LITERATURE REVIEW

The Cancer Mycobiome: A Highlight to Lung Cancer

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ABSTRACT

Microbiota is a collection of microorganisms such as bacteria, fungi, viruses, and archaea, with bacteria being the most numerous in the human body. Meanwhile, the mycobiome consists of commensal fungi, which are a small part of the microbiota. Examples found in the human body, from the skin to the internal organs, play a role in the immune response, homeostasis, metabolism, and disease. The composition of mycobiome varies over time, and the human intestine is the most studied organ due to the diversity of microbiota. Furthermore, lung cancer is the leading cause of death in oncology patients worldwide. Various studies suggest that mycobiomes play a role in cancer associated with dysbiosis. Chronic inflammation, biofilm formation, and carcinogen products are parts of cancer pathogenesis. Metagenome analysis has provided information about the diversity of microbiota, specifically mycobiome in the human body. The common method for gene sequencing in the metagenome is next-generation sequencing (NGS). Analysis through metagenomes in chronic diseases such as cancer shows that mycobiomes play a role in the process of cancer formation. However, the causal relationship between mycobiomes and cancer remains unknown.

INTRODUCTION

The human body is composed of variant microorganisms called microbiota, of which more than 99% are bacteria. The others include fungi, viruses, protozoa, and parasites.[1,2] Although bacteria are the most abundant microorganisms identified in human health and diseases, fungi also have the same role as bacteria.[3,4] The collection of fungi in the human body is called mycobiomes, and only 0.1% have been identified with different distribution sites.[5] According to a study, fungi have carcinogenesis effects in chronic diseases such as cancer.[6]

As stated by the World Health Organization (WHO), cancer is the leading cause of death worldwide. In 2020, approximately 10 million deaths were recorded, with the most common cases being breast, lung, colorectal, and prostate.[3,7] Several factors influence cancer development, such as infection, chronic inflammation, genetics, environment, and dysbiosis.[3] Lung cancer is the leading cause of death in the majority of oncology patients worldwide, accounting for 1.7 million deaths in 2018.[8,9]

The majority of fungi in the human body are unculturable, while mycobiome analysis is required to identify the role of fungi in cancer. With advances in medical technology through molecular and metagenomic investigation, unculturable fungi could be detected in cancer.[6,10] This literature review examined the role of the mycobiome in cancer by showing a correlation between the mycobiome and lung cancer.

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Physiology of Mycobiome

Composition and Colonization of Mycobiome

The mycobiome is part of the microbiota and consists of commensal fungi from eucaryotic microorganisms. Commensal fungi have the potential to become pathogens, which are called pathobionts.[5] Based on morphology and life cycle, fungi are divided into yeast and mold.[6] The fungi species are estimated at 2.2-3.8 million, although only 8% have been fully recognized.[2] Mycobiome can be found in the oral cavity, respiratory tract, urogenital tract, and breast milk.[5,11] Some fungi, such as *Candida*, *Malassezia*, and *Saccharomyces*, are found in the human body (Figure 1).[6]

