Revista Colombiana de
Hematología y Oncología
Enero - Junio de 2025 • Volumen 12 - Número 1

ORIGINAL

piRNAs and PIWI-like proteins in cancer and their future as biomarkers and therapeutic targets in lung cancer: a systematic review

piARNs y proteínas tipo PIWI en cáncer y su futuro como biomarcadores y objetivos terapéuticos en cáncer de pulmón: una revisión sistemática

»Jheremy Sebastian Reyes Barreto [1]
»María Alejandra Rodríguez Brilla [1]
»Laura Lucia Páez García [1]
»Ana Margarita Baldión Elorza [1,2]

[1] Cancer and Molecular Medicine Research Group (CAMMO), Bogotá, Colombia.
[2] Fundación Santa Fe de Bogotá, Bogotá, Colombia

Recibido el 29 de agosto de 2024; aceptado el 09 de junio de 2025

https//doi.org/10.51643/22562915.717

Abstract

Introduction: This systematic review evaluates the current evidence on the role of PIWI-interacting RNAs (piRNAs) in lung cancer, emphasizing their diagnostic and therapeutic potential. Lung adenocarcinoma, a major global health concern, necessitates exploration of alternatives to traditional methods. piRNAs, small non-coding RNAs, are abnormally expressed in cancerous tissues and biological fluids, indicating their potential as biomarkers and therapeutic targets. **Methods:** A comprehensive search was performed in PubMed and ScienceDirect databases according to PRISMA guidelines. The search focused on studies examining piRNA expression, their diagnostic value in LUAD tissues and extracellular vesicles, and their therapeutic implications. Studies published from 2020 onward were included and evaluated for bias and quality. **Results:** Out of nineteen initially identified papers, five studies met the inclusion criteria. These studies identified specific piRNAs with elevated expression in LUAD, such as piR-hsa-26925 and piR-hsa-5444, which showed strong diagnostic performance (AUC = 0.833). Additionally, piRNAs from extracellular vesicles, including piR-hsa-164586, demonstrated potential for early detection of Non-Small Cell Lung Cancer (AUC = 0.624). **Conclusions:** piRNAs show promise as non-invasive biomarkers for early diagnosis and

*** Autor para correspondencia:** Jheremy Reyes. Cancer and Molecular Medicine Research Group (CAMMO), Bogotá, Colombia.
Correo electrónico: js.reyesb@uniandes.edu.co
Doi: https//doi.org/10.51643/22562915.717

potential therapeutic targets in lung cancer. Further research is needed to validate these findings and understand the underlying mechanisms to improve clinical applications.

Keywords: PIWI-interacting RNAs; piRNAs; lung cancer; biomarkers; therapeutic targets.

Resumen

Introducción: Esta revisión sistemática evalúa la evidencia actual sobre el papel de los ARN interactuantes con PIWI (piRNAs) en el cáncer de pulmón, con énfasis en su potencial diagnóstico y terapéutico. El adenocarcinoma de pulmón, una importante preocupación global de salud, requiere explorar alternativas a los métodos tradicionales. Los piRNAs, ARN no codificantes pequeños, se expresan de manera anormal en tejidos cancerosos y fluidos biológicos, lo que indica su potencial como biomarcadores y objetivos terapéuticos. **Métodos:** Se realizó una búsqueda exhaustiva en las bases de datos PubMed y ScienceDirect, siguiendo las directrices PRISMA. La búsqueda se centró en estudios que examinaran la expresión de piRNA, su valor diagnóstico en tejidos LUAD y vesículas extracelulares, y sus implicaciones terapéuticas. Se incluyeron estudios publicados a partir de 2020 y se evaluaron por sesgo y calidad. **Resultados:** De los diecinueve artículos inicialmente identificados, cinco estudios cumplieron con los criterios de inclusión. Estos estudios identificaron piRNAs específicos con expresión elevada en LUAD, como piR-hsa-26925 y piR-hsa-5444, que mostraron un fuerte rendimiento diagnóstico (AUC = 0.833). Además, los piRNAs derivados de vesículas extracelulares, incluyendo piR-hsa-164586, demostraron potencial para la detección temprana del cáncer de pulmón de células no pequeñas (AUC = 0.624). **Conclusiones:** Los piRNAs muestran promesa como biomarcadores no invasivos para el diagnóstico temprano y objetivos terapéuticos en el cáncer de pulmón. Se necesita más investigación para validar estos hallazgos y comprender los mecanismos subyacentes para mejorar las aplicaciones clínicas.

Palabras clave: proteínas similares a PIWI; piRNAs; cáncer de pulmón; biomarcadores; objetivos terapéuticos.

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all cases. Among its histological subtypes, lung adenocarcinoma (LUAD) is the most prevalent, and despite advances in diagnosis and treatment, the five-year survival rate remains below 20% in most populations [1–3]. These grim statistics highlight an urgent need for more sensitive, specific, and minimally invasive biomarkers that can support early detection, real-time monitoring, and individualized therapy. Current clinical biomarkers such as carcinoembryonic antigen (CEA) and cytokeratin-19 fragment (CYFRA21-1) have limited diagnostic sensitivity and prognostic power, especially in early stages of disease [4].

In this context, PIWI-interacting RNAs (piRNAs)—a class of small non-coding RNAs originally described in germline cells—have

emerged as promising molecular tools in cancer research. These RNAs, typically 24–32 nucleotides in length, exert epigenetic and post-transcriptional regulatory functions by interacting with PIWI-like proteins and modulating gene expression through transposon silencing, chromatin remodeling, and RNA stability [5,6]. Although initially believed to be restricted to reproductive tissues, piRNAs have been increasingly detected in somatic cells and, more importantly, in tumor tissues and extracellular fluids of cancer patients [7–8].

