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Original Article

Serum Exosomal MiR-874 as a Potential Biomarker for Nonsmall Cell Lung Cancer Diagnosis and Prognosis

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Abstract

Lung cancer, primarily nonsmall cell lung cancer (NSCLC), is a leading cause of cancer-related fatalities globally. Due to late detection, the 5-year survival rate for NSCLC remains low. Therefore, the current research aimed to assess the diagnostic and prognostic value of serum exosomal miR-874 levels in NSCLC patients. This study involved 161 NSCLC patients and 80 control subjects. Blood samples were collected from all participants, and serum exosomal MiR-874 levels were quantified using quantitative reverse transcription-polymerase chain reaction. The study revealed a significant decrease in MiR-874 levels among NSCLC patients compared to controls. The receiver operating characteristic analysis demonstrated the diagnostic value of serum exosomal MiR-874 in effectively distinguishing NSCLC patients from controls. Furthermore, associations were observed between serum exosomal MiR-874 expression and adverse clinical factors such as young age, male sex, smoking, high tumor grade, squamous cell carcinoma histopathology, advanced tumor stage, and lymphatic involvement. Patients with high levels of MiR-874 had significantly longer overall and disease-free survival compared to those with lower levels. The study demonstrates that levels of serum exosomal miR-874 are considerably lower in NSCLC patients, indicating its potential as a diagnostic biomarker. The study's findings suggest that the expression of MiR-874 may predict the prognosis of NSCLC patients based on clinical features.

Keywords

- nonsmall cell lung cancer
- miR-874 serum exosomes
- diagnostic prognostic biomarker
- clinical characteristics survival outcomes

Introduction

Lung cancer represents one of the most prevalent types of cancer globally and is regarded as a primary cause of cancer-related fatalities.[1] According to recent statistics, the 5-year survival rate for nonsmall cell lung cancer (NSCLC) in the United States from 2010 to 2016 was approximately 20.5%. This is a significant improvement compared to the earliest reported 5-year survival rate in 1975, which was only 11.5%.[2] Improved early detection and better treatment modalities, including targeted and immune therapies, may account for the

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increase in NSCLC survival rates. Access to the latest diagnostic and treatment tools may also contribute to higher survival rates in higher-income countries.[3]

Micro-ribonucleic acid (miRNA) consists of about 22 noncoding bases, and it influences gene activity following transcription.[4] It functions as a translation suppressor or accelerates the degradation of target messenger RNA (mRNA) molecules.[5] An intriguing aspect is that a single miRNA has the ability to target multiple mRNA molecules, thereby influencing the signal transduction of diverse biological pathways.[6] Studying miRNA expression can offer important information about how abnormal miRNA expression is connected to the start, advancement, and reaction to anticancer drugs in tumors. Protein-coding genes can be regulated by miRNAs when they adhere to the target mRNA's 3′ unaltered segment (3′-UTR). This binding can result in either mRNA degradation or prevention of translation, thereby controlling the production of proteins determined by these genes.[7]

MiR-874 is a miRNA molecule that is situated at the chromosomal region 5q31.2. This particular genomic region is frequently observed to undergo deletions in different malignant tumors.[8] Its impact on various human diseases has been studied, and it has been found to be involved in diverse biological mechanisms. Its role in regulating critical processes like cell division, apoptotic death, dissemination, and resistance to chemotherapy has been observed in different malignancies through the regulation of target genes. MiR-874 has been demonstrated to target several genes implicated in the progression and growth of cancer, namely, AQP3, PI3K/AKT, and YAP/TAZ. AQP3 is an aquaporin responsible for facilitating water transport within cells. In cases where miR-874 is downregulated, AQP3 expression is elevated, fostering tumor growth and facilitating metastasis.[9] miR-874 also targets PI3K/AKT, which is critical for cells to survive and proliferate. Diminished levels of miR-874 activate the PI3K/AKT axis, consequently promoting tumor growth.[10] Additionally, miR-874 controls the YAP/TAZ transcription factors essential in cell division and motility. Reduced expression of miR-874 leads to an increase in YAP/TAZ levels, further contributing to enhanced tumor growth and metastatic potential.[11] In addition to its role in oncogenesis and spread, miR-874 has been involved with the development of resistance to cytotoxic drugs.[12]