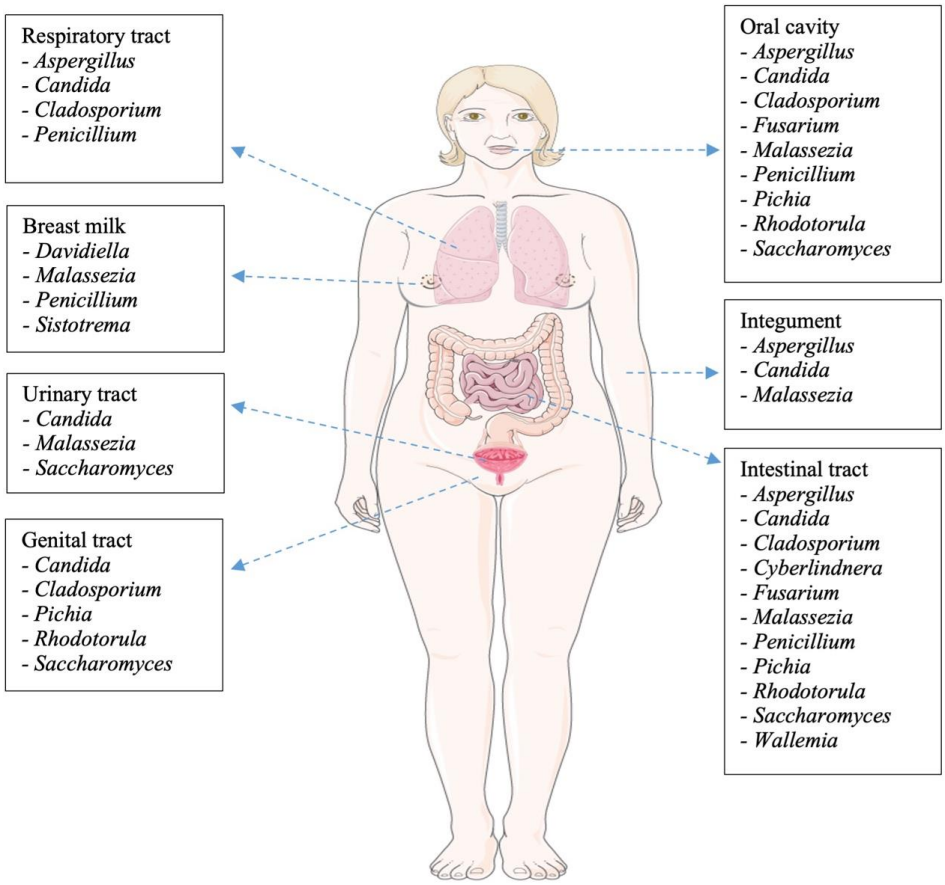

Figure 1. Mycobiome distribution throughout the human body[5]

According to previous studies, the composition of mycobiome is dynamic.[2,4] Mycobiome is formed since birth, with either the mother or the environment being the primary source. Colonization is influenced by gestational age, birth weight, and mode of delivery, while the composition and diversity are determined mostly by feeding methods. During adult life, diet and body weight are the main factors that influence mycobiome composition. Other factors are age, gender, lifestyle, antibiotic and antifungal use, geographic condition, and hygiene.[2]

Immune Response to Mycobiome

The mycobiome plays a significant role in the immune system and homeostasis of the human body. Fungi, as a source of antigens, stimulate the innate and adaptive immune systems to form memories after the recognition of commensal and pathogens.[2,3,12] Molecule structures such as chitin, mannan, and β-glucan are known as pathogen-associated molecular patterns (PAMPs) and will be recognized by immune systems through pattern recognition receptors (PRRs) located on the surface of neutrophils, monocytes, some B- and T-lymphocytes, antigen-presenting cells (APC), including dendritic cells (DC), and macrophages. C-type lectin receptors (CLRs), crucial for the recognition of fungi, trigger innate and adaptive immunity. The components include one or more carbohydrate recognition domains (CRD) to detect PAMPs.[12]

CLRs have different receptors to recognize PAMPs such as Dectin-1, Dectin-2, Macrophage inducible Ca^{2+}-dependent lectin (Mincle), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), and soluble mannose-binding lectin (MBL) which in turn activate neutrophils, monocytes, and natural killer (NK) cells. The activation leads to the formation of free radicals, along with pro-inflammatory cytokines and chemokines that kill fungi

and secrete perforin, as well as granulocyte-macrophage colony-stimulating factors (GM-CSF), to tumoricide.[3,12]

Mycobiome Role in Cancer Pathogenesis

The human intestine is the most studied organ due to the diversity of microbiota compared to other organs.[13] Several studies have been conducted to find a correlation between microbiota and various chronic diseases, such as cancer. The mycobiome is part of the microbiota in the human intestine and interacts with other commensal microorganisms.[2,4] The role of cancer is associated with dysbiosis. For example, antibiotic use could reduce commensal bacteria, which in turn increase fungi colonization. In the intestine, dysbiosis increases *Candida albicans* colonization, leading to fungi invasion into the bloodstream, which is subsequently related to irritable bowel syndrome, Chron disease, ulcerative colitis, and gastrointestinal cancer. In cancer pathogenesis, mycobiome causes chronic inflammation, biofilm formation, and production of carcinogenic metabolite.[3]

