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REVIEWS

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# Circulating microRNAs in Lung Cancer: Prospects for Diagnosis, Prognosis, and Prediction of Antitumor Treatment Efficacy

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**Abstract**—The review considers the main techniques to extract microRNA (miRNA) from various biological fluids (in particular, the serum and plasma), approaches to the analysis of miRNA concentration and composition, and methods to normalize the results in data analyses. Advantages and drawbacks of the methods are described. Special attention is given to circulating miRNAs, which can be used as markers for minimally invasive diagnosis, prediction of antitumor treatment efficacy, and disease prognosis in lung cancer. The review discusses the prospects and limitations that arise as the clinical significance is evaluated for miRNAs as potential tumor markers and a better understanding is gained for the roles various miRNAs play in the pathogenesis of lung cancer.

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*Keywords*: lung cancer, diagnosis, prognosis, tumor markers, circulating microRNAs

## INTRODUCTION

As is well known, gene expression is regulated epigenetically. Noncoding RNAs, including small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs), microRNAs (miRNAs), and long noncoding RNAs (lncRNAs), were recently identified as key regulators of cell processes. Of all noncoding RNA classes, miRNAs are the most widespread and best understood.

The miRNA class includes short (19- to 24-nt) noncoding RNAs that play an important role in regulating many biological processes, such as the cell cycle, cell growth, migration, apoptosis, differentiation, and stress response. According to various estimates, miRNAs regulate expression of 30–50% of all human genes [1]. To suppress posttranscription activity, miRNAs bind to the 3'-untranslated region of their target and thereby cause its degradation or reversible inactivation. Mechanisms of action other than transcriptional suppression were also described for miRNAs; e.g., miRNAs are capable of increasing mRNA translation and playing a role in maturation of other miRNAs [2, 3]. Many miRNAs and their families are highly conserved. For instance, the let-7 miRNA was identified in *Caenorhabditis elegans* [4], and mature miRNAs of the let-7 family proved to be highly conserved among vertebrates, including humans. Lee et al. [5] were the first to discover miRNAs (lin-4 miRNA) in 1993, but intense studies of short noncoding RNAs did not start until 2000, when another miRNA, let-7, was identified and a relationship was observed between the miRNA expression level and tumor development [6]. Now miRNAs are isolated in a separate class, which is currently the most abundant. The latest version (June 2014) of the mirbase.org database contains information on 35826 mature miRNAs of 223 species, including 2661 human miRNAs.

It is thought that miRNAs are among the key elements in the pathogenesis of cancer. Changes in miRNA expression profile were observed in almost all cancers, miRNAs acting both as oncogenes and as tumor suppressors [7]. Developing methods to diagnose cancer early by markers found in the blood and other biological fluids is an important element in fighting this plague of the 21st century. Liquid biopsy with detection of circulating extracellular nucleic acids, including DNA, RNA, and miRNA, is thought to be the most promising. The review discusses the methods and results of circulating miRNA profiling in lung cancer and the potential of circulating miRNAs as diagnostic, prognostic, and theranostic markers.

## METHODOLOGICAL APPROACHES TO CIRCULATING miRNA ANALYSIS

**Isolation.** Methods to isolate mRNA from various biological materials were developed. The methods

**Table 1.** Main commercial kits designed to isolate miRNA from biological fluids

| Kit | Principal methods | Advantages | Drawbacks |
|---|---|---|---|
| Trizol LS ("Life Technologies") | Phenol–guanidinium–chloroform, precipitation- or column-based purification (not included in the kit) | Sample size is not limited; the method can be combined with any purification technique | Phenol is used; enrichment in short RNAs is not achieved |
| miRVANA ("Life Technologies") | Phenol–guanidinium–chloroform, two-step column-based purification | Enrichment in short RNAs | Phenol is used; the sample size is limited by the column capacity |
| miRNeasy Serum/ Plasma kit ("Qiagen") | Phenol–guanidinium–chloroform, column-based purification | Enrichment in short RNAs | Phenol is used; the sample size is limited by the column capacity |
| TaqMan ABC miRNA Purification Kit ("Applied Biosystems") | Phenol–guanidinium–chloroform, purification using magnetic beads with immobil | Highly efficient isolation of preset target miRNAs | Phenol is used; only a preset range of miRNAs can be isolated |
| miRCURY Biofluids Kit ("Exiqon") | Denaturation and precipitation of plasma proteins and lipoproteins; column-based purification | Phenol is not used; the method is rapid and convenient; enrichment in short RNAs | The sample size is limited by the column capacity; miRNA losses due to coprecipitation are possible for samples with a high protein content |

