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The potential use of miRNAs in the diagnosis and prediction of metastatic lung carcinoma

Michaela Skaličanová[1], Tatiana Matáková[2], Anton Dzian[1], Erika Halašová[3], Marián Duffek[4], Mária Škereňová[5] and Miroslava Šarlinová[5]

[1] *Department of Thoracic Surgery, Jessenius Faculty of Medicine of Comenius University Bratislava and University Hospital Martin, Martin, Slovakia*
[2] *Institute of Medical Biochemistry, Jessenius Faculty of Medicine of Comenius University Bratislava, Martin, Slovakia*
[3] *Institute of Medical Biology, Biomedical Center, Jessenius Faculty of Medicine of Comenius University Bratislava, Martin, Slovakia*
[4] *Central Military Hospital, Faculty Hospital, Ružomberok, Slovakia*
[5] *Biomedical Center, Jessenius Faculty of Medicine of Comenius University Bratislava, Martin, Slovakia*

Abstract. Lung carcinoma is the "top killer" of all malignancies in the world. Early diagnosis of lung carcinoma significantly improves patient survival. Screening with biomarkers from peripheral blood could detect more patients at an early stage of the disease. MicroRNAs (miRNAs) could be a possible biomarker. These are 21–23 nucleotide long single-stranded RNA molecules playing an important role in the post-transcriptional regulation of gene activity. Individual miRNAs have the potential to regulate genes responsible for cell proliferation, differentiation, apoptosis, regulate cell cycle in cooperation with pro-oncogenes and tumor suppressor genes. In our study, we determined miRNA expression levels in individual samples of lung carcinoma patients and in a healthy control group. We used the reverse transcription method followed by qRT-PCR. The expression levels of the investigated miRNAs were evaluated in the QIAGEN GeneGlobe Data center software. We demonstrated the significance of miR-126 and let-7g as biomarkers of lung carcinoma in all clinical stages studied. We also observed significantly increased expression of miR-143 and miR-145 at the distant metastasis stage, and significantly decreased expression of miR-133a in the N2 disease group of lung carcinoma patients (N2 disease represents disease with metastases in the ipsilateral mediastinal and/or subcarinal lymph nodes or node). The investigated miRNAs showed no clear potential for detecting potentially resectable (N0–N1), locally advanced (N2) and distant organ metastatic (M1) lung carcinoma.

Key words: Lung carcinoma — Screening — miRNA — Biomarker — TNM classification

Introduction

Lung carcinoma is the leading cause of cancer-related deaths worldwide. Despite standard treatment, its aggressive behavior associated with invasion and migration causes rapid disease progression. MicroRNAs (miRNAs) regulate many genes and various signaling pathways. The increasing number of studies in this issue confirm that miRNAs significantly enter into different expression patterns in this disease. Dysregulation of miRNA expression results in the activation and regulation of epithelial-to-mesenchymal transition (EMT) and metastatic process at the level of different genes. Expression of different miRNAs either in tumor tissue or in blood serum causes metastasis at different sites. These miRNA profiles also correlate with prognosis and response

Correspondence to: Tatiana Matáková, Institute of Medical Biochemistry, Jessenius Faculty of Medicine of Comenius University Bratislava, Bratislava, Slovakia
E-mail: Tatiana.Matakova@jfmed.uniba.sk

to clinical therapy and may equally be potential therapeutic targets. However, in the area of miRNA-targeted therapy, research needs to be intensified to increase target specificity and efficacy on the one hand, and conversely reduce off-target efficacy and toxicity on the other hand (Han and Li 2018; Petrek and Yu 2019).

Metastasis is a hallmark of cancer, and distant metastasis often accompanies lung carcinoma, even at initial diagnosis, resulting in poor prognosis and high mortality. However, available biomarkers cannot reliably predict sites of metastasis. The metastatic cascade is a highly complex and intricate process that involves invasion, migration, angiogenesis, and EMT, which are tightly controlled by different pathways of genetic expression along with interactions between tumor cells and the extracellular matrix. It is the critical role of the EMT that provides the key to preventing tumor spread as well as identifying potential therapeutic targets. In particular, miRNAs, a group of small non-coding RNAs, influence transcriptional and post-transcriptional processes, with dysregulation of miRNA expression contributing to the regulation of metastasis. miRNAs primarily repress gene expression by direct interaction of target mRNAs (Huang 2018; Guo et al. 2019). Thus, the identification of different miRNA expression patterns supports the ability to classify tissue-specific miRNAs and predict disease stage. Aberrantly expressed miRNAs in various malignancies function as tumor suppressors or proto-oncogenes, regulating the biology of the tumor process by controlling the expression of target mRNAs to promote tumor growth, invasion, angiogenesis, and immune escape (Friedman et al. 2009; Di Leva et al. 2014). miRNAs regulate the actin cytoskeleton, the expression of extracellular matrix (ECM) receptors including integrins and ECM remodeling enzymes containing matrix metalloproteinases (MMPs), and regulate EMT, thereby modulating cell migration and invasiveness. Distant spread of the primary tumor represents the leading cause of death from non-small cell lung cancer (NSCLC), especially when metastasized to the brain (Celià-Terrassa and Kang 2016; Singh et al. 2018). Metastasis is a complex process in which cancer cells spread from a localized lesion to systemic disease. The "metastatic cascade" involves tumor cells crossing physical boundaries, invasion of the basement membrane and surrounding tissue, entry into the blood/lymphatic circulation (migration and intravasation), extravasation at secondary sites, and proliferation. EMT involves a variety of molecular factors, phenotypic changes, and genetic alterations in a multistep dissemination process. Conversely, cell spreading and proliferation as a metastatic lesion requires a transition from the mesenchyme to the epithelium (Dong et al. 2017).

Although many *in vitro* as well as *in vivo* studies have addressed the issue of miRNA-targeted therapy, this strategy is currently still very limited. Advances in understanding the molecular mechanisms of metastasis will provide another potential target in lung carcinoma therapy.

Table 1. Characteristics of the group of patients with lung carcinoma and control group of healthy subjects

		Lung carcinoma (n = 67)	Control (n = 70)
Age (years)		65.20 ± 8.03	67.07 ± 8.31
Sex	male	48 (72%)	45 (64%)
	female	19 (28%)	25 (32%)
Histology	Adenocarcinoma	38 (57%)	–
	Squamous cell carcinoma	29 (43%)	–
Stage	I	27 (40%)	–
	II	14 (21%)	–
	III	16 (24%)	–
	IV	10 (15%)	–

Material and Methods

Group characteristics

The study was approved by the Ethics Committee of Jessenius Faculty of Medicine in Martin and all individuals signed informed consent before participation. A total of 67 patients with lung carcinoma were included in the group. Patients were hospitalized at the Department of Thoracic Surgery, Jessenius Faculty of Medicine of Comenius University and University Hospital Martin in years 2017–2019. Patients enrolled in the study underwent radical surgical treatment for lung cancer or palliative surgery for metastatic involvement or generalization of malignant disease. Patients' demographic characteristics such as age and sex were monitored, and from clinical data we observed the stage of the disease and the histological type of the cancer. The individual clinical stages of lung cancer (TNM classification 8) were determined based on the pathological and clinical staging of the disease as follows: 27 patients were in the I. clinical stage of the disease at the time of sampling, 14 patients in the II. stage, in III. stage there were 16 patients and in VI. stage 10 patients. The control group consisted of apparently healthy subjects who were approached and consented to blood collection at the National Transfusion Station in Martin. The characteristics of the patient group and the control group are shown in Table 1. Patients with lung cancer were divided into individual groups according to the stage of the disease based on the 8th TNM classification and according to the histological type of lung cancer. Considering the goal of demonstrating the significance of miRNA as a possible biomarker in metastatic lung can-

cer, individual sets were divided according to N category (metastatic involvement of lymph nodes) and M category (metastasis to distant organs): set N0 are patients without metastatic involvement in the examined regional lymph nodes. The N1 group included patients with metastases in ipsilateral peribronchial and/or ipsilateral hilar nodes and intrapulmonary nodes. Group N2 is represented by patients with metastases to ipsilateral mediastinal and/or subcarinal lymph nodes. The M set included patients with the verified presence of distant metastases, including cases with tumor or nodules in the contralateral lung, verified pleural or pericardial carcinoma, with malignant pleural effusions, with solitary or multiple metastasis/metastases in one or more distant organs (Table 2).

