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Review Article

Research trends in microRNA profiling as a biomarker for lung adenocarcinoma via liquid biopsy: A bibliometric analysis

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Abstract

Research related to the development of diagnostic biomarkers in lung adenocarcinoma in various countries is important. Research on microRNA as a biomarker in lung adenocarcinoma varies depending on the population, specimen, and technology used for profiling and validation. The aim of this study was to map and analyze bibliometric data of publications related to the topic of microRNA as a candidate biomarker in lung adenocarcinoma and to determine any potential research gaps. A total of 8,506 articles were collected from Crossref, Google Scholar, Semantic Scholar, PubMed, and Scopus databases using Harzing's Publish or Perish platform. A systematic search was conducted using four keywords: “profiling,” “validating,” “microRNA,” and “lung adenocarcinoma,” and synonyms of these keywords based on the MeSH on NCBI. The data extraction process followed the chart from PRISMA-P. The article’s elimination was conducted using Mendeley Desktop and then was analyzed based on the authors' keywords using VOSviewer and Biblioshiny. A bibliometric analysis of 692 relevant articles identified four primary research clusters: (1) microRNA (19 keywords), which highlights its potential as a biomarker for early detection and diagnosis; (2) lung adenocarcinoma (18 keywords), reflecting advancements in lung cancer research; (3) liquid biopsy (19 keywords), emphasizing the growing interest in non-invasive diagnostic methods; and (4) bioinformatics (nine keywords), underscoring the role of computational approaches in transcriptomic analysis. As a primary topic, microRNAs have become a focal point of research for diagnosing lung cancer across various stages and as biomarkers for cancer cell proliferation, invasion, migration, and metastasis. Numerous studies have demonstrated the successful application of microRNAs in lung cancer diagnosis in the last decade, although the reported types of microRNAs are inconsistent. Therefore, further research on this topic should be continuously conducted, particularly to validate the types of microRNAs and the types of environments that influence them.

Keywords: Bibliometric, lung adenocarcinoma, liquid biopsy, microRNA, VOSviewer

Introduction

Lung cancer remains the leading cause of cancer incidence and mortality among men worldwide. Approximately 1.5 million individuals are diagnosed with lung cancer globally each

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year, with around 1.2 million deaths attributed to the disease [1] This high mortality rate underscores the current limitations in both diagnostic and therapeutic approaches. A major challenge lies in the fact that lung cancer often presents without specific symptoms in its early stages [2], leading to diagnoses at more advanced stages when treatment options are limited, resistance to chemotherapy is common, and the survival rate is significantly lower [3,4]. The gold standard for lung cancer diagnosis is tissue biopsy, an invasive procedure that carries risks, particularly in advanced stages where tissue necrosis may occur [5,6]. Moreover, tissue biopsy provides only a visualization of the tumor, lacking insight into the heterogeneity of cancer cells and limiting the ability to monitor disease progression. As a result, there is a critical need for non-invasive diagnostic biomarkers that can offer a more comprehensive view of clonal mutations and improve early detection.

Lung cancer is histopathologically classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [7,8]. NSCLC accounts for approximately 80% of all lung cancer cases worldwide [9], and it includes subtypes such as adenocarcinoma, large cell carcinoma, and squamous cell carcinoma [10]. Among these, adenocarcinoma is the most common, often originating from cells lining the alveoli and capable of producing mucus [11,12]. This subtype is strongly associated with cigarette smoking, which induces epigenetic changes that disrupt gene regulation, playing a pivotal role in lung cancer development [13-15]. These genetic and epigenetic alterations offer promising avenues for identifying diagnostic biomarkers [16-18]. particularly through the use of liquid biopsy.

Liquid biopsy is a minimally invasive technique that allows for the analysis of various biological specimens, including serum, plasma, urine, cerebrospinal fluid, and saliva [19,20]. These specimens contain a wealth of biological materials such as proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and non-coding RNA [21]. Among the most promising biomarkers identified through liquid biopsy are microRNAs (miRNAs), a class of non-coding RNA molecules that regulate gene expression. Each miRNA can target multiple genes, and their expression levels can fluctuate depending on the physiological or pathological state of the cell [22]. Given their involvement in key cancer pathways, miRNAs have garnered significant attention as potential diagnostic and prognostic biomarkers in lung cancer [23,24].

Studies on miRNAs in lung cancer, particularly adenocarcinoma, have advanced considerably, with numerous studies investigating their potential as diagnostics biomarkers [25-29]. However, miRNA expression is dynamic and influenced by environmental factors and disease pathology, raising questions about the robustness and feasibility of using miRNAs as reliable biomarkers in clinical practice [15,30-35]. Understanding the progression of miRNA studies in lung cancer is essential for identifying research trends and evaluating the clinical potential of these molecules [25-29]. Therefore, the aim of this study was to map the landscape of published studies, offering insights into the growing interest in miRNA-based diagnostic biomarkers for lung adenocarcinoma, as well as highlighting emerging areas of research that warrant further exploration.

Methods

Data resource and search strategy

Advanced keywords were developed based on the primary keywords, which were “profiling,” “validating,” “microRNA,” and “lung adenocarcinoma” for article searches. Each keyword was searched for synonyms using Medical Subject Headings (MeSH) from NCBI (https://ncbi.nlm.nih.gov/mesh). Based on the MeSH, a lexical analysis revealed that the keyword "profiling" is synonymous with "screening" in the context of this study. Further semantic exploration using a keyword co-occurrence analysis identified related terms such as "nanostring ncounter," "transcriptome," and "epigenetic expression." While the term "validating" remained unique, "microRNA" was found to be semantically related to both "epigenetic" and "liquid biopsy". The keywords "lung adenocarcinoma" were shown to be a more specific term for the broader categories of "non-small cell lung cancer," "small cell lung cancer," and "lung cancer."

Article searches were conducted as of April 23, 2024, by accessing Crossref, Google Scholar, Semantic Scholar, PubMed and Scopus databases through Harzing's Publish or Perish (PoP)

macOS GUI edition ver. 8.8.4275 (Harzing, London, UK) (https://harzing.com/resources/publish-or-perish/os-x) [36]. The searches were limited to cover publications between 2013 and 2024. The keywords "profiling," "validating," "microRNA," and "lung adenocarcinoma" were employed in Google Scholar. Scopus was queried using the terms "nanostring ncounter," "validating," "epigenetic," and "lung cancer." PubMed searches focused on "validating," "epigenetic" and "non-small cell lung cancer". Semantic Scholar was explored using "transcriptome," "validation," "microRNA," and "lung adenocarcinoma". Finally, Crossref was utilized with the keywords "epigenetic expression," "validation," "microRNA," and "small cell lung cancer."