Chronic Inflammation by Mycobiome

Fungi are renowned for causing the development and progressivity of cancer through inflammation. The invasion activates PRRs to recognize the carbohydrate cell wall of fungi, which is known as PAMPs. The interaction activates Toll/IL-1R domain-containing adaptor-inducing IFN-β (TRIF), spleen tyrosine kinase—caspase recruitment domain 9 (SYK-CARD9), and myeloid differentiation primary response gene 88 (MYD88) to induce the formation of various pro-inflammatory cytokines including interleukin 1β (IL-1β), IL-6, IL-12, IL-23, transforming growth factor-β (TGF-β), and interferon-gamma (IFN-γ). Consequently, anti-fungal response is formed through Th-1 and Th-17 together with phagocytes and neutrophils (Figure 2).[3,14]

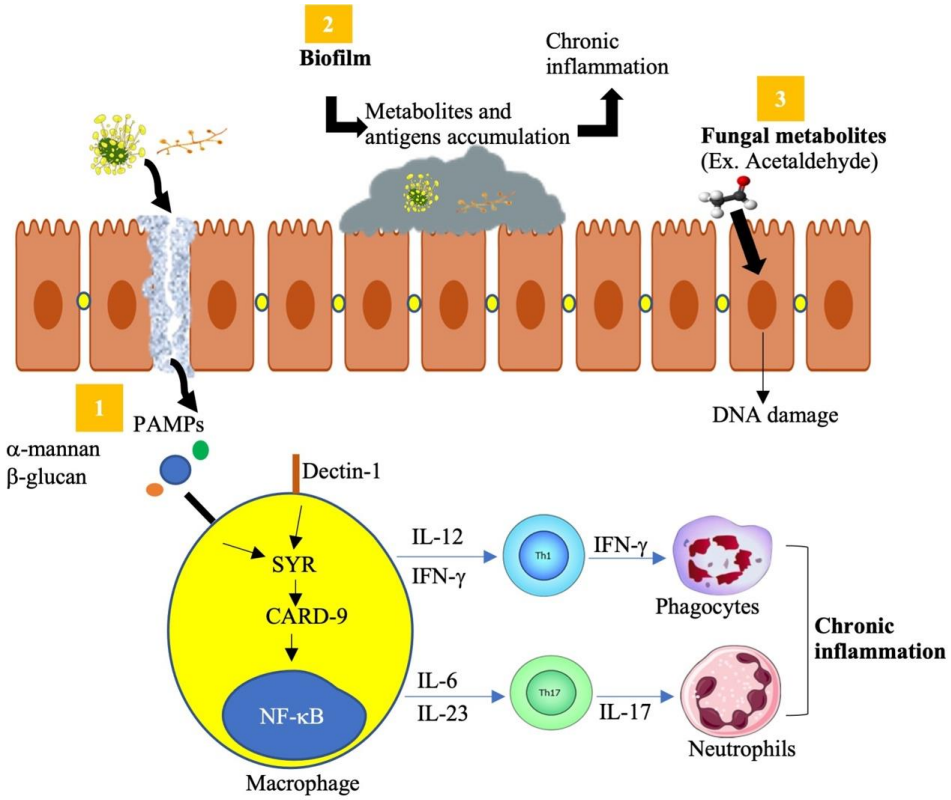

Figure 2. Mycobiome roles in cancer through (1) chronic inflammation, (2) biofilm formation, and (3) carcinogens product by fungi

PRRs function in the development of innate immunity and maintenance of epithelial barrier integrity. Carcinogenesis occurs when the epithelial barrier is damaged due to PRRs not being activated properly.[3,15] A decrease in CARD-9 expression or inherited CARD-9 deficiency, MYD88 gene and Mucin-2 (MUC2) gene, *C. albicans* toxin, as well as Malassezia translocation have been known to produce carcinogenesis through the breakdown of epithelial barrier integrity. In colorectal cancer (CRC) patients, there is a high burden of *Candida tropicalis* due to a decrease in CARD-9 expression. CARD-9 is a signaling protein that activates pro-inflammatory cytokines as a response to fungi, and high expression may reduce burden. *Candida tropicalis* causes immunosuppression and carcinogenesis through the increase of free radicals, cytokines, and nitric oxide (NO).[15]

