

POSTOPERATIVE DYNAMICS OF PLASMA MICRORNA IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Abstract

Relevance. Circulating microRNAs are promising minimally invasive biomarkers for the diagnosis of non-small cell lung cancer. However, their biological origin remains insufficiently understood. If the primary tumor represents their principal source, surgical tumor resection would be expected to reduce their plasma levels.

Objective. To determine whether plasma levels of previously identified diagnostic miRNAs decrease following surgical tumor resection in patients with non-small cell lung cancer.

Materials and methods. The study included 18 patients with histologically confirmed non-small cell lung cancer and 41 healthy controls who participated in our previous study. Paired patient plasma samples were collected before surgery and 14–119 days after surgery, prior to adjuvant therapy. The experimental workflow included magnetic bead-based RNA isolation, reverse transcription, TaqMan-based real-time PCR, and normalization to the exogenous spike-in control ath-miR-159a. Statistical analyses included correction for multiple comparisons.

Results. Seven miRNAs (miR-130b-3p, miR-1-3p, miR-423-5p, miR-200a-3p, miR-154-5p, miR-376a-3p, and miR-18b-5p) were significantly elevated in preoperative plasma compared with healthy controls. Five remained significantly elevated after surgery. No statistically significant postoperative decrease in NSCLC-associated candidate miRNAs was detected after correction for multiple comparisons. Three miRNAs (miR-495-3p, miR-191-5p, and miR-543) showed postoperative decreases at nominal $p < 0.05$, but these differences did not remain significant after correction. Their preoperative levels did not differ significantly from those of healthy controls. No statistically significant correlation was detected between postoperative miRNA levels and the time from surgery to postoperative blood sampling in this sample.

Conclusions. No statistically significant postoperative decrease in non-small cell lung cancer-associated candidate miRNAs was detected after correction for multiple comparisons. These findings do not establish their cellular origin or validate their utility for postoperative disease monitoring. Larger prospective studies are required.

Keywords: carcinoma, non-small-cell lung, circulating microRNA, plasma, biomarkers, tumor.

Introduction

Lung cancer (hereinafter – LC) remains one of the leading causes of cancer-related mortality worldwide and represents a major public health challenge [1; 2]. Despite advances in diagnostic imaging and therapeutic strategies, a substantial proportion of

patients are still diagnosed at advanced stages, resulting in poor clinical outcomes. Since prognosis is closely linked to disease stage at diagnosis, the development of reliable approaches for early detection remains a clinical priority. Current approaches to LC diagnosis, including imaging for lesion

detection and histopathological examination for diagnostic confirmation, have important limitations. Radiological findings alone are often insufficient to reliably distinguish malignant from benign pulmonary lesions as well as infectious and inflammatory diseases, making accurate differential diagnosis challenging [2; 3]. Although tissue biopsy remains the diagnostic gold standard, it is invasive and may not be feasible in all patients because of lesion location, procedural risks, or patient-related factors [4].

In this context, circulating microRNAs (miRNAs) have attracted considerable attention as potential minimally invasive biomarkers. miRNAs are small non-coding RNAs that regulate gene expression at the post-transcriptional level and are involved in key biological processes, including cell proliferation, apoptosis, and tumor progression. Numerous studies have demonstrated that miRNA expression profiles are altered in cancer and may serve as disease-specific signatures [5]. Importantly, miRNAs are remarkably stable in biological fluids, including plasma, due to their association with protein complexes or encapsulation in extracellular vesicles, which protects them from degradation. These characteristics make circulating miRNAs particularly attractive as liquid biopsy biomarkers [6; 7].

Several studies have identified miRNA-based biomarkers with promising diagnostic performance in LC using different biospecimens, including blood and exhaled breath condensate [8–10]. In particular, our previous work demonstrated that selected circulating miRNAs can effectively discriminate LC patients not only from healthy individuals but also from patients with tuberculosis, highlighting their potential clinical utility in differential diagnosis [11]. Nevertheless, despite the growing body of evidence supporting the diagnostic value of circulating miRNAs, important questions remain regarding their biological origin and dynamics in relation to tumor presence.

It is generally assumed that tumor cells contribute to the pool of circulating miRNAs through active secretion and passive release during cell death [12]. If tumor cells represent a major source of circulating tumor-associated miRNAs, surgical removal of the tumor would be expected to reduce their plasma levels. However, this hypothesis has not been consistently validated, and circulating miRNAs may also reflect systemic responses to malignancy and surgical intervention, including inflammation

and tissue remodeling, thereby complicating their interpretation.