Data collection and screening

An initial bibliometric analysis yielded 8,506 articles from five databases: Crossref (4,000), Google Scholar (2,500), Semantic Scholar (1,000), PubMed (587), and Scopus (419). The resulting dataset was subsequently exported in BibTeX format and imported into Mendeley Desktop version Desktop ver. 1.19.8. (Cambridge University, Cambridge, UK). This study focused on articles related to miRNA research in lung cancer. Duplicate entries were identified and merged within the software, resulting in 403 articles. Only research articles published between 2013 and 2024 were included, resulting in 7,406 articles continued for further selection. The article selection process adhered to predefined criteria that encompassed information about the title, author, publication year, abstract, and keyword analysis of original articles, then the articles with incomplete information were excluded. Additionally, articles with reviews, editorial letters, corrigenda, errata, or retractions were not considered. Subsequently, articles were screened based on type, corrigendum status, retraction notice, language, and completeness, yielding 4,914 articles. Selected articles exclusively investigated human lung cancer miRNAs. Therefore, studies involving animal models or plant subjects were excluded. Articles related to other cancer types or miRNAs associated with lung cancer chemotherapy were also removed from the analysis. A selection process was then applied to identify articles that aligned with the specified research topic, considering cancer type, biomarker diagnosis and detection, and study object. This resulted in 692 articles that were further analyzed using VOSviewer (Centre for Science and Technology Studies, Leiden University, Leiden, Netherlands) and RStudio (Posit, Massachusetts, USA) (**Figure 1**).

Data processing and analysis

Data were first converted into Research Information Systems (RIS) files and subsequently processed using VOSviewer version 1.6.20 (Centre for Science and Technology Studies, Leiden University, Leiden, The Netherlands). Article mapping was conducted based on bibliographic data, focusing on co-occurrence and co-authorship. A fractional counting method was employed to calculate the strength of relationships between keywords for the bibliometric analysis. Out of a total of 1,516 keywords, only those appearing in at least five articles were included in the co-occurrence analysis. The co-occurrence of these keywords within the same articles helped reveal trends in the research topics. Data normalization was performed using association strength [37], and then VOSviewer nodes were used to show the keywords used in the articles, while the frequency and trends of the research were presented as the size of the nodes. The larger the node, the more significant the keyword was included in the articles. The relationship between keywords that are used together in one article was illustrated as the connection line between nodes.

The strength of the relationship between keywords was presented as the thickness of the line, the thicker the line, the stronger the relationship between keywords. Meanwhile, the colors of the nodes and lines grouped the keywords into clusters. Each cluster represents a group of research trends that are more closely related to each other than to other clusters. The distance between nodes shows how strong the relationship is between keywords. Close nodes indicate that the keywords often appear together in publications. Additionally, bibliometric analysis was conducted using the RStudio package Bibliometrix: Biblioshiny (https://www.bibliometrix.org/home/) to examine trends in keyword usage and miRNA research related to lung adenocarcinoma from 2013 to 2024 [38].

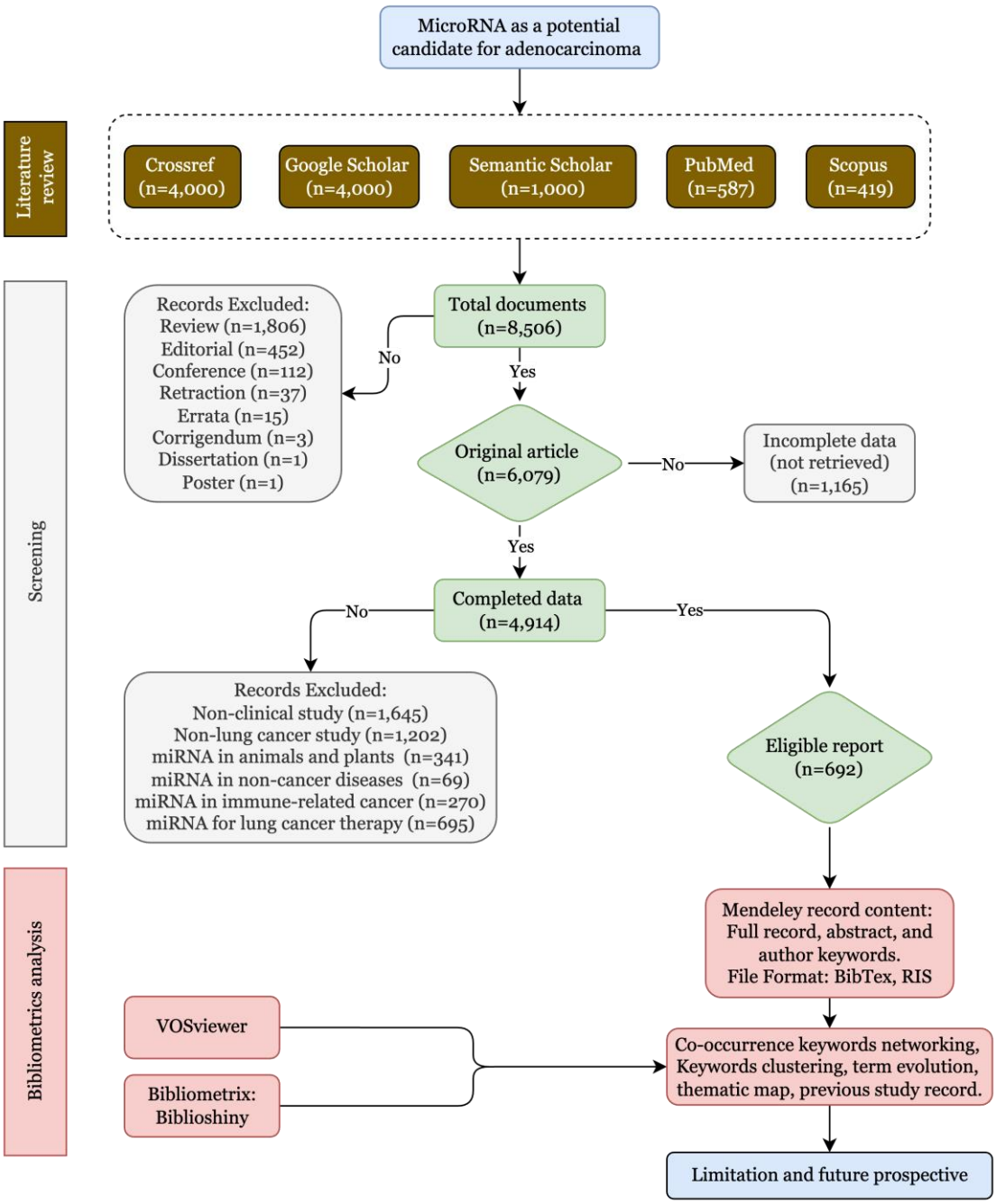

Figure 1. Number of original research articles used for analysis based on the PRISMA 2020 flow chart. The letter n in the flowchart indicates the number.

Results

Analysis of global publication trends and types of documents published

The bibliometric analysis related to the topic of miRNA as a candidate biomarker for lung adenocarcinoma identified 692 articles. Most of these articles were published in the journal Lung Cancer. The list of journals ranked in the top 10 article publications was Lung Cancer (31 articles), Oncology Letters (18 articles), Frontiers in Oncology (16 articles), International Journal of Molecular Sciences (16 articles), Translational Lung Cancer Research (16 articles), Oncotarget (14 articles), Cancers (10 articles), Chest (7 articles), Frontiers in Genetics (7 articles), and Nature Communications (7 articles) (**Figure 2**). The list of journal titles is in accordance with the topics analyzed: lung adenocarcinoma and miRNA.