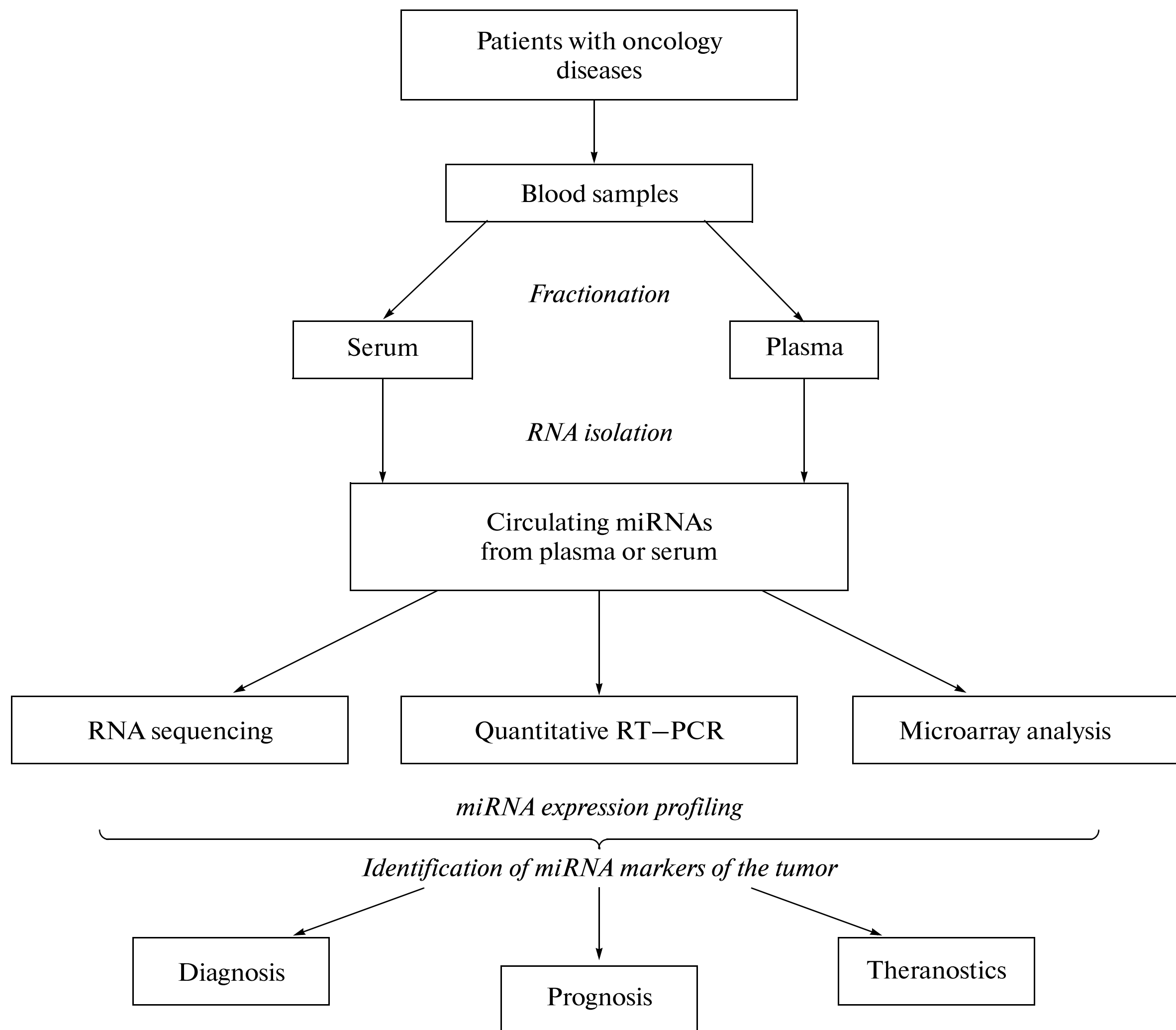
should be optimized for particular samples when working with biological fluids (the blood plasma, serum, urine, bronchial washing fluid, etc.). Depending on the study aim, the blood serum or plasma are examined for circulating miRNAs, each of the miRNA sources having its advantages and drawbacks. Several factors are to be considered when miRNA is to be quantitatively isolated from blood plasma or serum samples. The factors include nuclease activity of the blood, the circulating miRNA form, and several preanalytical variables (blood processing conditions, leukocyte and erythrocyte stability during sample processing, and sample storage duration and conditions).