Recent evidence has shown that specific piRNAs are aberrantly expressed in LUAD, with some displaying tumor-suppressive activity and others acting as oncogenic drivers depending on their molecular context. Their stability in circulation and presence in extracellular vesicles further enhances their attractiveness as non-invasive biomarkers for early detection [9–11]. Moreover, piRNAs have been implicated in crucial processes such as epithelial-mesenchymal transition, proliferation, immune evasion, and chemoresistance, positioning them as potential therapeutic targets [12].

Despite these promising findings, the role of piRNAs in lung cancer remains poorly characterized, and a comprehensive synthesis of available evidence is lacking. Given the rapid expansion of research in this field, a systematic review is warranted to critically evaluate their diagnostic accuracy, biological relevance, and translational potential.

This systematic review aims to (i) synthesize the available evidence regarding the expression profiles of piRNAs in LUAD and NSCLC, (ii) assess their diagnostic performance in tumor tissues and biological fluids, particularly serum-derived extracellular vesicles, (iii) explore their associations with clinical features such as disease stage and survival, and (iv) examine their proposed roles as therapeutic targets through modulation of PIWI proteins and downstream oncogenic pathways. In doing so, this review intends to contextualize piRNAs within the current diagnostic landscape, compare them with existing biomarkers, and identify knowledge gaps that require further exploration.

By consolidating current findings and highlighting the clinical implications of piRNAs in lung cancer, this work aspires to pave the way for future translational studies, the development of standardized detection assays, and the eventual integration of piRNA panels into liquid biopsy-based platforms for early diagnosis and personalized therapy.

Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines [13,14] and was registered in the PROSPERO database (Registration ID:1060469) to ensure transparency and methodological rigor. The aim was to comprehensively evaluate the role of PIWI-interacting RNAs (piRNAs) and PIWI-like proteins in lung cancer, with a focus on their diagnostic, prognostic, and therapeutic potential. All methodological phases—including literature search, study selection, data extraction, synthesis, and quality assessment—were performed systematically and independently by multiple reviewers, as detailed below.

Eligibility Criteria

Eligible studies included both experimental and observational designs that investigated the expression, function, or clinical relevance of piRNAs or PIWI-like proteins in lung tumors. We considered studies exploring their role in diagnosis, prognosis, or treatment of lung cancer.

Inclusion criteria:

- Studies reporting on the expression or function of piRNAs and/or PIWI-like proteins in lung cancer (LUAD, NSCLC, or general lung cancer).
- Articles evaluating diagnostic or prognostic value of piRNAs (e.g., sensitivity, specificity, AUC).
- Studies exploring the therapeutic relevance of piRNAs or PIWI proteins.
- Original articles published in peer-reviewed journals since January 2020.
- Publications available in English or Spanish.

Exclusion criteria:

- Studies not involving piRNAs or PIWI-like proteins.
- Articles not focused on lung cancer or lacking primary data.
- Case reports, case series, reviews, commentaries, editorials, abstracts, or letters to the editor.
- Preclinical studies without biomarker or therapeutic analysis.
- Articles published before 2020 or in languages other than English or Spanish.

Information Sources and Search Strategy.

The literature search was first conducted in June 2024 and updated in July 2024 using the following databases: PubMed, ScienceDirect, EMBASE, and Web of Science. Additionally, Google Scholar was used to identify potential grey literature. The search strategy combined Medical Subject Headings (MeSH) and free-text terms with Boolean operators: ("PIWI-like protein" OR "piwi" OR "piRNA" OR "PIWI-interacting RNA") AND ("cancer" OR "neoplasm" OR "tumor") AND ("lung cancer" OR "lung neoplasm" OR "pulmonary cancer" OR "pulmonary neoplasm") AND ("biomarker" OR "therapy target" OR "therapeutic target").

Selection Process.

The study selection process followed three phases: identification, screening, and inclusion. Titles and abstracts were screened independently by two reviewers (JSR and MAR) using Rayyan software[15]. Studies were categorized as "Include", "Maybe", or "Exclude". Disagreements were resolved through discussion or, when necessary, adjudicated by a third reviewer (AMB). Full texts of selected studies were then assessed for final eligibility. Inclusion decisions were made by consensus, and exclusion reasons were documented.

Data Collection Process and Extracted Items.

Data were extracted independently by two reviewers using a structured Excel spreadsheet. The following data were collected:

- Author(s), year of publication, and title.
- Study design and methodology.
- Tumor type and sample population (patients, cell lines, animal models).
- Type and identity of piRNAs or PIWI-like proteins analyzed.

– Diagnostic metrics (AUC, sensitivity, specificity, ROC curves).

– Functional findings and clinical implications.

Discrepancies in data extraction were resolved by consensus with the remaining authors.

Risk of Bias Assessment.

The methodological quality of included studies was evaluated using appropriate tools based on study design. Non-randomized studies were assessed using the Newcastle-Ottawa Scale (Table 1). AMSTAR, CONSORT, and SYRCLE scales were not used to evaluate the studies.[16,17,18] Quality assessment aimed to evaluate risk of bias, study validity, and overall reliability of findings

Table 1.

Newcastle-Ottawa Scale.

		Selection			Comparability			Exposure			
Author	Year	The case definition is adequate with independent validation	Consecutive or obviusly representative series of cases	Community controls	Controls with no history of disease (end point)	Cases and controls with comparable ages	Cases and controls with comparability on any other factors	Ascertainment of exposure using secure records (e.g. surgical records) or structured interviews with blinding to case/control statuses	Ascertainment of exposure using the same method for cases and controls	Ascertainment of exposure with non-response rate for both groups	Total Quality Score
Li J, Wang N	2021	*	*	*		*	*	*	*	*	8
Lin Y	2020	*	*	*	*		*	*	*	*	8
Cammarata G	2022	*	*	*	*	*	*	*	*		8
Li Y	2022	*	*	*	*	*	*	*	*	*	9
Xin XL	2022	*	*	*	*		*	*		*	7

Synthesis Methods and Evaluation of Evidence.