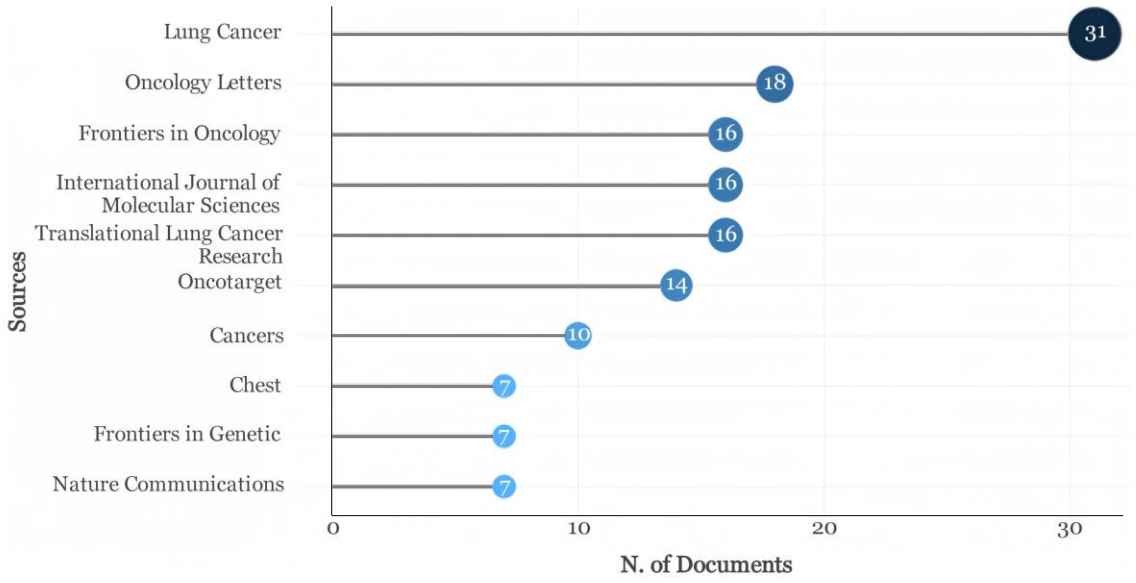

Figure 2. Top 10 journals that published articles related to lung adenocarcinoma and microRNA between 2013 and 2024.

Publication trends in microRNA and lung adenocarcinoma research using VOSviewer

The bibliometric analysis in VOSviewer was performed using the association strength method for normalization, with a minimum cluster size of 10 keywords, resulting in the identification of four distinct clusters. Based on the analysis, Cluster 1 consists of keywords related to "microRNA" marked with red nodes consists of 19 keywords: biomarker, circulating microRNA, COPD, diagnosis, differentially expressed genes, early detection, early diagnosis, gene expression, lung cancer, microRNA, miR-16, miR-21, plasma, profiling, RNA-seq, screening, serum, transcriptome, tumor suppressor (**Figure 3**). Cluster 2 related to "lung adenocarcinoma" marked with green nodes consisting of 18 keywords: apoptosis, bioinformatic analysis, deep learning, gene signature, immunotherapy, invasion, machine learning, metastasis, migration, nomogram, non-small cell lung cancer, overall survival, PD-L1, prognosis, proliferation, targeted therapy, tumor microenvironment. Furthermore, Cluster 3, related to "liquid biopsy" marked with blue nodes, consists of nine keywords: cancer, cell-free DNA, circulating tumor cells, ctDNA, early stage, exosome, extracellular vesicles, liquid biopsy, and next-generation sequencing. Cluster 4, related to "bioinformatics", marked with yellow nodes, also consisted of nine items, namely bioinformatics, circular RNA, LncRNA, lung squamous cell carcinoma, mRNA, small cell lung cancer, survival, TCGA, and treatment. The concurrent use of keywords within a single cluster indicates a stronger thematic relationship compared to the connections between different clusters. The keywords “microRNA,” “lung adenocarcinoma,” “liquid biopsy,” and “bioinformatics” appear larger than others, indicating their frequent usage in the reviewed articles (**Figure 3**).

Keywords frequently appearing in the context of miRNA research in lung adenocarcinoma are interconnected with keywords in different clusters, illustrating the relationships between terms used together within a single article. The keyword "microRNA" has the most connections to other clusters, with approximately 32 nodes (**Figure 4A**). "Lung adenocarcinoma" connects with keywords in other clusters through around 31 nodes (**Figure 4B**). "Liquid biopsy" is linked to different keyword clusters by 15 nodes (**Figure 4C**), while "bioinformatics" is connected to 7 clusters (**Figure 4D**). This suggests that "microRNA" has the widest range of connections with other keywords, while "bioinformatics" has the fewest.

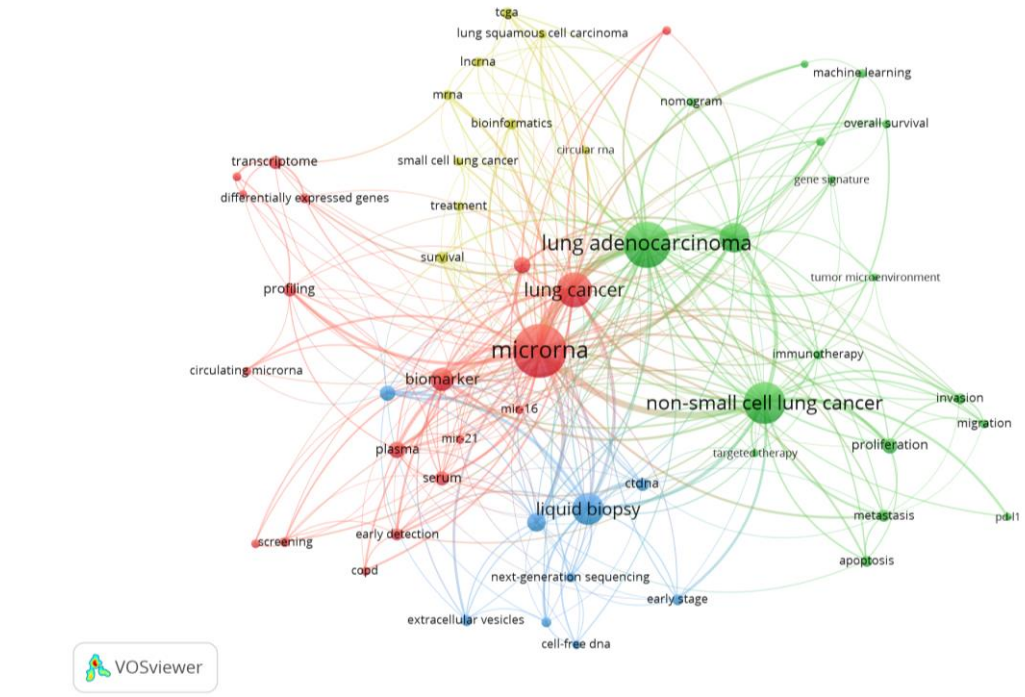

Figure 3. Keyword clustering based on the co-occurrence from published articles related to lung adenocarcinoma and microRNA between 2013 and 2024.

