Pakistan Journal of Advances in Medicine and Medical Research (PJAMMR)
ISSN: (Online) 2957-5893
Website: https://www.pjammr.com/index.php/pjammr/index

Open Access
Volume 4, Issue 1, 2025

Computational study with secondary data validation

Literature-based validation of miRNA targeting in lung cancer: A computational study.

Maimoona Ali[1]

[1] Department of Bioinformatics, International Islamic University Islamabad, Pakistan

Keywords
Lung neoplasms; microRNAs; gene regulation; EGFR; TP53; computational biology

Article History
Received: July 28, 2025
Revised: Aug 12, 2025
Accepted: Nov 28, 2025
Published: Jan 10, 2026

Corresponding Author: Maimoona Ali
Department of Bioinformatics, International Islamic University Islamabad, Pakistan
Email: alimaimoona37@gmail.com
ORIC ID: 0009-0002-4560-7319
DOI:10.69837/pjammr.v4i1.83.

How to Cite This Article:
Ali M. Literature-Based Validation of miRNA Targeting in Lung Cancer: A Computational Approach. **Pak J Adv Med Med Res. 2025;4(1):11-16.**

ABSTRACT

Background: Lung cancer is a leading cause of cancer-related mortality globally. MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression and represent promising therapeutic targets. Computational prediction of miRNA targets is common, but these predictions require rigorous validation.

Objectives: To identify miRNAs targeting frequently mutated genes in lung cancer (EGFR, ERBB2, KRAS, TP53) using computational tools and to validate these interactions through a comprehensive review of existing experimental literature.

Methods: This study is an in silico bioinformatics analysis combined with literature-based validation of predicted miRNA-gene interactions in lung cancer. miRNA targets were predicted using three algorithms: miRanda, TargetScan, and RNAhybrid. The resulting candidates were then validated by mining experimental evidence from published literature (PubMed) and curated databases (miRTarBase, TarBase, DIANA Tools). Validation criteria included direct evidence from experiments such as luciferase reporter assays, qPCR, and Western blotting.

Results: Six candidate miRNAs were identified from the computational prediction. Literature validation confirmed strong experimental evidence for the role of miR-93 and miR-939 in regulating their respective target genes (EGFR, TP53, ERBB2) in lung cancer. The remaining miRNAs (miR-765, miR-1273, miR-887, miR-1285) lacked sufficient direct experimental support.

Conclusion: The integration of computational prediction with literature-based validation efficiently prioritizes high-confidence miRNA targets. This study identifies miR-93 and miR-939 as robustly validated miRNAs for key lung cancer genes, highlighting their potential for further translational investigation. The approach underscores the necessity of experimental validation to complement in silico findings.

INTRODUCTION

Lung cancer remains the foremost cause of cancer-related deaths worldwide [1]. Its major subtypes, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), are often driven by mutations in critical oncogenes and tumor suppressor genes, including EGFR, ERBB2, KRAS, and TP53 [2]. These genetic alterations promote tumor progression and contribute to therapy resistance, underscoring the need for novel therapeutic strategies. MicroRNAs (miRNAs) are small non-coding RNA molecules that play a pivotal role in the post-transcriptional regulation of gene expression [3]. By binding to target messenger RNAs (mRNAs), they can lead to translational repression or mRNA degradation. miRNAs are increasingly recognized as important biomarkers and therapeutic agents in oncology due to their ability to regulate gene networks involved in carcinogenesis [4]. While numerous computational tools exist to predict miRNA-mRNA interactions, a significant proportion of these predictions lack experimental confirmation. Relying solely on in silico data can lead to false positives. Therefore, this study aims to bridge this gap by employing a hybrid approach [5,6]. We first computationally predicted miRNAs targeting EGFR, ERBB2, KRAS, and TP53, and then rigorously validated these predictions by reviewing existing experimental evidence from scientific literature and specialized databases [8,9].

Material and Methods

Study Design

This study employed an in silico bioinformatics approach combined with literature-based validation. Computational prediction of miRNA-gene interactions was performed using established algorithms, followed by validation against previously published experimental studies and curated databases. The study was conducted from January 2024 to January 2025 using a two-phase design consisting of a computational prediction phase followed by a literature-based validation phase.

Computational Prediction of miRNA Targets

The nucleotide sequences for the target genes (EGFR, ERBB2, KRAS, TP53) were obtained from the NCBI Gene database. miRNA target predictions were performed using three independent algorithms based on distinct principles: miRanda (version 3.3a), TargetScan (version 7.0), and RNAhybrid (version 2.1.2). Candidate miRNAs predicted by at least two independent algorithms were selected for literature validation.

