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Original Article

The Profiling of Apoptosis-Associated MicroRNA Expressions of the Lung Adenocarcinoma

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Abstract

OBJECTIVE: The most common histopathological subtype of lung cancer is adenocarcinoma. MicroRNAs are a class of non-coding RNAs that play roles in the regulation of gene expression. MicroRNAs affecting apoptosis may have different roles in lung adenocarcinoma development, progression, and differentiation. The objective of this study is to profile all known microRNAs linked to apoptosis in normal and lung cancer tissue using real-time polymerase chain reaction.

MATERIAL AND METHODS: Tissues with adenocarcinoma and healthy tissues were taken from the same lung. The degree of differentiation of all tumors was determined. Expressions of 84 apoptosis-associated microRNAs in both tissues were analyzed by real-time polymerase chain reaction array.

RESULTS: Eleven patients and 22 samples were included in the study. In the comparison of expression levels of apoptosis-associated microRNAs in normal and adenocarcinoma tissue, miR-134, miR-183-5p, miR-192-5p, miR-193b-3, miR-194-5p, miR-200c-3, miR-212-3p, miR-25-3p, miR-449a, and miR-9-5p showed significant difference in downregulation. Receiver operating characteristic curve analysis of 10 identified microRNAs was performed, and cut-off values, sensitivity, and specificity were determined. No significant difference was found between microRNA expression levels in adenocarcinoma tissues classified as moderate-well to poorly differentiated.

CONCLUSION: Differently, downregulated expressed apoptosis-associated microRNAs were detected in lung adenocarcinoma tissues. MicroRNAs can be used as biomarkers in the diagnosis in lung adenocarcinoma. The expression of microRNAs linked to apoptosis should be investigated in different lung cancer histological subtypes in order to identify potential biomarkers.

KEYWORDS: Apoptosis pathway, lung adenocarcinoma, miRNA expression profiles, tumor biomarker, experimental researches
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INTRODUCTION

Lung cancer is the most common cancer in men and has the highest mortality rate. The most common histopathological subtype of lung cancer is adenocarcinoma.[1,2] According to the World Health Organization's 2021 report, there were 2.21 million new cases and 1.80 million deaths in 2020 due to lung cancer.[3]

MicroRNAs (miRNAs) are a class of small, non-coding RNAs, with a length of 20-24 nucleotides, which play important roles in the regulation of gene expression. Increased and decreased expression of miRNAs may affect the behaviors of oncogenes and tumor-suppressor genes in lung cancers. MicroRNAs regulate the gene expression through post-transcriptional regulation of mRNA. MicroRNAs contribute to the process of carcinogenesis by regulating the cell cycle, metastasis, angiogenesis, metabolism, and apoptosis.[4] Abnormal expression of miRNAs occurs in many types of cancer, and many miRNAs function in tumor-suppressor genes or oncogene activations.[5] Recently, circulating extracellular miRNAs have been identified as non-invasive biomarkers for cancer diagnosis, prognosis, and the prediction of treatment response.[6,7] MicroRNAs can directly target pro- or anti-apoptotic mRNAs or their functional regulators to exert both anti- and pro-apoptotic effects.[8] Apoptosis-associated miRNAs may offer novel insights into lung adenocarcinoma prognosis and targeted therapeutics at the time of diagnosis.

The histological classification of the tumor is one of the critical parts of evaluating the prognosis of lung cancer.[9] The link between early diagnosis, treatment response, and the prognosis for various histological subgroups of lung cancer has been studied. A similar situation does not exist for adenocarcinoma subtypes and miRNAs.[10,11] MicroRNA expression patterns may differ among tissues with pathologically distinct lung adenocarcinoma differentiation.

The primary aim of this study is to compare all discovered miRNAs linked to the apoptotic pathway in healthy lung tissues with lung tumor tissues from the same lung using real-time (RT) polymerase chain reaction (PCR). In this way, we planned

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to determine the changing apoptosis-miRNA expressions between lung adenocarcinoma tissues and healthy tissues. One of the secondary goals of the study was to investigate the degree of histological differentiation and distinctions of miRNA expressions in lung adenocarcinomas.