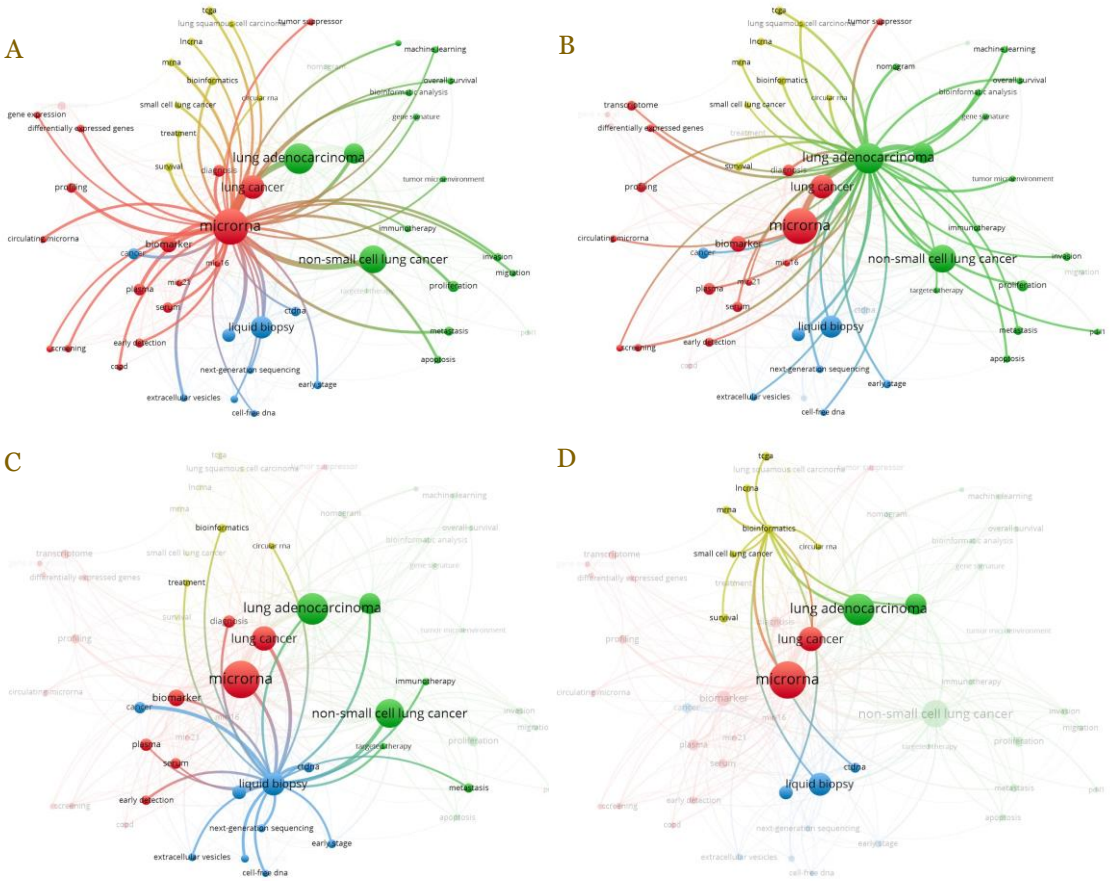

Figure 4. Network visualization of keyword clusters illustrating relationships among published articles on lung adenocarcinoma and microRNA between 2013 and 2024. The main keywords in each cluster are represented by the central interconnections within the clusters, including "microRNA," which is connected to 15 co-occurring keywords (A); "lung adenocarcinoma," connected to 15 keywords (B); "liquid biopsy," connected to nine keywords (C); and "bioinformatics," connected to eight keywords (D).

A bibliometric study using VOSviewer visualization based on keyword co-occurrence from 2013 to 2024 revealed publication trends in "microRNA"-related articles, with a notable increase in 2019 (**Figure 5A**). Research on miRNA "profiling and screening" in cancer, often utilizing "serum" and "plasma" specimens and examining effects on "invasion," "migration," and "proliferation," was common in articles published before 2018. Studies on "microRNA" in "liquid biopsy" particularly focusing on "exosomes" and "extracellular vesicles" in lung adenocarcinoma, were prominent from 2018 to 2021. More recently, from 2021 to 2024, the use of "microRNA" alongside keywords like "machine learning," "bioinformatics," "ctDNA," "immunotherapy," "tumor microenvironment," and "circular RNA" appeared in numerous studies.

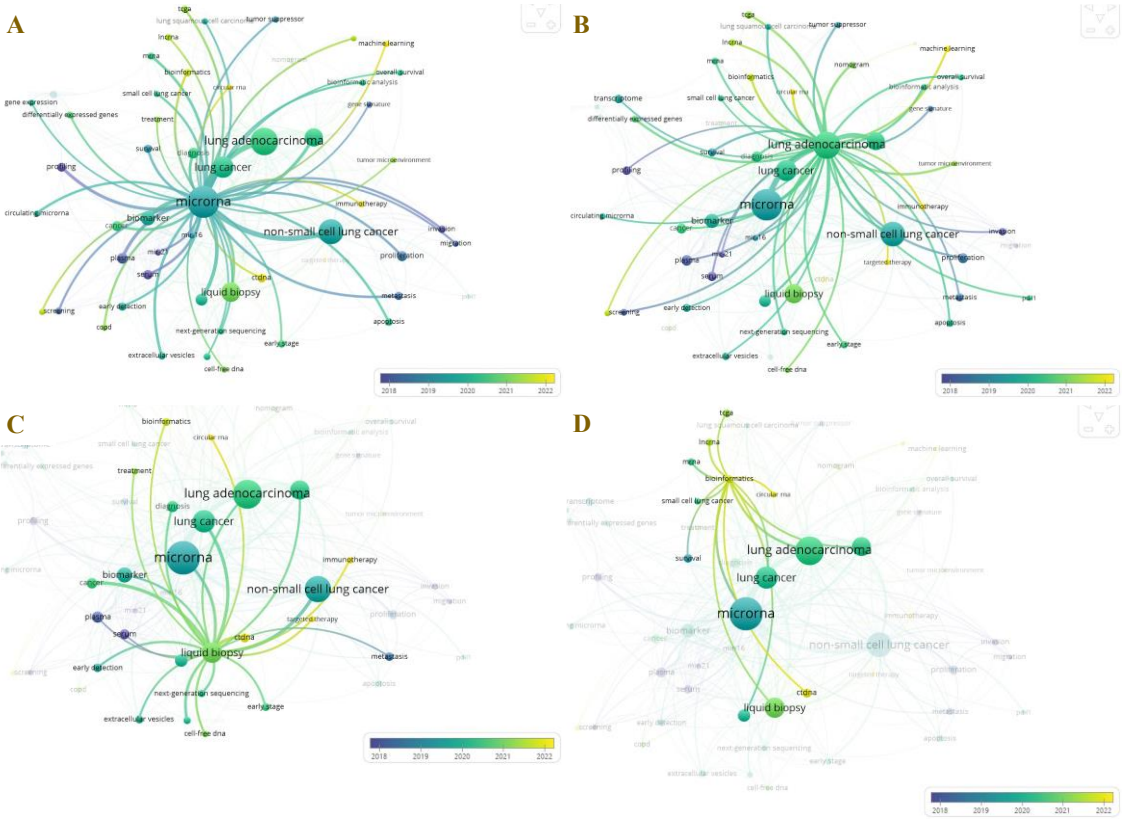

Figure 5. Overlay visualization depicting the temporal development of keywords and representing emerging research trends in published articles on lung adenocarcinoma and microRNA between 2013 and 2024. The interconnection use of the keywords based on the publication period focuses on microRNA (A), lung adenocarcinoma (B), liquid biopsy (C), and bioinformatics (D).

