involved in triggering the ERK and PI3K signaling pathways. Infections caused by *Mycobacterium tuberculosis* and *Helicobacter pylori*, associated with inflammation, enhance oncogenesis [25–27]. *Eubacterium xylanophilum, Eubacterium eligens,* and *Clostridium* also contribute to the most acute course of LC, their increased number is associated with the development of small cell lung cancer. Certain taxa (*Acidovorax*), in addition to cancers, can be involved in the development of other lung diseases. In the respiratory tract, *Propionibacterium* members contribute to the development of mild LC; however, their antitumor potential was demonstrated on laboratory mice [28]. Currently, sufficient data have been collected on the hypothetical effect of respiratory microbiota on LC development [29].

In LC, *Firmicutes* and *TM7* phyla and also *Eillonella, Megasphaera, Atopobium*, and *Selenomonas* genera are most often detected in lung microbiota. *Atopobium* and *Selenomonas* cause the development of milder oncogenic processes, and *Megasphaera* contributes to the development of acute LC. Representatives of this taxon, along with *Veillonella*, can be used as specific LC biomarkers for diagnosis and therapy of this pathology. *Filifactor* and *Treponema* genera were determined as significant markers of LC development using bronchoalveolar lavage fluid as a study material [30]. The number of *TM7* phylum members is increased in COPD and also in LC, which indicates possible development of oncogenic processes in case of increased inflammation.

As a result of metagenomics studies on LC using different material in combination with specific conditions, a significant increase in specific bacterial taxa and a simultaneous decrease in microbiota diversity were detected (Table).

Table

Bacterial communities detected in patients with lung cancer

Bacteria	Sample size	Sample type	References
H .influenzae *Enterobacter spp* *Escherichia coli*	216	Airway endoscopy	[31]
Granulicatella *Abiothrophia* *Streptococcus*	16	Sputum samples	[35]
Granulicatella *Streptococcus* *Mycobacterium*	10	Sputum samples	[36]
Acidovorax	176	Lung tissue	[38]
Brevundimonas *Acinetobacter* *Propionibacteium*	103	Bronchoalveolar lavage	[39]
Lactobacillus rossiae, Bacteroides pyogenes, Paenibacillus odorifer, Pseudomonas entomophila, Magnetospirullum gruphiwaldense	47	Bronchoalveolar lavage	[41]

The study of airway endoscopy material made it possible to evaluate pathogenic properties of gram-negative *H.influenzae*, *Enterobacter spp.*, *Escherichia coli* and gram-positive *Mycobacteria* [31]. In another study of bronchoalveolar lavage fluid, S.H. Le et al. found an increase in *Veillonella* and *Megaspahaera* genera during the development of LC [32]. Currently, researchers have no clear understanding of the role of *Streptococcus* and *Staphylococcus* in LC carcinogenesis. This may be due to difficulties of identifying other bacteria or due to the fact that bacteria may play a different role depending on a variety of conditions. The discrepancies in the data may be affected by lifestyle factors, specificity of environmental pollution (for example, the use of coal for heating), smoking, features of sampling the material for analysis and other factors [33]. It should be noted that an increase in *Streptococcus* members is typical of lung cancer [34].

It is known that exposure to chemical pollutants, in particular polycyclic aromatic hydrocarbons (PAHs), increases the risk of developing LC. H.D. Hosgood et al. examined the microbiota composition in non-smoking women who used coal as fuel at home. An increased content of *Granulicatella, Abiothrophia*, and *Streptococcus* was detected in the sputum samples [35]. An increase in *Granulicatella* (*Granulicatella adiacens*) was associated with LC progression [36]

Smoking can multiply the risk of LC formation. Currently, a lot of data have been collected about the molecular mechanisms underlying tobacco smoking. Smoking may contribute to the development of dysbiosis in different parts of the body, causing many diseases (asthma, COPD, and LC) [37]. Tobacco smoke directly interacts with the respiratory epithelium and contributes to impairment of immunological barriers. As a result, taxonomic composition and phylogenetic diversity of lung microbiota change (Fig. 1). The variability of *Firmicutes / Bacteroidetes* proportion in non-smoking / smoking patients should be taken into account to understand the role of microbiota in this case.