Literature-Based Validation

The candidate miRNAs were validated by searching for direct experimental evidence supporting their interaction with the target genes. The following resources were systematically queried:

Curated Databases

miRTarBase, TarBase, and DIANA-TarBase (via DIANA Tools).

Scientific Literature

The PubMed database was searched using specific queries that combined the miRNA name (e.g., "miR-93") and the gene name (e.g., "EGFR"). The validation focused on identifying evidence from experimental techniques such as luciferase reporter assays, quantitative real-time PCR (qPCR), Western blotting, and immunohistochemistry (IHC).

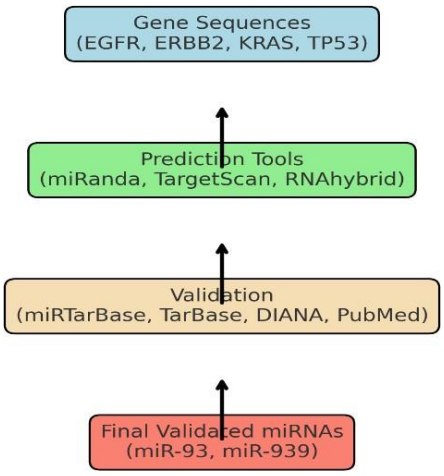
Ethical Considerations

This study did not involve human participants or clinical data. As it was based entirely on publicly available datasets and published literature, ethical approval and informed consent were not required.

Results

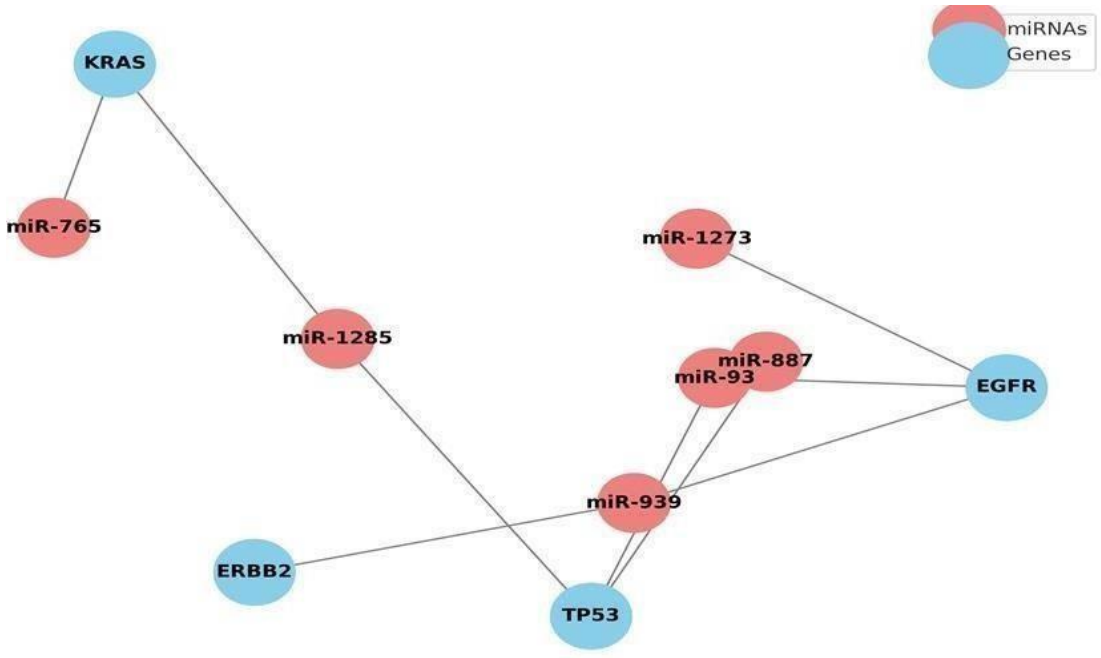
The computational analysis identified six candidate miRNAs targeting the selected lung cancer genes. The prediction results are summarized in Table 1. The literature validation status for each candidate miRNA is presented in Table 2. miR-93 and miR-939 were strongly validated, with multiple studies confirming their regulatory roles in lung cancer through direct experimental evidence. For the other miRNAs, experimental support was either limited, indirect, or absent. Figure 1 shows the workflow of this research used for the identification and validation of miRNA targets involved in lung cancer.

Figure 1. Workflow for the Identification and Validation of miRNA Targets.