The keyword "lung adenocarcinoma" was frequently used alongside "profiling" and "screening" methods, "plasma" and "serum" specimens, and cancer characteristics such as "proliferation," "metastasis," and "invasion" in articles published between 2013 and 2018 (**Figure 5B**). More recent research (2022–2024) connected "lung adenocarcinoma" with the identification of biomarkers like "lncRNA" and "circular RNA" as well as with "machine learning," "immunotherapy," "targeted therapy," and "bioinformatics". This trend indicated that recent studies increasingly used bioinformatics and machine learning to identify lung adenocarcinoma biomarkers, focusing on biomarkers involving lncRNA and circular RNA to advance immunotherapy and targeted therapy approaches.

The keyword "liquid biopsy" was most commonly found in articles published in 2021 (**Figure 5C**). The use of "liquid biopsy" with the keywords "plasma" and "serum" had developed in articles published before 2018, indicating that these two types of specimens had been widely used for a long time. Meanwhile, the use of "liquid biopsy" to search for miRNA biomarkers in lung cancer was frequently found in articles from 2019 to 2020. "Liquid biopsy," along with keywords such as "ctDNA," "targeted therapy," "immunotherapy," "circRNA," and "bioinformatics," appeared often in the latest articles, from 2021 to 2024 (**Figure 5D**). The keyword "bioinformatics" was most frequently found in articles in 2022, showing that bioinformatics analysis has become a common tool in contemporary research.

Trends in research topic

Bibliometric analysis of miRNA biomarkers in lung adenocarcinoma, conducted using the R-studio and Biblioshiny platforms (**Figure 6**), revealed 19 nodes representing research trends from 2013 to 2024. The keyword 'miR-21' appeared in articles from 2014 to 2018, with the highest frequency in 2017, indicating that miR-21 is an established research finding. Research involving plasma and serum specimens was conducted from 2016 to 2020, with the highest number of articles in 2018. Studies related to cancer characteristics such as metastasis, proliferation, and profiling were found in articles from 2016 to 2021, with a peak in 2019. The keywords "microRNA," "non-small cell lung cancer," and "exosome" followed similar trends, appearing in articles from 2018 to 2023, with the most frequent use in 2020. The keywords "microRNA" and "non-small cell lung cancer" had the largest node areas, indicating their widespread use in review articles compared to other keywords. The keywords "liquid biopsy," "lung cancer," and "lung adenocarcinoma" followed similar development trends, being present in articles from 2019 to 2023. Similarly, the keywords "TCGA," "ctDNA," and "biomarker" appeared in articles from 2021 to 2023. The keywords "COPD," "immunotherapy," and "machine learning" were most common in articles from 2023. This analysis highlights the consistency of keyword usage trends over the years, as shown by both VOSviewer and Biblioshiny bibliometric analysis [39].

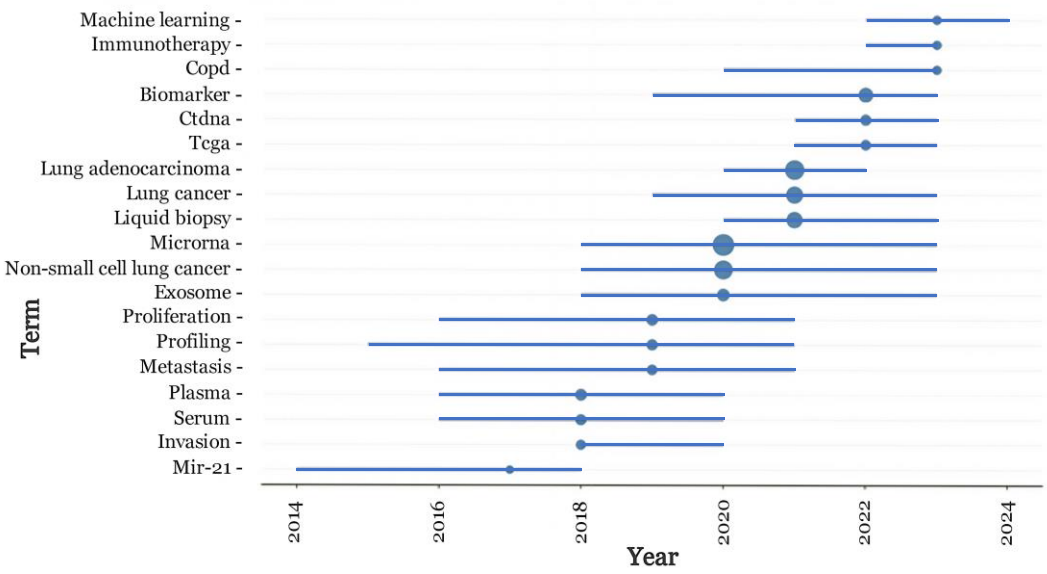

Figure 6. Research trends on the topic of miRNA biomarkers in lung adenocarcinoma between 2013 and 2024. The data reveal a strong association between microRNA, non-small cell lung cancer, and exosome, as evidenced by their frequent co-occurrence in numerous articles. Lung cancer, liquid biopsy, and lung adenocarcinoma also exhibit a high degree of co-occurrence, particularly during the 2018–2023 period, as indicated by their large node size and substantial number of publications. While machine learning, immunotherapy, and COPD demonstrate a growing interconnectedness, their relatively low frequency suggests ongoing development in this research area from 2022 to 2024.

Evolution of research topics based on keyword co-occurrence

Bibliometric analysis was conducted to understand the conceptual relationships between topics that appeared in articles using thematic evolution. This approach provided information on keyword changes from year to year (**Figure 7**). The keyword "non-small cell lung cancer" was widely used in articles from 2013 to 2018. These keywords evolved into "microRNA," "prognosis," and "profiling" in 2019–2020, while the keyword "microRNA" resulted from the evolution of several keywords from 2013 to 2018, including "non-small cell lung cancer," "early stage," "lung cancer," "metastasis," "microRNA," and "liquid biopsy". The keywords "microRNA" further evolved into four different keywords: "microRNA," "lung adenocarcinoma," "apoptosis," and "machine learning" from 2021 to 2024. This indicated that microRNA research was increasingly integrated with machine learning analysis. Meanwhile, the keyword "microRNA" in 2021–2024 resulted from the evolution of several keywords from 2019–2020, including "microRNA,"

"transcriptome," "ctDNA," "prognosis," "next-generation sequencing," "treatment," "digestion," "profiling," "non-small cell lung cancer," and "circulating microRNA". This showed that the keyword "microRNA" underwent the most evolution of all the keywords. The use of the keywords "liquid biopsy" and "transcriptome" remained unchanged from 2013 to 2024. Lastly, the keyword "lung adenocarcinoma" in 2021–2024 resulted from the development of several keywords from 2019–2020, including "microRNA," "prognosis," "digestion," and "RNA sequencing".

