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Prognostic Significance Of Circulating Mir-182-5p And Mir-218-5p As Liquid Biopsy Biomarkers In Advanced Non-Small Cell Lung Cancer

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Abstract

Background/Aims: *Advanced non-small cell lung cancer (NSCLC) carries a poor prognosis, and reliable non-invasive biomarkers for prognostication remain limited. Circulating microRNAs (miRNAs), such as miR-182 and miR-218, have shown potential in reflecting tumor behavior—miR-182 is often oncogenic, while miR-218 functions as a tumor suppressor. This study aims to evaluate the prognostic value of circulating miR-182-5p and miR-218-5p in patients with advanced NSCLC.*

Materials and methods: *In this prospective study, 60 patients with histologically confirmed advanced NSCLC and 30 healthy controls were enrolled. Serum levels of miR-182-5p and miR-218-5p were measured using quantitative real-time PCR at diagnosis and after four cycles of platinum-based chemotherapy. Expression patterns were correlated with clinical and pathological parameters.*

Results: *At diagnosis, miR-182-5p was downregulated in 68.3% and upregulated in 31.7%, while miR-218-5p was downregulated in 76.7% and upregulated in 23.3%. After chemotherapy, downregulation persisted in 75.0% for miR-182-5p and 77.1% for miR-218-5p. A significant association was found between miR-182-5p expression and lymph node metastasis (p = 0.008), with all node-negative patients exhibiting downregulation. No significant clinical correlations were observed for miR-218-5p.*

Conclusion: *miR-182-5p shows promise as a prognostic biomarker in advanced NSCLC, particularly for predicting lymph node involvement. The persistent downregulation of both miRNAs supports their utility in disease monitoring.*

Keywords: *NSCLC, circulating microRNA, miR-182, miR-218, liquid biopsy.*

INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide, accounting for approximately 1.8 million deaths annually. Non-small cell lung cancer (NSCLC) constitutes about 85% of all lung cancer cases and is often diagnosed at an advanced stage, where therapeutic options are limited, and prognosis is poor.[1] Despite advances in molecular diagnostics and targeted therapies, the 5-year survival rate for patients with advanced NSCLC remains dismal. Therefore, there is a critical need to identify novel, reliable, and non-invasive biomarkers that can aid in early detection, prognostication, and treatment monitoring.[2]

MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression at the post-transcriptional level. They play essential roles in various physiological and pathological processes, including cancer progression, metastasis, and resistance to therapy. In recent years, circulating cell-free miRNAs have emerged as promising liquid biopsy biomarkers due to their stability in blood and their potential to reflect tumor dynamics in real time.[3, 4] Among these, several miRNAs have shown differential expression patterns in NSCLC and have been linked to disease stage, histological subtypes, and overall survival.[5, 6]

Specifically, miR-182 and miR-218 have garnered attention for their opposing roles in cancer biology. miR-182 is generally considered an oncogenic miRNA, promoting tumor growth, invasion, and metastasis in various cancers, including NSCLC.[7] In contrast, miR-218 has been widely studied as a tumor suppressor, with evidence suggesting its involvement in inhibiting cancer cell proliferation and migration.[8]

However, the prognostic relevance of these two miRNAs in advanced NSCLC remains to be fully elucidated, particularly when assessed dynamically over the course of treatment.

Therefore, this study aims to investigate the clinical significance of circulating miR-182 and miR-218 in patients with advanced non-small cell lung cancer, focusing on their potential role as prognostic biomarkers.

MATERIALS AND METHODS

This study employed a mixed design with an initial cross-sectional component followed by a prospective cohort follow-up. It was conducted at the National Cancer Institute, Cairo University, Egypt, between February 2022 and December 2024. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Cairo University. Written informed consent was obtained from all participants before enrollment.

Inclusion criteria: The study included a total of 60 patients with histologically confirmed advanced non-small cell lung cancer (NSCLC) and 30 healthy controls. Eligible patients were aged over 18 years, had an Eastern Cooperative Oncology Group (ECOG) performance status between 0 and 2, and tested negative for EGFR and ALK mutations. All participants had adequate bone marrow, liver, and renal function.

Exclusion criteria: Patients with early-stage disease, ECOG performance status of 3 or 4, prior systemic anticancer therapy, or evidence of organ failure were excluded.

METHODS:

Clinical and Laboratory Evaluation

At baseline, all patients underwent a comprehensive clinical assessment, including a full medical history, physical examination, and routine laboratory testing. Radiological imaging, including CT scans of the chest, abdomen, and pelvis, was performed to determine tumor burden and metastatic spread. Blood samples were collected from patients at diagnosis and again after completing four cycles of first-line chemotherapy to assess circulating levels of miR-182-5p and miR-218-5p.