Three main problems are to be solved when isolating miRNA from biological fluids. First, miRNAs circulate as components of membrane vesicles (exosomes or microparticles) [8, 9] or complexes with proteins [10] or lipoproteins [11] according tomany studies, and all miRNA-containing entities should be disrupted to isolate miRNA. Second, endogenous RNases are highly active in the blood plasma and other biological fluids and can degrade circulating RNAs as native complexes are disrupted during isolation or degrade during long-term storage. Third, biological fluids abound in proteins, lipids, and their complexes, which associate with adsorbent surfaces and form vast interphases to complicate miRNA isolation. An optimal miRNA isolation protocol should meet the following requirements: be insensitive to ballast biopolymers, minimize the effect of endogenous nucleases, ensure efficient disruption of natural miRNA-containing complexes, and eventually yield a maximal amount of miRNA free of inhibitors that may affect the enzymes employed at subsequent steps.

Although the protocols available for miRNA isolation from biological fluids are apparently diverse, the vast majority of them are based on phenol–guanidinium extraction, which Chomczynski and Sacchi [12] proposed as early as 1987. The method ensures efficient separation RNA, DNA, and proteins and is considered to be the gold standard in RNA isolation. The phenol–guanidinium method with various modifications is often employed in miRNA isolation protocols with commercial kits, including TRIzol (Life Technologies, United States), miRVANA (Life Technologies, United States), etc. (Table 1). For instance, phenol–guanidinium extraction with subsequent purification on a column with a solid sorbent is used to obtain miRNA preparations enriched in short RNAs (Qiagen, United States) [13].

A phenol–chloroform–aqueous phase system is broadly used to extract RNA, although the method has several drawbacks. For instance, the procedure is laborious and time consuming (extraction takes 40–60 min), high-quality reagents (in particular, phenol) are necessary, and the results obtained with this intricate procedure depend on the researcher's skills. Phenol is highly toxic, requiring additional equipment, workplaces, and a waste disposal system. Thus, the method is difficult to use for routine miRNA isolation in medicine. A miRCURY kit (Exiqon, Denmark) is designed with an alternative approach to RNA isolation. In place of laborious organic extraction, complexes are denatured and proteins and lipids precipitated using patented reagents in this procedure, and miRNA is then isolated via sorption on a column. The procedure reduces the isolation time to 30 min and obviates the need of toxic reagents.

**An analysis of miRNA expression profiles.** The methods available for miRNA expression profiling can conventionally be divided into three groups: massive parallel sequencing of miRNA, microarray analysis,




Isolation, expression profiling, and possible application of circulating miRNAs as potential markers in medicine.

and quantitative RT–PCR (figure). Each of the approaches has its advantages and drawbacks.

Massive parallel sequencing makes it possible to examine the total set of miRNA sequences in a sample, including nonmature forms and miRNA precursors, and to search for unknown miRNAs. The drawbacks of the methods are that a large amount of starting material (1–5 μg of total RNA) is necessary, the procedure is time and labor consuming, and a bioinformatics analysis of the results is rather sophisticated and needs verification [14].