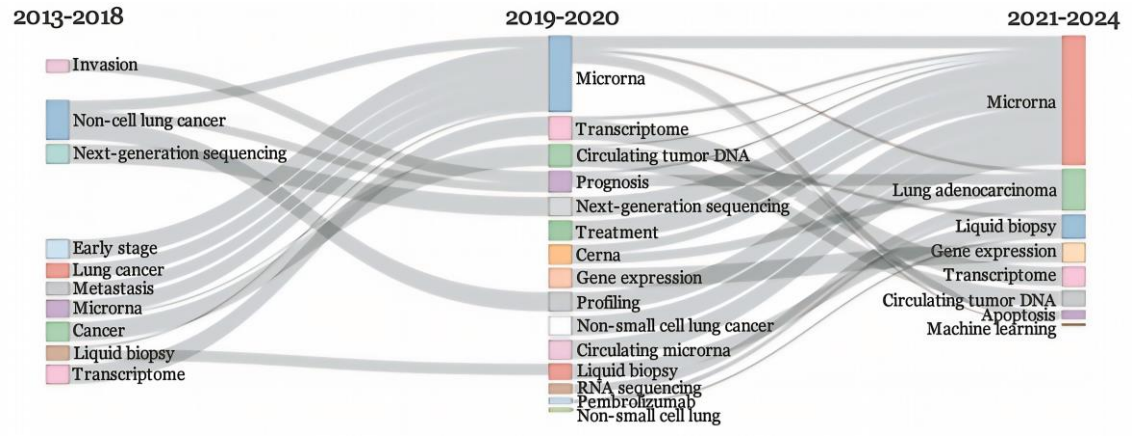

Figure 7. Themes evolution according to the shared keyword usage in the published articles on lung adenocarcinoma and microRNA between 2013 and 2024.

Keyword distribution in research networks: Motor, niche, emerging, and declining themes

Based on Biblioshiny analysis, eleven keyword clusters were identified and grouped into four thematic quadrants that reflect current trends and developments in the field. These quadrants categorize the topics into basic, motor, niche, and emerging or declining themes. The complexity, depth, and relevance of each topic as a research focus are depicted on the development degree line (**Figure 8**), while the relevance degree line on the thematic map measures the connection between topics.

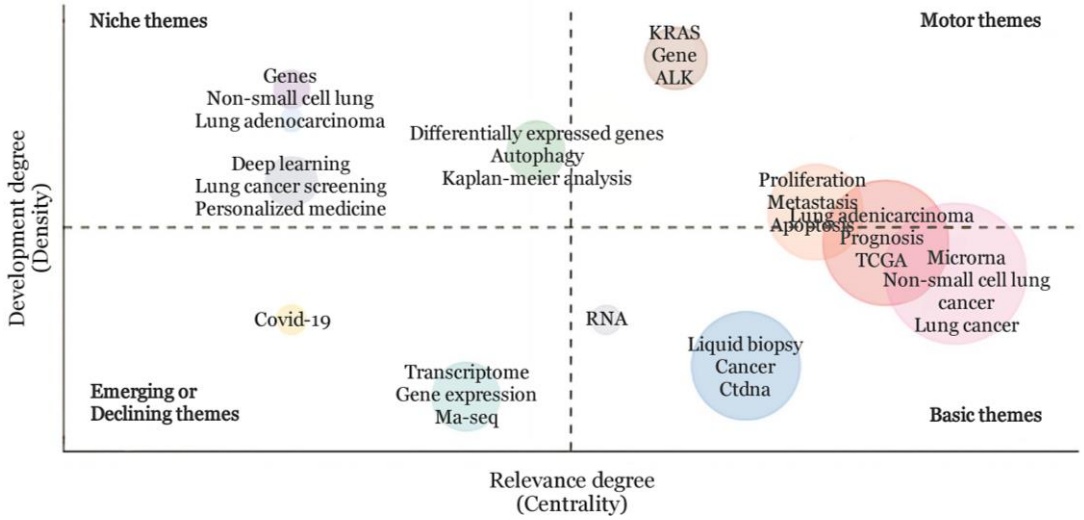

Figure 8. Bibliometric analysis using author keywords to describe the thematic map. The analysis quadrant showcases basic themes with high-density, yet low-centrality research topics, indicating in-depth study but limited interconnectivity. The clustering of liquid biopsy, cancer, and ctDNA within a single node underscores their relevance within a particular study. Node size reflects keyword frequency. In contrast, motor themes exhibit both high centrality and density, signifying their pivotal role in the broader research network and in-depth exploration. These themes drive research trends, underpin scientific advancement, and foster collaboration. Emerging themes represent nascent research areas with potential future prominence, while declining themes, once significant, now have diminished relevance due to low centrality and density.

Basic theme quadrant

This quadrant highlights four main topic groups with varying levels of complexity and interconnectedness: the first topic group consists of "microRNA," "non-small cell lung cancer," and "lung cancer". This group is noted for its high complexity and interconnectedness. The second topic group, "lung adenocarcinoma," "prognosis," and "TCGA," explains complex topics that are well-integrated. The third topic group consists of "proliferation," "metastasis," and "apoptosis". This group also shows high levels of depth and interconnectedness. The fourth topic group explains the latest studies on "liquid biopsy," "cancer," and "ctDNA," which are less complex than the previous thematic groups. The miRNA topic, specifically as a biomarker in lung adenocarcinoma, is prominent in the basic theme, aligning well with the focus of the article search.

Motor theme quadrant

This quadrant includes key driving topics that influence research directions in lung adenocarcinoma biomarkers: "KRAS" and "ALK". Both genes serve as major driving variables in lung adenocarcinoma biomarker research and have a high level of relevance to the overarching study topic. Furthermore, other keyword groups consisting of "proliferation," "metastasis," and "apoptosis" are quite relevant but have not yet become the core focus of emerging research. Additionally, studies related to these keywords are likely not deeply consolidated locally (low density), meaning that scientific discussions on this topic have not yet become highly specific or well-established. However, their movement toward motor themes indicates that proliferation, metastasis, and apoptosis are key mechanisms regulated by microRNA. In other words, microRNAs involved in these processes are beginning to emerge as a more dominant subject of study due to their relevance in molecular cancer therapy.

Niche theme quadrant

The niche theme quadrant for miRNA research in lung adenocarcinoma includes three specific topic groups that hold potential for targeted research: “differential gene expression,” “autophagy,” and “Kaplan-Meier analysis.” These topics are critical for understanding miRNA's role in lung adenocarcinoma, as differential expression and autophagy provide insights into cellular conditions that contribute to cancer development. Kaplan-Meier analysis is frequently used to conduct survival analysis in these studies. Research has shown that miRNA expression fluctuates according to cellular and environmental conditions, with specific miRNA profiles linked to malignancy. As such, miRNA is often used as a marker for early cancer diagnosis. The keywords "deep learning," "lung cancer screening," and "personalized medicine" are grouped into another topic that specifically has high relevance and complexity to the study.