MicroRNA Extraction and Quantification

Serum was separated from whole blood and subjected to total RNA extraction, including miRNAs, using the miRNeasy Mini Kit (Qiagen, Germany). RNA was isolated through phase separation using QIAzol Lysis Reagent and chloroform, followed by ethanol precipitation and purification through RNeasy Mini spin columns. The quality and concentration of extracted RNA were assessed using the NanoDrop spectrophotometer, with an A260/A280 ratio near 2.0 considered optimal.

Reverse Transcription and Real-Time PCR

Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using the miScript II RT Kit (Qiagen, Germany) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was then conducted using the miScript SYBR Green PCR Assay Kit (Qiagen, Germany) to quantify miR-182-5p and miR-218-5p expression levels. RNU6B was used as the endogenous control for normalization. All PCR reactions were performed in duplicate using a 384-well format and run on a real-time thermal cycler under standard cycling conditions.[9]

Data Analysis

Relative expression levels of miR-182-5p and miR-218-5p were calculated using the 2^−ΔΔCT method, comparing values at baseline and after chemotherapy. Expression patterns were categorized as upregulated or downregulated relative to the control group, and associations with clinical features such as lymph node metastasis were statistically analyzed.

Statistical analysis:

Statistical analysis was performed using IBM SPSS® Statistics version 26 (IBM® Corp., Armonk, NY, USA). Qualitative data were presented as frequencies and percentages. The association between miR-182-5p and miR-218-5p expression and clinical variables was assessed using Pearson's Chi-square test or Fisher's

exact test, as appropriate. Changes in miRNA expression before and after chemotherapy were evaluated using the McNemar test. A p-value < 0.05 was considered statistically significant.

RESULTS

General characteristics were presented in Table 1.

Concerning clinical and pathological features of advanced NSCLC patients, cough (35%) was the most common symptom, followed by pain (25%) and dyspnea (23.3%). Adenocarcinoma was the predominant histological subtype (78.3%), and most tumors were Grade II (58.3%) or Grade III (30%). Tumors typically measured 5–10 cm (68.3%), with 6.7% exceeding 10 cm. Lesions were mostly right-sided (53.3%), and lymph nodes were the most common metastatic site (80%), followed by lung (51.7%), bone (20%), pleura (23.3%), liver (18.3%), adrenals (11.7%), and brain (3.3%). Notably, 70% had one or two metastatic sites, while 30% had three or more.

Out of 60 patients, 53 received Gemcitabine/platinum-based chemotherapy (27 with cisplatin and 26 with carboplatin), while 7 received other platinum combinations. A total of 48 patients completed four chemotherapy cycles, including 21 on Gemcitabine/cisplatin, 21 on Gemcitabine/carboplatin, and 6 on other regimens. Among those who completed treatment, 14 patients experienced disease progression, while 34 showed regressive or stable disease.

The expression levels of miR-182-5p and miR-218-5p in the first tumor samples of NSCLC patients showed downregulation of miR-182-5p in 68.3% of patients, while 31.7% showed upregulation. Similarly, miR-218-5p was downregulated in a larger proportion of cases (76.7%) compared to 23.3% who exhibited upregulation. **Figure 1**

In the second sample, miR-182-5p remained predominantly downregulated in 75.0% of patients, while 25.0% exhibited upregulation. Similarly, miR-218-5p showed downregulation in 77.1% of cases and upregulation in 22.9%. **Figure 1**

Table 1: Baseline characteristics of Demographic data of a total of 60 advanced NCSLC.

		Count	%
Age 60	< 60 years	30	50.0%
	>= 60 years	30	50.0%
Sex	Male	49	81.7%
	Female	11	18.3%
Residence	Cairo	21	35.0%
	Giza	25	41.7%
	Other Gov	14	23.3%
Smoking	No	18	30.0%
	Yes	42	70.0%
Family history	No	58	96.7%
	Yes	2	3.3%
DM	No	53	88.3%
	Yes	7	11.7%
HTN	No	49	81.7%
	Yes	11	18.3%
PS	PS I	44	73.3%
	PS II	16	26.7%

NSCLC: Non-Small Cell Lung Cancer, DM: Diabetes Mellitus, HTN: Hypertension, PS: Performance Status