Due to heterogeneity in design, outcome measures, and biomarker evaluation techniques, a narrative synthesis approach was employed. Quantitative meta-analysis was not performed due to lack of standardized data; however, AUC values and other diagnostic metrics were reported where available. If feasible in future updates, meta-analysis of ROC curves using a random-effects model will be considered.

The certainty of the evidence was assessed using the GRADE approach, evaluating risk of bias, consistency, directness, precision, and publication bias.

Assessment of Publication Bias.

Given the small number of studies, formal funnel plot analysis was not feasible. Instead, we qualitatively evaluated the risk of publication bias by assessing study funding, selective outcome reporting, and availability of negative results.

Results

We searched two databases (PubMed and ScienceDirect) for relevant literature and identified 19 papers, of which only 8 met the inclusion criteria. After eliminating duplicates (n = 0), we screened titles and abstracts (n = 8), removing review articles and unrelated papers (n = 3). Ultimately, 5 studies were included in

this systematic review (Figure 1). The actual research analyzed 5 articles that revealed roles of piRNA and PIWI in Lung Cancer (Table 2).

Figure 1. PRISMA[13, 14] flow chart.

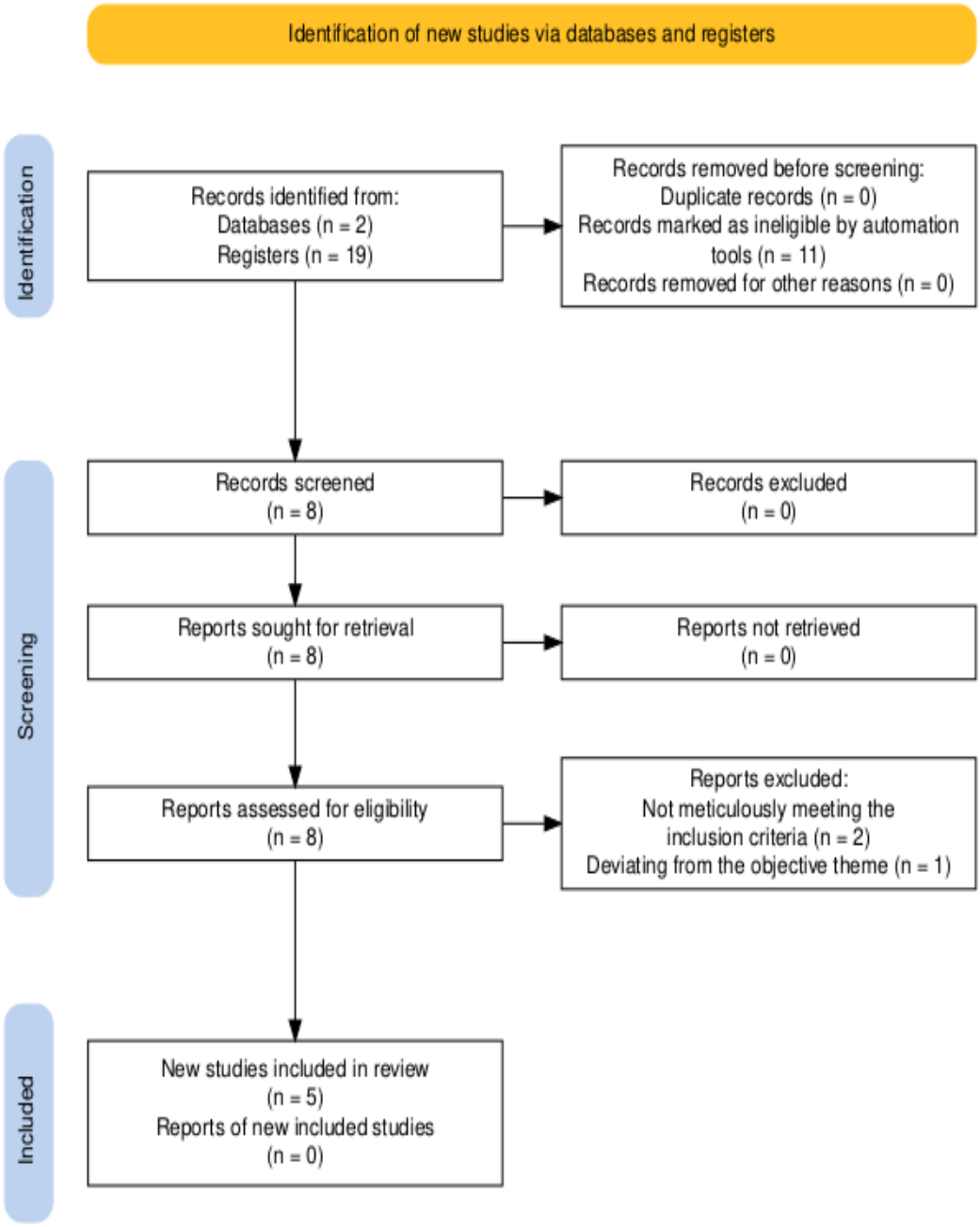


Table 2.
Characteristics of the reviewed studies

Author	piRNA	Type of Lung Cancer	Summary
Li J, Wang N	piR-hsa-26925, piR-hsa-5444	Lung Adenocarcinoma (LUAD)	The study investigated the expression profiles and diagnostic potential of piRNAs in LUAD. Small RNA sequencing identified 76 piRNAs with elevated expression and 9 with reduced expression in LUAD tissues. Notably, piR-hsa-26925 and piR-hsa-5444 were significantly upregulated in both LUAD tissues and serum exosome samples, showing promise as diagnostic biomarkers with an AUC of 0.833 for a 2-piRNA panel.
Lin Y	Various ncRNAs including piRNAs	General Lung Cancer	The study identified ncRNA profiles from bronchial epithelium of lung cancer patients versus cancer-free smokers using next-generation sequencing. It found significant differences in expression levels of various ncRNAs, including 13 piRNAs. SNHG9, an lncRNA, was highly expressed in lung tumor tissues and associated with poor overall survival.
Cammarata G	Various ncRNAs including piRNAs	General Lung Cancer	The review discussed the role of emerging regulatory ncRNAs in lung cancer. It highlighted the potential of ncRNAs, including piRNAs, as biomarkers for early detection, prognosis, and monitoring therapeutic strategies via liquid biopsy methods.
Li Y	piR-hsa-164586	Non-Small Cell Lung Cancer (NSCLC)	The study investigated serum-derived piRNAs from extracellular vesicles for early diagnosis of NSCLC. It identified piR-hsa-164586 as significantly upregulated in cancerous tissues and serum of NSCLC patients. The piR-hsa-164586 showed improved diagnostic performance over CYFRA21-1 with an AUC of 0.624 for stage I NSCLC.