The MYD88 gene is an important adaptor protein in PRRs that maintains the integrity of the intestine epithelial barrier. Low expression in mice was found to cause a decreased ability in epithelial regeneration. Furthermore, alterations occurred in the inflammation process, epithelial mutation, adenoma development, and progressivity of cancer. The MUC2 gene produces protective mucous, and a decrease in production causes chronic inflammation due to increased microbiota-mucosa contact. In murine models, chronic inflammation reportedly led to carcinogenesis.[3]

C. albicans produces candidalysin, which causes damage to the intestinal epithelial barrier. The toxin modulates immune response by neutrophil recruitment to the tumor site and correlates with poor prognosis.[3] Furthermore, Malassezia translocation from the intestine lumen to the pancreas has been known to induce pancreatic ductal adenocarcinoma in murine models. Engagement of MBL receptors with fungal cell walls activates complement 3 (C3), which induces carcinogenesis.[3,16]

Biofilm Formation

Biofilm formation, which causes chronic inflammation, is produced by interaction between bacteria and fungi to evade the human immune system.[3] A study by Tomkovich, *et al.* (2019) on three murine models inoculated with biofilm from healthy and cancer patients showed that biofilm was carcinogenic.[17] Biofilm formed by *C. albicans*, *Actinomyces naeslundii*, and *Streptococcus mutans* contributes to oral cancer.[14] Interaction between bacteria and fungi can be synergist or antagonist. For instance, synergist interaction between *Enterohaemorrhagic Escherichia coli* (EHEC) and *C. albicans* promotes EHEC to invade and damage enterocyte tissue infected by *C. albicans*. Meanwhile, antagonist interaction is *Acinetobacter baumanii*, *Serratia marcescens*, and *Salmonella typhimurium* known to have anti-fungal activity to *C. albicans.*[3]

Produce of Carcinogenic Metabolites

Mycobiome produces a metabolite that can damage deoxyribonucleic acid (DNA) and contributes to carcinogenesis.[3] According to a previous study, *C. albicans* plays a role in oral carcinoma through the production of the alcohol dehydrogenase enzyme, which converts alcohol to acetaldehyde.[6] Generally, acetaldehyde is a carcinogenic substance for humans, specifically in alcoholics. *C. albicans* also invade tissue and produce nitrosamine, which is known to be carcinogenic.[6] Aflatoxin B1 (AFB1) is a toxin produced by *Aspergillus* that induces hepatocellular cancer. Other toxins, such as fumonisin B1 produced by Fusarium, are associated with oesophageal cancer.[3]

Mycobiome in Lung Cancer

Investigations on the mycobiome in the respiratory tract are still limited. Previously, the lungs of healthy people were considered free from microbiota. Since the emergence of many studies, the mycobiome has been identified as playing a role in chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and bronchiectasis.[18,19] The respiratory mycobiome in healthy people was found in various genera and species through specimens such as bronchoalveolar lavage (BAL), sputum, and exhaled breath condensate (EBC) (Table 1).[2]

Table 1. The respiratory mycobiome found through different specimens[2]

Specimens	Genera	Species
Bronchoalveolar lavage (BAL)	*Cladosporium* sp.	Unclassified
	Candida sp.	*C. guilliermondii, C. albicans*
	Aspergillus sp.	*A. fumigatus, A. flavus*
	Penicillium sp.	Unclassified
	Saccharomyces sp.	Unclassified
Sputum	*Pichia* sp.	*P. jadini, P. brevicompactum*
	Saccharomyces sp.	Unclassified
	Candida sp.	*C. albicans, C. dubliniensis*
	Ganoderma sp.	Unclassified
	Malassezia sp.	Unclassified
	Aspergillus sp.	*A. flavus*
	Cryptococcus sp.	*C. magnus*
	Peniophora sp.	*P. incarnata, P. Cinerea*
	Daedaleopsis sp.	*D. confragosa*
	Sistotrema sp.	*S. brinkmanii*
Exhaled breath condensate (EBC)	*Stereum* sp.	*S. hirsutum*
	Cladosporium sp.	*C. herbarum*
	Aspergillus sp.	*A. sydowii*
	Penicillium sp.	*P. brevicompactum, P. expansum, P. glabrum, P. olsonii, P. bilaiae*
	Alternaria sp.	*A. alternata, A. infectoria*