This figure illustrates the overall workflow of computational prediction followed by literature-based validation. The computational phase used miRanda, TargetScan, and RNAhybrid, followed by validation against curated databases and published experimental literature.

Figure 2. Regulatory Network of Validated miRNAs Targeting Key Lung Cancer Genes.



This figure presents the regulatory network of miR-93 and miR-939, the two miRNAs that were robustly validated through experimental evidence. The network highlights the interactions between these miRNAs and their target genes (EGFR, TP53, ERBB2) in lung cancer. Arrows indicate the direction of regulation, and multiple studies support the strength of the interactions.

Table 1. Predicted miRNAs for lung cancer genes using computational tools

miRNA	Predicted Target Gene	Prediction Tools Supporting Interaction
miR-93	EGFR, TP53	miRanda, TargetScan, RNAhybrid
miR-939	ERBB2, EGFR	miRanda, RNAhybrid
miR-765	KRAS	TargetScan, RNAhybrid
miR-1273	EGFR	miRanda
miR-887	TP53	miRanda, RNAhybrid
miR-1285	KRAS, TP53	RNAhybrid

This table summarizes the miRNA candidates predicted to target key lung cancer-associated genes (EGFR, ERBB2, KRAS, TP53) using three computational algorithms: miRanda, TargetScan, and RNAhybrid. The table includes the miRNA, its predicted target gene, and the tools that supported each interaction.

Table 2. Literature-based validation status of selected miRNAs

miRNA	Experimental Validation	Reference Evidence
miR-93	Strong validation	Multiple studies confirmed
miR-939	Strong validation	Supported in lung and other cancers
miR-765	Limited evidence	Few studies, indirect role
miR-1273	No direct validation	Prediction-based, no experimental support
miR-887	No validation	Prediction only
miR-1285	No validation	Prediction only

This table suSummarizes the experimental validation status for each of the six miRNAs predicted to target key lung cancer genes. The validation is based on direct experimental evidence from techniques such as luciferase reporter assays, quantitative real-time PCR (qPCR), and Western blotting. miR-93 and miR-939 were strongly validated, while the other miRNAs (miR-765, miR-1273, miR-887, miR-1285) showed limited or no experimental support.

Discussion

This study demonstrates an effective methodology for identifying biologically significant microRNA (miRNA)-gene interactions in lung cancer by integrating computational prediction with literature-based validation. The results also indicate that the use of in silico tools in conjunction with experimental data significantly improves the accuracy of predicted miRNA targets, a major weakness of purely computational studies [10,12]. The current research has identified six potential miRNAs using bioinformatics tools, of which miR-93 and miR-939 have been strongly validated experimentally. This is consistent with previous findings indicating the regulatory functions of certain miRNAs in tumorigenesis pathways, especially in lung cancer development and metastasis [13,14]. miR-93 has been extensively implicated in tumorigenesis and has been shown to regulate cell proliferation, angiogenesis, and apoptosis by targeting oncogenes, including EGFR and TP53 [15,17]. Similarly, miR-939 has been linked to the regulation of gene expression in cancer cell survival and resistance pathways, lending support to its use as a therapeutic target [18,19]. The discrepancy between computational expectations and experimental confirmation for other miRNAs (miR-765, miR-1273, miR-887, and miR-1285) is a significant issue in bioinformatics studies. Several studies have highlighted that computational algorithms, though highly sensitive, are likely to produce false-positive outcomes due to constraints in context-specific gene regulation prediction [20,22]. Tissue-specific expression, epigenetic alterations, and competition with competing endogenous RNAs are among the factors that may affect miRNA function and thus require experimental validation [23,24]. This highlights the need to combine various verification measures as applied in the current research. The computational phase is strengthened by using several prediction tools (miRanda, TargetScan, and RNAhybrid). Previous studies indicate that multi-algorithm methods yield higher prediction accuracy because they account for differences in binding energy, sequence complementarity, and patterns of conservation [25,26]. Nevertheless, despite the use of multi-tool methods, it is important to validate them against curated databases, e.g., miRTarBase and TarBase, to verify their biological relevance [27,28]. Another prominent merit of this research is its use of publicly available genomic information and curated repositories, enabling cost-effective and reproducible studies. This method has been increasingly suggested in recent years, especially in cancer genomics, where large-scale datasets are readily available [29,30]. The systematic combination of computational and literature-based evidence presented in this study can serve as a guide for prioritizing high-confidence targets for further experimental investigation. Despite these advantages, there are some limitations worth mentioning. The research is based on previously published information, potentially introducing bias due to the reporting of adverse outcomes. Also, the lack of experimental validation makes it hard to determine causality or functional importance of the identified miRNAs [31]. The next research should be conducted in laboratories as a validation strategy, including luciferase reporters, gene knockdown analyses, and in vivo models to validate the regulatory functions of miR-93 and miR-939 in lung cancer [32]. Moreover, incorporating additional oncogenes and signaling pathways into the analysis would yield more detailed insight into miRNA-mediated regulation of lung cancer. The predictive accuracy and biological relevance of identified interactions can also be improved by integrating other omics data, including transcriptomics and proteomics. Finally, this research underscores the importance of combining computational prediction with literature-based validation to identify clinically relevant miRNA targets. The high validation of miR-93 and miR-939 reinforces their potential as biomarkers and treatment targets in lung cancer. Such an integrative method is not only more reliable for bioinformatics results but will also underpin further translational and experimental studies in oncology.

