

MicroRNA expression profiles predict progression and clinical outcome in lung adenocarcinoma

Kang Lin^{1,*}Tao Xu^{1,*}Bang-Shun He¹Yu-Qin Pan¹Hui-Ling Sun¹Hong-Xin Peng²Xiu-Xiu Hu²Shu-Kui Wang¹

¹Central Laboratory, Nanjing First Hospital, Nanjing Medical University,
²Medical School, Southeast University,
 Nanjing, Jiangsu, People's Republic of China

*These authors contributed equally to this work

Abstract: Lung cancer is one of the leading causes of cancer death worldwide. Accumulating evidence has indicated that microRNAs (miRNAs) can be proposed as promising diagnostic and prognostic markers for various cancers. The current study analyzed the miRNA expression profiles of 418 lung adenocarcinoma (LUAD) cases obtained from The Cancer Genome Atlas dataset, with the aim to investigate the relationship of miRNAs with progression and prognosis of LUAD. A total of 185 miRNAs were found to be differentially expressed between LUAD tumor tissues and adjacent normal tissues. Among them, 13, 10, 0, and 10 miRNAs were discovered to be associated with pathologic T, N, M, and Stage, respectively. Interestingly, mir-200 family (mir-200a, mir-200b, and mir-429) was shown to play a critical role in the progression of LUAD. In the multivariate Cox regression analysis, mir-1468 ($P=0.009$), mir-212 ($P=0.026$), mir-3653 ($P=0.012$), and mir-31 ($P=0.002$) were significantly correlated with recurrence-free survival. With regard to overall survival, mir-551b ($P=0.011$), mir-3653 ($P=0.016$), and mir-31 ($P=0.001$) were proven as independent prognostic markers. In summary, this study identified the cancer-specific miRNAs that may predict the progression and prognosis of LUAD.

Keywords: microRNA, progression, prognosis, lung adenocarcinoma

Introduction

Lung cancer is one of the most frequently diagnosed cancers and the leading cause of cancer death in both developed and developing countries.¹ Approximately 733,300 estimated new cases were diagnosed, and 610,200 patients died of lung cancer in 2015 in the People's Republic of China.² In contrast to the steady increase in survival for most cancers, the 5-year relative survival of lung cancer is just 18%.³ Among the various histological subtypes, lung adenocarcinoma (LUAD) is the most common type in females and nonsmoking males.¹ Despite many achievements made in LUAD treatment in the past decade, such as effective molecular targeted therapy, the prognosis of LUAD patients had no significant improvement.^{4,5} Thus, novel cancer-specific biomarkers are urgently needed for LUAD patients, which may help monitor the tumor progression and guide the clinical therapy according to the prediction of survival.

MicroRNAs (miRNAs) are small noncoding RNAs of 18–25 nucleotides, which can bind to the 3'-untranslated region leading to inhibition of translation or degradation of messenger RNAs (mRNAs) at the posttranslational level.⁶ Depending on the targeted mRNAs, miRNAs can act as either tumor oncogenes or tumor suppressors in cancers. The dysregulation of miRNAs in lung cancer may lead to alterations in cellular differentiation, proliferation, and apoptosis.^{7,8} Accumulating evidence has demonstrated that miRNAs play a critical role in either progression or prognosis of

Correspondence: Shu-Kui Wang
 Central Laboratory, Nanjing First Hospital, Nanjing Medical University,
 68 Changle Road, 210006 Nanjing,
 Jiangsu, People's Republic of China
 Tel/fax +86 25 5288 7003
 Email shukuiwang@163.com

LUAD.^{9–12} However, the majority of previous studies were based on small sample size, relatively limited numbers of miRNAs, or various miRNA detection methods.^{9,13–16}

In this study, the authors identified differentially expressed miRNAs between LUAD and adjacent normal tissues with miRNA expression profiles from The Cancer Genome Atlas (TCGA) project. The genome-wide miRNA expression profiles may help identify key miRNAs involved in LUAD carcinogenesis. Furthermore, the authors comprehensively investigated the association between miRNAs and progression and prognosis of LUAD, with the aim to find cancer-specific miRNAs, which may lay the foundation of molecular mechanisms and predict the prognosis of LUAD.

Materials and methods

Study population

From the TCGA data portal (<https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp>),¹⁷ 522 LUAD patients pathologically diagnosed from 1991 to 2013 were identified. The corresponding clinical information was downloaded (up to February 28, 2016) and filtered with the following exclusion criteria for further assessment: 1) subjects only with clinical data rather than miRNA expression data and 2) subjects with a history of other malignancies and/or neoadjuvant therapy. A total of 418 LUAD patients were enrolled in this study with complete clinical data, including age, sex, race, smoking status, the American Joint Committee on Cancer (AJCC) TNM staging system, vital status, and follow-up time. A total of 32 participants with adjacent normal tissues were included into Cohort N, while the whole 418 subjects were defined as Cohort T. As the data were downloaded from the TCGA project, all LUAD patients were well informed, and the study was performed in line with the TCGA publication guidelines (<http://cancergenome.nih.gov/publications/publicationguidelines>).

Processing miRNA expression profiles

The miRNA expression data (level 3) of 418 tumor tissues and 32 adjacent normal tissues were downloaded from the TCGA project (up to February 28, 2016). Among the 450 tissues, 400 cases and 50 cases were profiled using the Illumina HiSeq platform and the Illumina Genome Analyzer platform (Illumina Inc., San Diego, CA, USA), respectively. The expression levels of 1,046 miRNAs were presented as reads per million counts and processed with BRB-ArrayTools (Version 4.4.0; National Cancer Institute, Bethesda, MD, USA), which was developed by Doctor Richard Simon and BRB-ArrayTools Development Team.¹⁸ Before further

analysis, brief data filtering was performed according to the following criteria: 1) each miRNA with missing data >10% of all samples and 2) each miRNA with <1.5 fold change from the median expression value in at least 20% of total samples was excluded. Finally, the expression levels of retained miRNAs were log2 transformed.

Statistical analysis

Clinical variables were measured with chi-square test or Fisher's exact test. The miRNA expression levels between the two different groups (tumor and adjacent normal tissues, T3 + T4 and T1 + T2, N1 + N2 + N3 and N0, M1 and M0, stage III + IV and stage I + II) were evaluated with two-sample *t*-test, while the significance level was set as $P=0.01$ as default to control the false discovery rate. The leave-one-out cross-validation method was utilized to compute misclassification rate of differently expressed miRNAs between tumor and adjacent normal tissues. Unsupervised hierarchical cluster analysis was performed with one minus the centered Pearson correlation and average linkage methods.

