

Expression, Clinical Significance, Immune Infiltration, and Regulation Network of miR-3940-5p in Lung Adenocarcinoma Based on Bioinformatic Analysis and Experimental Validation

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Background: Based on bioinformatics analysis and experimental validation, we investigated the expression, clinical significance, immune infiltration, and potential signaling pathways of miR-3940-5p in lung adenocarcinoma (LUAD).

Methods: 521 LUAD tissue samples and 46 normal lung tissue samples from The Cancer Genome Atlas (TCGA) database. We evaluated the relationship between clinical features and miR-3940-5p expression using Kruskal–Wallis, Wilcoxon sign-rank, and logistic regression, explored the relationship between miR-3940-5p expression and the prognosis of LUAD patients using Kaplan–Meier survival curve analysis. Several databases were used to identify miRNA targets. MiR-3940-5p target genes were analyzed based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. The significant role of miR-3940-5p in function was evaluated using immune infiltration analysis. LUAD cell lines were tested for miR-3940-5p expression using QRT-PCR.

Results: There was a significant association between high miR-3940-5p expression in LUAD and T stage ($P=0.005$), pathologic stage ($P=0.047$), race (White vs Asian & Black or African American) ($P=0.041$), residual tumor ($P=0.043$), and anatomic neoplasm subdivision2 ($P=0.030$). MiR-3940-5p expression predicted poor overall survival (HR: 1.35; 95% CI: 1.01–1.81; $P=0.045$), disease-specific survival (HR: 1.53; 95% CI: 1.05–2.23; $P=0.026$), and progression-free survival (HR: 1.35; 95% CI: 1.03–1.77; $P=0.032$). BAP1, BBS1, CCR2, KCNE3, PEBP1, and RABL2A were all associated with poor OS in LUAD patients with low miR-3940-5p expression levels. According to GO and KEGG analyses, miR-3940-5p may play a role in LUAD development by regulating pathways such as measles, PI3K-Akt signaling pathway, and p53 signaling pathway. There was a correlation between the expression level of miR-3940-5p and immune infiltration. LUAD cell lines showed significantly higher levels of miR-3940-5p than Beas-2B cells.

Conclusion: A high expression of miR-3940-5p is significantly associated with a poor prognosis in patients with LUAD, suggesting that it could be used as a prognostic biomarker.

Keywords: microRNA-3940-5p, lung adenocarcinoma, clinical significance, immune infiltration, regulation network

Introduction

Worldwide, lung cancer is a serious threat to human health. Lung cancer is a highly heterogeneous disease, accounting for nearly a quarter of cancer-related deaths.¹ Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases and is subdivided into adenocarcinoma of the lung and squamous lung cancer.² The incidence of lung adenocarcinoma (LUAD) is increasing rapidly, and it is the most aggressive type of lung cancer.³ Despite recent advances in diagnosis and treatment, the long-term clinical prognosis and overall survival of patients with LUAD remains unsatisfactory.⁴ To guide the clinical diagnosis of LUAD, it is urgent to find validated tumor markers.

MiRNAs are ubiquitous small non coding RNAs, ranging from 19–24 nucleotides in length.⁵ There is an association between aberrant miRNA expression and the diagnosis and prognosis of cancer.⁶ MiRNAs regulate the growth and proliferation of immune and stromal cells in the tumor microenvironment (TME).⁷ Serum hsa-miRNA-3940-5p is an independent prognostic factor in colorectal cancer (CRC).⁸ The miRNA index (miR.index), constructed from nine miRNAs (hsa-miR-3940 and eight other miRNAs), is an independent predictor of survival in LUAD patients.⁹ There is no clear understanding of miR-3940-5p's role in LUAD.

In this study, we explored the expression, clinical significance, immune infiltration, and regulation network of miR-3940-5p in lung adenocarcinoma based on bioinformatic analysis and experimental validation. It provides patients with LUAD with a reliable prognostic and diagnostic marker.

Materials and Methods

Patients

521 LUAD tissue samples and 46 normal lung tissue samples from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>). The clinical information included T stage, N stage, M stage, pathologic stage, primary therapy outcome (CR vs PD&SD&PR), gender, race, age, residual tumor, anatomic neoplasm subdivision (right vs left), anatomic neoplasm subdivision2 (peripheral lung vs central lung), number_pack_years_smoked (≥ 40 vs < 40), and smoker.