Emerging or declining themes quadrant

The analysis of emerging or declining themes could provide insight into evolving or potentially less-researched areas. The emerging or declining theme quadrant contains the topics "RNA-seq," "gene expression," and "transcriptome" which are emerging topics and have high relevance to the study.

The thematic analysis underscores the complex interconnections and the varied relevance of keywords within lung adenocarcinoma research. miRNA profiling in diseases like lung cancer varies across regions, reflecting different miRNA expression profiles under various conditions and reinforcing its potential as a biomarker [40,41]. Cell conditions that lead to malignancy have a specific profile of miRNA involved, so miRNA is often used as a marker for early diagnosis of cancer. miRNA profiling in several types of disease, especially lung cancer, in various regions is very varied (**Table 1**).

The research topic of using miRNA as a biomarker for lung adenocarcinoma has been extensively studied. Between 2013 and 2024, the scope of this research area has expanded, with an increasing focus on related keywords. Key areas of interest now include the use of liquid biopsy specimens and advancements in personalized medicine. Studies have thoroughly investigated the role of miRNA in determining proliferation, metastasis, and apoptosis. However, there remains potential for further research in areas such as gene expression analysis, transcriptomics, and RNA sequencing.

Table 1. Previous studies assessing the roles of microRNA (miRNA) and their profile in lung cancers

Role of microRNAs	Patients and number	Method	Reference
Four EV-derived miRNAs (hsa-miR-106b-3p, hsa-miR-125a-5p, hsa-miR-3615, and hsa-miR-450b-5p) can be used to develop early detection of lung adenocarcinoma	A total of 460 plasma samples from 254 stage one-lung adenocarcinoma and 76 benign pulmonary patients in a Chinese-Japanese population (Peking Union Medical College Hospital and China-Japan Friendship Hospital) were used for extracellular vesicles	miRNA sequencing	[42]
Serum miR-133a-3p, miR-584-5p, miR-10b-5p, and miR-221-3p could improve the diagnostic ability of lung adenocarcinoma	Twenty-four lung adenocarcinomas and 24 healthy controls from Nanjing Hospital and Jiangsu Cancer Hospital, China for profiling. Then, 110 lung adenocarcinomas vs 110 healthy controls for validation	Pooling profiling using Exiqon miRNA qPCR panel	[43]
Serum miR-1228-3p and miR-181a-5p have the potential to be non-invasive biomarkers for the diagnosis and prognosis of NSCLC patients	NSCLC patients (from Qilu Hospital, Shandong University) using serum specimens	In silico analysis (and validation using qRT-PCR	[44]
Exosome miRNAs (miR-151a-5p, miR-10b-5p, miR-192-5p, miR-106b-3p, and miR-484) have the potential to be developed as prognostic markers in lung adenocarcinoma	Six lung adenocarcinoma patients before and after surgery, and six healthy individuals taken from Chinese PLA General Hospital and Beijing Shijitan Hospital of Capital Medical University in Beijing	qRT-PCR	[45]
Twenty-one miRNAs (let-7b-5p, miR-197-3p, miR-513a-5p, miR-940, miR-3620-3p, miR-3679-3p and others) were upregulated and three (miR-4293, miR-3915, and miR-4476) were downregulated in lung adenocarcinoma	Stage IA lung adenocarcinoma patients from Zhejiang Rongjun Hospital (Jiaxing, China) and Shaoxing People's Hospital (Shaoxing, China)	TaqMan low-density array analysis	[46]
Upregulation of exosomal miRNAs (miR-23b-3p, miR-10b-5p and miR-21-5p) was associated with poor overall survival and had the potential to be developed as a non-invasive prognostic biomarker in NSCLC	Plasma exosomal miRNA from ten NSCLC patients and ten healthy controls were taken from Xinqiao Hospital of Third Military Medical	qPCR array panel to analyze 84 plasma exosomal miRNAs	[47]
Plasma exosomal miRNAs (miR-21 and miR-4257) had the potential to be developed as predictive biomarkers for NSCLC recurrence	Plasma exosomal miRNA of six patients from the Department of Surgery, Teikyo University, Japan. Validation of miRNA in 195 NSCLC compared to 30 healthy controls	Microarray-based expression profiling pada miRNA	[48]
miR-21 and miR-188 were overexpressed and associated with poor prognosis in NSCLC	Tumor tissue and healthy tissue (32 samples) from Consorcio Hospital General Universitario de Valencia, Spain	Sequencing oligo ligation detection technology	[49]
miR-411, miR-370, and miR-376a were associated with poor survival after lung cancer tissue harvesting	Ninety NSCLC patients and 10 normal tissues were taken from the University of Michigan Health System, USA	PCR-based array	[50]

EV: extracellular vesicles; NSCLC: non-small cell lung cancer; qRT-PCR: quantitative reverse transcription polymerase chain reaction

Discussion

MiRNAs (miRNAs) are non-coding RNAs typically 18–24 nucleotides in length [51,52]. They function as regulators of gene expression and can act as either oncogenic miRNAs (oncomiRs), which promote cancer progression, or tumor suppressor miRNAs, which inhibit cancer cell development [53]. OncomiRs typically target tumor suppressor genes, whereas tumor suppressor miRNAs target oncogenes. Notably, a single type of miRNA can regulate multiple mRNAs, and a single mRNA can be targeted by various miRNAs. Due to their regulatory roles and association with cancer progression, miRNAs are frequently used as biomarkers for disease, particularly in cancer diagnostics and prognosis [54].

MiRNA (miRNA) is a component of the transcriptome, with miRNA genes located in exons (10%), introns (40%), and intergenic regions (50%) within eukaryotic genomes. In eukaryotes, miRNA genes are transcribed by RNA polymerase II in the nucleus, producing primary miRNA (pri-miRNA). The stem-loop structure of pri-miRNA is recognized by the nuclear microprocessor complex, composed of two core proteins: Drosha and DiGeorge syndrome critical region gene (DGCR8) [55]. DGCR8 binds to the stem region of the pri-miRNA hairpin, facilitating Drosha's attachment, which cleaves pri-miRNA to generate precursor miRNA (pre-miRNA) [56,57]. DGCR8 binds to the stem region of the pri-miRNA hairpin, facilitating Drosha's attachment, which cleaves pri-miRNA to generate precursor miRNA (pre-miRNA) [58-60].

Gene regulation by miRNAs involves several mechanisms: inhibition of translation when the miRNA binds incompletely to the mRNA, mRNA degradation if binding is complete, and shortening of both the poly-A tail and the mRNA cap [61]. MiRNA expression is closely associated with pathophysiological processes in humans, making it a valuable biomarker for disease diagnosis, including in cancer. Cancer development, in particular, results from an imbalance between oncogenes and tumor suppressor genes, both of which are regulated by miRNAs [54,62-64]. Cancer cells express higher levels of oncomiRs compared to healthy cells. MiRNAs secreted by cancer cells can also enter healthy cells, influencing the tumor microenvironment. Once released, these miRNAs circulate through the bloodstream, allowing for detection via liquid biopsy. Liquid biopsy is a non-invasive method for collecting biological specimens through body fluids, such as serum, plasma, urine, saliva, semen, ascites, and cerebrospinal fluid (CSF).