As a continuation of our previous study [11], we investigated changes in circulating miRNA levels in patients with non-small cell LC (hereinafter – NSCLC) using a paired study design comparing plasma samples collected before treatment and after surgical tumor resection. We hypothesized that miRNAs previously identified as NSCLC-associated biomarkers would decrease in plasma following surgery if the primary tumor represented a major source of these circulating miRNAs. Testing this hypothesis may provide important insights into the biological origin of circulating miRNAs and further substantiate their application not only as diagnostic biomarkers but also for monitoring disease dynamics and treatment response.

Materials and methods

Type of study. This was an observational study comparing paired pre- and postoperative plasma samples from patients with NSCLC and plasma samples from healthy controls.

Ethics approval. The study protocol was approved by the Local Ethics Committee of the Aitkhozhin Institute of Molecular Biology and Biochemistry (Protocol No. 9, dated 08.12.2021). Written informed consent was obtained from all participants prior to sample collection.

Subjects. Blood samples from 18 patients with histologically confirmed NSCLC were collected at the Kazakh Research Institute of Oncology and Radiology (Almaty, Kazakhstan) before treatment between August 2022 and May 2023. A second blood sample was collected 14–119 days after surgical tumor resection, prior to the initiation of adjuvant chemotherapy or radiotherapy. Exclusion criteria were severe comorbidities, HIV infection, other malignancies, and mental disorders. Blood samples from 41 healthy donors (control group) were collected at the Almaty Multidisciplinary Clinical Hospital (Almaty, Kazakhstan) between February and April 2023. Blood was collected into vacuum tubes containing 3.2 % sodium citrate as an anticoagulant, which had demonstrated a high miRNA yield during preliminary RNA extraction experiments.

The preoperative patient samples and the samples from healthy controls were collected as part of our previous study [11], and the same miRNA panel and experimental workflow were retained.

Plasma preparation. Blood samples were stored at 4 °C for no longer than 2 h prior to plasma

separation. After gentle inversion, samples were centrifuged at $1000 \times g$ for 15 min at 4°C . The plasma supernatant was transferred to fresh tubes and centrifuged again at $2500 \times g$ for 15 min at 4°C . The resulting plasma was aliquoted into 100 μL portions and stored at -70°C until RNA isolation.

RNA isolation. Total RNA was isolated from 100 μL of plasma using the MagMAX™ mirVana™ Total RNA Isolation Kit (Thermo Fisher Scientific, Cat. No. A27828, Waltham, MA, USA) according to the manufacturer's instructions. Before RNA isolation, 2 fmol of the exogenous spike-in control ath-miR-159a (5'-phosphorylated RNA oligonucleotide; Thermo Fisher Scientific, Cat. No. 10620310) was added to each sample.

cDNA synthesis. cDNA was synthesized using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific, Cat. No. A28007) according to the manufacturer's instructions.

Quantitative PCR. Amplification was performed using TaqMan™ Advanced miRNA Assays (Thermo Fisher Scientific, Cat. No. A25576) together with TaqMan™ Fast Advanced Master Mix (Thermo Fisher Scientific, Cat. Nos. 4444556, 4444557, and 4444964) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's protocol.

Primary data were analyzed using StepOnePlus Software v2.2.2 and ExpressionSuite Software v1.3. Relative miRNA expression was expressed as ΔCt values, calculated as $\Delta\text{Ct} = \text{Ct}(\text{reference}) - \text{Ct}(\text{target})$, with the exogenous spike-in ath-miR-159a used as the normalization control, as described previously [13]. Effect estimates for group comparisons were calculated on the ΔCt scale and reported as log2 fold-change (log2FC) values with corresponding 95% confidence intervals. For independent-group comparisons, positive log2FC values indicated higher miRNA levels in the first group listed. For paired postoperative versus preoperative comparisons, negative log2FC values indicated a postoperative decrease in miRNA levels. For comparative visualization of miRNA levels between groups, boxplots were generated using the SRplot online platform [14].

Statistical analysis was performed using Jamovi v2.6.44 software [15]. Age was compared between independent groups using Student's t-test, while

independent-group comparisons of miRNA levels were performed using the Mann–Whitney U test. Categorical variables were analyzed using Fisher's exact test. Paired preoperative and postoperative miRNA levels were compared using a one-sided Wilcoxon signed-rank test because a postoperative decrease was prespecified as the direction of the primary hypothesis. Corresponding nonparametric effect estimates and 95% confidence intervals were reported. Given the relatively small number of paired observations, the results of the paired analyses were interpreted with consideration of effect estimates and confidence intervals rather than statistical significance alone. P-values < 0.05 were considered statistically significant. For multiple comparisons, p-values were adjusted using the Benjamini–Hochberg false discovery rate (hereinafter – FDR) procedure. Adjusted p-values < 0.05 were considered statistically significant [16].