Clinical Information

The analysis was performed according to the reference.^{10–12} Molecule: hsa-miR-3940-5p [MIMAT0019229]. In The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov/>), LUAD (lung adenocarcinoma) project, miRNAseq data and clinical data have been collected. The RPM (Reads per Million mapped reads) format of miRNAseq data is grouped according to molecular expression.

Differential Expression of miR-3940-5p

Unpaired Samples

In accordance with the reference, we conducted the analysis.^{10–12} Molecule: hsa-miR-3940-5p. In the TCGA LUAD project, miRNAseq data were collected from the level 3 BCGSC miRNA profiling. For inter-sample expression comparison, log₂ transformation of miRNAseq data in RPM format is used. Data filtering keeps paired samples.

Paired Samples

In accordance with the reference, we conducted the analysis.^{13,14} Molecule: hsa-miR-3940-5p. In the TCGA LUAD project, miRNAseq data were collected from the level 3 BCGSC miRNA profiling. For inter-sample expression comparison, log₂ transformation of miRNAseq data in RPM format is used. There is no data filtering.

ROC Curves

In accordance with the reference, we conducted the analysis.^{10–12} Molecule: hsa-miR-3940-5p. Clinical variables included Normal vs Tumor. In the TCGA LUAD project, miRNAseq data were collected from the level 3 BCGSC miRNA profiling.

The Correlation Between miR-3940-5p Expression and Clinical Information

In accordance with the reference, we conducted the analysis.^{10–12} Molecule: hsa-miR-3940-5p. Subgroup: median. Clinical variables: T stage, pathological stage, residual tumor, and anatomic neoplasm subdivision2. In the TCGA LUAD project, miRNAseq data were collected from the level 3 BCGSC miRNA profiling.

Patient Prognosis Based on miR-3940-5p Expression in LUAD

In accordance with the reference, we conducted the analysis.^{10–12} Molecule: hsa-miR-3940-5p. Prognosis type included overall survival (OS), disease specific survival (DSS), and progression-free survival (PFS). The time from randomization to progression or death is called the PFS. In the TCGA LUAD project, miRNAseq data were collected from the level 3 BCGSC miRNA profiling.

Prediction of miR-3940-5p Targets

TargetScan, miRanda, TarBase, miRTarBase, miR2Disease, miRecords, and miRWalk were used to predict the targets of miR-3940-5p.^{15–18} The website (http://bioinfo.life.hust.edu.cn/miR_path/download.html) was used to download the LUAD mRNA average expression data. LUAD downregulated genes and common genes predicted by databases were analyzed using Venn diagrams.

Expression of miR-3940-5p Target Genes Correlating with Prognosis

In accordance with the reference, we conducted the analysis.¹⁹ Molecules: BAP1[ENSG00000163930], BBS1[ENSG00000174483], CCR2[ENSG00000121807], KCNE3[ENSG00000175538], PEBP1[ENSG00000089220], and RABL2A[ENSG00000144134]. The prognosis type was OS. In the TCGA LUAD project, RNAseq data and clinical data are provided in level 3 HTSeq-FPKM format.

GO and KEGG Analysis of miR-3940-5p

GO and KEGG analyses were conducted on miR-3940-5p targets using the DAVID database,^{20,21} focusing with BP (biological process), MF (molecular function), CC (cellular component), and pathway analysis.

Immune Infiltration Analysis by ssGSEA

In accordance with the reference, we conducted the analysis.^{14,19,22,23} Target molecule: hsa-miR-3940-5p.

QRT-PCR

Cells originally purchased from the Chinese Academy of Sciences included BEAS-2B, A549, and PC9. At 37 °C, 5% CO₂ was introduced to Beas-2B, A549, and PC9 cells in high-glucose DMEM medium (SH30022.01B; HyClone, Beijing, China) containing 10% fetal bovine serum (11,011–8611; Sijiqing Biotechnology, Hangzhou, China) and 1% penicillin-streptomycin (3810–74-0; Sigma, USA). QRT-PCR was used to identify miR-3940-5p levels in BEAS-2B, A549, and PC9 cell lines. In accordance with the reference, the specific steps were performed.²⁴ Following are the primer sequences used: U6, forward: CTCGCTTCGGCAGCACA, reverse: AACGCTTCACGAATTTGCGT; miR-3940-5p, forward: 5'-TAAAAGTGGTTGGGGCGG-3', reverse: 5'-GTGCAGGGTCCGAGGT-3'.