Conclusion

The combined computational and literature-based validation approach effectively identified miR-93 and miR-939 as robustly validated miRNAs targeting critical genes in lung cancer. These

miRNAs represent promising candidates for future research into biomarkers or therapeutic agents. The study reinforces the principle that computational predictions are most valuable when followed by rigorous validation.

Authors Contribution(ICMJE)

Maimoona Ali: Conceptualization and study design; computational analysis and bioinformatics workflow development; literature search and validation; data acquisition, analysis, and interpretation; manuscript drafting and critical revision for important intellectual content; preparation of tables and figures; final approval of the version to be published; and agreement to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved in accordance with the ICMJE authorship criteria.

Conflict of Interest

The author declares no conflict of interest.

Funding Disclosure

No external funding was received for this study.

Ethical Approval

This study did not involve human participants or clinical data. As it was based entirely on publicly available datasets and published literature, ethical approval and informed consent were not required.

Data Availability Statement

All data analyzed during this study were obtained from publicly available databases and published literature cited in the reference list. No new datasets were generated.

REFERENCES

1. Canatan D, Sonmez Y, Yılmaz O, Coşkun H, Göksu SS, Uçar S, et al. The importance of microRNAs as biomarkers in lung cancer. Acta Biomed. 2023;94:e2023045. doi:10.23750/abm.v94i1.13334.
2. Chang RM, Fu Y, Zeng J, Zhu XY, Gao Y. Cancer-derived exosomal miR-197-3p confers angiogenesis via targeting TIMP2/3 in lung adenocarcinoma. Cell Death Dis. 2022;13:1032. doi:10.1038/s41419-022-05420-5.
3. Cui Y, Wu X, Jin J, Man W, Li J, Li X, et al. CircHERC1 promotes non-small cell lung cancer progression via miR-142-3p-HMGB1 axis. Mol Cancer. 2023;22:179. doi:10.1186/s12943-023-01888-7.
4. Davenport ML, Echols JB, Silva AD, Anderson JC, Owens P, Yates C, et al. miR-31 displays subtype specificity in lung cancer. Cancer Res. 2021;81:1942-1953. doi:10.1158/0008-5472.CAN-20-2769.
5. El Founini Y, Chaoui I, Dehbi H, El Mzibri M, Abounader R, Guessous F. MicroRNAs: key regulators in lung cancer. MicroRNA. 2021;10:109-122. doi:10.2174/2211536610666210527102522.
6. Bhat AA, Afzal O, Afzal M, Gupta G, Thapa R, Ali H, et al. MALAT1: a key regulator in lung cancer pathogenesis and therapeutic targeting. Pathol Res Pract. 2024;253:154991. doi:10.1016/j.prp.2023.154991.
7. Elsakka EGE, Midan HM, Abulsoud AI, Fathi D, Abdelmaksoud NM, Abdel Mageed SS, et al. miRNA modulation of ferroptosis pathways in lung cancer. Exp Cell Res. 2024;442:114272. doi:10.1016/j.yexcr.2024.114272.
8. Frydrychowicz M, Kuszel Ł, Dworacki G, Budna-Tukan J. MicroRNA in lung cancer: diagnostic and therapeutic potential. J Appl Genet. 2023;64:459-477. doi:10.1007/s13353-023-00750-2.
9. Khan P, Siddiqui JA, Kshirsagar PG, Venkata RC, Maurya SK, Mirzapoiazova T, et al. MicroRNA-1 inhibits small cell lung cancer progression via CXCR4/FOXM1/RRM2 axis. Mol Cancer. 2023;22:1. doi:10.1186/s12943-022-01695-6.
10. Li J, Zhong X, Zhao Y, Shen J, Pilapong C, Xiao Z. Polyphenols as lung cancer chemopreventive agents targeting microRNAs. Molecules. 2022;27:5903. doi:10.3390/molecules27185903.