Results

To test the hypothesis that the tumor represents the primary source of circulating miRNA biomarkers, we compared preoperative and postoperative miRNA levels in patients with NSCLC. In addition, both preoperative and postoperative miRNA levels were compared with those of healthy controls to confirm the cancer-associated alterations in circulating miRNA expression before surgery and to evaluate whether miRNA levels shifted toward those observed in healthy individuals following tumor resection.

The demographic and clinical characteristics of the study groups are summarized in Table 1. Patients and controls did not differ significantly in age, sex distribution, smoking status, alcohol consumption, or family history of cancer ($p = 0.982, 1.000, 0.422, 0.322$, and 0.578 , respectively). The mean time from surgery to postoperative blood sampling was 54.72 ± 25.24 days (range 14–119 days).

The results of the quantitative analysis are summarized in Table 2. Representative examples are shown in Figures 1 and 2. Preoperative plasma levels of seven miRNAs (miR-130b-3p, miR-1-3p, miR-423-5p, miR-200a-3p, miR-154-5p, miR-376a-3p, and miR-18b-5p) were significantly higher in NSCLC patients than in healthy controls, with log2FC values ranging from 1.12 to 2.61. All of these differences remained statistically significant after correction for multiple comparisons.

Table 1. Demographic and clinical characteristics of the study groups

Characteristics	Controls	NSCLC patients
Total	41	18
Male/Female	30/11	13/5
Never-smokers/Former smokers/Current smokers*	23/4/14	7/2/9
Non-drinkers/Occasional drinkers/Frequent drinkers	33/8/0	12/6/0
Family history of cancer (Yes/No)	2/39	2/16
Lung adenocarcinoma/Squamous cell lung carcinoma	-	10/8
Tumor stage (I/II/III)	-	6/4/8
Time from surgery to postoperative blood sampling, days	-	54.72 ± 25.24
Age, years (mean ± SD)	58.46 ± 8.09	58.41 ± 8.83

* Former smokers who had quit smoking less than 3 years before enrollment were classified as current smokers;
Source: Compiled by the authors.

At postoperative sampling, five of the seven miRNAs remained significantly elevated compared with healthy controls, even after FDR correction for multiple comparisons. In contrast, postoperative levels of miR-154-5p and miR-18b-5p were no longer significantly different from those of the control group.

Table 2. Comparison of plasma miRNA levels between pre- and postoperative NSCLC patients and healthy controls

miRNA	Pre-op vs. Controls ¹		Post-op vs. Controls ¹		Post-op vs. Pre-op ²	
	log ₂ FC (95 % CI) [†]	p-value	log ₂ FC (95 % CI) [†]	p-value	log ₂ FC (95 % CI) [†]	p-value
miR-130b-3p	2.20 (1.37; 2.99)	4.8×10 ⁻⁷ *	1.71 (0.95; 2.64)	3.2×10 ⁻⁴ *	-0.62 (-Inf; 0.18)	0.111
miR-1-3p	2.61 (1.33; 3.48)	1.7×10 ⁻⁵ *	2.80 (2.09; 3.52)	3.8×10 ⁻⁷ *	0.38 (-Inf; 1.19)	0.842
miR-423-5p	1.55 (0.95; 2.25)	2.9×10 ⁻⁵ *	2.00 (1.35; 2.69)	5.0×10 ⁻⁸ *	0.50 (-Inf; 1.10)	0.959
miR-200a-3p	1.45 (0.76; 2.10)	7.4×10 ⁻⁵ *	1.52 (0.73; 2.07)	4.8×10 ⁻⁴ *	0.02 (-Inf; 0.50)	0.517
miR-154-5p	2.26 (0.76; 5.04)	0.0024*	0.88 (-0.67; 3.22)	0.269	-1.26 (-Inf; 0.13)	0.058
miR-376a-3p	1.44 (0.58; 2.42)	0.0012*	1.57 (0.75; 2.40)	2.6×10 ⁻⁴ *	0.04 (-Inf; 0.79)	0.552
miR-18b-5p	1.12 (0.34; 1.90)	0.010*	0.86 (-0.05; 1.70)	0.064	-0.32 (-Inf; 0.53)	0.169
miR-191-5p	0.73 (-0.08; 1.79)	0.066	0.27 (-0.92; 0.95)	0.642	-0.92 (-Inf; -0.33)	0.0083 [#]
miR-375-3p	0.74 (-0.11; 1.56)	0.082	0.66 (-0.03; 1.33)	0.055	-0.14 (-Inf; 0.83)	0.380
miR-29b-2-5p	1.06 (-0.53; 2.65)	0.222	0.70 (-1.18; 2.25)	0.508	-0.92 (-Inf; 0.81)	0.204
miR-382-5p	0.86 (-0.71; 2.52)	0.235	-0.38 (-2.88; 1.67)	0.788	-1.11 (-Inf; 0.88)	0.180
miR-543	0.95 (-0.77; 2.95)	0.248	-0.76 (-4.25; 1.04)	0.508	-2.70 (-Inf; -0.57)	0.025 [#]
miR-15b-5p	0.87 (-0.72; 2.30)	0.276	-1.20 (-3.14; 0.50)	0.151	-1.45 (-Inf; 0.62)	0.096
miR-495-3p	0.69 (-0.78; 2.42)	0.339	-2.19 (-4.35; 0.13)	0.066	-2.37 (-Inf; -0.97)	0.0045 [#]
miR-204-5p	-0.62 (-2.11; 0.90)	0.419	-0.49 (-2.27; 1.17)	0.610	-0.05 (-Inf; 1.59)	0.483