Xin XL	PIWIL4, PIWIL2	Non-Small Cell Lung Cancer (NSCLC)	The study re-analyzed gene expression profiles from peripheral blood mononuclear cells of NSCLC patients treated with Liujunzi decoction. It identified central protein-coding genes, including PIWIL4 and PIWIL2, associated with the anticancer mechanisms of the treatment. The results suggest gene silencing by RNA and piRNA-related processes are involved in the decoction's therapeutic effects.

Li J and colleagues [19] conducted a transcriptomic study to explore the diagnostic utility of piRNAs in LUAD, using small RNA sequencing on tumor tissues and adjacent non-neoplastic lung samples. They identified a total of 76 overexpressed and 9 underexpressed piRNAs in LUAD tissues. Among the most significantly upregulated were piR-hsa-26925 and piR-hsa-5444, both of which were selected for further validation. These piRNAs were subsequently measured in serum-derived exosomes from LUAD patients and healthy controls using quantitative real-time PCR (qRT-PCR), confirming their elevated levels in circulation. A diagnostic panel combining both piRNAs was evaluated using receiver operating characteristic (ROC) analysis, yielding an area under the curve (AUC) of 0.833, demonstrating high sensitivity and specificity for LUAD detection. The study highlights the potential of circulating piRNAs as non-invasive biomarkers and supports their use in early detection strategies for LUAD [19].

In a study by Lin Y et al. [20], the authors analyzed the non-coding RNA (ncRNA) expression profiles in bronchial epithelial cells obtained from sputum samples of 32 lung cancer patients and 33 cancer-free smokers. Using next-generation sequencing (NGS), the authors identified significant expression changes in multiple classes of ncRNAs, including 13 piRNAs, 108 miRNAs, and 25 long non-coding RNAs (lncRNAs). Of particular interest, the lncRNA SNHG9 was markedly overexpressed in lung tumor tissues and was shown to inversely correlate with overall survival. Functional assays performed in vitro revealed that SNHG9 knockdown led to reduced cancer cell proliferation, growth, and invasion, suggesting a functional role in tumor progression. Although piRNAs were not functionally characterized in this study, their altered expression patterns support their potential involvement in lung carcinogenesis, especially when considered alongside deregulated ncRNA networks [20].

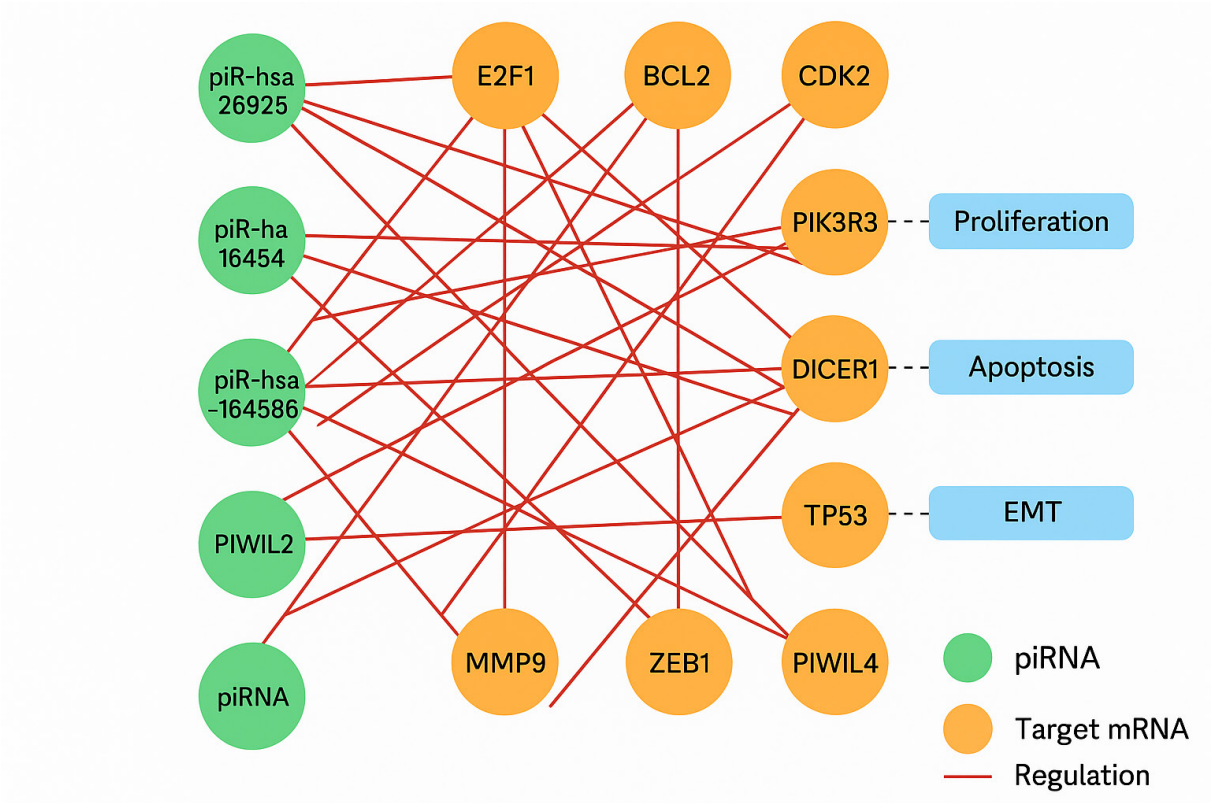
Cammarata G and colleagues [21] performed a systematic review of the role of extracellular vesicle (EV)-associated non-coding RNAs in lung cancer, emphasizing the diagnostic and prognostic potential of piRNAs among other regulatory ncRNAs. The review analyzed published studies focusing on liquid biopsy (LB) platforms, highlighting the use of EVs, circulating tumor cells, cell-free nucleic acids, and tumor-educated platelets as analytes. piRNAs were identified as emerging biomarker candidates due to their presence in exosomes and stability in peripheral blood. The authors discussed the integration of piRNAs into minimally invasive diagnostic workflows, especially in combination with other RNA species like miRNAs and circular RNAs. The review concluded that piRNAs may play a critical role in monitoring therapeutic response and disease progression, supporting further exploration of piRNA signatures in EVs as part of lung cancer management strategies [21].