MATERIAL AND METHODS

Sampling

This cross-sectional study included 11 patients who were newly diagnosed with lung adenocarcinoma, accepted to participate in the study, and had surgery for lung cancer between February 2020 and July 2021. The resection specimens were promptly sent to the pathology unit following the procedure without the addition of any fixative solutions. Pathologists collected fresh lung parenchyma samples and non-neoplastic lung tissues from neoplastic tissues for the PCR analysis. Following the removal of fresh tissue samples, formaldehyde was added to the resection materials to allow for routine macroscopic and micromorphological analysis. For cross-checking purposes, the preoperative and final pathology diagnoses were compared. This study used healthy and malignant tissues from the same lungs of 11 patients.

All participants did not receive any adjuvant therapies before the surgery. All patients were staged according to the International Association for the Study of Lung Cancer (IASLC) 8th Edition, and the grade of adenocarcinoma differentiation was made according to the IASLC, American Thoracic Society, and European Respiratory Society's classifications. According to these classifications, the lepidic pattern was well-differentiated, the acinar and papillary patterns were intermediate, and the solid or micropapillary patterns were poorly differentiated.

This study was conducted with permission numbered 2020/75 from the Gaziantep University's ethics committee, and informed consent forms were received from all participants. The study was conducted in accordance with the Declaration of Helsinki.

Acquisition of the Lung Tissues

Following a diagnosis of adenocarcinoma lung cancer, the researchers weighed 100 mg of tumors and healthy tissue components from the same lung samples that would otherwise be thrown away. The tissues were maintained in a special liquid (RNA later) at –80°C to ensure that the RNA in the tissues was stable and undamaged until the isolation.

MAIN POINTS

- Detection of the differentially expressed apoptosis-associated microRNAs (miRNAs) between lung adenocarcinoma and healthy tissues was done. All of the apoptosis-associated miRNAs were downregulated in lung adenocarcinoma tissues.
- No significant difference was found between the levels of apoptosis-related miRNA expressions in adenocarcinoma tissues pathologically classified as moderately well differentiated to poorly differentiated.
- For biomarker determination, the expression of apoptosis-associated miRNAs in other histopathological subtypes of lung cancer should be examined.

Isolation of the MicroRNA

The tissues that were withdrawn from the RNA were treated with the solution and remained on the ice block, and then, they were treated with QIAzol® Lysis Reagent. Tissues were homogenized quickly using Tissue Ruptor II, which smashed them at the greatest speed possible (Qiagen, Hilden, Germany). Filtering and removing of the homogenized tissue particles from the medium were executed before adding chloroform to the remaining liquid and starting the miRNA isolation procedure. The miRNA simple Mini Kit (cat. No. 217004; Qiagen) procedure was followed to the letter, and the quality and concentration of the miRNAs collected were determined using a micro-drop plate reader. Good-quality RNAs were defined with an absorbance value of 260/280, 1.8, or higher.

cDNA Synthesis From the MicroRNA

Before converting mature miRNAs to cDNA, all samples were exposed to 50 ng concentration to ensure standardization between the samples. MyScript II reverse-transcriptase RT-kit (cat. No. 218161; Qiagen) was used for cDNA synthesis from samples with a working concentration of 50 ng. The reaction content and experimental stages in the cDNA synthesis were carried out according to the manufacturer's instructions. MicroRNA samples were converted to cDNA by incubating in a PCR device at 37ºC for 60 minutes and at 95ºC for 5 minutes with the kit mix.

Measuring and Evaluating the Expressions of MicroRNAs

Variations between human apoptosis miRNA expression profiles were discovered using the miScript miRNA PCR Array (cat. No. MIHS-1 14Z; Qiagen). Prepared PCR buffer, SYBR Green, and master mix containing 50 ng cDNA were added to qPCR plates containing 84 specific microRNA primers and 12 internal controls. Quantitative PCR procedure was performed for 40 cycles of 94°C for 15 s, 55 s, and 70 s. Real-time polymerase chain reaction array plates were made using 84 miRNAs whose functions are known to be associated with apoptosis.