Abbreviations: Pre-op, preoperative patient samples; Post-op, postoperative patient samples.

¹ Mann–Whitney U test for comparisons between independent groups;

² One-sided Wilcoxon signed-rank test with corresponding one-sided 95 % confidence intervals to assess postoperative decreases in miRNA levels; -Inf indicates an unbounded lower confidence limit;

[†] log₂FC values are positive when miRNA levels are higher in the first group listed in each comparison;

[#] Significant at $p < 0.05$;

* Significant after false discovery rate (FDR) correction for multiple comparisons (adjusted $p < 0.05$).

Source: compiled by the authors.

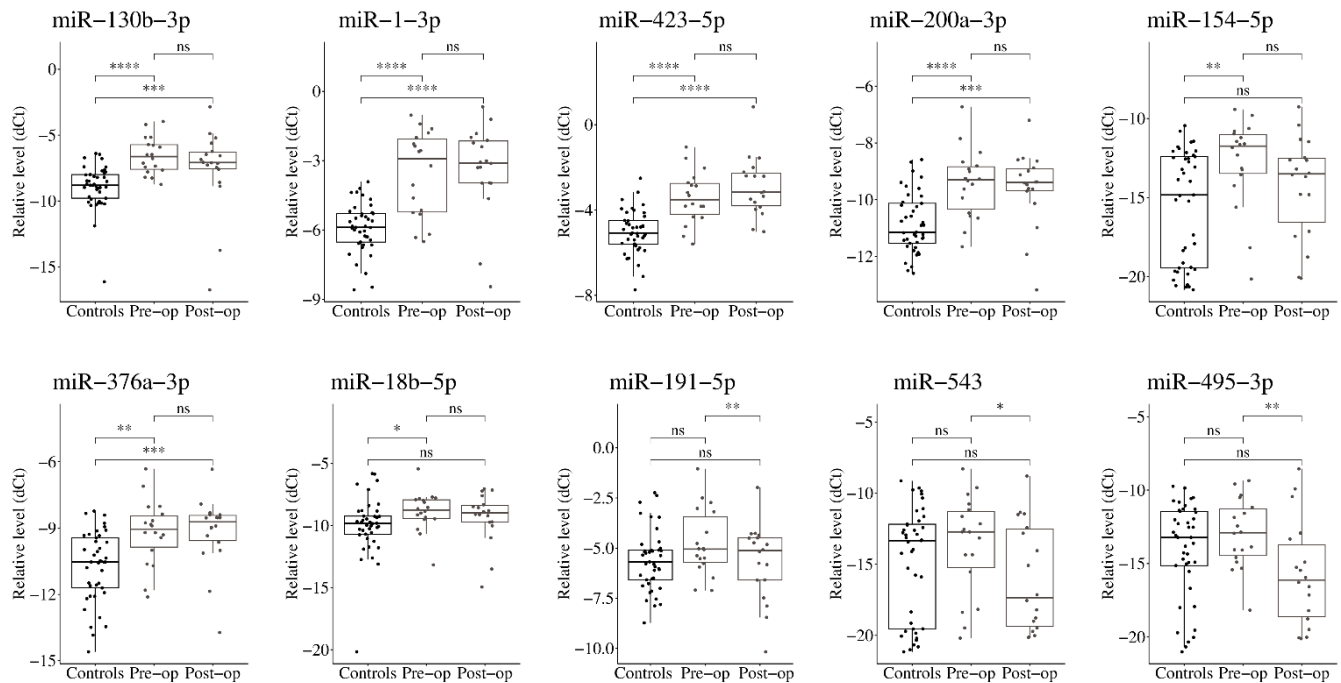


Figure 1. Relative expression of selected circulating miRNAs in healthy controls and NSCLC patients before and after surgery.