Bibliometric analysis shows that most studies identifying miRNAs in lung adenocarcinoma patients have used serum and plasma specimens (**Figure 3** and **Table 1**). Several studies on lung cancer have identified similar miRNAs, notably miR-10b-5p, miR-21, and miR-106b-3p (**Figure 3** and **Table 1**). These miRNAs have potential as candidate biomarkers for lung cancer diagnosis, though they must be used in conjunction with other miRNAs for greater diagnostic accuracy. Bibliometric findings on miRNA research in lung cancer suggest further opportunities, such as identifying miRNA profiles that are specific to certain environments and populations. Lung cancer, which has the highest incidence and mortality rate in Indonesia, is often diagnosed at an advanced stage, where treatment options are limited, resistance to chemotherapy is common, and the prognosis is poor.

Lung cancer histopathology is divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC having a higher global incidence. NSCLC includes subtypes such as adenocarcinoma, large cell carcinoma, and squamous cell carcinoma, with lung adenocarcinoma being the most common. Lung adenocarcinoma typically develops in cells near the alveoli and may produce mucus [65]. The incidence of lung adenocarcinoma is associated with both active and passive smoking, as well as former smokers. Chemical compounds in tobacco, including tobacco-specific nitrosamines (TSNAs) and nicotine-derived NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), induce epigenetic changes such as hypermethylation in tumor suppressor gene promoters and alterations in miRNA expression [66-70]. One example is the tumor suppressor gene TP53, which often mutates in ways that disrupt cell cycle regulation in lung adenocarcinoma. Additionally, lung adenocarcinoma frequently features mutations in KRAS, a gene encoding the GTPase RAS protein. When activated (GTP-RAS), this protein stimulates various isoforms involved in cell growth [71,72]. However, certain tumor suppressor miRNAs, such as let-7a and let-7b, can inhibit KRAS, thereby potentially countering cancer progression [73].

One hallmark of cancer is increased cell proliferation, often driven by the mitogen-activated protein kinase (MAPK) pathway, which is regulated by miR-3613-5p in lung adenocarcinoma [74]. The expression of miR-3613-5p is induced by nuclear factor kappa B (NF-κB) through its interaction with JUN, leading to an upregulation of v-akt murine thymoma viral oncogene homolog (AKT)/MAPK expression [74]. Along with sustained proliferation, lung adenocarcinoma cells also exhibit anti-apoptotic characteristics [75]. This is due to the downregulation of miRNAs that normally regulate apoptosis-related genes, often resulting from gene deletion, epigenetic silencing, or loss of expression in transcription factors.

For example, transfection of miR-7 in the A549 cell line has been shown to reduce B-cell lymphoma 2 (BCL-2) mRNA, thereby increasing the expression of caspase 3 and caspase 7. However, miR-7 is typically downregulated in lung adenocarcinoma, impairing its role in promoting apoptosis in cancer cells [76]. Apoptosis and suppression of invasion can also be regulated by miR-335, which targets BCL-2 and directly targets the specificity protein 1 (SP1) gene. SP1, a member of the Sp/Kruppel-like family of transcription factors, activates promoters of various genes involved in proliferation, apoptosis, and the cell cycle [77]. In addition, miR-608 regulates cell death in the A549 cell line by targeting BCL-XL [78,79]. miR-26a is associated with genes involved in metastasis, particularly through its interaction with matrix metalloproteinases (MMPs), which play key roles in cellular adhesion, invasion, and migration in cancer cells. MMP2, for instance, shows elevated expression in cancer cells and is critical to metastatic behavior [80].

MiRNA expression in lung adenocarcinoma can be analyzed in relation to targeted genes using machine learning. Bioinformatics applications in pathway analysis for lung adenocarcinoma carcinogenesis enhance research precision by focusing on specific target markers. Liquid biopsy, a non-invasive method for collecting specimens from body fluids, is widely used in cancer diagnosis, particularly for analyzing circulating tumor cells, ctDNA, RNA, and miRNAs, as well as material found in extracellular vesicles [81]. Common specimen sources include serum, plasma, saliva, urine, pleural fluid, peritoneal fluid, nasal fluid, and cerebrospinal fluid (CSF) [82]. Although liquid biopsies generally yield lower quantities of biological material than tissue biopsies, this limitation can be addressed by selecting an appropriate extraction method [83]. Additionally, targeting extracellular vesicles as an extraction focus is advantageous because they protect biological content from degradation [84]. EVs, which include RNA, DNA, non-coding RNAs, and proteins, provide valuable material for cancer screening and early diagnosis [85,86]. One subtype of extracellular vesicle, the exosome, is approximately 30–150 nm in size and can traverse the blood-brain barrier and enter recipient cells. Exosomes facilitate cell-to-cell communication in both healthy and cancerous cells; when exosomes from cancer cells enter healthy cells, they release molecular content that disrupts the genetic and epigenetic balance in these recipient cells [87-91].

Liquid biopsies are also essential for monitoring cancer progression, evaluating treatment response, and detecting recurrence, enabling analysis of tumor clonal evolution over time and across different body regions [92]. This minimally invasive approach also reduces the risk of necrosis associated with specimen collection in advanced cancer stages [93]. The US Food and Drug Administration (FDA) has approved the Therascreen PIK3CA RGQ PCR assay for detecting phosphoinositide-3-kinase catalytic subunit alpha (PIK3CA) mutations through liquid biopsy, following clinical trials demonstrating its efficacy for patients with hormone receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated, or metastatic breast cancer. Liquid biopsy thus serves as a viable alternative to traditional tissue biopsy, bringing cancer diagnostics closer to routine clinical practice [94-96].

Conclusion

Our bibliometric analysis highlights miRNA, lung adenocarcinoma, liquid biopsy, and bioinformatics as the most extensively researched areas in the study of miRNA as a candidate biomarker for lung adenocarcinoma. The scope of miRNA research is vast and is expected to continue expanding through 2024, with significant potential in areas such as lung cancer screening, differentially expressed genes, personalized medicine, and deep learning. The development of miRNA biomarkers offers promising applications in the screening and early diagnosis of adenocarcinoma. The ability to measure miRNA expression through liquid biopsy

specimens enhances its clinical utility, as exosome-derived miRNAs are protected from degradation and allow for a minimally invasive approach. While miRNA profiling in adenocarcinoma has demonstrated the potential to produce specific molecular signatures, challenges remain, particularly with the variation in miRNA expression across different populations. This gap underscores the need for further research, especially in the context of personalized medicine, to ultimately achieve precision.

Ethics approval

Not required.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies in manuscript writing support: AI-based language models using DeepL, were employed for Language refinement (improving grammar, sentence structure, and readability of the manuscript).

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