The relationship between mycobiome and lung cancer remains unknown. Available studies did not find significant results regarding mycobiome in lung cancer.[13] Narunsky-Haziza, *et al*. (2020) stated that lung cancer patients were found to have a significant number of *Aspergillus* genera and *Agaromycetes* class.[20] Meanwhile, Dohlman, *et al*. (2022) and Zhao, *et al*. (2023) reported the abundance of *Blastomyces* sp. and *Alternaria arborescens*, respectively.[21,22] The three studies clearly showed that the mycobiome plays a role in the development of cancer, either through interaction with bacteria or through response to the human immune system.[13]

Mycobiome Analysis Detection

Metagenome Approach

The metagenome approach reads all genetic material of unlimited ecosystem DNA from one organism. This approach is very useful for analyzing the diversity of microbiota that cannot be cultured.[23] According to previous studies, the majority of commensal fungi could not be cultured.[24,25] The metagenome principle involves analyzing and identifying community DNA using phylogenetic markers such as 18S rRNA (ribosome-ribonucleic acid) and internal transcribed spacer (ITS), which are commonly used for fungal identification.[23,26]

Next-Generation Sequencing (NGS)

NGS is a frequently used method for gene sequencing in metagenomes. Its purposes are to identify microorganism species from isolated specimens, determine taxonomy from the whole ecosystem, assess DNA diversity from the same species, interpret the entire genome from specific DNA, and estimate point mutations from minority populations.[24] There are a few steps in metagenome analysis, including (1) collection of specimens from tissue biopsy and feces using a combination of skin scrapping and skin swabbing, mouth swab, throat swab, trachea aspirate, sputum, and BAL; (2) DNA isolation and extraction from specimens; (3) library preparation and sequencing of DNA; and (4) bioinformatic analysis.[24,27]

Narunsky-Haziza, *et al*. (2020) conducted mycobiome analysis using the metagenome approach on 17,401 cancer specimens from 35 cancer types.[20] The results showed that fungi were found in 35 cancer types, and the majority were located in cell tumors and macrophages. Higher fungi loads were found in all cancer types, with Ascomycota and Basidiomycota phylums abundant in most colon cancer. Furthermore, interactions were found between fungi, bacteria, and the immune system. Statistical analysis was used as a diagnostic and prognostic tool to examine the association between cancer and fungi.[20] Another study by Dolhman, *et al*. (2022) on mycobiome in gastrointestinal and lung cancer reported that (1) fungal DNA was abundant in specimens of head-neck, gastric, and colorectal cancer compared to brain and esophagus cancer; (2) fungal diversity in gastrointestinal cancer was dominated by *Candida* sp., lung cancer by *Blastomyces* sp., and breast cancer by *Malassezia* sp; (3) an increase in *Candida* sp. colonization was related to the elevated expression of pro-inflammatory cytokines; (4) the existence of *Candida* sp. can predict metastatic incidents in colon cancer; and (5) increased colonization may suggest poor prognosis in head-neck and gastric cancer.[21] A study by Zhao, *et al*. (2023) in non-small-cell lung cancer (NSCLC) patients showed that there was a different diversity of mycobiome between NSCLC and non-NSCLC patients.[22] A high burden of *Botrytis fragariae* was found in BAL specimens of NSCLC patients. Increased interaction was found between fungi in the lung and the environment of BAL specimens in NSCLC patients. Additionally, a significant increase in the number of *A. aborescens* was found in tissue specimens of NSCLC. These three studies showed that mycobiome has a potential role in carcinogenesis, although the relationship between fungi and cancer remains unknown.[13,20,21]

SUMMARY

In conclusion, the mycobiome is a part of the microbiota. It consists of commensal fungi found in all parts of the human body, playing a crucial role in immunity and homeostasis. The variability in the body is influenced by composition and colonization. Dysbiosis tends to make commensal fungi become pathobiont. The development of cancer by mycobiome is through chronic inflammation, biofilm formation, and carcinogenic products. The metagenome approach with the NGS method can help assess the diversity of mycobiome in cancer patients. Although several studies have found mycobiome diversity and an increase in fungal colonies that have the potential for carcinogenesis in several types of cancer, the causal mechanism remains unknown.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Authors' Contributions
Writing the manuscript: EY. Co-coordinating study design and collecting data: EY, JZ, AR. Data analysis and interpretation: EY. Revising: EY, JZ, AR. Reviewing: JZ, AR. All authors contributed and approved the final version of the manuscript.

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