Microarray technology provides for a more rapid miRNA expression profiling, but requires a considerable amount of starting material, like sequencing, and allows only a limited (though large) number of targets to be examined [14]. Several commercial microarray variants are now available, including GeneChip miRNA arrays on the Affymetrix platform, Human miRNA arrays on the Agilent platform, miRCURY LNA miRNA arrays on the Exiqon platform, and Human miRNA v2 Panel on the Illumina platform. The platforms differ in the amount of starting material required (from 100 ng to 1 μg of RNA), analysis time (1.5–2 days), analysis cost, and labor intensity.

Quantitative RT–PCR is used to analyze the expression pattern of known miRNAs. Several main protocols were developed for the reactions. Compared with the above techniques, RT–PCR is cheap and has a high sensitivity, allowing small miRNA amounts to be used. A drawback is that a limited number of miRNAs can be examined simultaneously by quantitative RT–PCR. A solution to the problem was provided by preformed PCR microarrays, which are designed for a quantitative PCR analysis of numerous miRNAs in separate wells of a 96- or 384-well plate. The method combines the advantages of the two related techniques and seems to be the most promising for both research and clinical applications. Examples are provided by TaqMan arrays (Applied Biosystems, United States) and LNA qPCR panels (Exiqon, Denmark).

**Normalization of miRNA expression data.** A feature of studies of circulating miRNAs is that the results are difficult to interpret and findings from different studies are difficult to compare adequately. The difficulty arises because the number of miRNA molecules per cell is infeasible to correctly estimate in the case of biological fluids. Hence, a normalizer should be used when comparing samples. Data are normalized by the $2_T^{-\Delta C}$ method, which estimates the expression levels of particular miRNAs relative to certain internal standards. Unfortunately, "housekeeping miRNAs" are still unknown, while they might be used similarly to mRNAs of housekeeping genes, such as *GAPDH*. Results are sometimes normalized to the sample volume or, as characteristic of sequencing, to the total RNA amount [14]. Another common method is normalizing to exogenous miRNAs, e.g., synthetic oligonucleotides with *C. elegans* miRNA sequences, which are added to the sample at the isolation step [15]. Various short RNAs, such as constitutively expressed small nuclear RNAs (snRNU6 or snRNU48) can be used as standards. The main drawback that limits their use is that their size (>100 nt) is far greater than the miRNA size. Consequently, the two RNA types differ in the efficiency of isolation and require different RT–PCR conditions to be amplified [14].

Certain miRNAs can be employed in normalization given that their tissue concentration is constant. In the case of blood samples, miRNA-16 is thought to meet this requirement, although there is evidence that its concentration fluctuates depending, for instance, on the sample storage conditions [10]. Normalization of data on blood circulating miRNAs is further complicated by erythrocyte lysis or microclotting because blood cell miRNAs may find their way into the sample. To minimize the contribution of cell miRNAs to the circulating miRNA pool, miRNAs characteristic of erythrocytes of platelets can be included in the analysis [16].

## CIRCULATING miRNAs AS DIAGNOSTIC MARKERS

There is convincing evidence that miRNA expression patterns differ substantially between normal and tumor tissues in various oncology diseases, in particular, lung cancer. It was observed that tumor miRNAs occur in the blood plasma or serum in detectable concentrations and are sufficiently stable during clinical sample preparation and storage, thus attracting particular interest as cancer markers [17].

An approach to validation of miRNA markers is translating the data of tissue comparisons to the blood. In other words, the serum or plasma concentrations are determined for the miRNAs that proved to differ in expression profile between tumor and normal tissues. Shen et al. [18] observed a difference in expression between tumor and adjacent normal lung tissues for 12 miRNAs of patients with non-small cell lung cancer (NSCLC), and expression of the miRNAs was compared in paired tissue and blood plasma samples. A correlation between changes in tissue expression and changes in blood concentration was observed for only five out of the 12 miRNAs (Table 2). Zheng et al. [24] selected 15 target miRNA based on the published results of a microarray analysis of lung tumor tissues. The miRNA profile was obtained for plasma samples from patients with NSCLC and small cell lung cancer (SCLC). Only three miRNAs—155, 197, and 182—showed a substantial increase in lung cancer patients compared with healthy subjects. The data indicate that only some of the tumor cell miRNAs find their way in circulation.

