Literature-based validation of miRNA targeting in lung cancer: a Computational study.

Maimoona Ali[1]

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

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) 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.

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

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Corresponding Author: Maimoona Ali

Department of Bioinformatics, International Islamic University Islamabad, Pakistan

Email: alimaimoona37@gmail.com

ORCID: https://orcid.org/0009-0002-4560-7319

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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 such as 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 networks of genes 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 through analysis of previously published experimental studies. 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 conducted using three independent algorithms based on distinct principles: miRanda (version 3.3a), Target Scan (version 7.0), and RNAhybrid (version 2.1.2). miRNAs predicted by at least one tool were shortlisted as candidate interactions for further 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:

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

Scientific Literature

PubMed database was searched using specific queries combining 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).

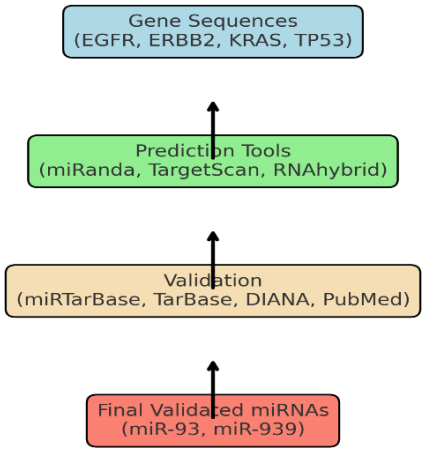
Ethical Considerations

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

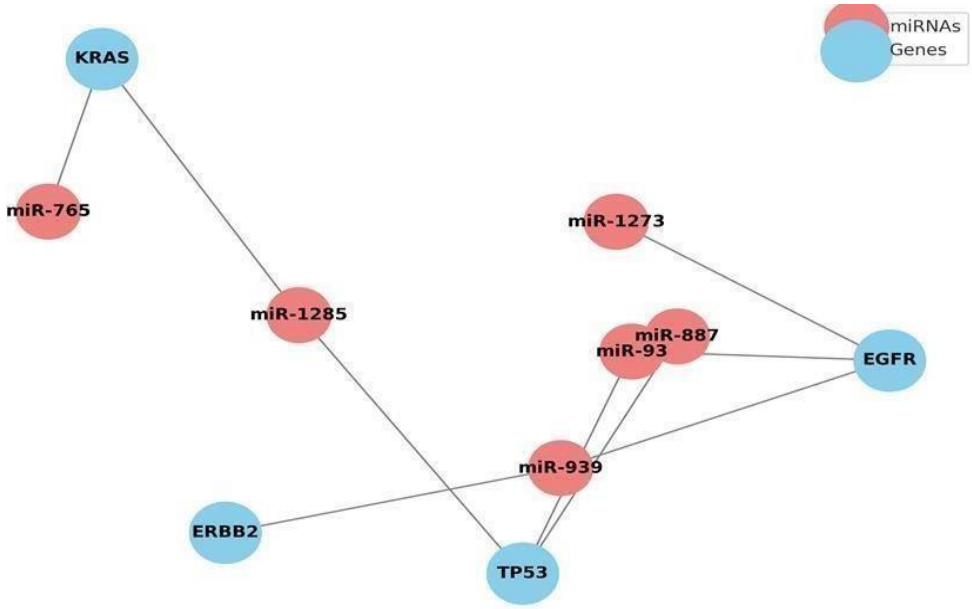
The computational analysis identified six candidate miRNAs targeting the selected lung cancer genes. The prediction results are summarized in Table 1. The literature validation status for each candidate miRNA is presented in Table 2. miR-93 and miR-939 were strongly validated, with multiple studies confirming their regulatory roles in lung cancer through direct experimental evidence. For the other miRNAs, experimental support was either limited, indirect, or entirely absent. Figure 1 shows the workflow of this research used for 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 bioinformatics workflow used in this study, which includes two phases: computational prediction of miRNA targets and literature-based validation. The computational phase involved the use of miRanda, TargetScan, and RNAhybrid to identify potential miRNAs, followed by validation through the review of experimental evidence from scientific literature and curated databases.

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 the strength of the interactions is supported by multiple studies.

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 provides a summary of the experimental validation status for each of the six miRNAs predicted to target key lung cancer genes. The validation is based on the presence of direct experimental evidence obtained from various 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, which is a significant weakness of purely computational studies [10,12].The current research has been able to identify six potential miRNAs using bioinformatics tools out of which miR-93 and miR-939 have been strongly validated through experimental means. 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 extensively been involved 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, which adds credence to its use as a therapeutic target [18,19].The difference between computational expectations and experimental confirmation of other miRNAs (miR-765, miR-1273, miR-887 and miR-1285) is a significant issue in bioinformatics studies. A number of studies have highlighted that computational algorithms, though very sensitive, are likely to produce false-positive outcomes because of constraints in context-specific gene regulation prediction [20,22]. Tissue-specific expression, epigenetic alterations, and competition with competing endogenous RNAs are some of the factors that may affect the functionality of miRNAs and hence 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 the use of several prediction tools (miRanda, TargetScan, and RNAhybrid). Previous studies indicate that multi-algorithms yield higher prediction accuracy as they consider the differences in binding energy, sequence complementarity, and patterns of conservation [25,26]. Nevertheless, despite multi-tool methods, it is important to validate predictions using curated databases,e.g. miRTarBase and TarBase, to verify that it is biologically relevant [27,28].The other prominent merit of this research is that it makes use of publicly available genomic information and curated repositories that enable cost-effective and reproducible studies. This method has been progressively suggested in recent years, especially in cancer genomics, where big data are easily available [29,30]. The systematic combination of computational and literature- based evidence presented in this study can be used as a guide to the prioritization of high-confidence targets to be further investigated in experiments.In spite of these advantages, there are some limitations that should be mentioned. The research is based on the already published information, potentially causing bias because of reporting adverse outcomes. Also, The study is limited by reliance on previously published experimental evidence rather than independent laboratory validation[31]. The next research ought to be conducted in the laboratories, as a method of validation, including the use of luciferase reporters, gene knockdown analysis, and in vivo models to validate the regulatory functions of miR-93 and miR-939 in lung cancer [32]. Moreover, incorporation of additional oncogenes and signaling pathways in the analysis would yield a 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 supports the significance of using a combination of computational prediction and literature-based validation to determine clinically relevant miRNA targets. The high miR-93 and miR-939 validation reinstate their potential as biomarkers and treatment targets in lung cancer. Such an integrative method is not only more reliable in the bioinformatics results, but also will underpin further translational and experimental studies in the field of 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

Concept & Design of Study: Maimoona Ali

Drafting: Maimoona Ali

Data Collection & Critical Review: Maimoona Ali

Final Approval of Version: Maimoona Ali

CONFLICT OF INTEREST

Not applicable.

FUNDING DISCLOSURE

No external funding was received for this study.

ETHICAL STATEMENT

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.

INFORMED CONSENT: Not applicable.

AI USAGE STATEMENT

AI tools (**e.g., ChatGPT**) were used for language editing and structuring of the manuscript. The authors take full responsibility for the content and accuracy of the

manuscript.

DATA AVAILABILITY STATEMENT

All data analyzed in this study were derived from publicly available databases and published literature, all of which are cited within the manuscript.

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