Abbreviations: Pre-op, preoperative patient samples; Post-op, postoperative patient samples.

Comparisons between independent groups were performed using the Mann–Whitney U test.

Postoperative decreases were evaluated using a one-sided Wilcoxon signed-rank test.

The significance annotations show nominal, unadjusted p-values. FDR-adjusted significance is indicated in Table 2. ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Source: compiled by the authors.

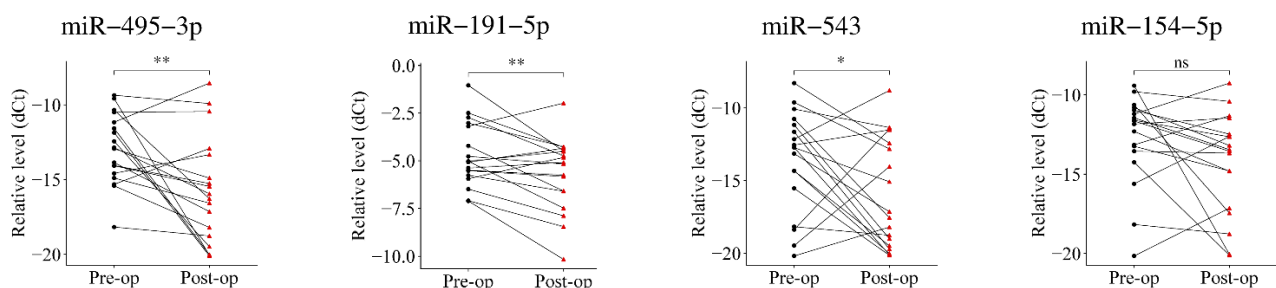


Figure 2. Individual changes in plasma levels of selected miRNAs in NSCLC patients before surgery and at postoperative sampling.

Abbreviations: Pre-op, preoperative patient samples; Post-op, postoperative patient samples.

Statistical comparisons were performed using a one-sided Wilcoxon signed-rank test.

The significance annotations show nominal, unadjusted p-values. FDR-adjusted significance is indicated in Table 2. ns, not significant; * $p < 0.05$; ** $p < 0.01$.

Source: compiled by the authors.

In the unadjusted paired analyses, postoperative decreases were observed for miR-495-3p, miR-191-5p, and miR-543 (nominal $p < 0.05$). However, none of these associations remained statistically significant after correction for multiple comparisons. miR-154-5p showed an estimated

postoperative decrease ($\log_2FC = -1.26$); however, the statistical evidence for this difference was inconclusive ($p = 0.058$).

No statistically significant correlations were detected between the time from surgery to postoperative blood sampling and either postoperative

miRNA levels or postoperative levels normalized to the corresponding preoperative values.

Exploratory secondary analyses were performed to examine potential associations between miRNA levels and selected demographic, lifestyle, and clinicopathological characteristics.

Neither preoperative nor postoperative miRNA levels differed significantly between patients with adenocarcinoma and those with squamous cell carcinoma.

Postoperative levels of miR-18b-5p, miR-1-3p, and miR-376a-3p were significantly higher in men than in women ($p = 0.0022$, 0.016 , and 0.022 , respectively). In addition, normalized postoperative levels of miR-18b-5p, miR-130b-3p, and miR-29b-2-5p were also significantly higher in men ($p = 0.019$, 0.035 , and 0.035 , respectively). However, after correction for multiple comparisons, only the difference in postoperative miR-18b-5p levels remained statistically significant (adjusted $p = 0.033$).

Smokers exhibited significantly higher postoperative and normalized postoperative levels of miR-18b-5p than non-smokers ($p = 0.040$ and 0.032 , respectively); however, these differences were no longer significant after correction for multiple comparisons.

Occasional alcohol drinkers showed significantly higher postoperative levels of miR-1-3p, miR-130b-3p, miR-376a-3p, and miR-18b-5p ($p = 0.0063$, 0.018 , 0.031 , and 0.034 , respectively), as well as higher normalized postoperative levels of miR-18b-5p, miR-130b-3p, and miR-15b-5p ($p = 0.0032$, 0.0069 , and 0.013 , respectively), compared with non-drinkers. After correction for multiple comparisons, only normalized postoperative miR-18b-5p levels remained significantly different (adjusted $p = 0.048$).