Recent studies focused on the mechanisms whereby miRNA-containing complexes appear in circulation, circulate, accumulate in organs and tissues, and are eliminated from the body. The results will help to understand how a correlation arises between tissue and blood miRNA profiles. It should be noted that a high level of cancer-specific miRNAs circulating within exosomes of a tumor origin was observed when studying circulating miRNAs in plasma samples from squamous cell carcinoma (SCC) patients. Some of these miRNAs were secreted into the medium of cultured lung cancer cell. The findings led to a conclusion that miRNA profiling of plasma exosomes will help to identify reliable cancer markers [26].

An alternative method to identify potential miRNA markers is a large-scale analysis of the composition and concentrations of circulating miRNAs in the blood plasma or serum by high-throughput techniques, such as sequencing, microarray studies, and PCR. Selected miRNAs are then tested for diagnostic significance via quantitative PCR in an independent sample of patients. Chen et al. [22] were the first to perform a large-scale miRNA analysis in the blood serum of NSCLC patients, using sequencing on the Solexa platform. The study involved 11 NSCLC patients, 11 healthy males, and 10 healthy females. In total, 63 out of 132 serum miRNAs were detected in the patients, but not in the healthy subjects, while 28 miRNAs were observed only in the healthy subjects. In addition, 76 miRNAs found in the serum samples of the NSCLC patients were not detected in blood cells of the same patients, suggesting tumor cells or their microenvironment to be a source of the miRNAs.

A further study was performed with an independent sample of 152 lung cancer patients and 75 healthy subjects by RT–PCR and showed that two specific miRNAs, miRNA-25 and miRNA-223, significantly differ in serum concentration between the patients and healthy subjects. Chen et al. [28] used their previous Solexa sequencing data [22] to verify a panel of 91 miRNAs on serum samples from 300 NSCLC patients and 220 healthy subjects. An extensive diagnostic model was designed to include ten miRNA markers and proved to differentiate lung cancer patients and healthy subjects with high sensitivity (93%) and high specificity (90%) [28].

**Table 2.** Circulating miRNAs in diagnosis and prognosis of lung cancer

| miRNA panel | Detectable changes | Sample | Clinical application | Detection method | Reference |
|---|---|---|---|---|---|
| miRNA-21, -126, 210, 486-5p | 86% sensitivity and 97% specificity in NSCLC patients vs. HSs; 73% sensitivity and 97% specificity in stage I NSCLC patients vs. HSs | Plasma | Diagnosis | Quantitative RT–PCR | [18] |
| miRNA-486, 30d, 1, 499 | Differentiation of HSCLC patients with favorable or poor prognosis (↑ miRNA-486, -30d; ↓ miRNA-1, -499 | Serum | Prognosis | Solexa sequencing, quantitative RT–PCR | [19] |
| miRNA-126, 183 | significant difference in average expression level of miRNA-26 (↑) and miRNA-183 (↓) between stage I/II NSCLC pa | Serum | Diagnosis | Quantitative stem–loop RT–PCR | [20] |
| 34-miRNA signature | 71% sensitivity and 90% specificity in patients with suspected NSCLS vs. HSs; 59% sensitivity and 90% specificity in stage I NSCLC patients vs. HSs; | Serum | Diagnosis | Quantitative RT–PCR | [21] |
| panel of 91 miRNAs | 28 miRNAs detected only in HSs; 63 miRNAs, only in NSCLC patients | Serum | Diagnosis | Solexa sequencing | [22] |
| miRNA-25, 223 | Significant difference in average expression level of miRNA-25 ($P = 8.78$ E–08) and miRNA-223 ($P = 1.56$ E–05) be | | | Quantitative RT–PCR | |
| miRNA-1254, 574-5p | 73% sensitivity and 71% specificity in stage I/II NSCLC patients vs. HSs | Serum | Diagnosis | Microarrays, quantitative RT–PCR | [23] |
| miRNA-155, 197, -182 | 81% sensitivity and 87% specificity in LC patients vs. HSs | Plasma | Diagnosis | Quantitative RT–PCR | [24] |
| miRNA-15b, 27b | 100% sensitivity and 84% specificity in NSCLC patients vs. HSs | Serum | Diagnosis | Quantitative RT–PCR | [25] |
| miRNA-146b, 221, let-7a, 155, 17-5p, 27a, 106a, 29c | Expression level significantly decreases for all but one miRNA and increases for miRNA-29c in NSCLC ($P < 0.05$) | Serum | Diagnosis | Quantitative RT–PCR | [17] |
| miRNA-205, 19a, 19b, 30b, 20a | Expression level significantly decreases after tumor resection ($P < 0.05$) | Plasma | Prognosis | Microarrays, quantitative RT–PCR | [26] |
| miRNA-21, 205, 30d, 24 | Significant difference in expression level between LC patients and HSs; | Serum | Diagnosis | Quantitative R–PCR | [27] |
| miRNA-21, 24 | Significant difference in expression level between LC patients before and after surgery ($P < 0.05$) | | Prognosis | | |
| miRNA-20a, 24, 25, 145, 152, 199a-5p, -221, 222, 223, 320 | 93% sensitivity and 90% specificity in NSCLC patients vs. HSs; 6/7 NSCLC cases identified prior to diagnosis | Serum | Diagnosis | Quantitative stem–loop RT–PCR | [28] |