Methods

Peripheral blood (2.5 ml) was collected before therapy using PAXgene Blood RNA Tubes (Qiagen, Hilden, Germany) for *in vitro* diagnostic purposes. After blood collection, the tubes were kept for two hours at room temperature. The RNA concentration and purity were confirmed by the spectrofotometric ratio using absorbance measurements at wavelengths of 260 nm and 280 nm on a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington. MA, USA). Isolated RNA was stored at –80°C.

Reverse transcription (RT) was performed with 2 ng total RNA in a 10-µl reaction using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer′s instructions. RT primers were included in TAgMan MicroRNA Assay Kit (Applied Biosystems). cDNA samples were stored at –20°C until subsequent quantitative polymerase chain reaction (qPCR).

The 20-µl reaction mixture required for the qPCR included RT product, TaqMan Universal Master Mix no UNG (Applied Biosystems), Nuclease-free water and probe mixture of the TaqMan MicroRNA Assay Protocol (Applied Biosystems). The qPCR cycling conditions were: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. All reactions were performed in duplicite.

The qPCRs were performed using an IQ5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Relative quantification (RQ) was carried out using the $2^{-\Delta\Delta Ct}$ comparative method.

Normalization and data analysis

In the context of finding appropriate endogenous controls, 60 samples were tested. This group consisted of 30 patients and 30 controls. The expression levels of the investigated miRNAs, which were normalized by the expression level of the endogenous control RNU48, were then evaluated using the QIAGEN GeneGlobe Data center software.

Analysis and statistical evaluation of the results were performed with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism 5 software (GraphPad softwere Inc., La Jolla, CA, USA). Differences between miRNA levels and clinicopathological parameters were evaluated using two tests: for testing of two groups, the Mann-Whitney U-test was used, and for testing of three or more groups, the Kruskal-Wallis test was used. A value of $p < 0.05$ was considered significant.

The rate of expression of statistically significant miRNAs in relation to lung cancer was evaluated by reciever operating characteristic (ROC) analysis.

Results

Results of expression of monitored miRNAs

We determined the expression levels of miRNAs: miR-126, miR-155, miR-221, miR-133a, let-7a, miR-182, miR-21, let-7g, and miR-19b in peripheral blood samples collected from lung carcinoma patients compared with a control group of apparently healthy subjects (Table 3). Using statistical analysis, we found significantly decreased expression for these miRNAs, especially the expression of miR-126 in the blood in all patient groups. In the adenocarcinoma patient group (Table 4), miR-126 had the lowest expression level at stages N1 and N2. Statistically significantly decreased expression of miR-126 was at stages N0 to N2. In the squamous cell carcinoma patient group (Table 5), miR-126 had statistically significantly lowest expression at stage N0. miR-133a in the group of all patients regardless of disease stage relative to the control group had a reduced expression level, which was statistically significant, at stage N2 (Table 3).

Table 2. Classification of patients based on N and M categories

	Files with the number of probands/N a M factor			
	T1-4N0M0	T1-4N1M0	T1-4N2M0	T1-4N2M1
Lung carcinoma ($n = 67$)	31	12	14	10
Adenocarcinoma ($n = 38$)	16	7	9	6
Squamous cell carcinoma ($n = 29$)	15	5	5	4

Table 3. Results of statistical analysis of differences in miRNA expression levels between patients with lung carcinoma according to N and M categories and healthy controls

miRNA	N0		N1		N2		M1	
	+/–	*p*	+/–	*p*	+/–	*p*	+/–	*p*
RNU48	1	N/A	1	N/A	1	N/A	1	N/A
miR-126	**– 28.81**	**0.000**	**– 49.28**	**0.024**	**– 28.24**	**0.007**	– 11.18	0.062
miR-155	**– 2.43**	**0.000**	**– 3.09**	**0.002**	**– 2.68**	**0.000**	**– 1.89**	**0.028**
miR-221	**– 1.97**	**0.010**	– 1.67	0.094	– 1.65	0.120	– 1.45	0.138
miR-21	**– 7.47**	**0.001**	**– 13.09**	**0.001**	**– 10.4**	**0.000**	– 3.12	0.140
mir-143	**+ 2.36**	**0.004**	**+ 1.87**	**0.012**	+ 1.57	0.211	**+ 5.33**	**0.000**
miR-145	**+ 1.49**	**0.009**	**+ 1.98**	**0.001**	+ 1.27	0.525	**+ 1.99**	**0.000**
miR-133a	– 1.53	0.076	– 1.31	0.810	**– 2.22**	**0.037**	– 1.38	0.289
let-7a	**–2.38**	**0.006**	– 3.83	0.061	– 2.52	0.052	– 1.51	0.221
miR-146a	– 1.48	0.248	– 1.36	0.313	– 1.37	0.641	+ 1.13	0.709
miR-31	+ 1.02	0.235	– 1.23	0.259	– 1.08	0.496	+ 1.09	0.579
miR-182	**– 5.73**	**0.005**	**– 8.91**	**0.018**	**– 5.24**	**0.005**	– 2.62	0.382
let-7g	**– 10.19**	**0.001**	**– 13.81**	**0.043**	**– 11.13**	**0.022**	– 6.34	0.088
miR-19b	**– 5.66**	**0.005**	**– 7.37**	**0.007**	**– 7.86**	**0.003**	– 3.71	0.559

+, up regulation (comparing to control group); –, down regulation (comparing to control group); N/A, not applicable. Values highlighted in bold are statistically significant results.

Expression of miR-143 and miR-145

We further determined the relative expression of miR-143 and miR-145 in peripheral blood samples collected from lung carcinoma patients compared to a control group of apparently healthy subjects (Table 3). We observed statistically significantly increased expression of these investigated miRNAs in the blood of patients with both adenocarcinoma and squamous cell carcinoma of the lung compared to control subjects. For miR-143, the *p*-values were statistically significant in the adenocarcinoma patient group at all stages of disease except stage N2 (Table 4). In the squamous cell carcinoma patient group, statistical significance was only at distant metastasis stage M1 (Table 5).

Table 4. Results of statistical analysis of differences in miRNA expression levels between patients with lung adenocarcinoma according to N and M category and control group

miRNA	N0		N1		N2		M1	
	+/–	*p*	+/–	*p*	+/–	*p*	+/–	*p*
RNU48	1	N/A	1	N/A	1	N/A	1	N/A
miR-126	**– 13.15**	**0.029**	**– 137.18**	**0.044**	**– 20.79**	**0.043**	– 10.39	0.190
miR-155	– 1.77	0.114	**– 4.28**	**0.003**	**– 3.2**	**0.004**	– 1.48	0.201
miR-221	– 1.64	0.306	– 2.12	0.064	– 1.71	0.264	– 1.53	0.152
miR-21	– 4.28	0.190	**– 29.03**	**0.004**	**– 9.24**	**0.008**	– 2.39	0.202
mir-143	**+ 3.03**	**0.000**	**+ 1.76**	**0.007**	+ 1.79	0.326	**+ 6.29**	**0.000**
miR-145	**+ 1.4**	**0.028**	**+ 1.65**	**0.021**	+ 1.47	0.242	**+ 2.14**	**0.001**
miR-133a	– 1.26	0.481	– 1.09	0.957	– 1.49	0.256	– 1.08	0.548
let-7a	– 2.03	0.131	– 5.46	0.094	– 2.77	0.118	– 1.24	0.434
miR-146a	– 1.37	0.700	– 1.76	0.228	– 1.04	0.144	+ 1.12	0.999
miR-31	– 1.14	0.288	– 1.27	0.323	– 1.22	0.410	+ 1.23	0.906
miR-182	– 5.09	0.097	**– 18.95**	**0.022**	**– 4.81**	**0.047**	– 3.04	0.435
let-7g	– 7.46	0.054	– 23.81	0.083	– 10.99	0.087	– 5.33	0.232
miR-19b	– 4.1	0.284	**– 10.04**	**0.022**	**– 6.17**	**0.039**	– 3.82	0.665

+, up regulation (comparing to control group); –, down regulation (comparing to control group); N/A, not applicable. Values highlighted in bold are statistically significant results.