Kaplan–Meier method and univariate Cox proportional hazards regression analysis were used to find out the potential prognostic factors for recurrence-free survival (RFS) and overall survival (OS). The median value was used as the cutoff point of miRNA expression levels. Days from initial treatment to new tumor event were defined as RFS, while the total follow-up time was OS. Meanwhile, the permutation test method with 10,000 permutations was used to ensure the robust relationship between miRNAs and survival. Subsequently, the multivariate Cox proportional hazards regression analysis was carried out to identify the independent prognostic markers. A two-sided P -value of <0.05 was regarded as significant. Statistic analysis was conducted with SPSS 20.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Characteristics of patients

The complete clinical and pathologic variables of all 418 patients are shown in Table 1. The mean age of all these patients was 64.6 years, and the median follow-up time was 23.5 months (inter quartile range, IQR: 14.3–39.9 months). Summarily, 149 patients (35.6%) suffered the recurrence after a median follow-up time of 14.6 months (IQR: 8.3–14.6 months), and 150 patients (35.9%) died after a median follow-up time of 23.2 months (IQR: 11.4–39.9 months). In addition, no significant difference was presented in the distribution of age, sex, race, AJCC TNM staging system,

Table I Clinical characteristics of patients with lung adenocarcinoma

Categories	Cohort N (n=32)	Cohort T (n=418)	P-value
Age (years), mean \pm SD	65.0 \pm 10.2	64.6 \pm 10.0	0.823
Sex, n (%)			0.714
Male	16 (50.0)	195 (46.7)	
Female	16 (50.0)	223 (53.3)	
Race, n (%)			0.099
Asian	0 (0.0)	6 (1.4)	
White	27 (84.4)	314 (75.1)	
African	5 (15.6)	46 (11.0)	
NA	0 (0.0)	52 (12.5)	
Pathologic Stage, n (%)			0.811
Stage I	20 (62.5)	226 (54.1)	
Stage II	7 (21.8)	93 (22.2)	
Stage III	5 (15.6)	70 (16.7)	
Stage IV	0 (0.0)	22 (5.3)	
NA	0 (0.0)	7 (1.7)	
Pathologic T, n (%)			0.900
T1	13 (40.6)	137 (32.8)	
T2	16 (50.0)	223 (53.3)	
T3	2 (6.3)	39 (9.3)	
T4	1 (3.1)	16 (3.8)	
TX	0 (0.0)	3 (0.8)	
Pathologic N, n (%)			0.520
N0	21 (65.6)	269 (64.4)	
N1	4 (12.5)	75 (17.9)	
N2	5 (15.6)	62 (14.8)	
N3	0 (0.0)	2 (0.5)	
NX	2 (6.3)	9 (2.2)	
NA	0 (0.0)	1 (0.2)	
Pathologic M, n (%)			0.152
M0	18 (56.3)	284 (68.0)	
M1	0 (0.0)	21 (5.0)	
MX	14 (43.8)	110 (26.3)	
NA	0 (0.0)	3 (0.7)	
Smoking status, n (%)			0.113
Nonsmoker	3 (9.7)	67 (16.0)	
Reformed smoker	14 (43.8)	235 (56.2)	
Current smoker	13 (40.6)	104 (24.9)	
NA	2 (5.9)	12 (2.9)	
Vital status, n (%)			0.376
Alive	23 (71.9)	268 (64.1)	
Dead	9 (28.1)	150 (35.9)	

Notes: MX, metastasis status unknown; n, number of patients; NX, regional lymph node unknown; TX, tumor invasion unknown.

Abbreviations: NA, not available; SD, standard deviation.

smoking status, and vital status between Cohort T and Cohort N ($P>0.05$).

Differentially expressed miRNAs between LUAD and adjacent normal tissues

After analysis of 1,046 miRNAs expression in LUAD tumor tissues (Cohort T) and adjacent normal tissues (Cohort N), a total of 185 miRNAs were found to be differentially

expressed by class comparison (Table S1). Among these 185 miRNAs, 133 miRNAs (71.9%) were upregulated, and the remaining 52 miRNAs (28.1%) were downregulated. In relation to the fold change, 48 differentially expressed miRNAs showed more than threefold change or <0.33 -fold change. Among these 48 miRNAs, 33 miRNAs (68.8%) were upregulated, while 15 miRNAs (31.2%) were downregulated (Figure 1A). Meanwhile, to examine the reliability of class comparison, class prediction was conducted among all subjects with these 185 miRNAs. A total of 97.1% (437/450) of these subjects were classified correctly into the unpaired condition. Furthermore, the unsupervised hierarchical clustering was conducted with the 185 miRNAs to separate the tumor and adjacent normal tissues (Figure 1B).

MiRNAs related to progression of LUAD

Among these 185 differentially expressed miRNAs, class comparison analysis was performed to determine miRNAs associated with AJCC TNM staging system. Overall, 13, 10, 0, and 10 miRNAs were discovered to be associated with pathologic T, N, M, and Stage, respectively (Tables S2–S4). As described in a Venn diagram (Figure 2), mir-429 was the only one that significantly associated with three variables (pathologic T, pathologic N, and pathologic Stage). Additionally, two miRNAs (mir-30e and mir-3200) were correlated with pathologic T and pathologic Stage, while four miRNAs (mir-600, mir-200a, mir-200b, and mir-548b) were linked to pathologic N and pathologic Stage.

MiRNAs with prognostic value in LUAD

To identify miRNAs with prognostic value in LUAD, the 185 differentially expressed miRNAs were subjected to univariate Cox proportional hazard regression analysis. Five miRNAs (mir-548v, mir-1468, mir-212, mir-3653, and mir-31) were significantly associated with RFS (Table 2), while eight miRNAs (mir-548v, mir-30e, mir-1468, mir-551b, mir-212, mir-3607, mir-3653, and mir-31) were significantly related to OS (Table 3). Furthermore, Kaplan–Meier survival analysis and log-rank test were conducted (Figures 3 and 4). After adjusted by multivariate Cox proportional hazards regression analysis, pathologic T ($P=0.001$), N ($P=0.003$), mir-1468 ($P=0.009$), mir-212 ($P=0.026$), mir-3653 ($P=0.012$), and mir-31 ($P=0.002$) were found to be independent prognostic factors for RFS (Table 2). With regard to OS, pathologic T ($P=0.002$), Stage ($P=0.008$), mir-551b ($P=0.011$), mir-3653 ($P=0.016$), and mir-31 ($P=0.001$) were proven as independent prognostic markers (Table 3).

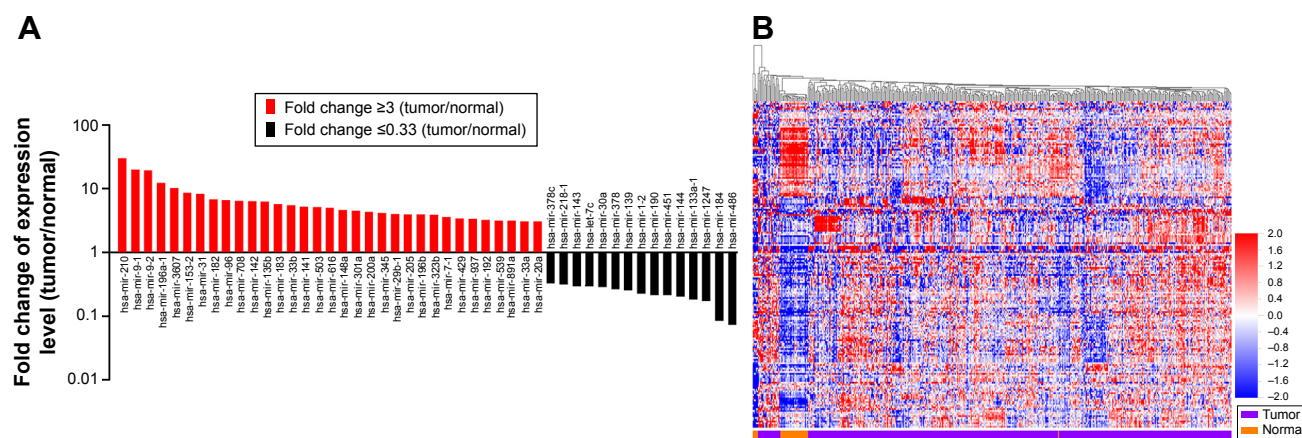


Figure 1 Differentially expressed microRNAs between lung adenocarcinoma and adjacent normal tissues.