Exosomes are tiny vesicles released by living cells and carry many molecules, including miRNAs. These exosomal miRNAs exhibit distinct patterns indicative of various diseases and reflect the underlying pathological processes. This unique feature makes them highly valuable as diagnostic and prognostic markers, providing significant insights for detecting and assessing a range of diseases.[13] MiR-874 has been found in exosomes and has several functions, such as regulating cell growth, differentiation, and apoptosis. In cancer, miR-874 is downregulated, which may promote tumor growth and progression.[14] Studies have shown that miR-874 has oncosuppressive effects on different malignancies such as colorectal carcinomas (CRCs), stomach adenocarcinoma, hepatic carcinoma (HCC), breast carcinoma, and osteosarcoma.[15–21]

Our study aimed to assess the level of miR-874 in the serum exosomes of NSCLC patients as the primary objective. Our secondary objective was to study miR-874 as a diagnostic and prognostic biomarker for NSCLC.

Patients and Methodology

Study Design

A case–control study was conducted at the Clinical Oncology Department of Zagazig University's Faculty of Medicine between February 2018 and January 2023.

Sample Size

The study included 161 individuals diagnosed with NSCLC, and 80 normal participants, matched to the NSCLC group in age and gender, were carefully selected as controls. The diagnosis of all patients was confirmed by studying their tissue pathology. The American Joint Committee on Cancer guidelines were used to determine the clinical stage of each patient.[22] To minimize the potential impact of clinical measures on the serum level of exosomal sRNA, we exclusively collected blood prior to patients undergoing tumor-invasive biopsy or any other tumor treatment, such as chemotherapy or radiotherapy.

Eligibility criteria:

1. Having NSCLC with stage I, II, or III
2. A performance status of 2 or less on the Eastern Cooperative Oncology Group scale[23]
3. To have normal hepatorenal, cardiac, and bone marrow functions
4. Satisfactory nutritional and auditory status

Exclusion criteria:

1. Previous chemo- or radiotherapy
2. Invasive medical procedure immediately before collection of blood specimens

Serum Exosome Purification and Exosome RNA Extraction

Two main phases are involved in isolating total RNA from extracellular vesicles (exosomes): exosome purification and total RNA extraction. The serum obtained from the patients and control subjects underwent cold centrifugation at around 4°C and 3,000 × *g* for about 10 minutes. This process helped to separate any debris that settled at the bottom. The clear fluid above was collected and filtered to eliminate any remaining particles. To purify exosomes and extract RNA, 1 mL of serum was processed with the exoRNeasy Midi Kit (No. 77144, Qiagen, Hilden, Germany) based on the directions supplied by the manufacturer. The RNA was measured at 200°C by the spectrophotometer NanoDrop ND of Thermo Fisher's. After quantification, the extracted RNA samples were stored at –80°C.

Quantitative Reverse Transcription-Polymerase Chain Reaction

We applied the miScript II RT kit (Qiagen GmbH) and followed the manual's directions to synthesize complementary deoxyribonucleic acid from the collected RNA. We utilized the miScript SYBR Green PCR kit from Qiagen GmbH to perform quantitative reverse transcription-polymerase chain reaction

Table 1 Primer sequences for qRT-PCR of MiR-874 and U6

	Primers	
miR-874	Forward primer	5′-GGCCCTGAGGAAGAACTGAG-3′
	Reverse primer	5′-TGAG ATCCAACAGGCCTTGAC-3′
U6	Forward primer	5′–GGGTGCTCGCTTCGGCAGC-3′
	Reverse primer	5′–CAGTGCAGGGTCCGAGGT-3′

Abbreviation: qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

(qRT-PCR) and repeated in triplicate using the Stratagene Mx3005P-qPCR system. To normalize the miR-874 level of expression, we employed the endogenous control U6. The steps of PCR were based on a first denaturation phase of 15 minutes at 95°C, subsequently followed by 40 cycles of 15 seconds of denaturation at 94°C, 30 seconds of annealing at 55°C, and 30 seconds of extension at 70°C. The primers used in the PCR were purchased from Qiagen GmbH and are listed in ►**Table 1**. The $2^{-\Delta\Delta Ct}$ equation was employed to quantitate miR-874 level.[24]

The primary outcome was to assess the level of miR-874 in the serum exosomes of NSCLC patients. The secondary outcome was to evaluate the diagnostic and prognostic value of miR-874 as a biomarker in NSCLC.