**Table 2.** (Contd.)

| miRNA panel | Detectable changes | Sample | Clinical application | Detection method | Reference |
|---|---|---|---|---|---|
| (1) miRNA signature including 16 ratios to estimate the disease risk | 80% sensitivity and 90% specificity in patients with suspected LC vs. HSs | Plasma | Diagnosis | Microarrays, quantitative RT–PCR | [29] |
| (2) miRNA signature including 16 ratios to diagnose the disease | 5% sensitivity and 100% specificity in LC patients vs. HSs | | | | |
| (1) miRNA signature including 10 ratios to estimate the risk of aggressive disease prior to diagnosis | 80% sensitivity and 100% specificity in patients with favorable prognosis vs. patients with poor prognosis | Plasma | Prognosis | | |
| (2) miRNA signature including 10 ratios to estimate the risk of aggressive disease at the time of diagnosis | 88% sensitivity and 100% specificity in LC patients with favorable prognosis vs. LC patients with poor prognosis | | | | |
| 17-miRNA signature (miRNA-16, 30e-3p, 106a, 148b, 26a, 106b, 301a, 27b, 152, 20a, 30a-5p, 148a, 145, 92a, let-7c, let-7b) | Predicts the 2-year survival in patients with advanced NSCLC | Serum | Prognosis | Microarrays, quantitative RT–PCR | [30] |
| 12-miRNA signature (miRNA-155-5p, 20a-5p, 25-3p, 296-5p, 191-5p, 126-3p, 223-3p, 152-3p, 145-5p, 199a-5p, 24-3p, let-7f-5p) | 85% sensitivity and 75% specificity in NSCLC patients vs. HSs | Plasma | Diagnosis | Quantitative stem–loop RT–PCR | [31] |
| miRNA-155-5p, 223-3p, 126-3p | ↑ miRNA-155-5p, 223-3p and ↓ miRNA-126-3p are associated with a high risk of progression in AC patients | | Prognosis | | |
| miRNA-20a-5p, 152-3p, 199a-5p | Predicts survival in SCLC (↑ miRNA-201-5p and ↓ miRNA-152-3p, 199a-5p) | | | | |

↑, increase in miRNA expression; ↓, decrease in miRNA expression; LC, lung cancer; NSCLC, non-small cell lung cancer; SCLC, squamous cell lung cancer; AC, lung adenocarcinoma; HSs, healthy subjects.

To estimate the clinical significance of blood biomarkers, it is important to determine how stable the miRNA profile is with time and how early changes in miRNA profile become detectable during cancer development and progression. Several studies were carried out to address these issues. Keller et al. [32] used microfluidic chips to study the profile of 904 miRNAs in 29 serum samples, which were collected over several years at various intervals (from 2 to 32 days). The samples were collected from eight patients before and after they were diagnosed with lung cancer and from six healthy subjects. The miRNA profile remained stable over several years, and substantial changes became detectable close to the time of diagnosis. However, it was observed that some of the changes in miRNA profile are not related to carcinogenesis. Chen et al. [28] used the original panel of ten miRNAs (see above) to test seven serum specimens, which were collected 7–33 days before the donor was diagnosed with lung cancer. Estimation of the serum concentrations for the ten miRNAs proved to identify the patients with suspected lung cancer in 86% (6/7) of cases.