Statistical Analysis

The conformity of the data to the normal distribution was tested with the Shapiro–Wilk test, and the Wilcoxon test was used to compare the non-normally distributed data on miRNAs in 2 dependent groups. Relationships between numerical variables were evaluated with Spearman's correlation coefficient. Threshold values for significant miRNAs were determined by using the receiver operating characteristic (ROC) curve analysis; sensitivity, specificity, and 95% CIs were calculated. As for descriptive statistics, mean ± SD for numerical variables, median (25%-75%), and number and percent values for categorical variables were presented. Statistical Package for Social Sciences (SPSS) for Windows version 24.0 package program (IBM Corp.; Armonk, NY, USA) was used for statistical analysis, and the *P*-value of <.05 was regarded as statistically significant.

RESULTS

Characteristics of the Participants

The study included 11 patients with lung adenocarcinoma, of which 72.7% of them were men, with a mean age of 63.7 ± 7.9 years. Except for one, all of the patients were smokers. In the study, 45.4% (n = 5) of them were in stage I, 27.3% (n = 3) of them were in stage II, 27.3% (n = 3) of them were in stage III, and 18.2% (n = 2) of them were in stage IV lung cancer and 1 had single brain metastasis. Pathological examinations of tumor differentiations revealed that 9.1% (n = 1) of them had well-differentiated tissues, 45.4% (n = 5) of them had moderately differentiated tissues, and 45.4% (n = 5) of them had poorly differentiated adenocarcinoma (Table 1).

Table 1. Demographic and Main Clinical Characteristics

Lung Adenocarcinoma Patients		n	%
Gender	Female	3	27.3
	Male	8	72.7
Smoker (package/year)	0	1	9.1
	30	2	18.2
	40	1	9.1
	50	2	18.2
	55	1	9.1
	60	2	18.2
	80	2	18.2
T	1a	1	9.1
	1b	1	9.1
	1c	1	9.1
	2	1	9.1
	2a	1	9.1
	3	4	36.4
	4	2	18.2
N	0	7	63.6
	1	2	18.2
	2	2	18.2
M	0	9	81.8
	1	2	18.2
Metastasis	0	10	90.9
	Brain	1	9.1
Pathology	Well-differentiated	1	9.1
	Intermediate-differentiated	5	45.4
	Poorly differentiated	5	45.4
TNM	Stage I	5	45.4
	Stage II	3	27.3
	Stage III	3	27.3
	Stage IV	2	18.2
Survey	Alive	11	100

TNM, tumor node metastasis.

Adenocarcinoma and Normal Lung Tissue's MicroRNA Real-Time-Polymerase Chain Reaction Expressions

In the comparison of miRNA expression levels by RT-PCR of normal and lung adenocarcinoma tissues, significant differences were observed in the expression of miR-134 ($P < .017$), miR-183-5p ($P < .016$), miR-192-5p ($P < .050$), miR-193b-3 ($P < .012$), miR-194-5p ($P < .050$), miR-200c-3p ($P < .021$), miR-212-3p ($P < .041$), miR-25-3p ($P < .050$), miR-449a ($P < .028$), and miR-9-5p ($P < .05$). All apoptosis-associated miRNAs which were differentially expressed were downregulated in adenocarcinoma tissue versus healthy tissues. Although not statistically significant, all apoptosis-associated miRNAs were less expressed in adenocarcinoma tissues (Table 2).

Cutoff values, sensitivity (95% CI), and specificity (95% CI) were calculated using ROC curve analysis of 10 discovered miRNAs. The correlation coefficients of 10 miRNAs that were calculated were strongly or very strongly correlated (Table 3, Figure 1).

MICRORNA EXPRESSIONS IN LUNG ADENOCARCINOMA'S TUMOR DIFFERENTIATIONS

In the present study, there was no statistically significant difference between miRNA expression levels by RT-PCR of adenocarcinoma tissues, which were pathologically classified as well-moderately and poorly differentiated.