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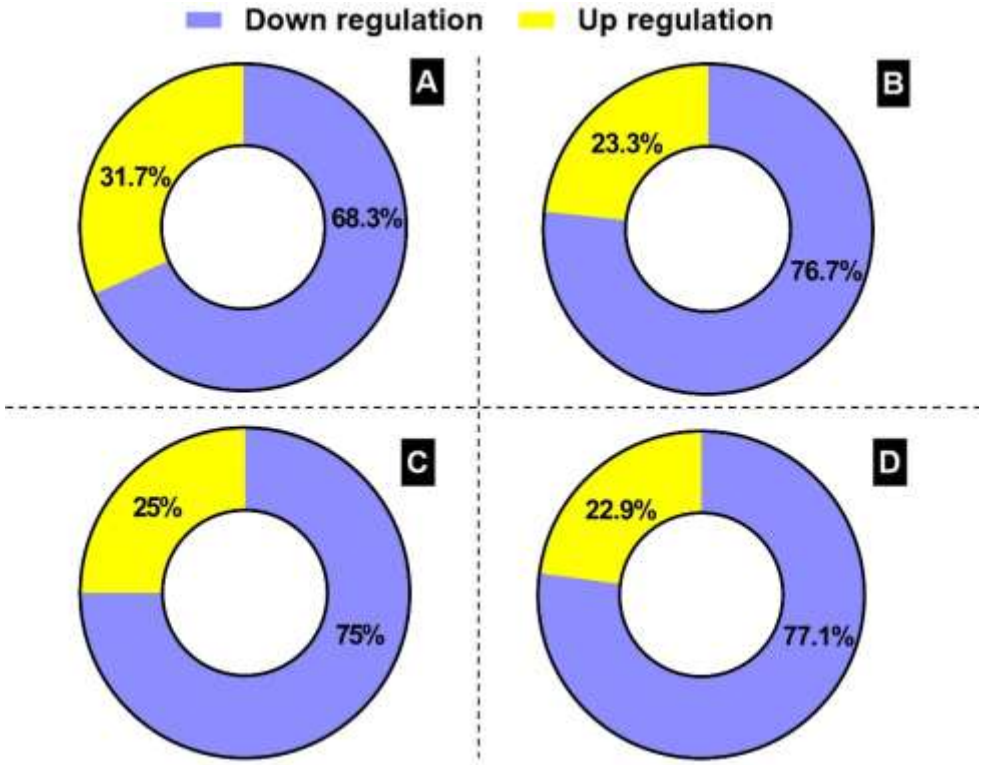

Figure 1. Expression levels of (A) miR-182-5p and (B) miR-218-5p in first samples and (C) miR-182-5p and (D) miR-218-5p in second samples of NSCLC patients

The association between the expression levels of miR-182-5p and miR-218-5p with all clinical and pathological variables was thoroughly analyzed; however, a statistically significant correlation was observed only between miR-182-5p expression and lymph node metastasis. **Figure 2**

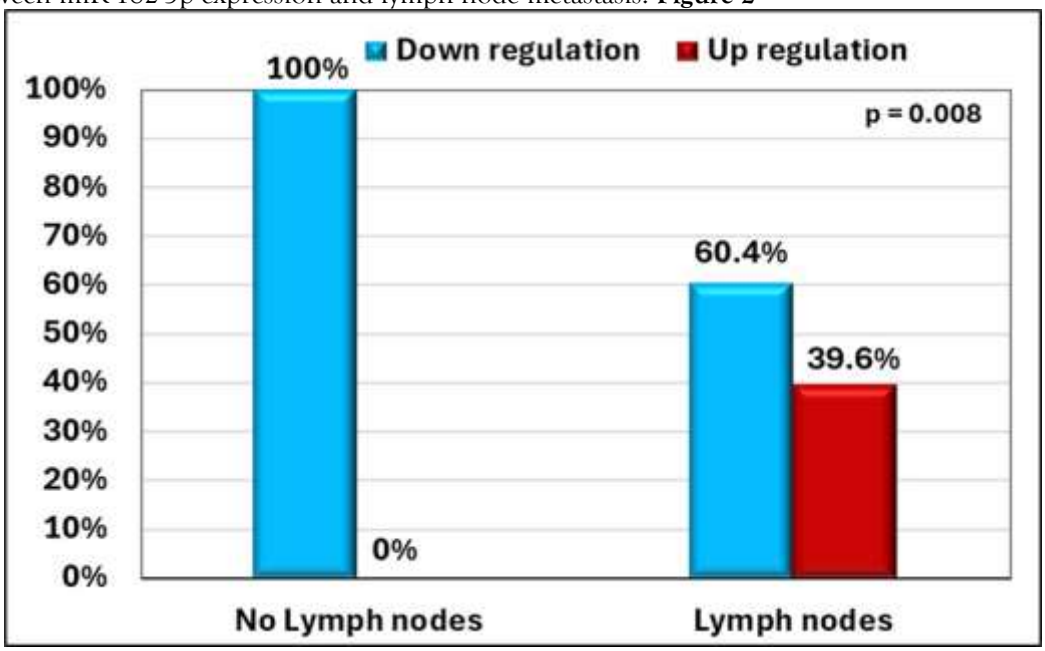

Figure 2. Relation of miR182 5P expression and lymph node positivity

DISCUSSION

MiRNAs have emerged as promising diagnostic, therapeutic, and prognostic biomarkers for NSCLC, complementing other established biomarkers such as DNA, RNA, proteins, epigenetic modifications, and glycoproteins.[10] So we aim to evaluate the prognostic value of circulating miR-182-5p and miR-218-5p in patients with advanced NSCLC.