Table 5. Results of statistical analysis of differences in miRNA expression levels between patients with squamous cell carcinoma of the lung according to N and M categories and the control group

miRNA	N0		N1		N2		M1	
	+/–	*p*	+/–	*p*	+/–	*p*	+/–	*p*
RNU48	1	N/A	1	N/A	1	N/A	1	N/A
miR-126	**– 55.34**	**0.008**	– 13.06	0.197	**– 49.97**	**0.121**	– 5.56	0.251
miR-155	**– 3.37**	**0.000**	– 2.02	0.086	**– 2.36**	**0.044**	– 1.77	0.134
miR-221	**– 2.5**	**0.008**	+ 1.08	0.892	**– 1.52**	**0.441**	+ 1.03	0.814
miR-21	**– 11.51**	**0.000**	– 4.31	0.066	**– 11.58**	**0.034**	– 2.04	0.653
miR-143	+ 2.1	0.055	+ 1.88	0.425	+ 1.38	0.196	+ 3.4	0.002
miR-145	+ 1.53	0.098	**+ 2.18**	**0.010**	+ 1.01	0.722	**+ 2.25**	**0.013**
miR-133a	– 1.97	0.081	– 1.44	0.576	– 4.3	0.093	– 1.3	0.528
let-7a	**– 2.83**	**0.040**	– 1.87	0.312	**– 2.06**	**0.370**	– 1.41	0.452
miR-146a	– 1.63	0.055	+ 1.2	0.867	– 2.05	0.186	+1.53	0.324
miR-31	1.13	0.537	– 1.19	0.494	+ 1.32	0.793	– 1.13	0.503
miR-182	**– 7.23**	**0.004**	– 3.54	0.239	**– 6.02**	**0.083**	– 1.42	0.910
let-7g	**– 13.36**	**0.022**	– 5.59	0.223	**– 9.61**	**0.200**	– 4.99	0.286
miR-19b	**– 7.35**	**0.004**	– 4.93	0.086	**– 13.65**	**0.052**	– 2.28	0.952

+, up regulation (comparing to control group); –, down regulation (comparing to control group); N/A, not applicable. Values highlighted in bold are statistically significant results.

Expression of miR-146a and miR-31

Based on the results of statistical analyses, we detected changes in the expression levels of miR-146a and miR-31 in individual patient groups compared with the control group (Tables 3, 4, 5). These changes, however, are not statistically significant.

In the group of lung carcinoma patients (Table 3), we observed several-fold decreased expression levels of miR-126, miR-155, miR-21, miR-182, let-7g and miR-19b, which were statistically significant at all stages except M1, and for miR-155 statistically significant at all stages including M1. Furthermore, we measured decreased miR-133a expression levels, but these were statistically significant only at the N2 stage. We also measured reduced expression levels of miRNA let-7a that were statistically significant only at the N0 stage. For miR-143 and miR-145, we measured several-fold increased expression levels that were statistically significant at the N0 and N1 stages; there was no statistical significance at the N2 stage, but at the M1 stage the increased expression levels were again statistically significant.

In the group of patients with lung adenocarcinoma (Table 4), we observed several-fold decreased expression levels of miR-126, which were statistically significant at all stages except M1 distant metastasis. Furthermore, we observed decreased expression levels of miR-155, miR-21, miR-182, and miR-19b in the samples of patients with adenocarcinoma, with statistical significance at stages N1 and N2. For miR-143 and miR-145 miRNAs, we observed statistically significantly increased expression levels at all stages except stage N2. The altered expression levels of miR-221, miR-133a, let-7a, miR-146a, miR-31 and let-7g in adenocarcinoma patients were not statistically significant.

In the group of patients with squamous cell carcinoma of the lung (Table 5), as in the group of patients with adenocarcinoma, we observed several-fold decreased miR-126 expression levels, but in this group they were statistically significant only at the N0 stage. Other miRNAs also had statistically significantly reduced expression at stage N0, namely miR-221, let-7a, miR-182, let-7g and miR-19b. For miR-155 and miR-21, we measured reduced expression levels in patients with squamous cell carcinoma of the lung, which were statistically significant at stages N0 and N2, respectively. We observed increased expression levels for miR-143 and miR-145, with miR-143 having statistical significance only at the M1 stage of distant metastasis and miR-145 being statistically significant at the N1 and M1 stages.

At stage N0, we observed statistically significantly decreased miR-126 expression levels overall in all lung cancer patients. In the adenocarcinoma patient group, increased expression levels of miR-143 and miR-145 were statistically significant. In the group of patients with stage N0 squamous cell carcinoma of the lung, the expression levels of miR-155, miR-221, miR-21, let-7a, miR-182, let-7g and miR-19b were statistically significant decreased.

At stage N1, we observed statistically significantly reduced let-7g expression levels in all lung carcinoma patients overall. Increased expression levels of miR-143 were significant in adenocarcinoma and in the all lung carcinoma patients group, whereas miR-145 was significantly increased in all