DISCUSSION

In the current study, it was determined that 10 apoptosis-associated miRNAs were significantly downregulated in lung adenocarcinoma tissues versus healthy tissues. The expressions of miRNAs were altered in adenocarcinoma tumor tissues compared to healthy tissues. This leads us to think that decreased tumor miRNA expression can have a significant positive impact on adenocarcinoma patient identification. The fact that miRNAs acting on the apoptotic pathway are less expressed in lung adenocarcinoma tissues suggests that these miRNAs act more on tumor-suppressor genes. The link between apoptotic pathways and miRNAs requires more research.

miR-134 and miR-449a are involved in the epidermal growth factor receptor (EGFR) of p53/p73/p63 and Kirsten rat sarcoma virus (KRAS) pathways.[12] In addition to apoptosis, miR-134 is known to contribute to carcinogenesis, cancer cell proliferation, invasion, treatment resistance, and metastases. miR-134 promotes non-small cell lung cancer (NSCLC) cell apoptosis by increasing caspase-3 and caspase-7 and decreasing the expression of Bcl2 protein.[13] miR-134 expression was associated with the invasive potential and epithelial–mesenchymal transition (EMT) phenotype of the NSCLC cells. miR-134 has been shown to inhibit EMT by targeting Forkhead Box M1 in the NSCLC cells.[14] In the present study, miR-134 and miR-449a were downregulated in adenocarcinoma tissues versus healthy tissues. The effects of miR-134 on prognosis in the NSCLC should also be investigated.

Overexpression of the miR-183-5p reduces cell proliferation, tumor growth, migration, and invasion, and the miR-183-5p

Table 2. Comparison of miRNA Expressions in Normal and Lung Adenocarcinoma Tissue

		Normal	Adenocarcinoma	
miRNA	**n**	**Median (25%-75%)**	**Median (25%-75%)**	***P***
hsa-let-7a-5p	11	21.26 (19.27-22.94)	19.02 (18.29-23.07)	.657
hsa-let-7c	11	22.13 (20.3-23.72)	20.12 (19.37-23.47)	.594
hsa-let-7e-5p	11	24.09 (22.2-25.71)	21.81 (21.12-25.75)	.477
hsa-let-7g-5p	11	21.75 (20.13-23.64)	19.32 (18.52-23.34)	.424
hsa-miR-1	11	27.4 (26.04-30.62)	27.21 (26.15-27.91)	.594
hsa-miR-101-3p	11	19.54 (19.03-24.85)	19.39 (18.48-23.29)	.722
hsa-miR-106b-5p	11	22.88 (21.64-25.88)	20.26 (18.9-23.45)	.131