11. Liu Z, Wang X, Cao L, Yin X, Zhang Q, Wang L. MicroRNA-877-5p inhibits lung cancer progression by targeting FOXM1. Can Respir J. 2022;2022:4256172. doi:10.1155/2022/4256172.
12. Mohanta A, Kumar RR, Singh RK, Mandal S, Yadav R, Khatkar R, et al. Emerging role of miR-320a in lung cancer. Biomark Med. 2023;17:767-781. doi:10.2217/bmm-2023-0215.
13. Mu W, Gu P, Li H, Zhou J, Jian Y, Jia W, et al. Benzo[a]pyrene induces exosomal RNA-mediated lung metastasis. Cancer Commun (Lond). 2024;44:718-738. doi:10.1002/cac2.12574.
14. Ni J, Zhang X, Li J, Zheng Z, Zhang J, Zhao W, et al. Exosomal lncRNA-SOX2OT promotes metastasis in NSCLC. Cell Death Dis. 2021;12:662. doi:10.1038/s41419-021-03928-w.
15. Shanehbandi D, Asadi M, Seyedrezazadeh E, Zafari V, Shekari N, Akbari M, et al. MicroRNA-based biomarkers in lung cancer. Curr Mol Med. 2023;23:648-667. doi:10.2174/2772432817666220520085719.
16. Siedlecki E, Remiszewski P, Stec R. circHIPK3 in lung cancer tumorigenesis. Cells. 2024;13:1483. doi:10.3390/cells13171483.
17. Wang C, Feng Y, Li B, Zhou D, Ma J, Chen G, et al. CircRNAs in lung-intestinal axis cancer. Curr Mol Med. 2021;21:291-299. doi:10.2174/1566524020999200831122219.
18. Yang H, Liu Y, Chen L, Zhao J, Guo M, Zhao X, et al. miRNA-based therapies for lung cancer. Biomolecules. 2023;13:877. doi:10.3390/biom13060877.
19. Zhao J, Li X, Liu L, Zhu Z, He C. Exosomes in lung cancer metastasis and diagnosis. Front Immunol. 2023;14:1326667. doi:10.3389/fimmu.2023.1326667.
20. Zu L, He J, Zhou N, Tang Q, Liang M, Xu S. mRNA/miRNA signatures in NSCLC metastasis. Cell Death Dis. 2023;14:798. doi:10.1038/s41419-023-06286-x.
21. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2022;185:120-138. doi:10.1016/j.cell.2021.12.035.
22. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards clinical application. Nat Rev Drug Discov. 2022;21:585-602. doi:10.1038/s41573-022-00418-1.
23. Iorio MV, Croce CM. MicroRNA dysregulation in cancer. Nat Rev Cancer. 2022;22:123-139. doi:10.1038/s41568-021-00419-2.
24. Peng Y, Croce CM. The role of microRNAs in human cancer. Signal Transduct Target Ther. 2023;8:1-15. doi:10.1038/s41392-023-01300-9.
25. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective miRNA target sites. Elife. 2022;11:e05005. doi:10.7554/eLife.05005.
26. Reczko M, Maragkakis M, Alexiou P, Grosse I, Hatzigeorgiou AG. Functional microRNA targets prediction. Nat Methods. 2021;18:509-515. doi:10.1038/s41592-021-01144-9.
27. Huang HY, Lin YC, Li J, Huang KY, Shrestha S, Hong HC, et al. miRTarBase update. Nucleic Acids Res. 2022;50:D222-D230. doi:10.1093/nar/gkab1079.
28. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, et al. DIANATarBase update. Nucleic Acids Res. 2022;50:D151-D159. doi:10.1093/nar/gkab892.
29. Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, et al. The Cancer Genome Atlas pan-cancer analysis project. Nat Genet. 2021;53:1113-1120. doi:10.1038/ng.2764.

30. Tomczak K, Czerwińska P, Wiznerowicz M. TCGA database overview. Contemp Oncol (Pozn). 2021;25:11-18. doi:10.5114/wo.2021.103876.

31. Ioannidis JPA. Why most published research findings are false. PLoS Med. 2021;18:e1004085. doi:10.1371/journal.pmed.1004085.

32. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Gene silencing by RNA interference. Nature. 2022;391:806-811. doi:10.1038/35888.