Statistical Analyses

Results were analyzed using IBM SPSS Statistics (Version 27). The Student's *t*-test and the analysis of variance test were used to compare the means and standard deviations of the groups. The diagnostic accuracy of miR-874 in serum exosomal samples was determined through receiver operating characteristic (ROC) analysis. The Kaplan–Meier curve and log-rank test were used to assess the overall and disease-free survival rates of NSCLC patients.

Ethics

The study was approved by the ethical committee of the Faculty of Medicine at Zagazig University (2018-Feb-225). All participants involved provided written informed consent. The study measures were conducted following the ethical norms of the responsible committee on human experimentation and the Helsinki Declaration of 1965, revised in 2013.

Results

In this research, the patients' age was between 28 and 66 years, the mean was 48.25 ± 11.27 years, and the median age was 51. There were 106 (65.8%) male and 55 (34.2%) female patients. Among the patients, 128 (79.5%) were smokers.

Assessing the Diagnostic Value and Potential Biomarkers of Serum Exosomal mir-874 in Nonsmall Cell Lung Cancer

There is a significant difference between the levels of miR-874 found in serum exosomes of NSCLC patients compared to those found in controls. The unpaired *t*-test result indicates a *p*-value of less than 0.0001, which is highly significant. The mean value of miR-874 in patients (0.8178) was noticeably lower when compared to controls (1.084) (►**Fig. 1A**).

Exploring the Association and Implications of Serum Exosomal miR-874 Expression in Nonsmall Cell Lung Cancer

►**Table 2** presents data on miR-874 levels in the serum exosomes of NSCLC patients based on various clinical factors. These factors include age, gender, smoking, tumor grade,

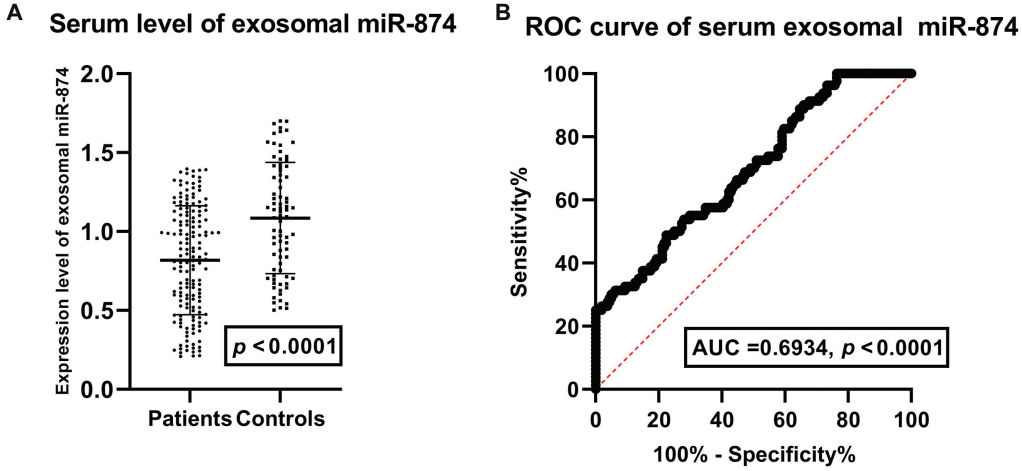

Fig. 1 **A** Serum exosomal MiR-874 as a diagnostic marker for nonsmall cell lung cancer: differential levels and **B** receiver operating characteristic (ROC) analysis.