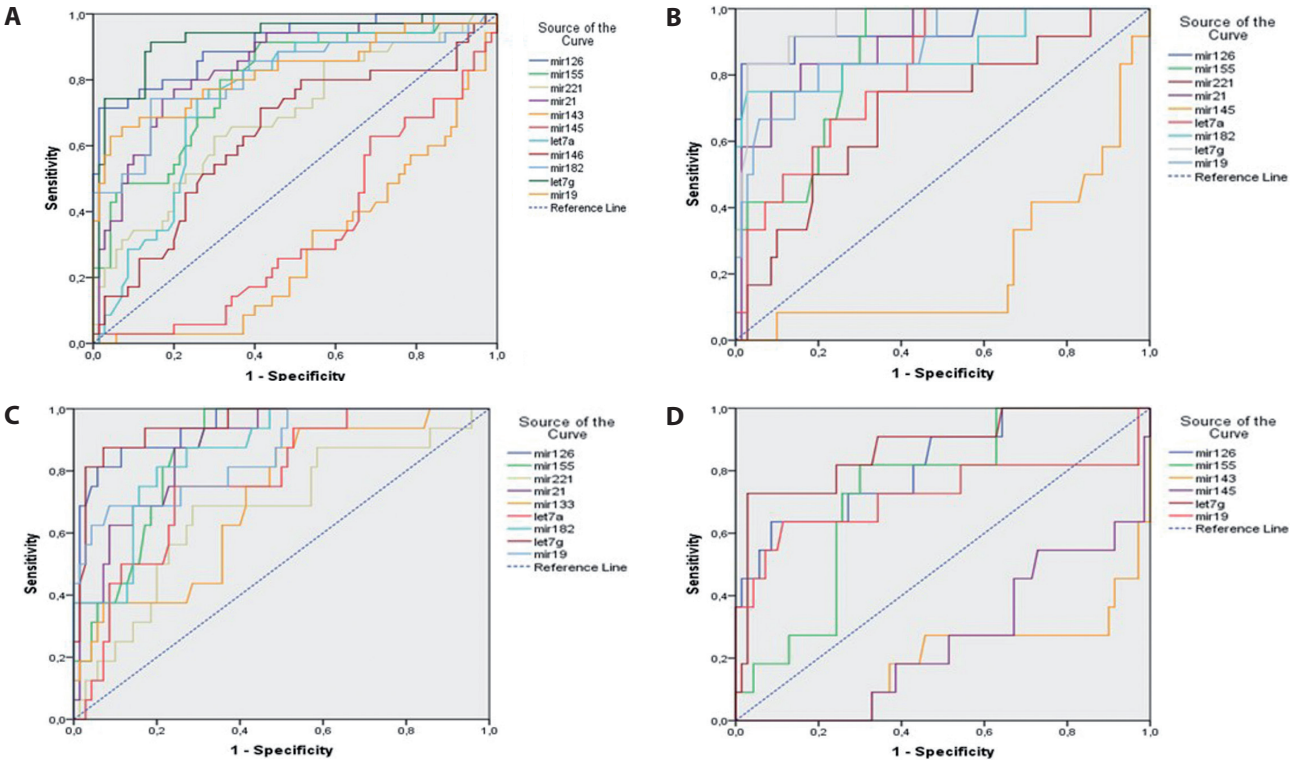
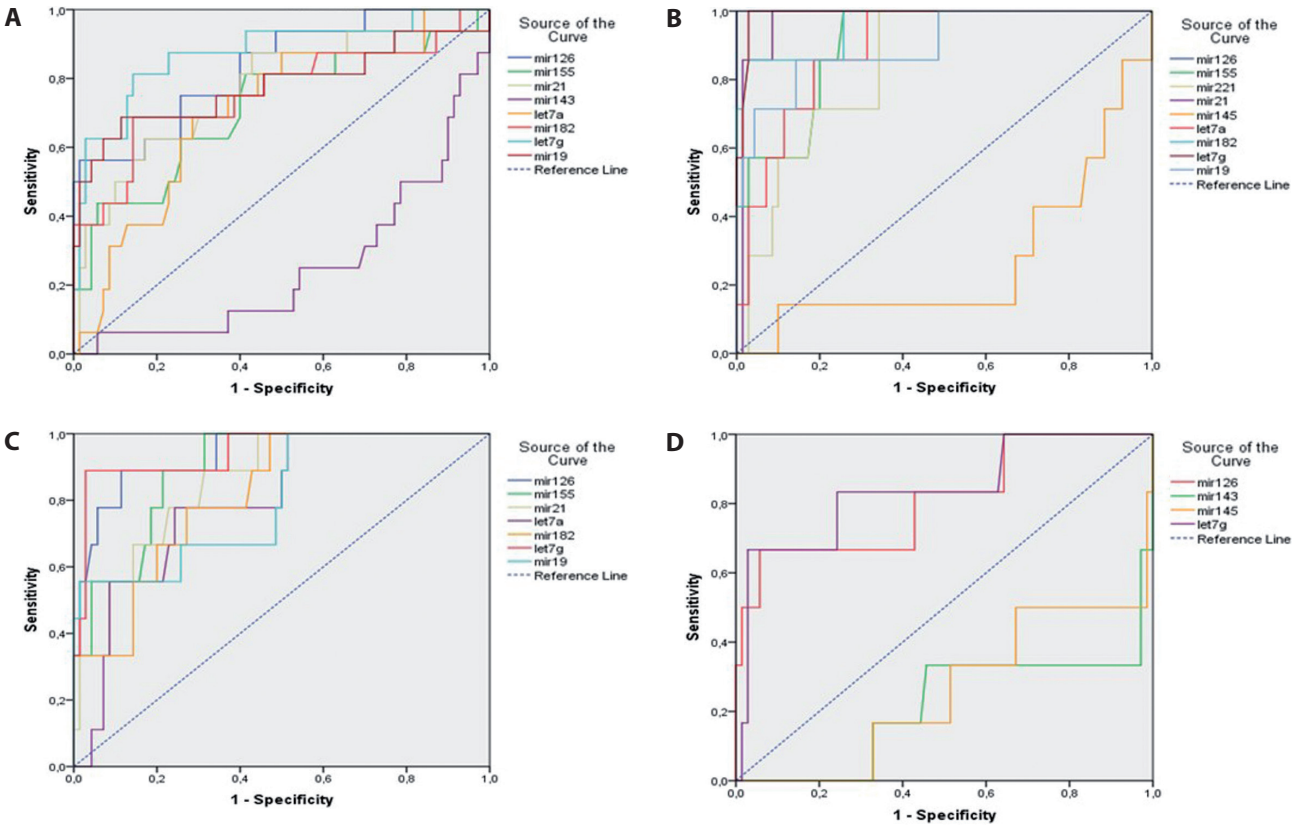


Figure 1. ROC curves of individual miRNAs at stage N0 (**A**) N1 (**B**), N2 (**C**) and M1 (**D**) in the group of lung carcinoma patients.



groups. The decreased expression levels that were statistically significant at stage N1 in the overall lung carcinoma and adenocarcinoma patient groups were miR-126, miR-155, miR-21, miR-182, and miR-19b.

At stage N2, we observed statistically significantly decreased expression levels of miR-133a and let-7g only in the group of all lung carcinoma patients. In all patient groups, there were statistically significant decreased expression levels of miR-155 and miR-21, while the decreased expression levels of miR-126, miR-182 and miR-19b were statistically significant at stage N2 in the all lung cancer group and the adenocarcinoma group.

For stage M1, we observed a statistically significant decrease in miR-155 expression level in the group of all lung carcinoma patients. Increased expression levels of miR-143 and miR-145, which were statistically significant at stage M1, were observed in all patient groups.

ROC analysis of examined miRNAs

The results of ROC analysis of individual miRNAs in the group of lung cancer patients are shown in Figure 1 according to the stages of the disease. For stage N0, the following miRNAs have diagnostically significant sensitivity and specificity: let-7g, miR-126, miR-21, miR-19b, miR-182, miR-155, and let-7a (Fig. 1A). For stage N1, we observed an excellent discriminating test with an AUC (area under the curve) value above 0.9 for let-7g, miR-126, miR-21 and a very good discriminating test for miR-19b, miR-182, miR-155 and miR-21 (Fig. 1B). For stage N2, let-7g, miR-126, miR-21, miR-155, miR-182, miR-19b and let-7a are diagnostically significant (Fig. 1C). At M1 stage, we observed a very well discriminating test with an AUC value of 0.8–0.9 for let-7g and miR-126 (Fig. 1D). The results of ROC analysis of individual miRNAs in the group of patients with lung adenocarcinoma are shown in Figure 2 according to the stages of the disease. For stage N0, the following miRNAs have diagnostically significant sensitivity and specificity: let-7g, miR-126, miR-19b, miR-21 and miR-182 (Fig. 2A). For stage N1, we observed an excellent discriminating test with an AUC value of 1 for miR-126, further between 0.9–1.0 for let-7g, miR-21, miR-182, miR-155, a very good discriminating test for miR-221 and let-7a (Fig. 2B). For stage N2, let-7g, miR-126, miR-155, miR-21, miR-182, miR-19b and let-7a are diagnostically significant (Fig. 2C). At M1 stage, we observed a very well discriminating test with an AUC value of 0.8-0.9 for let-7g and miR-126 (Fig. 2D). The results of ROC analysis of individual miRNAs in the group of patients with squamous cell carcinoma of the lung are shown in Figure 3 according to the stages of the disease. For stage N0, the following miRNAs have diagnostically significant sensitivity and specificity: let-7g, miR-126, miR-182, and miR-21 with excellent discriminatory test (Fig. 3A). Furthermore, miR-155, miR-19b, let-7a with a very well discriminating test and miR-221 with a well discriminating test. For the N1 stage, we observed an excellent discriminating test for let-7g, a very good discriminating test for miR-19b, miR-126, and a good discriminating test for miR-21 and miR-155 (Fig. 3B). For stage N2, miR-19b, miR-126, let-7g, miR-133a and miR-182 are diagnostically significant with an excellent discriminating test, miR-21 and miR-155 with a very well discriminating test and still miR-146 with a well discriminating test (Fig. 3C). At the M1 stage, we observed a very well discriminating test with an AUC value of 0.8–0.9 for let-7g and a very well discriminating test for both miR-126 and miR-155 (Fig. 3D).

Discussion

Lung carcinoma is the second most commonly diagnosed cancer. Unfortunately, up to 70% of patients are currently diagnosed at late stages with metastases. A possible way to influence this is to introduce lung carcinoma screening. A promising group of potential biomarkers, also based on recent studies that could be part of lung cancer screening and possibly correlate with the extent of disease are miRNAs (Asakura et al. 2020; Lampignano et al. 2020; Maldonado et al. 2020; Xie et al. 2021).

The main aim of the present work was to evaluate the diagnostic significance of the investigated miRNAs for lung cancer and its individual histological types with a focus on their potential in metastatic disease.