hsa-miR-122-5p	2	32.02 (31.87-32.08)	33.11 (32.83-33.71)	.655
hsa-miR-125a-5	11	19.9 (19.24-21.87)	18.82 (18.14-22.03)	.424
hsa-miR-125b-5	11	19.59 (18.39-21.14)	18.43 (17.64-21.64)	.534
hsa-miR-128	8	29 (28.82-31.25)	27.27 (25.35-32.04)	.161
hsa-miR-1285-3p	9	31.79 (31.27-32.53)	28.46 (28.27-30.26)	.110
hsa-miR-133a	10	27.02 (26.29-30.63)	26.59 (25.95-27.49)	.241
hsa-miR-133b	10	27.99 (27.25-32.02)	27.3 (26.5-27.61)	.333
hsa-miR-134	10	28.1 (27.53-32.02)	26.92 (26.04-27.93)	.017*
hsa-miR-141-3p	11	20.41 (19.22-25.03)	19.15 (16.25-22.38)	.248
hsa-miR-143-3p	11	19.41 (19.12-22.3)	19.32 (18.1-21.81)	.657
hsa-miR-144-3p	9	25.06 (22.82-29.66)	23.27 (22.84-23.68)	.110
hsa-miR-145-5p	11	18.68 (18.24-20.02)	18.55 (18.05-20.05)	1.000
hsa-miR-146a-5p	11	22.62 (21.53-25.47)	21.31 (19.35-22.56)	.075
hsa-miR-149-3p	4	32.62 (32.42-33.52)	32.27 (31.98-33.45)	1.000
hsa-miR-153	8	29.25 (29.07-31.11)	28.64 (26.34-30.89)	.263
hsa-miR-15a-5p	11	21.09 (20.65-25.12)	20.52 (18.89-23.1)	.248
hsa-miR-15b-5p	11	23.33 (22.45-25.8)	21.85 (20.89-26.02)	.374
hsa-miR-16-5p	11	18.26 (17.09-20.27)	16.84 (15.59-21.66)	.477
hsa-miR-17-5p	11	26.49 (25.17-29.22)	23.91 (22.64-29.52)	.286
hsa-miR-181a-5	11	21.75 (20.92-22.89)	19.92 (19.25-22)	.131
hsa-miR-181b-5	11	22.75 (21.7-23.91)	20.54 (19.9-23.05)	.182
hsa-miR-181c-5	10	28.39 (26.77-31.42)	26.2 (25.14-27.02)	.059
hsa-miR-181d	11	27.17 (26.26-29.62)	25.36 (23.92-28.06)	.213
hsa-miR-183-5p	11	26.78 (26.03-28.57)	24.1 (20.27-26.28)	.016*
hsa-miR-185-5p	11	22.3 (22.02-26.31)	21.48 (20.55-23.27)	.131
hsa-miR-186-3p	4	32.44 (31.2-32.59)	29.96 (28.77-31.96)	.068
hsa-miR-192-5p	11	25.45 (25.08-27.14)	22.64 (21.82-26.22)	.050*
hsa-miR-193a-5	11	25.18 (24.99-27.91)	24.41 (23.64-27.19)	.248
hsa-miR-193b-3	10	26.39 (25.31-28.78)	23.92 (22.59-24.36)	.012*
hsa-miR-194-5p	11	24.51 (23.68-26.05)	21.67 (21.11-25.38)	.050*
hsa-miR-195-5p	11	20.11 (19.8-21.38)	19.91 (19.12-22.15)	.929
hsa-miR-200c-3p	11	20.67 (19.52-21.49)	17.8 (16.47-20.29)	.021*
hsa-miR-203a	11	24.04 (22.58-24.99)	23.15 (21.33-24.68)	.182
hsa-miR-204-5p	11	26.61 (26.1-28.55)	27.88 (25.98-29.11)	.722
hsa-miR-205-5p	11	27.37 (25.14-28.8)	24.49 (20.62-26.36)	.155
hsa-miR-206	8	32.36 (31.22-32.95)	33.12 (31.36-33.27)	.401