Notes: (A) Differentially expressed microRNAs with more than threefold change or <0.33 -fold change. (B) Unsupervised hierarchical cluster analysis of the 185 microRNAs expression data.

Discussion

miRNAs show aberrant expression profiles in various cancers, and dysregulation of miRNAs may alter the neoplastic progression and prognosis of human malignancies.^{19,20} As the previous studies described, miRNA expression data were open access in the TCGA data portal, and analyzing the TCGA data has already become an important approach to investigate the association between miRNA expression patterns and pathologic characteristics and survival in

various cancer types, including lung cancer,¹² hepatocellular carcinoma,²¹ renal cell carcinoma,²² and bladder cancer.²³ For LUAD patients, Li et al¹² identified an eight-miRNA signature for predicting the OS by mining the TCGA data before. However, subjects with a history of other malignancies and/or neoadjuvant therapy were enrolled into that study, which may alter the miRNA expression levels in these LUAD subjects compared to others. In terms of the TCGA LUAD cohort, patients with a history of other malignancies and/or neoadjuvant therapy approximately accounted for 20%, which should not be ignored. Furthermore, cases with OS <1 month were removed, which may affect the results of survival analysis. Ge et al²² divided cases into two groups (follow-up time >30 days and <30 days) in the previous study and found that the survival-related miRNAs differed according to whether these cases with OS <1 month were included. Thus, given the differences in the approaches for the selection of cases and miRNAs, the authors identified differentially expressed miRNAs between LUAD and adjacent normal tissues with the TCGA dataset following the strict exclusion criteria and explored the cancer-specific miRNAs for predicting the development and survival in patients with LUAD.

In the present study, 185 miRNAs were identified to be differentially expressed between LUAD tumor tissues and adjacent normal tissues, which clearly separated the tumor and adjacent normal tissues in the unpaired condition. Subsequently, 13, 10, and 10 miRNAs were, respectively, discovered to be associated with pathologic T, N, and Stage among these 185 differentially expressed miRNAs. Unfortunately, none was significantly related to tumor metastasis status, which may be explained by the small number of M1 patients accounting for only 5% in this study. Interestingly, mir-200

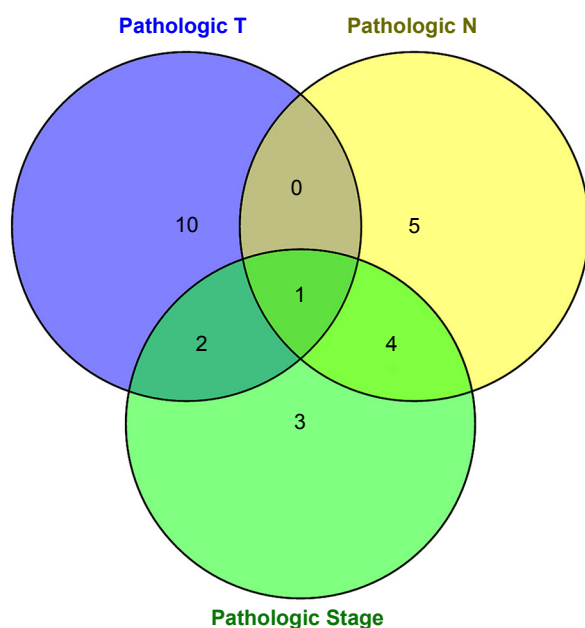


Figure 2 Venn diagram of microRNAs related to progression of lung adenocarcinoma.

Notes: A total of 13, 10, and 10 microRNAs were discovered to be associated with pathologic T, N, and Stage among the 185 differentially expressed miRNAs, respectively.

Abbreviations: N, regional lymph node invasion; T, tumor invasion.

Table 2 Univariate and multivariate Cox regression analyses of recurrence-free survival in lung adenocarcinoma

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value ^a	HR (95% CI)	P-value ^a
Age (≥ 65 years vs < 65 years)	1.002 (0.720–1.392)	0.992		
Sex (male vs female)	0.961 (0.692–1.333)	0.810		
T (T3 + T4 vs T1 + T2)	2.336 (1.533–3.560)	0.000	2.312 (1.380–3.874)	0.001
N (N1 + N2 + N3 vs N0)	2.403 (1.728–3.343)	0.000	1.882 (1.237–2.866)	0.003
M (M1 vs M0)	2.426 (1.347–4.369)	0.003		
Stage (III + IV vs I + II)	2.409 (1.710–3.394)	0.000		
hsa-mir-548v (high vs low)	0.601 (0.424–0.852)	0.004		
hsa-mir-1468 (high vs low)	0.661 (0.473–0.923)	0.015	0.550 (0.351–0.861)	0.009
hsa-mir-212 (high vs low)	1.520 (1.094–2.112)	0.013	1.624 (1.059–2.490)	0.026
hsa-mir-3653 (high vs low)	0.596 (0.428–0.831)	0.002	0.572 (0.371–0.882)	0.012
hsa-mir-31 (high vs low)	1.559 (1.109–2.191)	0.011	2.000 (1.286–3.111)	0.002

Note: ^aStatistical significant results in bold.

Abbreviations: CI, confidence interval; HR, hazard ratio.

family (mir-200a, mir-200b, and mir-429) was shown to play a critical role in the progression of LUAD. Finally, these 185 differentially expressed miRNAs were subjected to univariate and multivariate Cox proportional hazard regression analyses. Four (mir-1468, mir-212, mir-3653, and mir-31) and three (mir-551b, mir-3653, and mir-31) miRNAs were validated as independent predictors for RFS and OS of LUAD patients, respectively. Overall, the findings of this study can be a complement to the previous studies.^{9,12,14}

mir-200 family consisting of five (mir-200a, mir-200b, mir-200c, mir-141, and mir-429) miRNAs acts as either oncogenes or tumor suppressors in various human cancers and is significantly involved in the processes of epithelial-to-mesenchymal transition, cell invasion, and metastasis.^{24–27} Based on the chromosomal locations, the mir-200 family can be divided into two clusters. One is the mir-200ba/429 cluster containing mir-200a, mir-200b, and mir-429, which is

located on chromosome 1p36.²⁸ This study has demonstrated that the mir-200ba/429 cluster was significantly associated with three pathologic variables (pathologic T, pathologic N, and pathologic Stage) in LUAD. Interestingly, upregulated expression levels of these three miRNAs were observed between tumor and normal tissues, which indicated the oncogene role of this cluster. Inversely, this cluster declined during tumor progression acting as the tumor suppressor. As a previous study reported,²⁹ these three miRNAs could be expressed in a wavelike manner during carcinogenesis. Specifically, these three miRNAs were upregulated in tumor initiation but possibly downregulated during tumor progression. In addition, it has been noted that low expression level of mir-200 family could predict poor survival and serve as a prognostic marker for various cancers.^{30,31} Unfortunately, this study failed to elucidate the prognostic role of the mir-200ba/429 cluster in LUAD.