Statistical Analysis

According to the reference, a statistical analysis was conducted.¹⁹

Results

Clinical Characteristics

This study included 521 patients, as shown in Table 1. The T stage was made up of 173 T1 (33.4%), 277 T2 (53.5%), 49 T3 (9.5%), and 19 T4 (3.7%). The N stage consisted of 336 N0 (66.3%), 95 N1 (18.7%), 74 N2 (14.6%), and 2 N3 (0.4%). The M stage consisted of 351 M0 (93.9%), and 23 M1 (6.1%). The pathological stage consisted of 283 Stage I (55.1%), 123 Stage II (23.9%), 84 Stage III (16.3%), and 24 Stage IV (4.7%). The primary therapy outcome consisted of 68 PD (15.7%), 35 SD (8.1%), 6 PR (1.4%), and 324 CR (74.8%). The gender consisted of 278 females (53.4%) and 243 males (46.6%). The race consisted of 8 Asian (1.8%), 53 Black or African American (11.6%), and 394 White (86.6%). The age consisted of 240 patients (≤65, 47.8%), and 262 patients (>65, 52.2%). The residual tumor consisted of 342 R0 (95.3%), 13 R1 (3.6%), and 4 R2 (1.1%). The anatomic neoplasm subdivision consisted of 202 left (39.9%) and 304 right (60.1%). The anatomic neoplasm

Table I Relationships Between miR-3940-5p Expression and Clinical Characteristics

Characteristic	Overall	Low Expression of hsa-miR-3940-5p	High Expression of hsa-miR-3940-5p	P value	Method
n	521	260	261		
T stage, n (%)				< 0.001	Chisq.test
T1	173 (33.4%)	113 (21.8%)	60 (11.6%)		
T2	277 (53.5%)	122 (23.6%)	155 (29.9%)		
T3	49 (9.5%)	18 (3.5%)	31 (6%)		
T4	19 (3.7%)	5 (1%)	14 (2.7%)		
N stage, n (%)				0.939	Fisher.test
N0	336 (66.3%)	169 (33.3%)	167 (32.9%)		
N1	95 (18.7%)	46 (9.1%)	49 (9.7%)		
N2	74 (14.6%)	35 (6.9%)	39 (7.7%)		
N3	2 (0.4%)	1 (0.2%)	1 (0.2%)		
M stage, n (%)				0.070	Chisq.test
M0	351 (93.9%)	168 (44.9%)	183 (48.9%)		
M1	23 (6.1%)	6 (1.6%)	17 (4.5%)		
Pathologic stage, n (%)				0.102	Chisq.test
Stage I	283 (55.1%)	151 (29.4%)	132 (25.7%)		
Stage II	123 (23.9%)	62 (12.1%)	61 (11.9%)		
Stage III	84 (16.3%)	38 (7.4%)	46 (8.9%)		
Stage IV	24 (4.7%)	7 (1.4%)	17 (3.3%)		
Primary therapy outcome, n (%)				0.016	Fisher.test
PD	68 (15.7%)	24 (5.5%)	44 (10.2%)		
SD	35 (8.1%)	22 (5.1%)	13 (3%)		
PR	6 (1.4%)	4 (0.9%)	2 (0.5%)		
CR	324 (74.8%)	173 (40%)	151 (34.9%)		
Gender, n (%)				0.124	Chisq.test
Female	278 (53.4%)	148 (28.4%)	130 (25%)		
Male	243 (46.6%)	112 (21.5%)	131 (25.1%)		
Race, n (%)				0.124	Fisher.test
Asian	8 (1.8%)	5 (1.1%)	3 (0.7%)		
Black or African American	53 (11.6%)	34 (7.5%)	19 (4.2%)		
White	394 (86.6%)	196 (43.1%)	198 (43.5%)		
Age, n (%)				0.154	Chisq.test
≤65	240 (47.8%)	112 (22.3%)	128 (25.5%)		
>65	262 (52.2%)	140 (27.9%)	122 (24.3%)		
Residual tumor, n (%)				0.101	Fisher.test
R0	342 (95.3%)	171 (47.6%)	171 (47.6%)		
R1	13 (3.6%)	3 (0.8%)	10 (2.8%)		
R2	4 (1.1%)	1 (0.3%)	3 (0.8%)		
Anatomic neoplasm subdivision, n (%)				0.928	Chisq.test
Left	202 (39.9%)	102 (20.2%)	100 (19.8%)		
Right	304 (60.1%)	151 (29.8%)	153 (30.2%)		
Anatomic neoplasm subdivision2, n (%)				0.043	Chisq.test
Central Lung	62 (33.3%)	37 (19.9%)	25 (13.4%)		
Peripheral Lung	124 (66.7%)	53 (28.5%)	71 (38.2%)		

(Continued)

Table 1 (Continued).