(*Continued*)

Table 2. Comparison of miRNA Expressions in Normal and Lung Adenocarcinoma Tissue (*Continued*)

		Normal	Adenocarcinoma	
miRNA	**n**	**Median (25%-75%)**	**Median (25%-75%)**	***P***
hsa-miR-20a-5p	11	21.98 (20.07-24.23)	18.52 (17.46-24.22)	.248
hsa-miR-21-5p	11	16.1 (15.51-19.11)	13.39 (12.08-18.25)	.091
hsa-miR-210	8	27.32 (24.13-28.58)	23.36 (19.42-25.36)	.327
hsa-miR-212-3p	11	29.1 (28.83-31.01)	26.34 (26.14-28.62)	.041*
hsa-miR-214-3p	11	24.7 (23.17-25.16)	22.64 (21.62-23.45)	.286
hsa-miR-218-5p	11	22.24 (21.14-22.95)	21.55 (20.67-23.81)	.929
hsa-miR-221-3p	8	25,72 (24.05-27.68)	23.06 (21.06-25.95)	.263
hsa-miR-222-3p	9	22.97 (21.44-24.44)	20.82 (18.23-26.29)	.594
hsa-miR-23a-3p	11	18.21 (17.72-19.38)	17.45 (16.54-19.48)	.477
hsa-miR-24-3p	11	17.51 (16.65-19.07)	16.16 (15.68-18.6)	.248
hsa-miR-25-3p	11	21.28 (20.91-22.49)	19.07 (18.31-21.35)	.050*
hsa-miR-26a-5p	11	19.5 (18.35-20.86)	18.18 (17.32-22.08)	.657
hsa-miR-26b-5p	11	21.62 (20.43-23.22)	19.94 (19.37-24.29)	.722
hsa-miR-27a-3p	11	18.77 (18.12-20.95)	17.75 (16.9-21.02)	.424
hsa-miR-29a-3p	11	21.33 (20.44-24.04)	20.06 (18.84-22.77)	.110
hsa-miR-29b-3p	11	22.13 (20.92-24.25)	20.91 (18.75-21.17)	.155
hsa-miR-29c-3p	11	20.56 (20.09-23.43)	19.46 (18.12-22.01)	.131
hsa-miR-30a-5p	11	19.99 (19.36-21.04)	20.04 (19.04-22.42)	.424
hsa-miR-30b-5p	11	19.53 (18.91-22.54)	18.87 (17.58-20.86)	.424
hsa-miR-30c-5p	11	20.04 (19.3-22)	18.95 (18.38-20.86)	.477
hsa-miR-30d-5p	11	20.21 (19.21-21.53)	19.32 (18.43-20,27)	.155
hsa-miR-30e-5p	11	21.21 (20.29-24.61)	20.6 (18.54-24.69)	.533
hsa-miR-31-5p	11	26.43 (25.53-31.17)	23.76 (19.13-26.03)	.062
hsa-miR-32-5p	10	26.84 (25.36-29.28)	24.19 (22.6-25.66)	.262
hsa-miR-338-3p	8	25.15 (22.98-26.99)	24.75 (22.3-25.96)	1.000
hsa-miR-34a-5p	10	23.77 (22.26-30.1)	22.06 (19.95-23.59)	.074
hsa-miR-34c-5p	8	28.29 (27.1-29.31)	25.14 (24.49-31.17)	.327
hsa-miR-365a-3p	10	26.42 (25.64-30.78)	24.3 (22.58-27.09)	.093
hsa-miR-378a-3	11	22.82 (21.95-27.06)	21.79 (21.01-25.49)	.213
hsa-miR-409-3p	11	28.28 (27.65-31.17)	26.32 (25.41-28.61)	.075
hsa-miR-449a	7	28.91 (28.35-31.28)	25.29 (24.55-26.4)	.028*
hsa-miR-451a	11	20.16 (19.08-24.54)	20.14 (19.39-20.79)	.657
hsa-miR-491-5p	11	28.05 (27.63-34.66)	26.15 (25.34-30.05)	.062
hsa-miR-497-5p	8	24.3 (23.79-26.85)	23.55 (21.84-24.7)	.263
hsa-miR-512-5p	0		34.68 (33.3-36.06)	N/A
hsa-miR-542-3p	11	27.24 (27.08-29.13)	25.01 (24.06-28.28)	.155
hsa-miR-7-5p	9	27.05 (26.79-29.94)	24.68 (21.15-28.01)	.173
hsa-miR-708-5p	11	26.08 (25.73-29.09)	23.47 (21.68-28.63)	.110
hsa-miR-9-5p	11	28.42 (27.35-30.37)	24.03 (21.94-31.56)	.050*
hsa-miR-92a-3p	11	21.59 (21.12-24.08)	20.1 (19.07-22.74)	.131
hsa-miR-98-5p	9	25.83 (25.71-28.56)	25.24 (23.37-28.61)	.515

*Significant at .05 level, Wilcoxon test.
miRNA, microRNA; N/A, not applicable.

Table 3. miRNAs ROC Curve Analysis

miRNA	AUC ± SE	*P*	Cutoff	Sensitivity (95% CI)	Specificity (95% CI)
hsa-miR-134	0.805 ± 0.10	.003	≤27.34	70 (34.8-93.3)	90.91 (58.7-99.8)
hsa-miR-183-5p	0.843 ± 0.09	.001	≤24.51	72.33 (39.0-94.0)	90.91 (58.7-99.8)
hsa-miR-192-5p	0.793 ± 0.11	.010	≤24.19	72.73 (39.0-94.0)	100.00 (71.5-100)
hsa-miR-193b-3	0.882 ± 0.08	.001	≤24.36	80 (44.4-97.5)	90.91 (58.7-99.8)
hsa-miR-194-5p	0.777 ± 0.120	.021	≤23.44	72.73 (39.0-94.0)	100 (71.5-100)
hsa-miR-200c-3p	0.826 ± 0.09	.001	≤19.15	72.73 (39.0- 94.0)	90.91 (58.7-99.8)
hsa-miR-212-3p	0.835 ± 0.095	.001	≤28.62	81.82 (48.2- 97.7)	81.81(48.2-97.7)
hsa-miR-25-3p	0.785 ± 0.109	.009	≤19.12	63.64 (30.8- 89.1)	100 (71.5-100.0)
hsa-miR-449a	0.903 ± 0.09	.001	≤26.95	87.50 (47.3- 99.7)	88.89 (51.8-99.7)
hsa-miR-9-5p	0.760 ± 0.119	.029	≤25.37	63.64 (30.8- 89.1)	100.00 (71.5-100.0)