Table 3 Univariate and multivariate Cox regression analyses of overall survival in lung adenocarcinoma

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value ^a	HR (95% CI)	P-value ^a
Age (≥ 65 years vs < 65 years)	0.990 (0.711–1.377)	0.951		
Sex (male vs female)	0.918 (0.661–1.275)	0.610		
T (T3 + T4 vs T1 + T2)	2.372 (1.552–3.623)	0.000	2.617 (1.406–4.871)	0.002
N (N1 + N2 + N3 vs N0)	2.555 (1.833–3.561)	0.000		
M (M1 vs M0)	2.342 (1.308–4.194)	0.004		
Stage (III + IV vs I + II)	2.615 (1.853–3.691)	0.000	2.004 (1.195–3.361)	0.008
hsa-mir-548v (high vs low)	0.589 (0.415–0.835)	0.003		
hsa-mir-30e (high vs low)	0.681 (0.490–0.945)	0.022		
hsa-mir-1468 (high vs low)	0.668 (0.478–0.933)	0.018		
hsa-mir-551b (high vs low)	0.646 (0.457–0.913)	0.013	0.550 (0.346–0.874)	0.011
hsa-mir-212 (high vs low)	1.501 (1.080–2.086)	0.016		
hsa-mir-3607 (high vs low)	0.666 (0.478–0.927)	0.016		
hsa-mir-3653 (high vs low)	0.559 (0.401–0.780)	0.001	0.562 (0.351–0.899)	0.016
hsa-mir-31 (high vs low)	1.544 (1.097–2.172)	0.013	2.181 (1.382–3.440)	0.001

Note: ^aStatistical significant results in bold.

Abbreviations: CI, confidence interval; HR, hazard ratio.

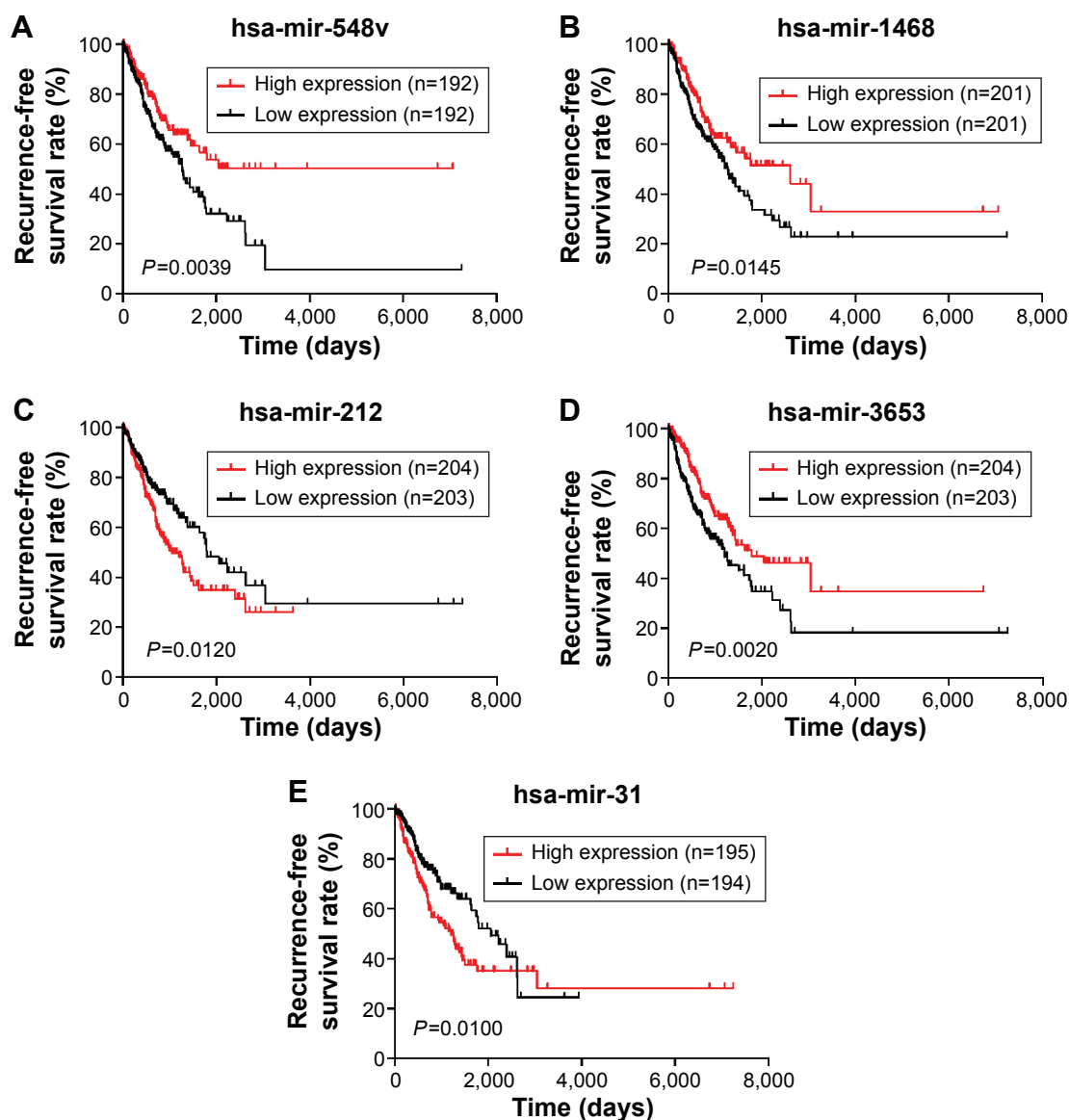


Figure 3 Kaplan-Meier survival curves for recurrence-free survival of lung adenocarcinoma patients.

Notes: (A) hsa-mir-548v; (B) hsa-mir-1468; (C) hsa-mir-212; (D) hsa-mir-3653; and (E) hsa-mir-31.

Among the five survival-related miRNAs, two miRNAs (mir-212 and mir-31) were negative prognosticators and the remaining three miRNAs (mir-551b, mir-1468, and mir-3653) were positive prognosticators according to the association between their expression level and patient survival. With regard to the two risky miRNAs, the role of mir-212 remains to be controversial in lung cancer. Li et al³² found that mir-212 displayed tumor-promoting properties by targeting the Hedgehog signaling pathway in non-small-cell lung cancer. In contrast, Incoronato et al³³ revealed the tumor suppressor role of mir-212 by promoting cancer cell apoptosis in non-small-cell lung cancer. However, the findings of this study were in-line with the former. Upregulated mir-212 expression was identified in the comparison between LUAD and adjacent

normal tissues, and the highly expressed mir-212 was associated with poor RFS. Meng et al³⁴ showed that mir-31 increased cell migration, invasion, and proliferation in an ERK1/2 signaling-dependent manner, and the low expressed mir-31 predicted excellent survival. Furthermore, a recent meta-analysis confirmed that high mir-31 expression was associated with poor survival in general cancers, and mir-31 may be a useful clinical prognostic biomarker.³⁵ Fortunately, mir-31 predicted poor RFS and OS in this study, which was in agreement with previous studies. Regarding the protective miRNAs, to the authors' knowledge, this is the first time to report the association of mir-551b, mir-1468, and mir-3653 with OS or RFS in LUAD, and the functions of these three miRNAs in cancers were poorly understood.