Boeri et al. [29] used a microarray technique and quantitative RT–PCR to estimate the concentrations of 100 miRNAs in plasma samples collected during computed tomography from patients who were more recently diagnosed with lung cancer ($N = 41$). Data were normalized by miRNA expression by a ratios method. The expression level of each particular miRNA was related to the expression levels of the other 99 miRNAs. The 4950 resulting ratios were used to identify the clinically significant differences between samples of the two groups. Data processing yielded a miRNA signature (16 ratios involving 15 different miRNAs), which differentiated preclinical patients (1–2 years before a diagnosis of lung cancer) from heavy smokers without oncology diseases. The method showed 80% sensitivity, 90% specificity, and 85% accuracy. Bianchi et al. [21] identified a 34-miRNA signature, which reported NSCLC at an early stage, before clinical signs appear, with 80% accuracy.

Thus, the available data indicate that cancer-related miRNAs appear in circulation long before a clinical manifestation of the tumor. Although an increasing number of studies focus on circulating miRNAs, there is still no consensus as to the miRNAs that should be included in a panel suitable for clinical diagnosis of lung cancer. Several technical and general factors underlie this circumstance, i.e., miRNA detection should be accurate; the methods used to isolate and analyze miRNAs should be standardized; and test and reference subject samples should be formed correctly, with due regard to the sex and age compositions, tumor histology, disease stage, concomitant disorders, and certain particular nontumor pathologies.

## CIRCULATING miRNAs AS PROGNOSTIC MARKERS

There is evidence that miRNAs are possibly involved in the signaling pathways that regulate various biological processes determining the tumor aggressiveness and sensitivity to chemotherapeutics. The processes include cell differentiation, proliferation, motility, epithelial–mesenchymal transition, DNA repair, apoptosis, and survival [33–36]. Examination of biopsy and surgery materials fails to report in full the clonal molecular changes that accompany tumor progression and metastasis. Circulating miRNA profiling is of particular interest in terms of disease prognosis, prediction of the response to therapy, monitoring, and estimation of treatment efficacy [15].

To identify the prognostic markers, Hu et al. [19] compared the serum miRNA profiles for NSCLC patients with high or low survival parameters (30 patients per group); the profiles were obtained by Solexa sequencing. Four miRNAs—486, 30d, 1, and 499—were found to significantly differ in expression level between the two groups. Serum samples of 243 patients with NSCLC stages I–IIIA were examined. NSCLC patients with a high mortality rate displayed higher expression of miRNA-486 and miRNA-30d and lower expression of miRNA-1 and miRNA-499 in the serum.

Based on the above approach, Boeri et al. [29] identified a miRNA signature of risk to have or to develop aggressive cancer; the signature included ten ratios between miRNA expression levels. The signature of risk to develop aggressive cancer was identified by analyzing the plasma samples collected from 19 NSCLC patients prior to diagnosis (a training sample). Examination of a test sample (22 patients) showed that the signature is suitable for differentiating between lung cancer patients with a poor or favorable prognosis (sensitivity, 80%; specificity, 100%). The signature of having aggressive cancer was tested with plasma samples collected from patients at the time of diagnosis. The signature proved to identify the lung cancer patients having a poor prognosis with 88% sensitivity and 100% specificity. To verify the clinical significance of the relevant miRNAs as prognostic markers, the results should be verified with independent representative samples.

Le et al. [27] used quantitative RT–PCR to estimate the expression levels of four miRNAs (miRNA-21, -205, -30d, and -24) in the blood serum for 82 NSCLC patients before treatment and 50 healthy subjects. Expression of the four circulating miRNAs in the lung cancer patients was significantly higher than in the healthy subjects even at early cancer stages. Two miRNAs, 21 and 30d, were identified as markers whose expression levels before treatment is associated with a low survival.