In our study miR-182-5P was mainly downregulated and associated with advanced clinical stage especially extensive lymph node metastasis. MiR-182, a member of the miR-183 family [11], has been frequently reported to be upregulated in various malignancies [12], including non-small cell lung cancer (NSCLC), supporting its potential classification as an oncomir.[11, 12] Nonetheless, its function remains complex and warrants further exploration due to its documented opposing effects in different contexts.[12] Notably, studies have demonstrated a correlation between miR-182 levels in plasma-derived exosomes and corresponding tissue levels in stage I NSCLC.[13] Additionally, circulating miR-182 was found to be significantly elevated in NSCLC patients compared to healthy controls, and its expression was higher in HPV-DNA positive NSCLC cases than in HPV-DNA negative ones.[13] Likewise, Zhang et al.[14] Yang et al.[15], and Shi et al.[16] reported findings that contradict our results, as they found that miR-182-5p was predominantly upregulated. Moreover, extensive lymph node metastasis was associated with increased miR-182-5p expression. Also, Gao et al.[17] reported that miR-182-5p was significantly upregulated in NSCLC tissues compared to non-cancerous lung tissues, indicating its oncogenic role in NSCLC. This suggests its potential utility as a biomarker for distinguishing malignant from non-malignant lung tissues. However, they also found that miR-182-5p expression did not have a significant association with the survival outcomes of NSCLC patients.

Regarding miR-218-5p, our study found that it was predominantly downregulated, consistent with the findings of Chen et al.,[18] who reported that miR-218 expression levels were significantly reduced in NSCLC cell lines. The expression levels of miR-218 in non-small cell lung cancer cell lines were lower compared to normal human lung epithelial cells ($P < 0.05$ and $P < 0.001$, respectively). Similarly, Yun et al.[19] highlighted that miR-218-5p was primarily downregulated in the plasma and tissues of NSCLC patients, suggesting its potential as a diagnostic marker with high specificity and sensitivity. Another study by Liu et al.[20] described the negative role for miR-218-5p in regulating the oncogene Diphthamide biosynthesis 1 (DPH1), with miR-218-5p downregulation driving DPH1's oncogenic function [20]. In another study by Meng et al.,[21] miR-218-5p overexpression in SW1417 human colorectal cancer (CRC) cells inhibited cell viability and induced cell apoptosis, Furthermore, CRC cells with induced high miR-218-5p expression exhibited a downregulation in cellular Fas-associated death domain-like interleukin-1β-converting enzyme inhibitory protein (c-FLIP), insinuating c-FLIP as a direct target of miR-218-5p. This was in concordance with a study by Shaath et al.,[22] which showed the upregulation of miR-218-5p to suppress tumor formation potential in CRC.

However, our study did not yield any significant findings correlating miR-218-5p with other reported studies.

This study has a few limitations. The sample size was relatively small, which may limit the generalizability of the findings. Additionally, the study focused only on two microRNAs, potentially overlooking the contribution of other relevant biomarkers. Finally, long-term survival data were not assessed, restricting evaluation of the prognostic value over extended follow-up periods.

CONCLUSION

Circulating miR-182-5p and miR-218-5p demonstrate differential expression in advanced NSCLC and persist after chemotherapy. Notably, miR-182-5p shows potential as a prognostic biomarker, particularly in relation to lymph node involvement. These findings support the utility of miRNA profiling as a non-invasive tool for disease monitoring and prognostication in advanced NSCLC.

ABBREVIATIONS:

NSCLC: Non-Small Cell Lung Cancer, miRNA: MicroRNA, miR-182-5p: MicroRNA-182-5p, miR-218-5p: MicroRNA-218-5p, HPV: Human Papillomavirus, EGFR: Epidermal Growth Factor Receptor, ALK: Anaplastic Lymphoma Kinase, ECOG: Eastern Cooperative Oncology Group, IRB: Institutional Review Board, CT: Computed Tomography, qRT-PCR: Quantitative Real-Time Polymerase Chain Reaction, cDNA: Complementary DNA, RNA: Ribonucleic Acid, PCR: Polymerase Chain Reaction, SYBR: SYnergy Brands, RNU6B: RNA, U6 Small Nuclear 2, SPSS: Statistical Package for the Social Sciences, USA: United States of America, A260/A280: Absorbance Ratio at 260 and 280 nm, DPH1: Diphthamide Biosynthesis 1, c-FLIP: Cellular Fas-Associated Death Domain-Like IL-1β-Converting Enzyme Inhibitory Protein, CRC: Colorectal Cancer.

Conflict of interest:

None to be declared.

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