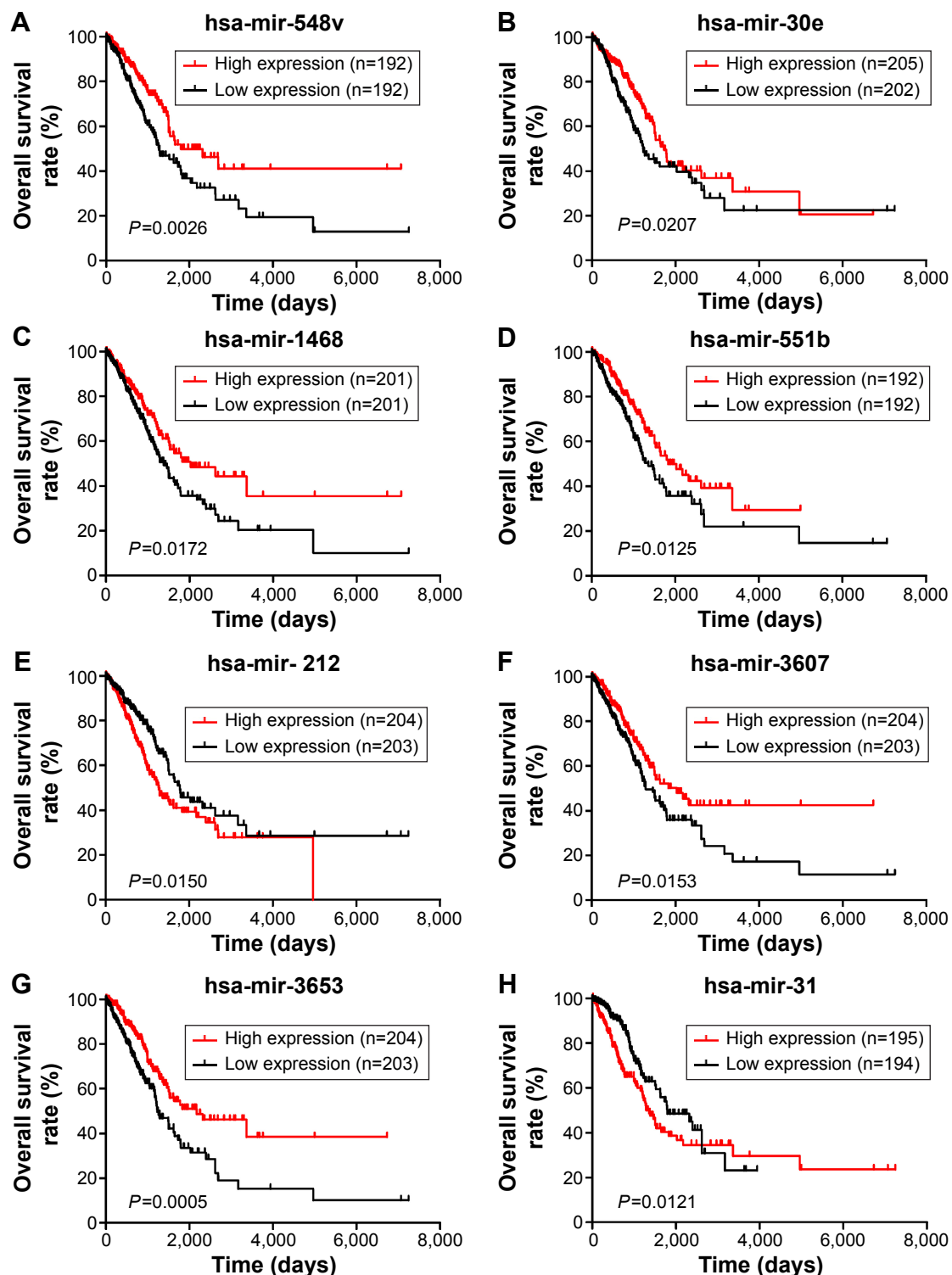


Figure 4 Kaplan-Meier survival curves for overall survival of lung adenocarcinoma patients.

Notes: (A) hsa-mir-548v; (B) hsa-mir-30e; (C) hsa-mir-1468; (D) hsa-mir-551b; (E) hsa-mir-212; (F) hsa-mir-3607; (G) hsa-mir-3653; and (H) hsa-mir-31.

Limitations

However, several limitations in this study should be noted. First, the majority of patients enrolled in this study were Caucasians and subjects from Asia accounted for only 1.4%, which affected the generalizability of the findings of the

study. Additional sets with samples from non-Caucasians will be needed to confirm these findings. Second, only 1,046 human miRNAs were analyzed. So, miRNAs identified here may not represent all candidates that associated with tumor progression and prognosis of LUAD. Third, the follow-up

data downloaded from TCGA had a relatively high censored rate, which may influence the survival analysis.

Conclusion

This study identified 185 differentially expressed miRNAs between LUAD tumor tissues and adjacent normal tissues, and further confirmed the progression-related and prognosis-related miRNAs that may show the underlying molecular mechanism and predict the prognosis of LUAD. Given that false-positive results do potentially exist, these findings require further validation with large-scale independent cohorts.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Differentially expressed microRNAs between lung adenocarcinoma and adjacent normal tissues