We observed several statistically significant changes in miRNA expression in the group of all lung cancer patients. The malignant profile at stages N0, N1 and N2 showed miR-126, miR-155, miR-21, miR-182, let-7g and miR-19b, respectively. However, we could not distinguish metastasis between the different stages of the lymphatic system by these miRNAs. However, their malignant profile was not demonstrated at the distant metastasis M1 stage. The values of the multiples of reduced miRNA expression were no longer statistically significant, as they were close to those of the apparently healthy individuals in the control group. These miRNAs are probably related to metastasis to the lymphatic system and not to haematogenous spread to distant organs. However, an interesting result was the statistical significance of miR-133a only at the N2 stage and statistical significance for let-7a, miR-221 only at the N0

◀ **Figure 2.** ROC curves of individual miRNAs at stage N0 (**A**) N1 (**B**), N2 (**C**) and M1 (**D**) in the group of patients with lung adenocarcinoma.

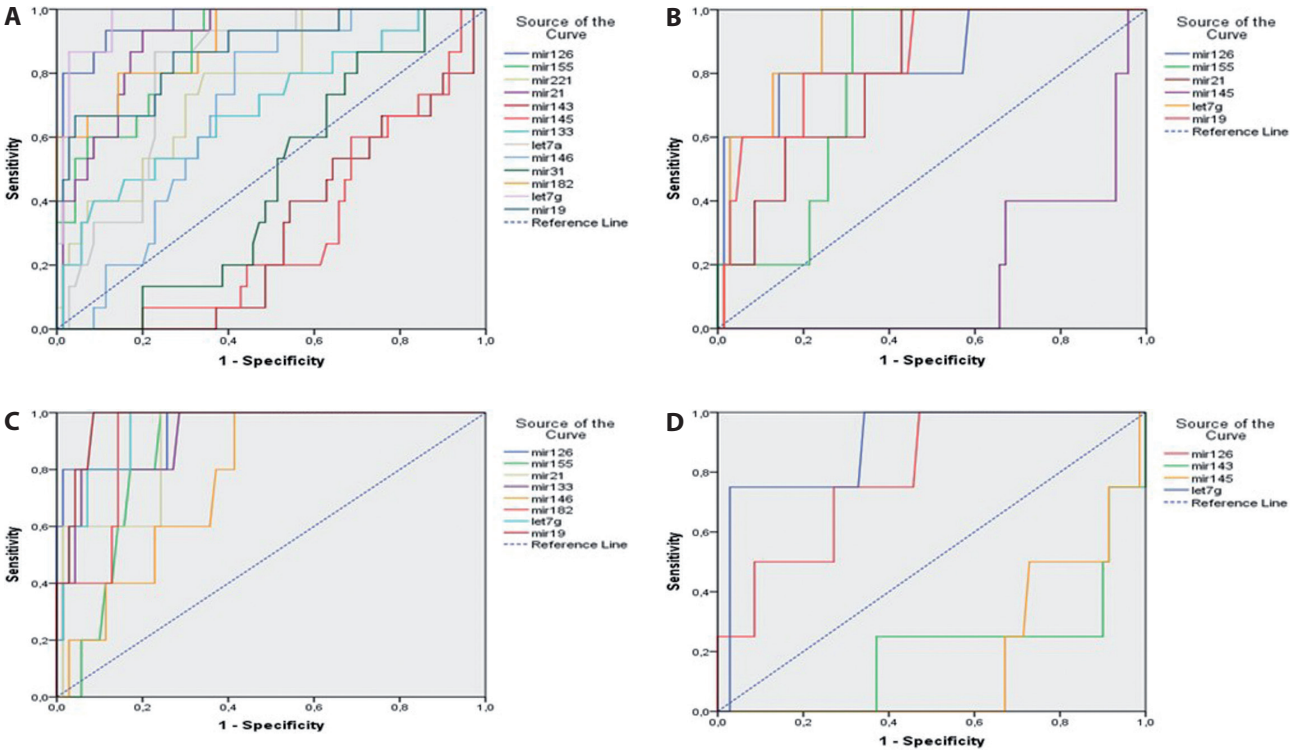


Figure 3. ROC curves of individual miRNAs at stage N0 (**A**) N1 (**B**), N2 (**C**) and M1 (**D**) in the group of patients with squamous cell carcinoma of the lung.

stage, by which we could distinguish the early resectable stage (N0) from the locally advanced stage (N2). When the expression levels of miR-143 and miR-145 were increased, they were statistically significant at the N0 and N1 stages, i.e. at the early stage, but also at the M1 stage. Notably, the levels were several-fold higher at the distant metastasis M1 stage. In the group of patients with lung adenocarcinoma, miR-126 showed the most reduced multiples of its expression at the N0, N1 and N2 stages, especially at the N1 stage, which represents the early stage. Statistically significant decreased expression levels were observed in stages N1 and N2 for miR-155, miR-21, miR-182 and miR-19b, but they could not distinguish between early and advanced stage lung carcinoma. For miR-143 and miR-145, we again observed increased expression levels with statistical significance at the N0 and N1 stages, but also multiple increased expression levels just at the distant metastasis M1 stage. In the group of squamous cell lung cancer patients, most of the statistically significant miRNAs were in the N0 stage, namely miR-126, miR-155, miR-221, miR-21, let-7a, miR-182, let-7g, and miR-19b, i.e., they could help to distinguish patients at an early stage in this histological type of lung carcinoma. However, we also observed statistical significance for miR-155 and miR-21 at stage N2. Of note was the multiple decreased expression level of miR-126, which could help us to distinguish early resectable stage. miR-143 and miR-145 had a significantly higher fold of expression in the distant metastasis M1 stage.

Using ROC analysis of individual miRNAs in patient groups, we observed their sensitivity and specificity, i.e. their diagnostic significance as biomarkers in clinical stages of lung cancer. The unequivocal markers for all stages of lung carcinoma are miR-126 and let-7g. But to distinguish metastasis in the lymphatic system, there were no differences between N0, N1 and N2 stage. In N2 disease, we observed significantly decreased expression of only miR-133a.

In the literature, miR-126 is referred to as a tumor suppressor miRNA that plays a role in the metastasis of various types of cancer, including liver cancer, colorectal cancer, malignant melanoma, and lung carcinoma as well. miR-126 is encoded by intron 7 of a gene containing an epidermal growth factor-like domain. Studies have shown that miR-126 is a key regulator that controls EMT in lung carcinoma and also has a critical role in the metastasis process (Chen et al. 2020). EMT is characterized by the loss of cell junctions and the acquisition of migratory functions, which plays a key role in the early process of cancer cell metastasis. Thus, miR-126 suppresses tumor cell invasion and migration in lung carcinoma. Overexpression of miR-126 inhibits EMT. Suppression of EMT may be an important mechanism ac-

counting for miR-126-mediated inhibition of lung carcinoma (Jia et al. 2018). Thus, reduced miR-126 expression in lung cancer tumor tissue was a negative prognostic factor for disease progression-free survival of patients (Chen et al. 2020). According to Tafsiri et al. (2015), there is a correlation between miR-126 and TNM staging of NSCLC (TNM Classification of malignant tumours, T category describes the primary tumour site and size, N category describes the regional lymph node involvement, M category describes the presence or otherwise of distant metastatic spread). This suggests that miR-126 can be used as a biomarker for the early diagnosis of NSCLC. It has been shown that in NSCLC patients, those with high miR-126 expression had a better prognosis than those with low expression (Sun et al. 2020). In NSCLC patients, the level of miR-126 is reduced not only in tumor tissue compared to healthy tissue but also in body fluids such as blood, plasma, and sputum (Jiao et al. 2020). The same results were also reached by Sun et al. (2020) who described that miR-126 is downregulated in NSCLC tissues and cells and this reduced expression was associated with tumor size and poor overall survival of NSCLC patients. In our study, miR-126 miRNA appeared to be the most downregulated in all lung cancer patient samples. In the adenocarcinoma patient group, miR-126 had the lowest expression level in N1 and N2 stages. The results for stages N0 to N2 were statistically significant. In the second group of squamous cell carcinoma patients, there was statistically significant decreased miR-126 expression in the N0 group. When comparing all patients *versus* apparently healthy subjects, there were equally statistically significant reduced levels in stages N0, N1, and N2. Thus, we can confirm that miR-126 could be used in clinical practice as a biomarker for early stages of lung carcinoma.