In context of studying microbiota, a hypothesis was put forward about the synergistic effect of somatic mutations and disruption of the epithelial barrier due to tobacco smoking in the development of LC. A study was conducted to understand the effect of *TP53* gene mutations on the composition of lung microbiota in in order to prove this hypothesis [38]. Initially, when

comparing the samples obtained from tumor tissues with those obtained from healthy ones, an increase in the number of *Proteobacteria* and a decrease in the number of *Firmicutes* were detected. The presence of *Acidovorax, Ruminococcus, Oscillospira, Duganella, Ensifer,* and *Rhizobium* genera was associated with smoking. In particular, members of *Acidovorax* were the most common taxa in smokers. The association of *Acidovorax* members with LC development was revealed in the presence of mutations in the *TP53* gene.

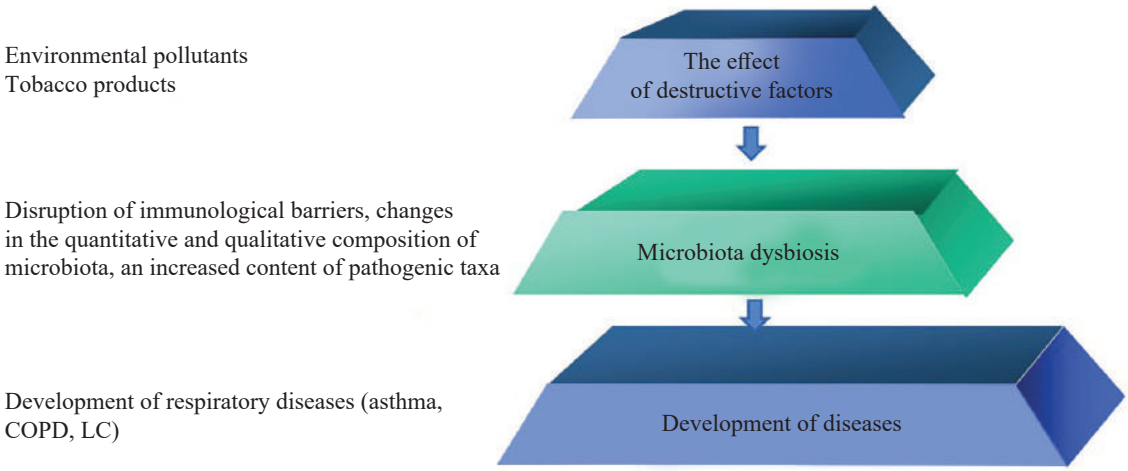

Fig. 1. Development of microbiota dysbiosis

An important issue is differences in microbiota depending on the histopathological type of LC. S. Gomes et al. revealed an increase in the proportion of *Brevundimonas, Acinetobacter,* and *Propionibacteium* taxa in patients with lung adenocarcinoma [39]. The presence of *Enterobacter* was characteristic of squamous cell LC. The development of non-small cell LC may also be accompanied by an increase in the activity of intestinal microbiota. In one study, bacteria of *Phascolarctobacterium* genus were strongly associated with the development of squamous cell LC [40]. *Phascolarctobacterium faecium* and *Phascolarctobacterium succinatutens* species detected in this study belong to microbiota in the gastrointestinal tract. Despite their unique properties, lung microbiota in tumors may have similarities with healthy tissues. At the same time, the presence of such rare bacterial species as *Lactobacillus rossiae, Bacteroides pyogenes, Paenibacillus odorifer, Pseudomonas entomophila,* and *Magnetospirullum gruphiwaldense* was observed in non-small cell LC [41].

Patients with LC are most often characterized by a decrease in the alpha diversity of lung microbiota [42]. A similar phenomenon was observed in the analysis of lung adenocarcinoma [43]. Regarding beta diversity, there are data about the absence of differences between malignant and healthy tissues. When assessing the overall diversity of microbiota, an increase in the Shannon diversity index was also noted in LC patients compared with healthy individuals.