MicroRNA	Expression level ^a		Fold change	P-value	FDR
	LUAD	Normal			
hsa-mir-210	9.54±1.69	4.62±1.58	30.17	<1e-07	<1e-07
hsa-mir-9-1	8.68±2.36	4.38±1.97	19.81	<1e-07	<1e-07
hsa-mir-9-2	8.67±2.35	4.39±2.09	19.47	<1e-07	<1e-07
hsa-mir-196a-1	3.76±3.39	0.14±1.86	12.33	<1e-07	<1e-07
hsa-mir-3607	4.73±1.86	1.38±1.87	10.21	<1e-07	<1e-07
hsa-mir-153-2	3.33±1.74	0.23±1.27	8.61	<1e-07	<1e-07
hsa-mir-31	2.39±2.71	-0.66±1.68	8.32	0.0000001	0.000000391
hsa-mir-182	14.35±0.92	11.58±1.48	6.84	<1e-07	<1e-07
hsa-mir-96	4.46±1.11	1.72±1.42	6.67	<1e-07	<1e-07
hsa-mir-708	6.52±1.26	3.83±1.43	6.45	<1e-07	<1e-07
hsa-mir-142	11.14±1.26	8.47±2.21	6.36	<1e-07	<1e-07
hsa-mir-135b	5.54±1.71	2.88±1.75	6.29	<1e-07	<1e-07
hsa-mir-183	13.17±1.06	10.66±0.59	5.71	<1e-07	<1e-07
hsa-mir-33b	1.72±1.44	-0.75±1.25	5.53	<1e-07	<1e-07
hsa-mir-141	10.54±0.91	8.15±1.92	5.23	<1e-07	<1e-07
hsa-mir-503	2.74±1.41	0.37±1.13	5.17	<1e-07	<1e-07
hsa-mir-616	1.34±1.43	-0.99±1.14	5.02	<1e-07	<1e-07
hsa-mir-148a	15.36±0.98	13.15±1.06	4.63	<1e-07	<1e-07
hsa-mir-301a	3.61±1.27	1.44±1.21	4.5	<1e-07	<1e-07
hsa-mir-200a	9.62±1.07	7.51±1.03	4.34	<1e-07	<1e-07
hsa-mir-345	3.94±1.21	1.87±1.27	4.2	<1e-07	<1e-07
hsa-mir-29b-1	8.66±0.99	6.66±2.01	3.99	<1e-07	<1e-07
hsa-mir-205	6.39±2.92	4.41±2.62	3.97	0.0002091	0.000506
hsa-mir-196b	4.66±2.47	2.68±1.59	3.95	0.0000099	0.0000293
hsa-mir-323b	0.9±2.04	-1.07±1.59	3.92	0.0000997	0.000253
hsa-mir-7-1	4.3±0.88	2.44±1.67	3.62	<1e-07	<1e-07
hsa-mir-429	6.73±1.21	4.94±2.15	3.44	<1e-07	<1e-07
hsa-mir-937	1.82±1.82	0.07±1.57	3.36	0.0000005	0.0000018
hsa-mir-192	9.13±2	7.43±1.84	3.24	0.0000044	0.0000135
hsa-mir-539	1.35±1.68	-0.3±1.14	3.15	0.0000017	0.00000567
hsa-mir-891a	1.42±2.26	-0.23±1.9	3.15	0.0005067	0.00115
hsa-mir-33a	4.19±1.23	2.56±1.07	3.09	<1e-07	<1e-07
hsa-mir-20a	7.63±0.97	6.01±2.2	3.08	<1e-07	<1e-07
hsa-mir-130b	4.92±1.03	3.35±0.81	2.96	<1e-07	<1e-07
hsa-mir-3648	0.9±1.34	-0.66±1.63	2.93	<1e-07	<1e-07
hsa-mir-548v	0.59±1.5	-0.95±1.11	2.91	0.0000028	0.00000906
hsa-mir-199b	11.06±0.99	9.57±1.09	2.81	<1e-07	<1e-07
hsa-mir-450a-1	1.75±1.46	0.27±1.17	2.78	0.0000004	0.00000147
hsa-mir-424	6.19±1.24	4.73±1.09	2.76	<1e-07	<1e-07
hsa-mir-29b-2	8.8±0.97	7.34±0.99	2.74	<1e-07	<1e-07
hsa-mir-187	3.63±2.3	2.17±1.53	2.74	0.0005938	0.00132
hsa-mir-2355	5.78±1.04	4.33±2	2.73	<1e-07	<1e-07
hsa-mir-675	2.56±2.66	1.12±2.05	2.7	0.0035632	0.00664
hsa-mir-455	7.19±1.08	5.77±1.32	2.68	<1e-07	<1e-07
hsa-mir-224	4.97±1.86	3.55±0.99	2.68	0.0000233	0.0000648
hsa-mir-193b	5.69±1.4	4.28±1.22	2.67	<1e-07	<1e-07
hsa-mir-450a-2	1.7±1.48	0.29±1.2	2.66	0.0000017	0.00000567
hsa-mir-642a	1.84±1.4	0.46±2.12	2.6	0.0000008	0.00000282
hsa-mir-103-2	3.19±0.8	1.85±1.35	2.54	<1e-07	<1e-07
hsa-mir-93	11.78±0.98	10.45±0.49	2.52	<1e-07	<1e-07

(Continued)

Table S1 (Continued)

MicroRNA	Expression level ^a		Fold change	P-value	FDR
	LUAD	Normal			
hsa-mir-1307	10.31±0.92	8.98±1.53	2.5	<1e-07	<1e-07
hsa-mir-409	3.76±1.54	2.45±1.25	2.48	0.0000034	0.0000108
hsa-mir-181b-2	2.54±1.09	1.24±1.06	2.46	<1e-07	<1e-07
hsa-mir-19b-1	2.65±1	1.35±1.31	2.45	<1e-07	<1e-07
hsa-mir-199a-1	9.89±0.94	8.6±0.8	2.44	<1e-07	<1e-07
hsa-mir-199a-2	10.66±0.94	9.37±0.82	2.44	<1e-07	<1e-07
hsa-mir-188	1.24±1.19	-0.03±1.3	2.42	<1e-07	<1e-07
hsa-mir-940	1.04±1.41	-0.23±1.58	2.42	0.0000032	0.0000102
hsa-mir-1301	3.68±1.13	2.47±1.18	2.32	<1e-07	<1e-07
hsa-mir-542	7.77±1.21	6.55±1.75	2.32	0.0000002	0.000000755
hsa-mir-323	0.54±1.9	-0.65±1.45	2.28	0.0039901	0.00719
hsa-mir-194-1	6.77±1.88	5.59±1.83	2.27	0.0006354	0.0014
hsa-mir-19b-2	6.5±0.88	5.35±2.38	2.23	<1e-07	<1e-07
hsa-mir-26a-1	0.22±1.26	-0.94±1.04	2.22	0.0000063	0.0000192
hsa-mir-760	0.77±1.54	-0.38±1.45	2.21	0.0001207	0.000301
hsa-mir-425	7.4±0.85	6.27±0.71	2.2	<1e-07	<1e-07
hsa-mir-550a-1	1.69±1.16	0.58±1.43	2.16	0.0000006	0.00000214
hsa-mir-34a	7.65±0.88	6.54±0.92	2.15	<1e-07	<1e-07
hsa-mir-550a-2	1.32±1.19	0.23±1.27	2.13	0.0000015	0.00000517
hsa-mir-628	3.9±1.12	2.82±1.66	2.12	0.0000011	0.00000383
hsa-mir-136	4.27±1.42	3.19±1.33	2.11	0.000042	0.000111
hsa-mir-106a	3.42±1.31	2.35±1.9	2.09	0.0000374	0.0000996
hsa-mir-551b	0.99±1.89	-0.06±1.44	2.08	0.006576	0.0115
hsa-mir-194-2	6.93±1.9	5.89±1.8	2.06	0.0027277	0.0053
hsa-mir-939	-0.09±1.16	-1.12±1	2.05	0.0000155	0.0000447
hsa-mir-629	6.83±0.92	5.81±0.68	2.04	<1e-07	<1e-07
hsa-mir-200b	9.26±1.08	8.24±1.09	2.04	0.0000003	0.00000112
hsa-mir-625	7.93±0.94	6.93±0.7	2.01	<1e-07	<1e-07
hsa-mir-592	0.9±1.42	-0.09±1.4	1.99	0.0018302	0.00372
hsa-mir-17	9.34±0.84	8.35±1.26	1.98	<1e-07	<1e-07
hsa-mir-22	16.17±0.65	15.19±0.84	1.97	<1e-07	<1e-07
hsa-mir-766	3.06±1.12	2.08±1.91	1.97	0.0000103	0.0000302
hsa-mir-375	14.04±1.81	13.06±1.3	1.97	0.0027744	0.00536
hsa-mir-454	2.89±0.87	1.93±1.23	1.95	<1e-07	<1e-07
hsa-mir-3127	2.3±1.21	1.35±1.29	1.93	0.0000253	0.0000685
hsa-mir-3676	1.39±1.6	0.44±1.39	1.93	0.0019053	0.00382
hsa-mir-3200	1.41±1.57	0.47±1.41	1.92	0.0013135	0.00278
hsa-mir-331	5.39±0.93	4.46±1.13	1.91	0.0000001	0.000000391
hsa-mir-493	2.9±1.46	1.97±1.15	1.9	0.0005135	0.00115
hsa-mir-660	5.28±1.1	4.36±2.66	1.89	0.000098	0.000251
hsa-mir-217	4.08±1.63	3.15±1.94	1.89	0.0032523	0.00614
hsa-mir-590	4.01±0.96	3.1±0.75	1.88	0.0000018	0.00000594
hsa-mir-576	3.08±0.98	2.19±1.34	1.86	0.0000017	0.00000567
hsa-mir-19a	4.13±1.18	3.26±1.22	1.84	0.0001625	0.000396
hsa-mir-320b-2	1.9±1.17	1.04±1.06	1.82	0.0000604	0.000157
hsa-mir-374a	9.39±0.87	8.53±3.24	1.82	0.0001007	0.000253
hsa-mir-382	3.27±1.5	2.41±1.38	1.82	0.0016885	0.00345
hsa-mir-487b	1.47±1.61	0.61±1.35	1.82	0.0036683	0.0068
hsa-mir-582	7.83±1.61	6.97±2.27	1.82	0.0046804	0.00834
hsa-mir-191	8.76±0.71	7.9±0.78	1.81	<1e-07	<1e-07
hsa-mir-1287	5.7±1.06	4.84±0.8	1.81	0.0000097	0.000029
hsa-mir-1271	0.74±1.21	-0.11±1.14	1.8	0.0002572	0.000608
hsa-mir-3647	2.71±1.24	1.87±1.72	1.79	0.0003578	0.000834