Another tumor suppressor miRNA in lung cancer described in the literature is miR-133a. Zhang et al. (2021) found that the level of miR-133a is significantly reduced in tumor tissue compared to healthy tissue. They report that miR-133a exhibits a tumor suppressive effect by targeting various mRNAs in lung tumor tissue. They confirmed the low expression of miR-133a in lung cancer patients, which correlated with poor prognosis. They further found that upregulation of miR-133a plays a role in tumor growth inhibition as well as metastasis formation. Patients with low miR-133a expression had a shorter survival time compared to patients with high miR-133a expression. Thus, reduced miR-133a expression level may be a potential adverse prognostic factor for NSCLC patients (Ghasemkhani et al. 2016). In a study by Shen et al. (2020) it was found that miR-133a was downregulated in NSCLC cells and tissues, and when its expression was increased, the proliferation of NSCLC cells was inhibited. Similarly, our study confirmed statistically significantly decreased miR-133a expression in blood samples from lung carcinoma patients.

Another miRNA monitored in our study was miR-143. Mataki et al. (2016) and Misono et al. (2018) described miR-143 and its tumor suppressive functions in lung adenocarcinoma cells in their study. Their study confirmed the tumor suppressive effect in all miR-143/miR-145 family members (miR-143-5p, miR-143-3p, miR-145-5p, and miR-145-3p). In the work of Yang et al. (2019), they describe consistently that miR-143 was downregulated more than two-fold in NSCLC. But Zhou et al. (2020) suggest that miR-143-3p expression is different in different cancer types (it can be both increased and decreased). However, up-regulation of miR-143 is associated with a worse prognosis in lung carcinoma as it promotes metastasis formation. According to their findings, miRNA-143 promotes the growth of lung carcinoma tumor cells. Also Wang et al. (2019) described an increase in miR-143 expression in patients with metastatic lung carcinoma. They argue that increased miR-143 expression in the tumor stroma promotes tumorigenesis of lung carcinoma, such that miR-143 triggers EMT and angiogenesis. Our study confirmed the increased expression of miR-143. In the literature, we found studies supporting the claim that miR-143 is an oncogenic microRNA, but also papers on its tumor suppressor role. Expression was statistically significantly increased in adenocarcinoma and in all stages of the disease except N2. The expression of miR-143 was increased several-fold in M1 stage of distant metastasis. The same result was also found for miR-145.

For miR-145, its tumor suppressor function has been described in the literature in various cancer types, including NSCLC, gastric cancer, breast cancer, and colorectal cancer (Ding et al. 2017; Lei et al. 2017; Sheng et al. 2017; Liu Q et al. 2018). Similarly, Cui et al. (2014) describe downregulation of miR-145 in various tumor types. Reduced miR-145 expression in lung carcinoma cells promotes proliferation and metastasis formation and decreases the rate of apoptosis. Therefore, restoring the expression of this miRNA could be considered as a therapeutic target in the treatment of lung carcinoma.

The results of the study by Sadeghiyeh et al. (2019) are consistent with the studies by Zhang and Wang (2015), which demonstrated the antimetastatic effect of miR-145 in 2015. Similarly, Xie et al. (2021) in their study confirm the reduced expression of miR-145 in NSCLC patients and also, inhibition of the epithelial-mesenchymal transition process of NSCLC cells. In 2021, Zhu et al. described miR-145 as a tumor suppressor gene that has significant clinical value in the diagnosis and treatment of NSCLC. They found decreased miR-145 expression in NSCLC tissues compared with expression in adjacent non-tumor tissues. And also that its reduced expression is closely related to metastasis to lymph nodes. Some studies have suggested a relationship between reduced miR-145 levels and increased bone metastasis formation (Sadeghiyeh et al. 2019). For miR-145,

we found increased expression levels in all participating patients. Our findings do not correlate with data in the literature where miR-145 is described as a tumor suppressor microRNA. Patients with lung adenocarcinoma had statistically significantly increased levels at all stages except stage N2 and, in the case of squamous cell carcinoma, at stages N1 and M1.

miR-221 has been implicated as an oncogenic miRNA playing a role in the carcinogenesis of several tumor types, including lung carcinoma (Xiang et al. 2020). Yin et al. (2019) found that miR-221 was overexpressed in non-small cell lung carcinoma tissues and cell lines compared to healthy tissue cells. They also found that downregulation of miR-221 suppressed the proliferation and metastasis formation of NSCLC cells. In contrast, the increased expression of miR-221 promoted the growth of NSCLC cells, so miR-221 could be considered a promising anticancer target in the treatment of NSCLC. Increased expression level of miR-221 inhibits apoptosis of tumor cells and promotes their cell proliferation (Lu et al. 2020; Xiang et al. 2020). In most of our patient blood samples, we measured decreased expression levels of miR-221. In the group of patients with squamous cell carcinoma of the lung, expression was statistically significantly decreased only at the N0 stage.

In the case of miR-21 and miR-155, the literature describes their increased expression in various types of solid malignancies such as breast, gastric, liver, colorectal, B-cell lymphoma as well as lung carcinoma (Xu and Shi 2019; Zhu Z et al. 2020). In addition, reduced expression levels of miR-21 and miR-155 can induce apoptosis and inhibit cell proliferation and invasion (Xu and Shi 2019). miR-155 represents an important therapeutic target as it is involved as an oncogene in several types of cancer (Mahesh and Biswas 2019; Shao et al. 2019). miR-155 positively correlates with lung cancer pathogenesis, suggesting that miR-155 acts as an oncogene here (Shao et al. 2019). Abnormally elevated miR-155 is found in tissues, plasma and sputum of lung cancer patients, making miR-155 a potential molecular marker for its early diagnosis. In addition, lung cancer patients with high miR-155 expression have a short survival time and poor prognosis (Dezfuli et al. 2020; Zhu HZ et al. 2020). In our study, we did not confirm the increased miR-155 expression from blood of lung carcinoma patients.

The let-7a miRNA, which is a member of the let-7 family, is thought to have tumor suppressor effects. Thus, it inhibits the expression of genes that promote tumor tissue growth. Several studies have observed its downregulation in peripheral blood samples from patients (Liu JK et al. 2018; Zhang et al. 2018; Zhao et al. 2018; Baran et al. 2019; Duan et al. 2019). Baran et al. (2019) in their work measured decreased let-7a expression levels in the serum of lung carcinoma patients but also in tumor tissue. However, significant downregulation was observed in patients with lymph node metastases. The study by Zhao et al. (2018) focused on patients with adenocarcinoma and observed decreased expression in tumor tissue. In our work, let-7a is downregulated in blood samples in the whole group of lung carcinoma patients, consistent with the results reported in the literature. In the case of squamous cell carcinoma, the expression was statistically significantly reduced in the N0 stage. The literature describes both oncogenic and tumor suppressor effects of let7-g (Sathipati and Ho 2017; Qu et al. 2018; Sim et al. 2018; Ciu et al. 2020). In our study, we found decreased expression of both let-7a and let-7g in the overall group of lung cancer patients. We also confirmed statistically significant expression for stages N0, N1 and N2.

In recent years, a number of peer-reviewed publications have emerged demonstrating the potential importance of miRNAs as biomarkers for lung carcinoma. However, the agreement between the results of different studies is low because there are no standardized analytical procedures and data normalization followed by all sites in the same way. Based on the data published so far, it is plausible that investigation of microRNAs as biomarkers may in the future, after validation by prospective studies, complement imaging, sputum cytology and biopsy examinations in the diagnosis of lung carcinoma or be a potential screening method.

Conclusion

Lung cancer is the leading cause of cancer-related mortality worldwide. Early diagnosis could improve patient survival. Although in some countries at-risk patients undergo radiological screening using low-dose computed tomography, due to high costs and false-positive results, this screening is not satisfactory enough. The availability of screening with a peripheral blood biomarker could increase the uptake of patients with this diagnosis.

The present article demonstrated the importance of miR-126 and let-7g as important biomarkers of lung cancer in all monitored clinical stages. However, based on our results, we cannot unambiguously state that in the analysis of individual stages we found characteristic miRNAs that would speak of an important biomarker for metastasis. Although miR-143 and miR-145 were significantly shown in increased expressions only in the stage of distant metastases, and in N2 disease we observed significantly reduced expression of miR-133a in the group of all lung cancer patients. Due to the significant limitations of our study, the miRNAs monitored by us did not show a clear potential for the detection of potentially resectable (N0–N1), locally advanced (N2) and distant organ metastatic (M1) lung cancer.