No significant differences in postoperative or normalized postoperative miRNA levels were observed according to the presence or absence of a family history of cancer.

No significant associations were found between normalized postoperative miRNA levels and disease stage.

Discussion

Whether diagnostic circulating miRNAs originate predominantly from the primary tumor remains an important but insufficiently explored question. The present study addressed this issue by investigating whether circulating miRNAs previously identified as diagnostic biomarkers of NSCLC decrease

following surgical tumor resection. The principal findings can be summarized as follows. First, most miRNAs with previously demonstrated diagnostic performance remained significantly elevated in the present subset of patients compared with healthy controls. Second, contrary to our prespecified hypothesis, the majority of these biomarkers did not demonstrate a clear and consistent decline following surgical resection of the primary tumor. Finally, no statistically significant association was detected between postoperative miRNA levels and the time from surgery to postoperative blood sampling in this sample, although the limited sample size precludes excluding a time-dependent association.

In an earlier study, we identified a panel of circulating miRNAs capable of discriminating LC patients from both healthy individuals and patients with tuberculosis, including subjects with stage I disease [11]. The present study included a subset of patients from that cohort for whom paired postoperative plasma samples were available. Despite the reduction in the number of patients from 68 to 18, seven of the previously identified biomarkers remained significantly elevated in preoperative plasma compared with healthy controls. Thus, the present subset largely reproduced the diagnostic differences observed in the original cohort.

In this sample, we did not find statistically significant evidence that surgical tumor resection was followed by a consistent reduction in circulating levels of NSCLC-associated miRNAs. None of the miRNAs that were consistently elevated in patients compared with healthy controls demonstrated a statistically significant postoperative decrease after correction for multiple comparisons. Although miR-495-3p, miR-191-5p, and miR-543 exhibited significant postoperative reductions before correction for multiple comparisons, none of them had shown elevated preoperative levels relative to healthy controls, and they were therefore not considered candidate biomarkers. Among the miRNAs elevated before surgery, miR-154-5p showed an estimated postoperative decrease ($\log_2FC = -1.26$); however, the statistical evidence for this difference was inconclusive ($p = 0.058$). Collectively, these findings leave the cellular origin of elevated circulating miRNAs in NSCLC patients unresolved.

According to published studies, all four of the aforementioned miRNAs are involved in cancer development. miR-191-5p has been described as a circulating diagnostic and prognostic biomarker in

several malignancies [17], and we previously reported its diagnostic value in breast cancer in the Kazakh population [18]. miR-495-3p is generally regarded as a tumor suppressor and has been proposed as a potential diagnostic, prognostic, and therapeutic biomarker in multiple cancers [19; 20]. miR-543 has also been implicated in cancer biology; however, its function appears to be highly context-dependent, with both oncogenic and tumor-suppressive roles reported in different tumor types [21]. miR-154-5p has been associated with multiple cancer-related processes and is generally considered a tumor-suppressive miRNA [22].

One possible explanation for the absence of substantial postoperative changes is that circulating miRNAs originate from multiple cellular sources rather than exclusively from the primary tumor. In addition to malignant cells, circulating miRNAs may be released from platelets, leukocytes, endothelial cells, fibroblasts, and other non-malignant cells through both passive mechanisms, including apoptosis, necrosis, and inflammation, and active secretion in extracellular vesicles or protein complexes [23–25]. Consequently, circulating miRNA profiles may reflect systemic host responses to malignancy rather than direct tumor shedding alone. Our findings are consistent with this concept and suggest that elevated plasma levels of NSCLC-associated miRNAs may reflect persistent systemic processes associated with malignancy, such as chronic inflammation, immune activation, angiogenesis, or tissue remodeling, rather than solely direct release from the primary tumor. This may explain why the diagnostic differences between patients and healthy controls persisted despite surgical tumor resection.

An alternative explanation for the lack of postoperative normalization is that circulating miRNA levels may require a longer period to return toward baseline after surgery. In the present sample, we did not detect statistically significant associations between the time from surgery to postoperative blood sampling and either postoperative miRNA levels or levels normalized to preoperative values over a postoperative sampling interval of 14–119 days. However, given the small sample size and heterogeneous timing of postoperative sampling, a time-dependent association cannot be excluded.

From a clinical perspective, our results suggest that diagnostic and monitoring biomarkers should not necessarily be considered interchangeable. Although the investigated circulating miRNAs

remain promising candidates for the early and differential diagnosis of NSCLC, their utility for postoperative disease monitoring, particularly as direct markers of primary tumor removal, appears to be limited. Further studies are required to elucidate the mechanisms underlying these observations.