AUC, area under the ROC curve; miRNA, microRNA; ROC, receiver operating characteristic; SE, sedimentation equilibrium.

induces cell cycle arrest and increases apoptosis.[15] It has been presented that miR-183-5p can suppress p53 and activate protein kinase B signaling through phosphorylation. miR-183 is effective in DNA damage, cell metabolism, and apoptosis pathway.[16] In the current study, miR-183-5p expression was decreased in adenocarcinoma tissues. Interventions that increase the miR-183-5p expression may be involved in the treatment of lung adenocarcinoma.

The miR-192-5p overexpression plays a potential role in suppressing tumorigenesis and metastases through different mechanisms. Overexpression of the miR-192-5p in the studies was thought to suppress tumor growth in vivo.[17] Exosomal miR-192-5p has been found as a potential prognostic marker for lung adenocarcinoma.[18] In the present study, the miR-192-5p is downregulated in the lung adenocarcinoma tissues. However, in this study, no comparison was made between serum and healthy controls.

The miR-193 is involved in suppressing cell proliferation, cell cycle progression, cell migration, and invasion in lung cancer.[19] It has been shown that the nuclear factor kappa B (NF-κB) signaling contributes to cancer progression by controlling the EMT and metastasis.[20] miR-193b-3p has been previously identified as a diagnostic biomarker for the NSCLC.[21] The NF-κB can bind to the promoter region of the miR-194-5p and regulate the miR-194-5p expression in ovarian cancer cells.[22] Lung cancer's cell viability and proliferation are inhibited by the miR-194-5p. CircCHST15 in the cytoplasm has been presented to promote tumor growth by the programmed death ligand-1-mediated escape from the immune system, downregulating the expression of miR-194-5p.[23] Therefore, low expression of the miR-194-5p could be a risk factor for cancer prognosis.

miR-200c-3p is involved in the EGFR–tyrosine kinase inhibitor (TKI) resistance by regulating the EMT modulations. miR-200c-3p was downregulated in the EGFR–TKI-resistant cell lines. Patients with high miR-200c-3p expression had a higher progression-free survival with EGFR–TKI treatment. The miR-200c-3p overexpression promoted cell death by inhibiting the EMT process in the EGFR–TKI-resistant cell lines.[24] Increased expression of the miR-200c-3p appears to be an increasing factor for apoptosis. In the present study, the miR-200c-3p expression was downregulated in the tissues with adenocarcinoma.