Our further research is necessary to focus on expanding the spectrum of monitored miRNAs by the methodology of NGS analyzes, increasing the number of patients in the

monitored cohort, taking into account all parameters of TNM classification and clinical data.

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References

Asakura K, Kadota T, Matsuzaki J, Yoshida Y, Yamamoto Y, Nakagawa K, Takizawa S, Aoki Y, Nakamura E, Miura J, et al. (2020): A miRNA-based diagnostic model predicts resectable lung cancer in humans with high accuracy. Commun. Biol. **3,** 134
https://doi.org/10.1038/s42003-020-0863-y

Baran K, Kiszałkiewicz J, Migdalska-Sęk M, Jabłoński S, Kordiak J, Antczak A, Góralska K, Brzeziańska-Lasota E (2019): An assessment of the relationship between the expression of CCR7/CCL19 axis and selected regulatory miRNAs in non-small cell lung cancer. Mol. Biol. Rep. **46,** 5389-5396
https://doi.org/10.1007/s11033-019-04993-3

Celià-Terrassa T, Kang Y (2016): Distinctive properties of metastasis-initiating cells. Genes Dev. **30,** 892-908
https://doi.org/10.1101/gad.277681.116

Chen Q, Chen S, Zhao J, Zhou Y, Xu L (2020): MicroRNA-126: A new and promising player in lung cancer. Oncol. Lett. **35,** 12296
https://doi.org/10.3892/ol.2020.12296

Cui F, Zhou Q, Xiao K, Ma S (2020): The MicroRNA hsa-let-7g promotes proliferation and inhibits apoptosis in lung cancer by targeting HOXB1. Yonsei Med. J. **61,** 210-217
https://doi.org/10.3349/ymj.2020.61.3.210

Cui SY, Wang R, Chen LB (2014): MicroRNA - 145: a potent tumor suppressor that regulates multiple cellular pathways. J. Cell. Mol. Med. **18,** 1913-1926
https://doi.org/10.1111/jcmm.12358

Dezfuli NK, Adcock IM, Alipoor SD, Seyfi S, Salimi B, Mafi Golchin M, Dalil Roofchayee N, Varhram M, Mortaz E (2020): The miR-146a SNP rs2910164 and miR-155 SNP rs767649 are risk factors for non-small cell lung cancer in the Iranian population. Can. Respir. J. **2020,** 8179415
https://doi.org/10.1155/2020/8179415

Di Leva G, Garofalo M, Croce CM (2014): MicroRNAs in cancer: Annu. Rev. Pathol. **9,** 287-314
https://doi.org/10.1146/annurev-pathol-012513-104715

Ding Y, Zhang C, Zhang J, Zhang N, Li T, Fang J, Zhang Y, Zuo F, Tao Z, Tang S, et al. (2017): miR-145 inhibits proliferation and migration of breast cancer cells by directly or indirectly regulating TGF-beta1 expression. Int. J. Oncol. **50,** 1701-1710
https://doi.org/10.3892/ijo.2017.3945

Dong Y, Jin X, Sun Z, Zhao Y, Song X (2017): MiR-186 inhibited migration of NSCLC via targeting cdc42 and effecting EMT process. Mol. Cells **40,** 195-201

Duan S, Li J, Tian J, Yin H, Zhai Q, Wu Y, Yao S, Zhang L (2019): Crosstalk between let-7a-5p and BCL-xL in the initiation of toxic autophagy in lung cancer. Mol. Ther. Oncolytics **15,** 69-78
https://doi.org/10.1016/j.omto.2019.08.010

Friedman RC, Farh KK, Burge CB, Bartel DP (2009): Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. **19,** 92-105
https://doi.org/10.1101/gr.082701.108

Ghasemkhani N, Shadvar S, Masoudi Y, Talaei AJ, Yahaghi E, Goudarzi PK, Shakiba E (2016): Down-regulated microRNA 148b expression as predictive biomarker and its prognostic significance associated with clinicopathological features in non-small-cell lung cancer patients. Diagn. Pathol. **11,** 110
https://doi.org/10.1186/s13000-016-0562-7

Guo T, Li J, Zhang L, Hou W, Wang R, Zhang J, Gao P (2019): Multidimensional communication of microRNAs and long non-coding RNAs in lung cancer. J. Cancer Res. Clin. Oncol. **145,** 31-48
https://doi.org/10.1007/s00432-018-2767-5

Han Y, Li H (2018): miRNAs as biomarkers and for the early detection of non-small cell lung cancer (NSCLC). J. Thorac. Dis. **10,** 3119-3131
https://doi.org/10.21037/jtd.2018.05.32

Huang Q (2018): Predictive relevance of ncRNAs in non-small-cell lung cancer patients with radiotherapy: a review of the published data. Biomark. Med. **12,** 1149-1159
https://doi.org/10.2217/bmm-2018-0004

Jia Z, Zhang Y, Xu Q, Guo W, Guo A (2018): miR-126 suppresses epithelial-to-mesenchymal transition and metastasis by targeting PI3K/AKT/Snail signaling of lung cancer cells. Oncol. Lett. **5,** 7369-7375
https://doi.org/10.3892/ol.2018.8207

Jiao Z, Yu A, He X, Xuan Y, Zhang H, Wang G, Shi M, Wang T (2020): Bioinformatics analysis to determine the prognostic value and prospective pathway signaling of miR-126 in non-small cell lung cancer. Ann. Transl. Med. **24,** 1639
https://doi.org/10.21037/atm-20-7520

Lampignano R, Kloten V, Krahn T, Schlange T (2020): Integrating circulating miRNA analysis in the clinical management of lung cancer: Present or future. Mol. Aspects Med. **72,** 100844
https://doi.org/10.1016/j.mam.2020.100844

Lei C, Du F, Sun L, Li T, Li T, Min Y, Nie A, Wang X, Geng L, Lu Y, et al. (2017): miR-143 and miR-145 inhibit gastric cancer cell migration and metastasis by suppressing MYO6. Cell Death Dis. **8,** e3101
https://doi.org/10.1038/cddis.2017.493

Liu JK, Liu HF, Ding Y, Gao GD (2018): Predictive value of microRNA let-7a expression for efficacy and prognosis of radiotherapy in patients with lung cancer brain metastasis. Medicine (Baltimore) **97,** 12847
https://doi.org/10.1097/MD.0000000000012847

Liu Q, Chen J, Wang B, Zheng Y, Wan Y, Wang Y, Zhou L, Liu S, Li G, Yan Y (2018): miR-145 modulates epithelial-mesenchymal transition and invasion by targeting ZEB2 in non-small cell lung cancer cell lines. J. Cell Biochem. **120,** 8409-8418
https://doi.org/10.1002/jcb.28126

Lu X, Yin B, Wang X, Wang F, Li Y, Wang N, Yang X, Jiang W (2020): Long non-coding RNA-ZNF281 upregulates PTEN expression via downregulation of microRNA-221 in non-small cell lung cancer. Oncol. Lett. **20,** 2962-2968 https://doi.org/10.3892/ol.2020.11821

Mahesh GA, Biswas R (2019): MicroRNA-155: A master regulator of inflammation. J. Interferon Cytokine Res. **39,** 321-330 https://doi.org/10.1089/jir.2018.0155

Maldonado SG, Motsch E, Trotter A, Kauczor HU, Heussel CP, Hermann S, Zeissig SR, Delorme S, Kaaks R (2020): Overdiagnosis in lung cancer screening: Estimates from the German lung cancer screening intervention trial. Int. J. Cancer **148,** 1097-1105 https://doi.org/10.1002/ijc.33295

Qu TT, Chen F, Wang J, Zhang YJ, Cheng MB, Sun WZ, Shen YF, Zhang Y (2018): PCAF-mediated acetylation of Lin28B increases let-7 biogenesis in lung adenocarcinoma H1299 cells. BMC Cancer **18,** 27 https://doi.org/10.1186/s12885-017-3959-0