MICROBIOTA AND IMMUNITY IN THE LUNGS

Studies of the past decade showed that lung microbiota maintains resistance of the lungs to bacterial colonization and plays an important role in providing a balanced immune response in the lungs. The composition of lung microbiota and its relationship with human immunity change with age, probably due to environmental effects. The evolution of these relationships leads to the development of regulatory processes that determine resistance to host antigens and non-dangerous agents and ensure exclusion of pathogens and transformed cells [44].

The pathogenesis of many diseases can largely be determined by specific interactions of bacterial communities from different ecological niches of the human body (Fig.2). Interaction of microbiota components from different parts of the body occurs during circulation of metabolic products, proinflammatory cytokines, and other signaling molecules. Bacterial translocation is an important property which is manifested by migration of viable resident bacteria from one niche to the other. In this regard, hypotheses were formed about axes of interaction between microbial communities.

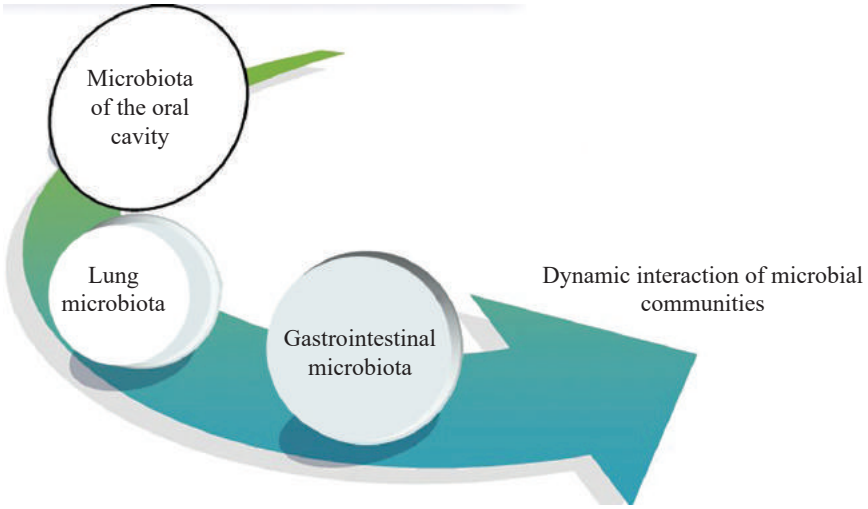

Fig. 2. Interaction between microbial communities from different ecological niches of the host organism

These include the "microbiota – brain – gut", "microbiota – gut – liver", and "microbiota – gut – skin" axes [45–47]. Despite the fact that these hypotheses are largely contradictory, nevertheless they reveal some features of the microbiota effect on physiological processes. This is mainly associated with the activation of innate and adaptive immunity mechanisms.

Lung microbiota closely interacts with other niches of the host organism. Therefore, the development of pulmonary diseases can be determined by impaired stability of gut microbiota composition. Lung microbiota and gut microbiota are currently thought to function together, refuting previous ideas about the presence of a "barrier" between them. Gut microbiota stimulates production of various regulatory cytokines and maturation of T and B cells, which provides enhanced protection of the mucous membrane. This effect not only persists in the gut, but also spreads to other mucous membranes through lymphatic and hematopoetic systems, affecting the immune response in remote organs [48].

Gut microbiota is involved in synthesis of biologically active molecules (mostly short-chain fatty acids and vitamins), which can reduce inflammatory processes. In particular, *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* express anti-inflammatory interleukin 10 (IL-10) and an IL-12 inhibitor, which can stop the severe course of allergic asthma. Since malignant transformation is associated with inflammation, inhibitory properties of *Faecalibacterium prausnitzii* may have therapeutic effects on the development of LC. This feature was demonstrated on cancer A549 cell line with a decrease in the expression of proinflammatory cytokines (IL-1, TGF-B2, IL-1RA) [49]. According to the results of these studies, it becomes possible to form the hypothesis about the "microbiota – gut – lungs" axis to reveal the etiology of pulmonary diseases.