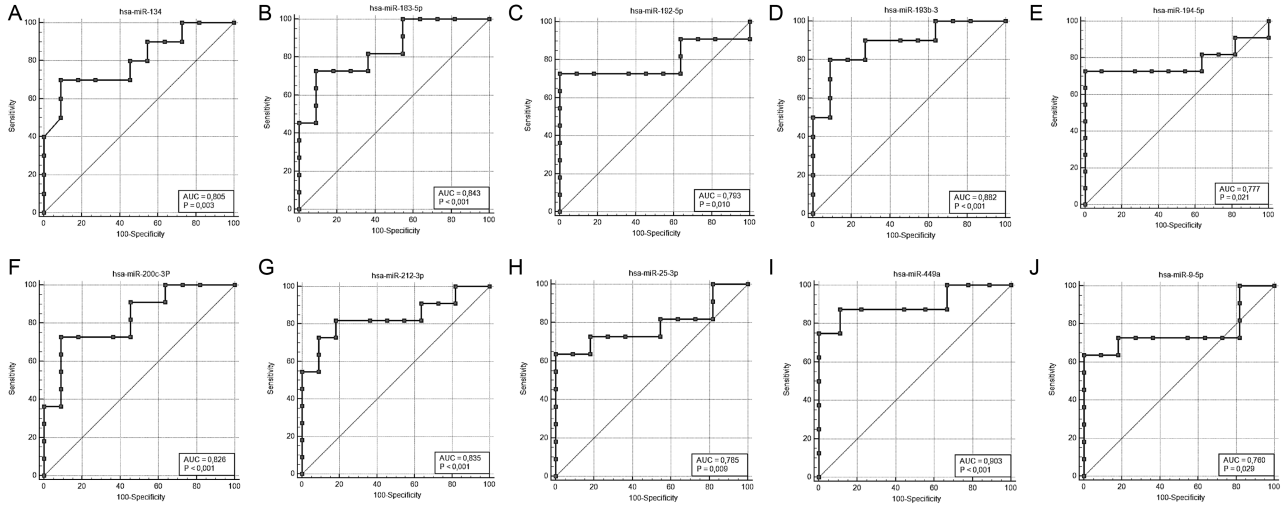

Figure 1. MicroRNA receiver operating characteristic curve analysis results [(A) miR-134, (B) miR-183-5p, (C) miR-192-5p, (D) miR-193b-3, (E) miR-194-5p (F) miR-200c-3p, (G) miR-212-3p, (H) miR-25-3p, (I) miR-449a, (J) miR-9-5p]. AUC, area under the curve.

miR-212-3p plays a suppressive role in the malignant invasion by targeting the disintegrin and metalloproteinase 10 (ADAM10) in the NSCLC.[25] Non-small cell lung carcinoma is involved with the miR-212-3p, X-inactive specific transcript, and Casitas B-lineage proto-oncogene like 1. X-inactive specific transcript knockdown has been shown to suppress the NSCLC cell proliferation, migration, invasion, and EMT in vitro, and it reduces tumor growth in vivo.[26] As in our study, the miR-212-3p was downregulated in the NSCLC tissues in previous studies, as well. The expression levels of miR-212-3p should be investigated for the determination of lung adenocarcinoma prognosis.

miR-25-3p is associated with cell proliferation, migration, and invasion in the NSCLC primary tumors and cell lines. High miR-25-3p expression levels were positively correlated with lung cancer patients' survival.[27] miR-25-3p affects the NSCLC progression by targeting the Forkhead Box P2.[28]

miR-449a has been downregulated in the NSCLC tissues and cell lines in the studies. When miRNA-449a was upregulated in the NSCLC cells, the ability of the cells to invade and migrate was weakened, and the ADAM10 expression was presented to be decreased.[29] There are promising studies on the use of exosome engineering for the treatment of cancer.[30] miR-25-3p and miR-449a could be used in lung adenocarcinoma in line with the targeted drug therapies. The upregulation of miR-9-5p in the NSCLC promotes the growth, invasion, and metastasis of cancer cells by suppressing the expression of transforming growth factor-β receptor 2.[31] However, miR-9-5p was downregulated in adenocarcinoma tissues in the present study. Different NSCLC histological subtypes might express the miR-9-5p differently.

The insufficient number of samples prevented us from obtaining any useful information about the relationship between the histological grades and microRNA expressions. However, although there are not enough studies on this subject in the literature, we believe that the relationship between pathological grading and miRNA expression levels might be crucial for early diagnosis, treatment response, and prognosis follow-up.

The current study has a number of limitations. Although the characteristics of gender and stage of the patients reflect society, the findings need to be supported by a larger number of cases. Larger sampling and demographic diversity are needed to examine the difference between the degree of differentiation and miRNA expressions of lung adenocarcinoma. Another limitation of the study is that the miRNA expressions linked to apoptosis of the tissues were not investigated in serum. Serum and tissue miRNA correlations could not be demonstrated. In addition, serum miRNA levels should be compared with completely healthy volunteers.

CONCLUSION

The detection of apoptosis-associated miRNAs, which were expressed differently in lung adenocarcinoma, was presented. The identification of these miRNAs that may be associated with the activation of tumor-suppressor genes could inspire new research and target therapeutics in this area. The histological subtype of lung adenocarcinoma's apoptosis-associated miRNAs can be employed as biomarkers for prognosis and targeted treatments. To fully understand the function of miRNAs in cancer biology, further studies on different histological subtypes of lung cancer are needed, and these studies should be conducted with more patients.

Ethics Committee Approval: This cross-sectional study was conducted with permission numbered 2020/75 from the Gaziantep University Ethics Committee.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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