Table 2 Clinical correlates of exosomal miR-874 serum levels in nonsmall cell lung cancer patients

		MiR-874			
	Number	Mean	SD		*p*
Age					
< 50 ≥ 50	78 83	0.509 1.108	0.181 0.159	22.37[a]	< 0.0001
Gender					
Male Female	106 55	0.62 1.199	0.245 0.109	16.71[a]	< 0.0001
Smoking					
Yes No	128 33	0.701 1.271	0.286 0.075	11.34[a]	< 0.0001
Tumor grade					
I II III IV	42 38 41 40	1.241 0.9832 0.6785 0.3587	0.0883 0.0733 0.0973 0.0971	737.0[b]	< 0.0001
Histopathology					
Adeno. SCC	92 69	0.565 1.16	0.21 0.14	20.3[a]	< 0.0001
T (Tumor)					
T1 T2 T3	40 56 65	1.25 0.94 0.46	0.083 0.111 0.148	542.6[b]	< 0.0001
N (nodes)					
N0 N1 N2	48 49 64	1.22 0.9 0.45	0.098 0.106 0.146	565.2[b]	< 0.0001
TNM stage					
I II III	48 63 50	1.22 0.84 0.4	0.097 0.14 0.12	535.1[b]	< 0.0001

Abbreviations: Adeno., adenocarcinoma; SCC, squamous cell carcinoma; SD,, standard deviation.
[a]Student's *t*-test.
[b]One-way analysis of variance (ANOVA).

histopathology, T (tumor) stage, N (nodes) stage, and TNM stage.

Age was a significant variable, as there was a notable difference in serum exosomal miR-874 levels between patients below 50 year and those aged 50 or older ($p < 0.0001$). Similarly, gender was also a significant factor, with males having lower serum exosomal miR-874 levels than females ($p < 0.0001$).

Smoking was found to have a significant association with NSCLC patients, as smokers exhibited lower serum exosomal miR-874 levels compared to nonsmokers ($p < 0.0001$). Tumor grade was also significant; there was a significant association between lower expression of miR-874 and undifferentiated tumors ($p < 0.0001$).

The histopathology of the cancer was also a significant factor, as serum exosomal miR-874 levels differed significantly between adenocarcinoma and squamous cell carcinoma (SCC) ($p < 0.0001$) with lower levels in adenocarcinoma. The T (tumor) stage was found to have an association with miR-874 expression, with a higher T stage associated with lower serum exosomal miR-874 levels ($p < 0.0001$).

The N (nodes) stage was also significant, with lower miR-874 expression being correlated with a higher N stage, *p* less than 0.0001. Finally, serum exosomal miR-874 levels differed markedly among stages of the TNM classification system ($p < 0.0001$), with advanced TNM stages associated with lower miR-874 expression.

Impact of Serum Exosomal mir-874 Expression on Survival in Nonsmall Cell Lung Cancer Patients

We conducted a comparative analysis of the survival rates of NSCLC patients based on their serum exosomal miR-874 levels. The log-rank (Mantel–Cox) test results showed a significant difference in overall survival comparing the lower and high-expression patients. The chi-square value was 13.33 with 1 degree of freedom, and the *p*-value was 0.0003. The median overall survival was shorter in patients with low miR-874 levels (36 months) than those with high