(Continued)

Table S1 (Continued)

MicroRNA	Expression level ^a		Fold change	P-value	FDR
	LUAD	Normal			
hsa-mir-128-2	5.79±1.02	4.97±1.24	1.76	0.0000252	0.0000685
hsa-mir-671	2.77±0.88	1.98±1.3	1.73	0.000004	0.0000124
hsa-mir-3677	2.19±1.4	1.42±1.07	1.71	0.0037151	0.00685
hsa-mir-15a	6.97±0.72	6.2±2.07	1.7	0.0000025	0.00000817
hsa-mir-3653	2.49±1.1	1.73±1.84	1.7	0.0004719	0.00108
hsa-mir-3605	1.15±1.02	0.4±1.85	1.68	0.0002559	0.000608
hsa-mir-18a	3.44±1.2	2.72±1.61	1.66	0.0013779	0.00289
hsa-mir-505	5.28±0.87	4.56±1.24	1.65	0.000014	0.0000407
hsa-mir-337	3.64±1.37	2.93±0.85	1.64	0.0039896	0.00719
hsa-mir-186	8.03±0.58	7.36±0.61	1.59	<1e-07	<1e-07
hsa-mir-92b	7.33±1.15	6.67±1.28	1.58	0.0020063	0.00398
hsa-mir-324	5.4±0.89	4.75±1.13	1.56	0.0001217	0.000301
hsa-mir-30e	13.47±0.56	12.84±0.71	1.55	<1e-07	<1e-07
hsa-mir-212	2.8±1.25	2.17±0.99	1.55	0.0057283	0.0101
hsa-mir-130a	6.04±1.02	5.42±1.3	1.54	0.001206	0.00257
hsa-mir-335	6.79±0.94	6.17±2.02	1.53	0.0014444	0.00301
hsa-mir-155	8.6±1.06	7.98±0.88	1.53	0.0014863	0.00308
hsa-mir-659	0.39±1.06	-0.22±1.08	1.53	0.0023122	0.00455
hsa-mir-28	11.96±0.65	11.36±0.53	1.52	0.0000005	0.0000018
hsa-mir-181b-1	9.39±0.89	8.78±0.51	1.52	0.0001596	0.000392
hsa-mir-16-1	8.92±0.64	8.33±1.65	1.51	0.0000233	0.0000648
hsa-mir-148b	7.63±0.78	7.06±0.61	1.48	0.000069	0.000178
hsa-mir-128-1	6.21±0.94	5.65±0.63	1.47	0.0009856	0.00213
hsa-mir-200c	13.16±0.92	12.61±0.81	1.46	0.0011298	0.00242
hsa-mir-10a	14.35±0.97	13.8±1.14	1.46	0.0025228	0.00494
hsa-mir-589	6.52±0.85	6.06±0.96	1.37	0.0039521	0.00719
hsa-mir-106b	9.17±0.89	8.75±0.57	1.34	0.0079862	0.0138
hsa-mir-103-1	13.99±0.72	13.58±0.58	1.32	0.0020074	0.00398
hsa-mir-26b	9.33±0.7	8.94±0.89	1.31	0.0032529	0.00614
hsa-mir-27b	10.74±0.77	10.36±0.63	1.31	0.0055184	0.00977
hsa-mir-125a	9.25±0.69	9.68±1.3	0.74	0.0015033	0.00309
hsa-let-7g	9.28±0.68	9.77±1.24	0.72	0.0003311	0.000777
hsa-mir-320a	8.29±0.88	8.76±0.55	0.72	0.0031799	0.00607
hsa-mir-30d	13.34±0.96	13.84±0.6	0.71	0.003882	0.00711
hsa-mir-99a	7.97±1.07	8.5±1.18	0.69	0.0070379	0.0122
hsa-let-7b	13.79±0.72	14.36±0.99	0.67	0.0000312	0.0000838
hsa-mir-193a	7.38±1.08	7.97±1.1	0.67	0.0034317	0.00644
hsa-mir-30c-2	8.69±0.79	9.3±0.41	0.66	0.0000184	0.0000521
hsa-mir-340	4.53±1	5.14±1.87	0.65	0.0029427	0.00565
hsa-mir-1468	1.69±1.34	2.32±0.81	0.65	0.0091377	0.0157
hsa-mir-15b	7.67±0.77	8.31±0.72	0.64	0.0000075	0.0000226
hsa-mir-652	5.26±1.01	5.91±0.7	0.64	0.0003747	0.000867
hsa-let-7f-1	4.31±0.86	4.95±1.89	0.64	0.0005407	0.00121
hsa-mir-145	9.98±1.01	10.61±1.31	0.64	0.0008178	0.00179
hsa-mir-511-1	3.26±1.31	3.94±1.01	0.62	0.0044798	0.00802
hsa-mir-223	7.75±1.25	8.57±1.36	0.57	0.0004184	0.000961
hsa-mir-204	1.38±1.72	2.23±1.25	0.55	0.006179	0.0108
hsa-mir-548b	1.1±1.51	2.01±1.16	0.53	0.0018735	0.00378
hsa-mir-218-2	5.34±1.14	6.33±2.56	0.51	0.0000504	0.000132
hsa-let-7d	9.03±0.69	10.03±1.5	0.5	<1e-07	<1e-07
hsa-let-7f-2	12.98±1.03	13.97±2.24	0.5	0.000004	0.0000124
hsa-mir-3614	1.96±1.33	2.98±0.88	0.49	0.0000237	0.0000653
hsa-mir-140	9.69±0.83	10.76±0.41	0.48	<1e-07	<1e-07

(Continued)

Table S1 (Continued)