Li Y et al. [22] investigated piRNAs isolated from serum-derived extracellular vesicles in patients with non-small cell lung cancer (NSCLC). Using a two-stage study design, they conducted high-throughput sequencing followed by clinical validation in a cohort of 115 NSCLC patients, including 95 with stage I disease, and 47 healthy controls. Among the piRNA candidates, piR-hsa-164586 emerged as significantly overexpressed in both tumor tissue and patient serum compared to controls. Diagnostic accuracy analysis revealed that piR-hsa-164586 outperformed CYFRA21-1, a commonly used clinical biomarker, with an AUC of 0.624 for stage I NSCLC. Furthermore, piR-hsa-164586 levels correlated with clinical parameters such as age and TNM stage, suggesting its utility not only for early detection but also for risk stratification and disease monitoring. These findings support the use of piRNAs in blood-based diagnostics, particularly for early-stage NSCLC [22].

Xin XL et al. [23] sought to elucidate the mechanisms of action underlying the anticancer effects of Liujunzi decoction, a traditional Chinese herbal formula, in advanced-stage NSCLC patients following first-line chemotherapy. The study involved re-analysis of gene expression profiles from peripheral blood mononuclear cells (PBMCs), identifying 162 differentially expressed genes (DEGs) associated with RNA regulation and immune signaling. Among the central genes highlighted were PIWIL2, PIWIL4, and DICER1, all of which are implicated in piRNA biogenesis and gene silencing. Functional enrichment analyses revealed that cytokine–cytokine receptor interaction pathways and RNA interference processes were enriched in the treatment group, suggesting that piRNA–PIWI axis modulation may be part of the herbal decoction's mechanism of action. Although the study did not measure piRNAs directly, it provides indirect evidence of piRNA-related pathways in therapeutic modulation of lung cancer [23].

Figure 2.

Network diagram illustrating the predicted regulatory interactions between selected piRNAs and their target mRNAs in lung cancer. The green nodes represent piRNAs identified in the reviewed studies (e.g., piR-hsa-26925, piR-hsa-164586, PIWIL2), while the orange nodes correspond to known or predicted mRNA targets (e.g., BCL2, TP53, DICER1). Red edges indicate proposed regulatory interactions based on literature and computational predictions. Associated biological processes—including proliferation, apoptosis, and epithelial-to-mesenchymal transition (EMT)—are annotated with blue labels connected to their respective mRNA effectors.



Discussion

The growing body of evidence on piRNAs and PIWI-like proteins has opened new perspectives in cancer biology, particularly in lung cancer. These small non-coding RNAs, initially studied in the germline, have been increasingly recognized for their roles in somatic tissues and tumorigenesis, with implications for diagnosis, prognosis, and treatment. Our systematic review consolidates current findings on their expression patterns, clinical relevance, and functional roles in lung cancer, while identifying critical gaps that must be addressed to enable their clinical translation (Figure 2).

From a diagnostic standpoint, several studies have demonstrated the potential of piRNAs as non-invasive biomarkers. The study by Li J et al. [19] exemplifies this application, reporting two piRNAs—piR-hsa-26925 and piR-hsa-5444—significantly upregulated in LUAD tissues and serum-derived exosomes. The resulting diagnostic panel achieved an AUC of 0.833, reflecting strong discriminatory performance. These findings are reinforced by Li Y et al. [22], who identified piR-hsa-164586 as a circulating biomarker for early-stage NSCLC with diagnostic superiority over CYFRA21-1, a current standard serum marker. Such results highlight the feasibility of incorporating piRNAs into liquid biopsy platforms, particularly for early detection, a major challenge in lung cancer screening [19,22,24].