Characteristic	Overall	Low Expression of hsa-miR-3940-5p	High Expression of hsa-miR-3940-5p	P value	Method
Number_pack_years_smoked, n (%)				0.089	Chisq.test
<40	176 (49.7%)	96 (27.1%)	80 (22.6%)		
≥40	178 (50.3%)	80 (22.6%)	98 (27.7%)		
Smoker, n (%)				0.546	Chisq.test
No	76 (15%)	35 (6.9%)	41 (8.1%)		
Yes	431 (85%)	218 (43%)	213 (42%)		
Age, median (IQR)	66.5 (59, 72)	67 (60, 74)	65 (58, 72)	0.036	Wilcoxon

subdivision2 consisted of 62 central lungs (33.3%) and 124 peripheral lungs (66.7%). The number pack years smoked consisted of 176 patients (<40, 49.7%) and 178 patients (≥40, 50.3%). The smokers consisted of 76 no (15%) and 431 yes (85%). There was a range of 59 to 72 years of age, with a median age of 66.5 years.

Clinical Characteristics of LUAD are Negatively Correlated with MiR-3940-5p Expression

As shown in **Figure 1A**, LUAD tissues (0.631 ± 0.028 , $n=521$) expressed significantly higher miR-3940-5p levels than normal tissues (0.393 ± 0.128 , $n=46$) ($P<0.001$). As shown in **Figure 1B**, LUAD tissues (0.586 ± 0.085 , $n=46$) expressed significantly higher miR-3940-5p levels than matched normal tissues (0.393 ± 0.128 , $n=46$) ($P=0.02$). As shown in **Figure 1C**, the AUC of miR-3940-5p was 0.709. As shown in **Table 1**, miR-3940-5p expression was associated with T stage ($P<0.001$), primary therapy outcome ($P=0.016$), anatomic neoplasm subdivision2 ($P=0.043$), and median age ($P=0.036$; low miR-3940-5p expression 67 (60, 74) vs high miR-3940-5p expression 65 (58, 72)). As shown in **Figure 2** and **Table 2** (dichotomous logistic model), miR-3940-5p expression was significantly related to T stage ($P=0.005$), pathologic stage ($P=0.047$), race ($P=0.041$), residual tumor ($P=0.043$), and anatomic neoplasm subdivision2 ($P=0.030$). Overall, the findings indicated a link between high miR-3940-5p expression and a poor prognosis for LUAD patients.