Among the secondary findings, miR-18b-5p was the only miRNA that remained significantly associated with clinical characteristics after correction for multiple comparisons, showing higher postoperative levels in men and higher postoperative levels normalized to preoperative values in participants reporting occasional alcohol consumption. Although the biological basis of these associations remains unclear, they suggest that circulating miR-18b-5p may be influenced not only by tumor-associated processes but also by host-related factors. This is consistent with previous reports demonstrating that circulating miRNA profiles may be affected by lifestyle factors, including cigarette smoking, emphasizing the importance of considering these variables in biomarker studies [26].

The present findings should be interpreted in light of several potential study limitations. First, the number of patients with paired preoperative and postoperative samples was relatively small. The relatively small number of paired samples limited statistical power, particularly for detecting small-to-moderate postoperative changes. Therefore, the absence of statistically significant postoperative decreases should not be interpreted as evidence of absence of a true biological effect, and the findings require confirmation in larger prospective cohorts.

Second, analysis was performed using total plasma rather than isolated extracellular vesicles. It is possible that profiling extracellular vesicle-associated miRNAs would provide greater specificity for detecting tumor-related signals and their changes following surgical resection. However, isolation of extracellular vesicles introduces an additional pre-analytical step and substantially reduces the amount of available miRNA for analysis, thereby increasing the analytical demands on downstream detection methods.

Finally, interpretation of circulating miRNA studies remains challenging because of the limited methodological standardization across laboratories. Differences in sample type, pre-analytical handling, RNA isolation procedures, normalization strategies, and analytical platforms substantially contribute to the variability reported between studies [27];

28]. In the present study, these sources of technical variability were minimized by using a standardized workflow, including two-step centrifugation to obtain platelet-poor plasma, exclusion of hemolyzed samples, magnetic bead-based RNA isolation, TaqMan-based quantitative PCR, and normalization using the exogenous spike-in control ath-miR-159a. These measures minimized technical variability within the present study, although broader methodological standardization remains necessary to improve comparability across studies.

Conclusion

In this cohort, most circulating miRNAs previously identified as diagnostic biomarkers of NSCLC remained elevated at postoperative sampling after surgical tumor resection. No statistically significant postoperative decrease in the NSCLC-associated candidate miRNAs was detected after correction for multiple comparisons. These findings suggest that circulating miRNAs with diagnostic value may not directly reflect the presence or removal of the primary tumor and may therefore have limited utility as standalone biomarkers for postoperative disease monitoring. Given the small number of paired samples and variability in postoperative sampling time, these observations should be confirmed in larger prospective studies.

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ҰСАҚ ЖАСУШАЛЫ ЕМЕС ӨКПЕ ОЫРЫ БАР НАУҚАСТАРДАҒЫ ПЛАЗМАЛЫҚ МИКРОРНҚ-НЫҢ ОТАДАН КЕЙІНГІ ДИНАМИКАСЫ

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Андатпа

Өзектілігі. Циркуляциядағы микроРНҚ ұсақ жасушалы емес өкпе оырын диагностикалауға арналған перспективалы, аз инвазиялық биомаркерлер болып табылады. Алайда олардың биологиялық шығу тегі жеткілікті зерттелмеген. Егер олардың негізгі көзі бастапқы ісік болса, ісікті хирургиялық алып тастағаннан кейін олардың қан плазмасындағы деңгейі төмендеуі тиіс.

Мақсаты. ҮЖЕӨО бар пациенттерде ісікті хирургиялық алып тастағаннан кейін бұрын анықталған диагностикалық микроРНҚ-ның плазмадағы деңгейі төмендейтінін анықтау.

Әдістері. Зерттеуге алдыңғы зерттеуімізге қатысқан, гистологиялық тұрғыдан расталған ұсақ жасушалы емес өкпе оыры бар 18 пациент және 41 дені сау ерікті енгізілді. Пациенттердің жұпталған плазма үлгілері операцияға дейін және операциядан кейін 14–119 күн өткен соң, адьювантты ем басталғанға дейін алынды. Эксперимент РНҚ-ны магниттік бөлшектер арқылы бөліп алуды, кері

транскрипцияны, TaqMan технологиясына негізделген нақты уақыттағы ПТР-ды және ath-miR-159a экзогенді бақылауы бойынша қалыпқа келтіруді қамтыды. Статистикалық талдау көптік салыстыруларға түзету енгізу арқылы жүргізілді.