Beyond expression levels, some studies provide insights into the mechanistic roles of piRNAs and associated non-coding RNAs. Lin Y et al. [20] revealed significant dysregulation of piRNAs and lncRNAs in bronchial epithelial cells of lung cancer patients. Although the functional roles of piRNAs were not directly assessed, the inverse correlation between lncRNA SNHG9 and patient survival, as well as its impact on tumor cell behavior, suggests a broader regulatory network in which piRNAs may be involved [20,25]. These data point to the prognostic value of non-coding RNAs and call for further investigation into piRNA–lncRNA–mRNA axes.

The potential of extracellular vesicles (EVs) as carriers of piRNAs adds another dimension to their clinical utility. Cammarata G et al. [21] emphasized the relevance of EV-contained piRNAs in liquid biopsy approaches, where their stability and tumor-specificity make them ideal candidates for minimally invasive diagnostics. The use of serum-derived EVs to detect piRNAs like piR-hsa-164586 [22] demonstrates the practicality of this strategy and supports further development of EV-based piRNA detection kits [20,21,26].

From a therapeutic perspective, the work by Xin XL et al. [23] sheds light on the involvement of PIWI-like proteins (PIWIL2, PIWIL4) and related gene silencing machinery in mediating anticancer effects. Although piRNAs were not directly measured, the modulation of key genes involved in piRNA pathways suggests their role in mediating therapeutic responses. These observations align with emerging studies proposing the modulation of piRNA–PIWI complexes as a therapeutic strategy to inhibit tumor proliferation, angiogenesis, and immune evasion [22,23,27,28]. Nonetheless, experimental validation remains limited, and functional assays (e.g., knockdown/overexpression in LUAD models) are urgently needed to establish causality.

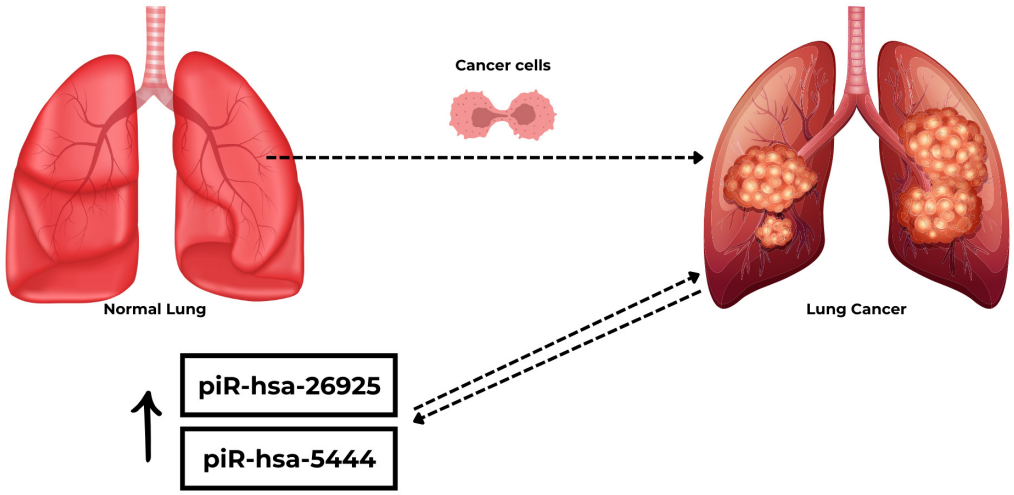
Despite promising findings, the field faces several limitations. Most included studies are preclinical and observational in nature, with small sample sizes and lack of external validation. There is also considerable heterogeneity in piRNA detection methods, RNA isolation protocols, and normalization strategies, which limit reproducibility and hinders meta-analytic synthesis. Only one study directly compared piRNAs with established biomarkers (e.g., CYFRA21-1) [22], and none explored longitudinal performance or predictive value for treatment response. Moreover, the biological functions

of many piRNAs remain inferred rather than demonstrated, underscoring the need for functional studies, CRISPR-based screens, and in vivo validation.

Finally, while the integration of piRNAs into clinical workflows holds promise, several steps are required: (i) development of standardized assays (e.g., qRT-PCR or droplet digital PCR kits), (ii) validation in multicenter cohorts with ethnic and biological diversity, (iii) stability testing under biobank and clinical conditions, and (iv) integration with existing panels (e.g., miRNAs, ctDNA) to enhance diagnostic precision.

Figure 3.

Schematic representation of the role of piRNAs in lung cancer. As the primary tumor focus develops, overexpression of piRNAs (piR-hsa-26925 and piR-hsa-5444) initiates, leading to hyperproliferation of tumor cells and generating a positive feedback loop. These piRNAs can be utilized as early biomarkers and for targeted regulatory and anti-tumor purposes.[19, 20, 21, 22, 23]



Limitations

This systematic review has several limitations that must be acknowledged. First, the number of eligible studies on piRNAs and PIWI-like proteins in lung cancer remains limited, with significant heterogeneity in study design, methodology, and reporting. Most included articles were preclinical, observational, or exploratory, with small sample sizes and a lack of longitudinal validation, limiting the generalizability of the findings. Second, the piRNA detection techniques varied widely, ranging from small RNA sequencing to qRT-PCR and bioinformatic re-analysis, often without consistent use of normalization controls or standard reference genes.

Third, functional characterization of the piRNAs was scarce: only one study evaluated direct effects on cell behavior, and most relied on correlative expression data. As such, the biological roles of specific piRNAs remain largely inferred, and the mechanisms linking piRNA

dysregulation to cancer phenotypes need confirmation through loss- and gain-of-function experiments in relevant lung cancer models. Fourth, although some studies reported diagnostic performance using AUC values, none provided complete diagnostic metrics (e.g., sensitivity, specificity, PPV, NPV), nor did they include external validation cohorts, making it difficult to estimate real-world clinical utility.