Microaspiration and aspiration are the most likely mechanisms in the association between gut microbiota and lung bacteria. The products of bacterial metabolism in the gastrointestinal tract can affect the intensity of differentiation of specific immunity components: T cells, regulatory T cells, and Th17s [50]. As a result, the immune response and systemic inflammation enhance, which reflects the way microbiota affects adaptive immune homeostasis in the development of diseases. There are also ways to transmit signals from the gastrointestinal tract to the pulmonary system through the bloodstream, which can affect the stability of respiratory microbiota composition [51]. Further research is needed to confirm the hypothesis about these mechanisms.

Metagenomics studies showed that the manifestation of abnormal immune activity occurs due to a decrease in the number of commensal bacteria, which have properties beneficial for the body. On the contrary, reproduction and activity of pathogens increase; *Gammaproteobacteria* use by-products of inflammatory responses for their growth [52]. Studying the features of lower respiratory tract microbiota composition revealed that pathological processes are largely associated with a decrease in the number of *Bacteroidetes* in healthy microbiota and a shift towards the spread of *Gammaproteobacteria.* Experimental studies conducted on humans and laboratory animals made it possible to determine members of this taxon as pulmonary pathogens [53].

As a part of the pulmonary system, alveolar macrophages and resident dendritic cells, as well as other components of immunity are a primary barrier for pathogenic microorganisms. They act as important mediators of immune responses in the lungs and are activated only if stimulated by harmful bacteria. Macrophages and dendritic cells stimulate division of regulatory T cells involved in the implementation of acquired immune responses. In addition, their crucial property is the ability to secrete signaling molecules: prostaglandin E2, tumor growth factor beta (TGF-B), and IL-10, which contributes to maintenance of homeostasis [54]. Performing the functions of antigen-presenting cells, alveolar macrophages, dendritic cells, and pulmonary epithelium cells provide recognition of pathogenic components (mainly of microbial origin) through a system of pattern recognition receptors (PPR). Activation of these receptors triggers subsequent expression of signaling molecules.

γδ T cells are important effectors and regulators of the innate immune response to pulmonary infections [55]. It was shown that inhaling non-pathogenic bacteria that do not cause dysbiosis or infections promotes activation of these cells, which prevents development of the abnormal inflammatory response. These cells also play a protective role against allergies [56–58]. Using laboratory mice, it was shown that airway colonization by certain bacterial strains in newborns protects against acute allergic reactions in the respiratory tract [59–61]. These and other studies convincingly demonstrate that a contact of the host organism with bacteria at early stages of development is crucial for formation of full-fledged and functional immunity in the lungs [62].

The development of LC due to changes in lung microbiota may be caused either by increased sensitivity of the immune system leading to chronic inflammation or by a disrupted mechanism of pathogen recognition. Sometimes healthy microbiota may contribute to formation of an environment favorable to malignant transformation of lung tissue cells. For example, some bacteria may contribute to colonization of the lung tissue by metastatic cancer cells. It was shown that local application of antibiotics reduces formation of metastases, which is associated with modulation of the immune response. This also suggests that bacteria should be used as therapeutic tools with caution.

CONCLUSION

Despite advancements in identifying the features of human microbiota, this technique has some limitations. For example, this method makes it possible to identify microorganisms mainly only at the genus level, since the analysis of short sequences of bacterial genomes is available. As an alternative, a method of whole genome sequencing was developed, which makes it possible to identify species in the microbiota. Despite the advantages of both approaches, they can only detect dominating species in the population.

Given that the lungs are always affected by upper respiratory tract bacteria and by the environment, it can be assumed that microorganisms promoting normal state of the human body sometimes can contribute to oncogenic cell transformation or development of other pathologies. Information about changes in lung microbiota and the influence of external factors on these processes will undoubtedly contribute to a better understanding of LC etiology, identify new targets for therapy, and help elaborate new immunotherapeutic approaches to the treatment of lung pathologies.

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Received 17.01.2022;
approved after peer review 04.03.2022;
accepted 19.03.2022