Нәтижелері. Жеті микроРНК-ның (miR-130b-3p, miR-1-3p, miR-423-5p, miR-200a-3p, miR-154-5p, miR-376a-3p және miR-18b-5p) операция алдындағы плазмадағы деңгейі дені сау еріктілермен салыстырғанда статистикалық тұрғыдан мәнді жоғары болды. Операциядан кейін олардың бесеуінің деңгейі мәнді жоғары болып қалды. Көптік салыстыруларға түзету енгізілгеннен кейін ұсақ жасушалы емес өкпе обырынмен байланысты кандидаттық микроРНК деңгейлерінің операциядан кейін статистикалық мәнді төмендеуі анықталмады. Үш микроРНК (miR-495-3p, miR-191-5p және miR-543) номиналды $p < 0,05$ кезінде операциядан кейін төмендегенімен, түзетуден кейін бұл айырмашылықтар статистикалық мәнділігін жоғалтты. Олардың операция алдындағы деңгейлері бақылау тобындағы деңгейлерден статистикалық тұрғыдан мәнді ерекшеленбеді. Осы іріктемеде операциядан кейінгі микроРНК деңгейлері мен операциядан кейінгі қан үлгісін алуға дейінгі уақыт арасында статистикалық мәнді корреляция анықталмады.

Қорытынды. Көптік салыстыруларға түзету енгізілгеннен кейін ұсақ жасушалы емес өкпе обырынмен байланысты кандидаттық микроРНК деңгейлерінің операциядан кейін статистикалық мәнді төмендеуі анықталмады. Бұл нәтижелер олардың жасушалық шығу тегін анықтауға немесе ауруды операциядан кейін бақылауға жарамдылығын растауға мүмкіндік бермейді. Ірі проспективті зерттеулер қажет.

Түйін сөздер: ұсақ жасушалы емес өкпе обыры; циркуляциядағы микроРНК; қан плазмасы; ісік биомаркерлері.

ПОСЛЕОПЕРАЦИОННАЯ ДИНАМИКА ПЛАЗМЕННЫХ МИКРОРНК У ПАЦИЕНТОВ С НЕМЕЛКОКЛЕТОЧНЫМ РАКОМ ЛЕГКОГО

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Аннотация

Актуальность. Циркулирующие микроРНК являются перспективными минимально инвазивными биомаркерами для диагностики немелкоклеточного рака лёгкого. Однако их биологическое происхождение остаётся недостаточно изученным. Если первичная опухоль является их основным источником, её хирургическое удаление должно приводить к снижению их уровней в плазме крови.

Цель. Определить, снижаются ли плазменные уровни ранее выявленных диагностических микроРНК после хирургического удаления опухоли у пациентов с НМРЛ.

Методы. В исследование включены 18 пациентов с гистологически подтверждённым немелкоклеточным раком лёгкого и 41 здоровый доброволец из нашего предыдущего исследования. Парные образцы плазмы пациентов получены до операции и через 14–119 дней после операции, до начала адъювантной терапии. Эксперимент включал выделение РНК с помощью магнитных частиц, обратную транскрипцию, ПЦР в реальном времени на основе технологии TaqMan и нормализацию по экзогенному контролю ath-miR-159a. Статистический анализ проводили с поправкой на множественные сравнения.

Результаты. Предоперационные уровни семи микроРНК (miR-130b-3p, miR-1-3p, miR-423-5p, miR-200a-3p, miR-154-5p, miR-376a-3p и miR-18b-5p) были статистически значимо выше, чем у здоровых добровольцев. После операции уровни пяти из них оставались значимо повышенными. После поправки на множественные сравнения статистически значимого послеоперационного снижения кандидатных микроРНК, ассоциированных с немелкоклеточным раком лёгкого, не выявлено. Три микроРНК (miR-495-3p, miR-191-5p и miR-543) показали послеоперационное снижение при номинальном $p < 0,05$, однако после поправки эти различия утратили статистическую значимость. Их предоперационные уровни статистически значимо не отличались от уровней в контрольной группе. В данной выборке не выявлено статистически значимой корреляции между послеоперационными уровнями микроРНК и временем от операции до послеоперационного забора крови.

Выводы. После поправки на множественные сравнения статистически значимого послеоперационного снижения кандидатных микроРНК, ассоциированных с немелкоклеточным раком лёгкого, не выявлено. Полученные результаты не позволяют установить их клеточное происхождение или подтвердить пригодность для послеоперационного мониторинга заболевания. Необходимы более крупные проспективные исследования.

Ключевые слова: немелкоклеточный рак лёгкого, циркулирующие микроРНК, плазма крови, опухолевые биомаркеры.

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