Finally, while some studies compared piRNAs with existing biomarkers (e.g., CYFRA21-1), no formal comparative effectiveness analyses were conducted, and integration into multivariable models or panels was not assessed. These limitations highlight the need for standardized protocols, robust validation, and interdisciplinary collaboration before piRNAs can be translated into clinical tools.

Conclusions

PIWI-interacting RNAs (piRNAs) and PIWI-like proteins represent a promising but still emerging class of molecular markers in lung cancer. The current evidence, though preliminary, suggests that certain piRNAs such as piR-hsa-26925, piR-hsa-5444, and piR-hsa-164586 show differential expression in tumor tissues and patient serum, with potential utility as non-invasive biomarkers for early detection and risk stratification. Additionally, the involvement of PIWI-related pathways in therapeutic modulation, as suggested by transcriptomic and functional analyses, supports the hypothesis that piRNA–PIWI complexes may influence tumor biology and response to treatment.

However, significant gaps remain. Larger, multicenter, and functionally oriented studies are needed to validate piRNA signatures, elucidate their molecular mechanisms, and define their added value over current clinical biomarkers. Integration of piRNAs into standardized liquid biopsy platforms, in combination with other RNA species and cell-free DNA, could eventually enhance early diagnosis, disease monitoring, and personalized therapy in lung cancer. Until such steps are taken, piRNAs should be considered promising molecular candidates whose full clinical potential is yet to be realized.

Abbreviation list:

piRNAs – PIWI-interacting RNAs

LUAD – Lung Adenocarcinoma

NSCLC – Non-Small Cell Lung Cancer

AUC – Area Under the Curve

CEA – Carcinoembryonic Antigen

CYFRA21-1 – Cytokeratin-19 Fragment

qRT-PCR – Quantitative Real-Time Polymerase Chain Reaction

ROC – Receiver Operating Characteristic

EVs – Extracellular Vesicles

NGS – Next-Generation Sequencing

lncRNAs – Long Non-Coding RNAs

ncRNAs – Non-Coding RNAs

PBMCs – Peripheral Blood Mononuclear Cells

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PROSPERO – International Prospective Register of Systematic Reviews

GRADE – Grading of Recommendations, Assessment, Development and Evaluations

CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats

Conflicts of interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Funding Sources

This study was not supported by any sponsor or funder.

Contributions

Contributors played a substantial role in conception, design, acquisition, analysis, interpretation, writing, and critical review of the manuscript. All authors approved the final content and accepted responsibility for its accuracy and integrity.

Acknowledgements

Thank you to my father for teaching me medicine, to my sister for always pushing me to be better, and to my mother for giving me life.

References

1. Reyes Barreto JS, Cabezas Varela CS, Girón Jurado LV, Baldión Elorza AM. piRNAs and PIWI-like proteins in cancer and their future as biomarkers and therapy targets in breast cancer. Rev colomb hematol oncol [Internet]. 2024;11(1):80-94. Available from: https://dx.doi.org/10.51643/22562915.701

2. Reyes Barreto JS, Giron Jurado LV, Montoya Estrada MP, Sánchez Moreno IL, Picón Moncada LT, Luna-Orozco K, et al. piRNAs and PIWI-like proteins in Multiple Myeloma and their future as biomarkers and therapy targets. Rev colomb hematol oncol [Internet]. 2024;11(1): 67-79 Available from: https://dx.doi.org/10.51643/22562915.697

3. Yan H, Wu Q-L, Sun C-Y, Ai L-S, Deng J, Zhang L, et al. piRNA-823 contributes to tumorigenesis by regulating de novo DNA methylation and angiogenesis in multiple myeloma. Leukemia [Internet]. 2015;29(1):196–206. Available from: http://dx.doi.org/10.1038/leu.2014.135

4. Paramanantham A, Asfiya R, Das S, McCully G, Srivastava A. Extracellular vesicle (EVs) associated non-coding RNAs in lung cancer and therapeutics. Int J Mol Sci [Internet]. 2022;23(21):13637. Available from: http://dx.doi.org/10.3390/ijms232113637

5. Cammarata G, Giovannelli P, Barbato F, Carè A. PIWI-interacting RNAs in lung cancer: A systematic review. Int J Mol Sci. 2020;21(23), 9143. Available from: https://dx.doi.org/10.3390/ijms21239143

6. Mai D, Ding P, Tan L, Zhang J, Pan Z, Bai R, et al. PIWI-interacting RNA-54265 is oncogenic and a potential therapeutic target in colorectal adenocarcinoma. Theranostics [Internet]. 2018;8(19):5213–30. Available from: http://dx.doi.org/10.7150/thno.28001

7. Jian Z, Han Y, Li H. Potential roles of PIWI-in-

teracting RNAs in lung cancer. Front Oncol [Internet]. 2022;12:944403. Available from: http://dx.doi.org/10.3389/fonc.2022.944403

8. Hu C, Zhou H, Liu Y, Huang J, Liu W, Zhang Q, et al. ROCK1 promotes migration and invasion of non-small-cell lung cancer cells through the PTEN/PI3K/FAK pathway. Int J Oncol [Internet]. 2019;55(4):833–44. Available from: http://dx.doi.org/10.3892/ijo.2019.4864

9. Zhang C, Yu Z, Yang S, Liu Y, Song J, Mao J, et al. ZNF460-mediated circRPPH1 promotes TNBC progression through ITGA5-induced FAK/PI3K/AKT activation in a ceRNA manner. Mol Cancer [Internet]. 2024;23(1):33. Available from: http://dx.doi.org/10.1186/s12943-024-01944-w