MicroRNA	Expression level ^a		Fold change	P-value	FDR
	LUAD	Normal			
hsa-let-7e	10.21±1.06	11.27±0.92	0.48	<1e-07	0.000000391
hsa-mir-598	3.56±1.24	4.61±2.09	0.48	0.000016	0.0000457
hsa-mir-1976	3.61±1.11	4.69±1.34	0.47	0.0000002	0.000000755
hsa-let-7a-2	13.83±0.75	14.97±0.35	0.46	<1e-07	<1e-07
hsa-mir-138-1	0.67±1.66	1.8±1.58	0.46	0.0002348	0.000564
hsa-mir-3912	-0.22±1.1	0.94±0.85	0.45	0.0000002	0.000000755
hsa-mir-2110	1.24±1.15	2.43±1.31	0.44	<1e-07	<1e-07
hsa-let-7a-1	12.63±0.71	13.89±0.42	0.42	<1e-07	<1e-07
hsa-let-7a-3	12.72±0.72	13.98±0.45	0.42	<1e-07	<1e-07
hsa-mir-195	5.07±1.05	6.49±1.45	0.37	<1e-07	<1e-07
hsa-mir-125b-2	3.2±1.31	4.63±0.62	0.37	<1e-07	<1e-07
hsa-mir-514-3	0.8±2.23	2.23±1.76	0.37	0.0008279	0.0018
hsa-mir-101-2	5.14±1.11	6.63±2.36	0.36	<1e-07	<1e-07
hsa-mir-584	4.43±1.45	5.97±0.72	0.34	<1e-07	<1e-07
hsa-mir-378c	1.96±1.25	3.59±1.13	0.32	<1e-07	<1e-07
hsa-mir-218-1	0.08±1.27	1.76±1.24	0.31	<1e-07	<1e-07
hsa-mir-143	15.86±1	17.64±1.82	0.29	<1e-07	<1e-07
hsa-let-7c	9.98±1.1	11.74±0.68	0.29	<1e-07	<1e-07
hsa-mir-30a	13.85±1.12	15.7±1.09	0.28	<1e-07	<1e-07
hsa-mir-378	7.65±1.09	9.57±0.97	0.26	<1e-07	<1e-07
hsa-mir-139	5.28±1.18	7.26±1.25	0.25	<1e-07	<1e-07
hsa-mir-1-2	2.78±1.76	4.93±2.4	0.22	<1e-07	<1e-07
hsa-mir-190	1.2±1.18	3.43±1.29	0.21	<1e-07	<1e-07
hsa-mir-451	8.35±1.6	10.61±2.22	0.21	<1e-07	<1e-07
hsa-mir-144	6.11±1.6	8.46±3.27	0.2	<1e-07	<1e-07
hsa-mir-133a-1	1.87±1.78	4.36±1.11	0.18	<1e-07	<1e-07
hsa-mir-1247	3.18±1.98	5.76±1.18	0.17	<1e-07	<1e-07
hsa-mir-184	1.07±2.11	4.66±1.78	0.083	<1e-07	<1e-07
hsa-mir-486	6.32±1.65	10.11±1.48	0.072	<1e-07	<1e-07

Note: ^aLog2-transformed miRNA expression data, presented as mean ± standard deviation.

Abbreviations: FDR, false discovery rate; LUAD, lung adenocarcinoma.

Table S2 MicroRNAs associated with the pathologic T of lung adenocarcinoma

MicroRNA	Expression level ^a		Fold change	P-value	FDR
	T3 + T4	T1 + T2			
hsa-mir-186	7.81±0.61	8.07±0.57	0.84	0.0022673	0.103
hsa-mir-103-2	2.93±0.79	3.23±0.80	0.81	0.0095228	0.216
hsa-mir-30e	13.19±0.53	13.51±0.56	0.8	0.0000848	0.0269
hsa-mir-26b	9.05±0.60	9.37±0.71	0.8	0.0016008	0.0846
hsa-mir-454	2.60±0.90	2.93±0.86	0.79	0.0086132	0.21
hsa-mir-3653	2.12±1.10	2.55±1.10	0.74	0.0074348	0.206
hsa-mir-190	0.79±1.29	1.24±1.14	0.73	0.0077873	0.206
hsa-mir-345	3.49±1.27	4.01±1.19	0.7	0.0032425	0.128
hsa-mir-429	6.24±1.46	6.81±1.15	0.67	0.0009922	0.0629
hsa-mir-142	10.62±1.31	11.22±1.23	0.66	0.0009389	0.0629
hsa-mir-3200	0.85±1.74	1.49±1.53	0.64	0.0054251	0.191
hsa-mir-301a	3.04±1.22	3.70±1.26	0.63	0.0002553	0.0405
hsa-mir-187	2.66±2.30	3.78±2.28	0.46	0.0007366	0.0629

Note: ^aLog2-transformed miRNA expression data, presented as mean ± standard deviation.

Abbreviation: FDR, false discovery rate.

Table S3 MicroRNAs associated with the pathologic N of lung adenocarcinoma

MicroRNA	Expression level ^a		Fold change	P-value	FDR
	N1 + N2 + N3	N0			
hsa-mir-196b	5.12±2.54	4.45±2.44	1.59	0.00993	0.236
hsa-mir-539	1.67±1.82	1.20±1.60	1.38	0.008377	0.236
hsa-mir-30c-2	8.54±0.80	8.77±0.77	0.85	0.004827	0.219
hsa-mir-660	5.06±1.17	5.38±1.06	0.8	0.00645	0.236
hsa-mir-30d	13.11±0.94	13.46±0.96	0.78	0.00045	0.0476
hsa-mir-200a	9.39±1.19	9.75±0.98	0.78	0.00121	0.0767
hsa-mir-200b	8.97±1.22	9.42±0.96	0.73	0.0000619	0.00981
hsa-mir-204	1.04±1.67	1.51±1.74	0.72	0.00969	0.236
hsa-mir-548b	0.77±1.53	1.26±1.48	0.71	0.002651	0.14
hsa-mir-429	6.40±1.36	6.91±1.10	0.7	0.000061	0.00981

Note: ^aLog2-transformed miRNA expression data, presented as mean ± standard deviation.

Abbreviation: FDR, false discovery rate.

Table S4 MicroRNAs associated with the pathologic Stage of lung adenocarcinoma

MicroRNA	Expression level ^a		Fold change	P-value	FDR
	III + IV	I + II			
hsa-mir-582	8.24±1.72	7.73±1.56	1.43	0.0064914	0.187
hsa-mir-30e	13.31±0.55	13.52±0.56	0.87	0.0017273	0.15
hsa-mir-29b-2	8.55±0.96	8.88±0.95	0.8	0.0037832	0.15
hsa-mir-29b-1	8.40±0.99	8.74±0.98	0.79	0.003783	0.15
hsa-mir-200b	9.00±1.26	9.35±1.01	0.78	0.0058496	0.185
hsa-mir-660	4.99±1.18	5.38±1.06	0.77	0.0031704	0.15
hsa-mir-200a	9.28±1.22	9.73±1.01	0.73	0.0003346	0.106
hsa-mir-429	6.37±1.43	6.84±1.13	0.72	0.0009152	0.145
hsa-mir-548b	0.71±1.55	1.22±1.48	0.7	0.0055601	0.185
hsa-mir-3200	0.99±1.56	1.55±1.53	0.68	0.0025169	0.15

Note: ^aLog2-transformed miRNA expression data, presented as mean ± standard deviation.

Abbreviation: FDR, false discovery rate.

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