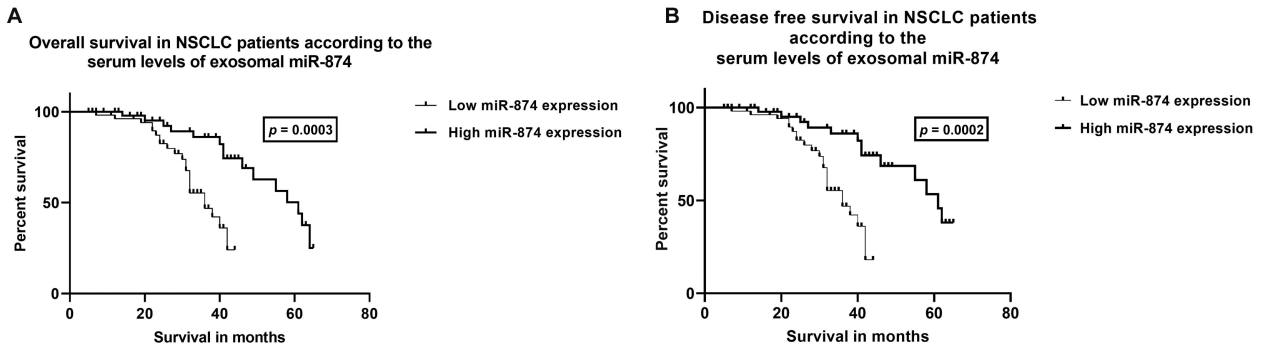

Fig. 2 **A** Prognostic significance of serum exosomal MiR-874 expression in nonsmall cell lung cancer: insights from overall and **B** disease-free survival analysis.

levels (61 months) (►**Fig. 2A**). Similarly, the log-rank (Mantel–Cox) test showed a significant difference in disease-free survival between the groups, with a chi-square value of 13.81 and a *p*-value of 0.0002. Patients with lower levels of miR-874 had a median disease-free survival of 36 months, whereas those with higher levels had a longer median disease-free survival of 61 months (►**Fig. 2B**).

Discussion

It is common for lung cancer to go undetected until it reaches an advanced stage, making it difficult to treat and resulting in a less favorable outlook for many patients.[25] Early detection and diagnosis of NSCLC are critical to improving survival rates.[26] Biomarkers are crucial for achieving the goal; various studies have identified potential biomarkers in serum peptides, urine metabolites, and miRNAs in sputum and plasma.[27–30] These biomarkers show promise in enhancing the accuracy and sensitivity of current NSCLC screening and diagnostic techniques.[31]

Studies have extensively investigated miRNA-874 expression and functionality in various cancer types. Although miR-874 has been examined as a potential biomarker for cancer, its impact on NSCLC has not been explored yet. It is worth noting that miR-874 expression is reduced in some types of cancer while elevated in others.[32–35] This intriguing observation highlights the cancer-specific nature of miR-874's role, emphasizing the need to consider the specific cancer type while evaluating its significance. MiR-874 is essential as an oncosuppressor in HCC by impeding cell division, dissemination, and invasion. However, its downregulation has been observed in HCC.[36] The low expression of miR-874 in CRC is also linked to tumor progression and an unfavorable prognosis.[32] In contrast, miR-874 is often overexpressed in breast carcinoma and is related to the aggressiveness of the tumor and metastasis.[34] Similarly, in adenocarcinoma of stomach, miR-874 upregulation has been involved in promoting cell division, metastases, and invasion.[35]

These findings highlight the variability of miR-874's role as a biomarker across different malignancies. Therefore, the aim of this study was to investigate the potential of miRNA-874 as a biological marker for the diagnosis, prognosis, and clinical implications of NSCLC patients.

We observed a significant downregulation of miR-874 in serum exosomes among patients with NSCLC compared to controls. In addition, miR-874 acts as oncosuppressor in NSCLC. The observation that miR-874 levels are lower in the serum exosomes of NSCLC patients than in controls is significant. Studies in other malignancies have also demonstrated the role of miR-874 as an oncosuppressor. MiR-874 was found to be low expressed and acts as a tumor suppressor in endometrial adenocarcinoma,[33] osteosarcoma,[37] gastric cancer,[18] and pancreatic adenocarcinoma.[38] The low level of miR-874 in NSCLC is consistent with the tumor suppressor function observed in other malignancies.

Examining miR-874 dysregulation in NSCLC and other cancer types can improve understanding its broader implications as a therapeutic target or biomarker.

In the current research, we evaluated how accurately serum exosomal miR-874 could distinguish between individuals with NSCLC and controls using ROC analysis. Our findings indicate that serum exosomal miR-874 is a good diagnostic marker, with an area under the curve value of 0.6934. Although a limited number of studies specifically focus on miR-874 in NSCLC, research findings related to miR-874 in other types of cancer can shed light on its potential diagnostic value. Serum miR-874 is a potential diagnostic marker for several types of cancer, including CRC, gastric cancer, HCC, pancreatic adenocarcinoma, endometrial carcinoma, osteosarcoma, and prostate cancer.[18,32,33,36–39] The downregulation of miR-874 in cancer patients compared to controls indicates its potential diagnostic value as a noninvasive biomarker.