10. Shaker FH, Sanad EF, Elghazaly H, Hsia S-M, Hamdy NM. piR-823 tale as emerging cancer-hallmark molecular marker in different cancer types: a step-toward ncRNA-precision. Naunyn Schmiedebergs Arch Pharmacol [Internet]. 2025;398(1):47–68. Available from: http://dx.doi.org/10.1007/s00210-024-03308-z

11. Hsu Y-L, Hung J-Y, Chiang S-Y, Jian S-F, Wu C-Y, Lin Y-S, et al. Lung cancer-derived galectin-1 contributes to cancer associated fibroblast-mediated cancer progression and immune suppression through TDO2/kynurenine axis. Oncotarget [Internet]. 2016;7(19):27584–98. Available from: http://dx.doi.org/10.18632/oncotarget.8488

12. Lu X, Shen J, Huang S, Liu D, Wang H. Tumor cells-derived exosomal PD-L1 promotes the growth and invasion of lung cancer cells in vitro via mediating macrophages M2 polarization. Eur J Histochem [Internet]. 2023;67(3). Available from: http://dx.doi.org/10.4081/ejh.2023.3784

13. Haddaway NR, Page MJ, Pritchard CC, McGuinness LA. PRISMA2020: An R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimised digital transparency and Open Synthesis. Campbell Syst Rev [Internet]. 2022;18(2). Available from: https://dx.doi.org/10.1002/cl2.1230

14. Moher D, Liberati A, Tetzlaff J, Altman DP. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6. Available from: https://dx.doi.org/10.1371/journal.pmed.1000097

15. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan—a web and mobile app for systematic reviews. Syst Rev [Internet]. 2016;5(1). Available from: https://dx.doi.org/10.1186/s13643-016-0384-4

16. Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol [Internet]. 2014;14(1). Available from: http://dx.doi.org/10.1186/1471-2288-14-43

17. Cuschieri S. The CONSORT statement. Saudi J Anaesth [Internet]. 2019;13(5):27. Available from: http://dx.doi.org/10.4103/sja.sja_559_18

18. Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, et al. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ [Internet]. 2017;j4008. Available from: http://dx.doi.org/10.1136/bmj.j4008

19. Li J, Wang N, Zhang F, Jin S, Dong Y, Dong X, et al. PIWI-interacting RNAs are aberrantly expressed and may serve as novel biomarkers for diagnosis of lung adenocarcinoma. Thorac Cancer [Internet]. 2021 [cited 2024 Jul 7];12(18):2468–77. Available from: https://doi.org/10.1111/1759-7714.14094

20. Lin Y, Holden V, Dhilipkannah P, Deepak J, Todd NW, Jiang F. A non-coding RNA land-

scape of bronchial epitheliums of lung cancer patients. Biomedicines [Internet]. 2020 [cited 2024 Jul 7];8(4):88. Available from: https://doi.org/10.3390/biomedicines8040088

21. Cammarata G, de Miguel-Perez D, Russo A, Peleg A, Dolo V, Rolfo C, et al. Emerging noncoding RNAs contained in extracellular vesicles: rising stars as biomarkers in lung cancer liquid biopsy. Ther Adv Med Oncol [Internet]. 2022 [cited 2024 Jul 7];14:175883592211312. Available from: https://doi.org/10.1177/17588359221131229

22. Li Y, Dong Y, Zhao S, Gao J, Hao X, Wang Z, et al. Serum-derived piR-hsa-164586 of extracellular vesicles as a novel biomarker for early diagnosis of non-small cell lung cancer. Front Oncol [Internet]. 2022 [cited 2024 Jul 7];12. Available from: https://doi.org/10.3389/fonc.2022.850363

23. Xin X-L, Wang G-D, Han R, Jiang Y, Liu C, Liu L-S, et al. Mechanism underlying the effect of Liujunzi decoction on advanced-stage non-small cell lung cancer in patients after first-line chemotherapy. J Tradit Chin Med [Internet]. 2022 [cited 2024 Jul 7];42(1):108–15. Available from: https://doi.org/10.19852/j.cnki.jtcm.2022.01.007

24. Taghizadeh M, Jafari-Koshki T, Jafarlou V, Raeisi M, Alizadeh L, Roosta Y, et al. The role of piRNAs in predicting and prognosing in cancer: a focus on piRNA-823 (a systematic review and meta-analysis). BMC Cancer [Internet]. 2024;24(1):484. Available from: http://dx.doi.org/10.1186/s12885-024-12180-2

25. Zhang Y, Liu S, Zhao T, Dang C. METTL3-mediated m6A modification of Bcl-2 mRNA promotes non-small cell lung cancer progression. Oncol Rep [Internet]. 2021;46(2). Available from: http://dx.doi.org/10.3892/or.2021.8114

26. Ding X, Li Y, Lü J, Zhao Q, Guo Y, Lu Z, et al. PiRNA-823 is involved in cancer stem cell regulation through altering DNA methylation in association with luminal breast cancer. Front Cell Dev Biol [Internet]. 2021;9:641052. Available from: http://dx.doi.org/10.3389/fcell.2021.641052

27. Sohn EJ, Han M-E, Park YM, Kim YH, Oh S-O. The potential of piR-823 as a diagnostic biomarker in oncology: A systematic review. PLoS One [Internet]. 2023;18(12):e0294685. Available from: http://dx.doi.org/10.1371/journal.pone.0294685

28. Jiang W, Cai F, Xu H, Lu Y, Chen J, Liu J, et al. Extracellular signal regulated kinase 5 promotes cell migration, invasion and lung metastasis in a FAK-dependent manner. Protein Cell [Internet]. 2020;11(11):825–45. Available from: http://dx.doi.org/10.1007/s13238-020-00701-1