Literature-Based Validation

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Literature-Based Validation of miRNA Targeting in Lung Cancer: A Computational Approach

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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: A systematic bioinformatics workflow was employed. miRNA targets were predicted using three algorithms: miRanda, target Scan, 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.

Keywords: Lung Neoplasms; MicroRNAs; Gene Regulation; EGFR; TP53; Computational Biology

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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 [5-7]. 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].

Material & Methods

Study Design

This study utilized a systematic, two-phase design consisting of a computational prediction phase followed by a literature-based validation phase.from jan 2024 to jan 2025.

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:

Curated Databases

miRTarBase [5], TarBase [6], 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).

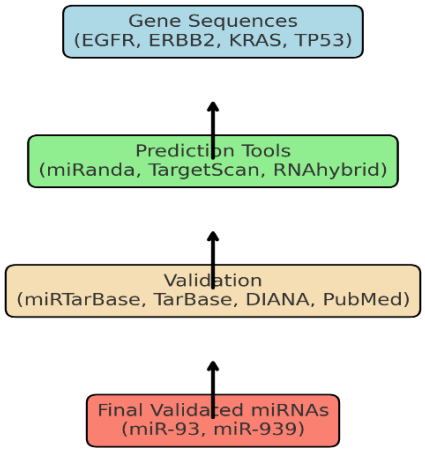
Ethical Considerations

This study did not involve human participants, animal experiments, or access to private clinical data. As it was based entirely on publicly available genomic data and previously published literature, ethical approval was not required.

RESULTS

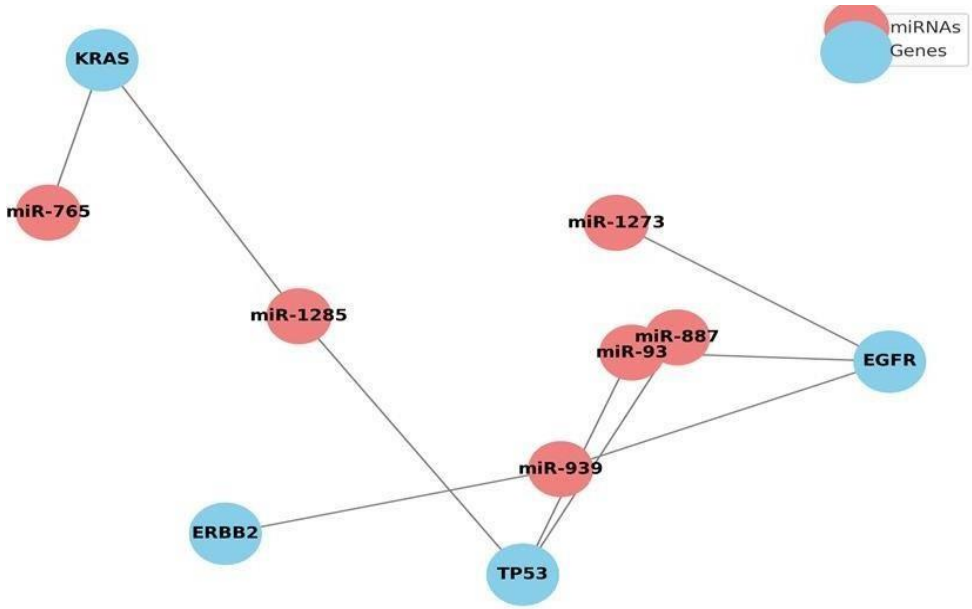
The computational analysis identified six candidate miRNAs targeting the selected lung cancer genes. The prediction results are summarized in Table 1. The literature validation status for each candidate miRNA is presented in Table 2. miR-93 and miR-939 were strongly validated, with multiple studies confirming 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 experimental 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 experimental 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 a practical workflow for prioritizing biologically relevant miRNA-target interactions by integrating computational prediction with existing experimental knowledge. Our findings highlight miR-93 and miR-939 as high-confidence regulators of key oncogenes in lung cancer. The strong validation for these miRNAs is consistent with previous reports of their tumor-suppressive roles [9-10].The lack of experimental support for the other predicted miRNAs (miR-765, miR-1273, miR-887, miR-1285) underscores a common challenge in computational biology: not all in silico predictions translate to biological reality. This discrepancy can arise from various factors, including context-specific gene expression, regulatory mechanisms, and limitations in prediction algorithms[11].A key strength of this approach is its efficiency in leveraging publicly available data to generate hypotheses and avoid redundant experimental work. However, a limitation is its dependence on the scope and quality of previously published studies; negative results are often underreported, creating a potential bias.Future work should focus on experimental functional validation of the top candidates, miR-93 and miR-939, and explore their combinatorial effects in lung cancer pathways. The figure 2 shows network of predicted and validated miRNAs targeting lung cancer genes.

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.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reason

Authors Contribution

Concept & Design of Study: A Maimoona

Drafting :A Maimoona

Data Collection & Critical Review: A Maimoona

Final Approval of Version:A Maimoona

Author contributed significantly to the study's conception, data collection, analysis, Manuscript writing, and final approval of the manuscript as per **ICMJE Criteria.**

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