Sanfiorenzo et al. [31] identified two different plasma miRNA signatures associated with the histo-

logical type of lung cancer that predict tumor progression with a high sensitivity. Changes in the concentrations of three miRNAs (152-3p, 199a-5p, and 20a-5p) were associated with a high risk of progression (recurrence) in patients with lung adenocarcinoma, while changes in the concentrations of three other miRNAs (155-5p, 233-3p, and 126-3p) were associated with a high risk of tumor progression in these patients. Wang et al. [30] examined serum samples of 391 patients with advanced NSCLC. The miRNA profiles of eight serum samples were obtained using a TaqMan human microRNA microarray card set v. 3.0 (Life Technologies, United States). Expression of 17 selected miRNAs was then studied in 192 (training sample) and 191 (test sample) serum samples of NSCLC patients by quantitative RT–PCR. Changes in expression of the 17 miRNAs in the serum were associated with the 2-year survival of NSCLC patients (Table 2). The panel of the 17 miRNAs was proposed as an efficient diagnostic test, which should be checked with an independent sample of subjects to evaluate its significance as a marker of disease prognosis.

## CIRCULATING miRNAs AS TREATMENT EFFICACY MARKERS

Circulating miRNAs are of interest not only as potential markers for diagnosis and prognosis evaluation in cancer, but also as markers of treatment efficacy for both drug therapy and radiotherapy. Clinically significant miRNA markers are identified by comparing cancer-related miRNA expression profiles obtained before and after treatment. Aushev et al. [26] estimated the plasma levels of 90 miRNAs with the use of a commercial kit for an analysis based on RT–PCR (miRCURY LNA, Exiqon, Denmark). The levels of five miRNAs (205, 191, 19b 30b, and 20a) were shown to significantly decrease after surgery. Le et al. [27] compared the profiles of four serum miRNAs (see the second section) in lung cancer patients. The profiles were obtained prior to and 10 days after combined treatment, which included chemotherapy and surgery). The expression levels of miRNA-21 and miRNA-24 decreased in the postsurgery period compared with the baseline.

An increasing number of studies support the association of changes in blood mRNA profile with drug resistance in patients with oncology diseases. The miRNA-21 expression level in the blood plasma of lung cancer patients was associated with sensitivity to chemotherapy with platinum drugs. Wei et al. [37] showed that the plasma miRNA-21 level in NSCLC patients with partial tumor regression after chemotherapy is several times lower than in NSCLC patients with a stabilized or progressive disease. Franchina et al. [38] showed that the miRN-22 level in the blood plasma correlates with the response to treatment with pemetrexed (a platinum drug). An increase in miRNA-22 expression in the plasma was observed in lung cancer patients with disease progression after chemotherapy. It is thought that the plasma miRNA-22 level can be used as a potential marker to predict the response to pemetrexed chemotherapy.

Circulating miRNAs may play a key role in the antitumor response due to activation of the immune system. Kumarswamy et al. [39] showed that STAT3 activation in cancer cells affects the immune cell recruitment, which is absolutely essential for successful T-cell therapy. The regulation of STAT3 activity is known to involve oncogenic miRNA-21, which is capable of stimulating the tumor cell growth and proliferation. Studying the expression level of circulating miRNA-21 in the blood provides a promising approach to designing new predictors of the response to antitumor immunotherapy.

## CONCLUSIONS

The published data considered above indicate that studying the role certain miRNAs play in the pathogenesis of cancer and, in particular, lung cancer is promising. Circulating miRNAs of the blood plasma or serum have a high potential as cancer markers. Changes in circulating miRNA profile were recently associated with lung cancer, its progression, and survival. In addition, miRNA profiling may be informative for differentiating lung cancers according to their histological type. When identified, specific regulatory miRNAs associated with drug and radiotherapy sensitivities of tumors will provide a basis for developing innovative strategies of individual therapy and post-treatment monitoring in lung cancer. Developing the protocols for efficient routine miRNA testing in clinical labs is still a problem unsolved. To introduce miRNA testing in medicine, efforts of several labs should be joined to develop the efficient standard protocols of blood sample preparation and storage and miRNA isolation and analysis, to search for new candidate markers, and to perform translation studies of known candidate markers with independent groups of healthy subjects and patients.

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