Mataki H, Seki N, Mizuno K, Nohata N, Kamikawaji K, Kumamoto T, Koshizuka K, Goto Y, Inoue H (2016): Dual-strand tumor-suppressor microRNA-145 (miR-145-5p and miR-145-3p) coordinately targeted MTDH in lung squamous cell carcinoma. Oncotarget **7,** 72084-72098 https://doi.org/10.18632/oncotarget.12290

Misono S, Seki N, Mizuno K, Yamada Y, Uchida A, Arai T, Kumamoto T, Sanada H, Suetsugu T, Inoue H (2018): Dual strands of the miR-145 duplex (miR-145-5p and miR-145-3p) regulate oncogenes in lung adenocarcinoma pathogenesis. J. Hum. Genet. **63,** 1015-1028 https://doi.org/10.1038/s10038-018-0497-9

Petrek H, Yu AM (2019): MicroRNAs in non-small cell lung cancer: Gene regulation, impact on cancer cellular processes, and therapeutic potential. Pharmacol. Res. Perspect. **7,** e00528 https://doi.org/10.1002/prp2.528

Sadeghiyeh N, Sehati N, Mansoori B, Mohammadi A, Shanehbandi D, Khaze V, Baradaran B (2019): MicroRNA-145 replacement effect on growth and migration inhibition in lung cancer cell line. Biomed. Pharmacother. **111,** 460-467 https://doi.org/10.1016/j.biopha.2018.12.094

Sathipati SY, Ho SY (2017): Identifying the miRNA signature associated with survival time in patients with lung adenocarcinoma using miRNA expression profiles. Sci. Rep. **7,** 7507 https://doi.org/10.1038/s41598-017-07739-y

Shao C, Yang F, Qin Z, Jing X, Shu Y, Shen H (2019): The value of miR-155 as a biomarker for the diagnosis and prognosis of lung cancer: a systematic review with meta-analysis. BMC Cancer **19,** 1103 https://doi.org/10.1186/s12885-019-6297-6

Shen Y, Yang Y, Li Y (2020): miR-133a acts as a tumor suppressor in lung cancer progression by regulating the LASP1 and TGF-β/Smad3 signaling pathway. Thorac. Cancer **12,** 3473-3481 https://doi.org/10.1111/1759-7714.13678

Sheng N, Tan G, You W, Chen H, Gong J, Chen D, Zhang H, Wang Z (2017): MiR-145 inhibits human colorectal cancer cell migration and invasion via PAK4-dependent pathway. Cancer Med. **6,** 1331-1340 https://doi.org/10.1002/cam4.1029

Sim J, Kim Y, Kim H, Shin SJ, Kim DH, Paik SS, Jang K (2018): Identification of recurrence-associated microRNAs in stage I lung adenocarcinoma. Medicine (Baltimore) **97,** 10996 https://doi.org/10.1097/MD.0000000000010996

Singh M, Venugopal C, Tokar T, McFarlane N, Subapanditha MK, Qazi M, Bakhshinyan D, Vora P, Murty NK, Jurisica I, et al. (2018): Therapeutic targeting of the premetastatic stage in human lung-to-brain metastasis. Cancer Res. **78,** 5124-5134 https://doi.org/10.1158/0008-5472.CAN-18-1022

Sun L, Zhou H, Yang Y, Chen J, Wang Y, She M, Li C (2020): Meta-analysis of diagnostic and prognostic value of miR-126 in non-small cell lung cancer. Biosci. Rep. **40,** 349 https://doi.org/10.1042/BSR20200349

Tafsiri E, Darbouy M, Shadmehr MB, Zagryazhskaya A, Alizadeh J, Karimipoor M (2015): Expression of miRNAs in non-small-cell lung carcinomas and their association with clinicopathological features. Tumour Biol. **36,** 1603-1612 https://doi.org/10.1007/s13277-014-2755-6

Wang H, Deng Q, Lv Z, Ling Y, Hou X, Chen Z, Dinglin X, Ma S, Li D, Wu Y, et al. (2019): N6-methyladenosine induced miR-143-3p promotes the brain metastasis of lung cancer via regulation of VASH1. Mol. Cancer **18,** 181 https://doi.org/10.1186/s12943-019-1108-x

Xiang C, Zhang Y, Zhang Y, Liu C, Hou Y, Zhang Y (2020): lncRNA LEF1-AS1 promotes proliferation and induces apoptosis of non-small-cell lung cancer cells by regulating miR-221/PTEN signaling. Cancer Manag. Res. **12,** 3845-3850 https://doi.org/10.2147/CMAR.S246422

Xie Y, Meng WY, Li RZ, Wang YW, Qian X, Chan C, Yu ZF, Fan XX, Pan HD, Xie C, et al. (2021): Early lung cancer diagnostic biomarker discovery by machine learning methods. Transl. Oncol. **14,** 100907 https://doi.org/10.1016/j.tranon.2020.100907

Xu S, Shi L (2019): High expression of miR-155 and miR-21 in the recurrence or metastasis of non-small cell lung cancer. Oncol. Lett. **18,** 758-763 https://doi.org/10.3892/ol.2019.10337

Yang Y, Li S, Cao J, Li Y, Hu H, Wu Z (2019): RRM2 regulated by LINC00667/miR-143-3p signal is responsible for non-small cell lung cancer cell progression. Onco. Targets Ther. **12,** 9927-9939 https://doi.org/10.2147/OTT.S221339

Yin G, Zhang B, Li J (2019): miR-221-3p promotes the cell growth of non-small cell lung cancer by targeting p27. Mol. Med. Rep. **20,** 604-612 https://doi.org/10.3892/mmr.2019.10291

Zhang L, Hao C, Zhai R, Wang D, Zhang J, Bao L, Li Y, Yao W (2018): Downregulation of exosomal let-7a-5p in dust exposed- workers contributes to lung cancer development. Respir. Res. **19,** 235 https://doi.org/10.1186/s12931-018-0949-y

Zhang B, Wang Q (2015): MicroRNA-based biotechnology for plant improvement. J. Cell Physiol. **230,** 1-15 https://doi.org/10.1002/jcp.24685

Zhang Q, Xu L, Wang J, Zhu X, Ma Z, Yang J, Li J, Jia X, Wei L (2021): KDM5C expedites lung cancer growth and metastasis through epigenetic regulation of microRNA-133a. Onco. Targets Ther. **14,** 1187-1204 https://doi.org/10.2147/OTT.S288799

Zhao W, Hu JX, Hao RM, Zhang Q, Guo JQ, Li YJ, Xie N, Liu LY, Wang PY, Zhang C, et al. (2018): Induction of microRNA-let-7a inhibits lung adenocarcinoma cell growth by regulating cyclin D1. Oncol. Rep. **40,** 1843-1854 https://doi.org/10.3892/or.2018.6593

Zhou JH, Yao ZX, Zheng Z, Yang J, Wang R, Fu SJ, Pan XF, Liu ZH, Wu K (2020): G-MDSCs-derived exosomal miRNA-143-3p promotes proliferation via targeting of ITM2B in lung cancer. Onco. Targets Ther. **13,** 9701-9719 https://doi.org/10.2147/OTT.S256378

Zhu HZ, Fang CJ, Guo Y, Zhang Q, Huang LM, Qiu D, Chen GP, Pang XF, Hu JJ, Sun JG, et al. (2020): Detection of miR-155-5p and imaging lung cancer for early diagnosis: in vitro and in vivo study. J. Cancer Res. Clin. Oncol. **146,** 1941-1951 https://doi.org/10.1007/s00432-020-03246-2

Zhu Z, Li Q, Xu M, Qi Z (2020): Effect of whole-brain and intensity-modulated radiotherapy on serum levels of miR-21 and prognosis for lung cancer metastatic to the brain. Med. Sci. Monit. **26,** 401-406 https://doi.org/10.12659/MSM.924640

Zhu Z, Wu Q, Zhang M, Tong J, Zhong B, Yuan K (2021): Hsa_circ_0016760 exacerbates the malignant development of non-small cell lung cancer by sponging miR-145-5p/FGF5. Oncol. Rep. **45,** 501-512 https://doi.org/10.3892/or.2020.7899

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