The current study revealed that a reduction in the level of serum exosome miR-874 was significantly associated with all the known negative prognostic factors in NSCLC. The decrease was found more commonly in males, younger age groups, smokers, higher grade tumors, histopathology of SCC, advanced tumors, extensive lymphatic involvement, and late stage of TNM. Low levels of miR-874 in serum exosomes were significantly related to the presence of metastatic spread, lymphatic metastases, undifferentiated pathology, and late stage of TNM in patients with CRC.[32]

In stomach adenocarcinoma, lower levels of miR-874 were related to advanced stage and lymphatic invasion, indicating its potential as a prognostic marker.[18] It has been observed that patients who suffer from advanced prostate carcinoma and have high Gleason scores exhibit decreased expression of miR-874.[39]

The expression of miR-874 demonstrates variability across different malignant tumors, which can be attributed to multiple mechanisms and pathways through which it operates. By directly targeting and downregulating various oncogenes involved in cancer progression and metastasis, miR-874 plays a pivotal role. For example, in HCC, miR-874 targets insulin-like growth factor 1 receptor, which is vital in the proliferation of cells as well as survival.[40] Additionally, miR-874 suppresses the protumorigenic effects of oncogenes, thereby contributing to tumor growth inhibition.[41] Furthermore, miR-874 was reported to impede epithelial-mesenchymal transition (EMT), a critical mechanism for cancer metastasis in different types of cancer.[42] In gastric cancer, miR-874 targets the EMT-inducing transcription factor Slug, effectively impeding EMT and suppressing tumor invasion and metastasis.[43] Notably, miR-874 also regulates signaling pathways that govern crucial cellular functions such as cell division, apoptotic death, and differentiation, ultimately suppressing tumorigenesis.[44] Lastly, miR-874 indirectly enhances tumor suppression by targeting and suppressing genes that act as negative regulators of tumor-suppressive pathways. For instance, in breast cancer, miR-874 targets CDK9, a cyclin-dependent kinase that promotes cell cycle progression and inhibits apoptosis. Through downregulating CDK9, miR-874 activates tumor suppressor pathways, effectively restraining cancer cell growth.[45]

This study revealed a significant association between lower levels of miR-874 and shorter overall and disease-free survival. This study suggests that low expression of miR-874 is associated with a lower overall survival rate (36 months) than those with higher expression (61 months), indicating that miR-874 may serve as a reliable prognostic indicator. Reports have revealed that lower miR-874 expression in malignant tumors is associated with worse outcomes and shorter overall survival in CRC,[32] brain gliomas,[46] breast carcinoma,[34,47] HCC,[48] renal cell carcinoma,[49] and nasopharyngeal carcinoma.[50] Our study is constrained by the limited number of participants and the inherent heterogeneity of NSCLC subtypes, each characterized by distinct molecular markers.

Conclusion

Our research findings indicate a noteworthy reduction in the levels of miR-874 present in the serum exosomes of patients diagnosed with NSCLC. The study findings suggest that miR-874 could be a noninvasive diagnostic biomarker for NSCLC. Additionally, miR-874 expression reduction is related to adverse prognostic characteristics, indicating that it could be used as a prognostic indicator for NSCLC.

Patient Consent

All participants provided written informed consent.

Note

The manuscript has been read and approved by all the authors.

Authors' Contributions

Conceptualization: A.F.G., W.H.E., A.A., and A.A.A. Data curation: A.A., M.A., and A.A.. Formal analysis: W.H.E. and A. A. Investigation: A.F.G. and W.H.E. Methodology: A.F.G., S.S.A-S, A.A., A.A., M.A., A.A., A.A.A., M.M.B., A.N.S.M., and W.H.E. Writing – review and editing: A.F.G., S.S.A-S., A.A., A.A., M.A., A.A., A.A.A., M.M.B., A.N.S.M., and W.H.E. Finally, the paper was revised and approved by all authors.

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Conflict of Interest

None declared.

Acknowledgments

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