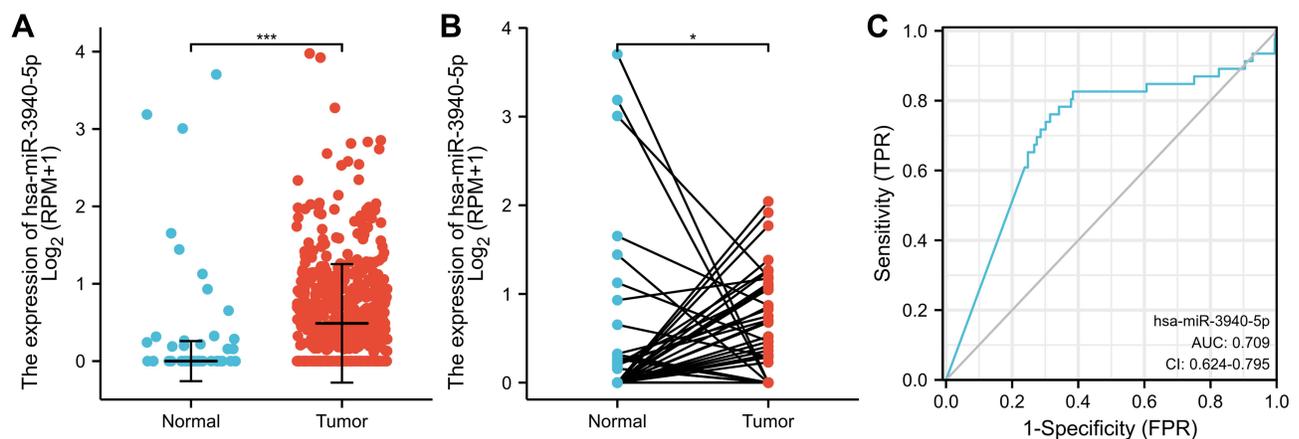


Figure 1 Expression of miR-3940-5p in LUAD and normal tissues. **(A)** MiR-3940-5p expression was significantly higher in 521 LUAD tissues (0.631 ± 0.028) compared with 46 normal tissues (0.393 ± 0.128) ($***P<0.001$). **(B)** MiR-3940-5p expression was significantly higher in 46 LUAD tissues (0.586 ± 0.085) compared with 46 normal tissues (0.393 ± 0.128) ($*P<0.05$). **(C)** ROC curve.

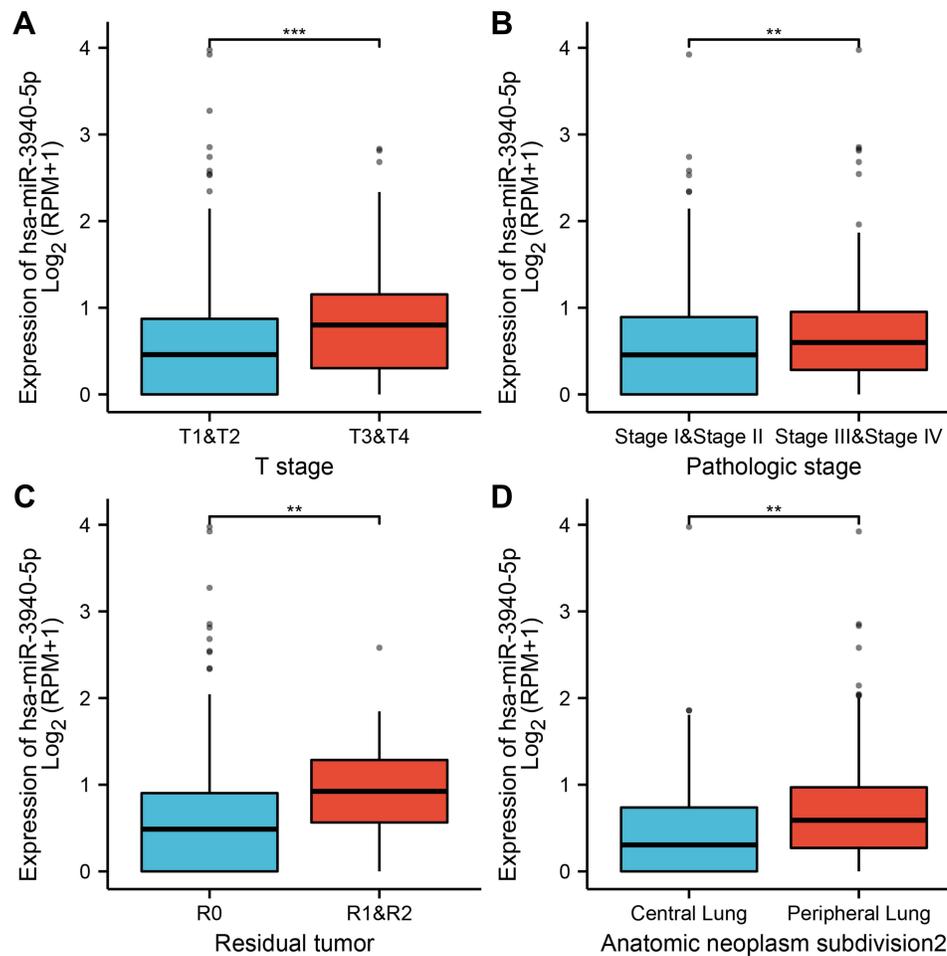


Figure 2 Association with miR-3940-5p and clinical characteristics in LUAD. (A) T Stage, (B) Pathologic stage, (C) Residual tumor, (D) Anatomic neoplasm subdivision 2. **P<0.01; ***P<0.001.

Survival of LUAD Patients is Influenced by miR-3940-5p

As shown in Figure 3, miR-3940-5p expression was negatively associated with poor OS (HR: 1.35; 95% CI: 1.01–1.81; P=0.045), DSS (HR: 1.53; 95% CI: 1.05–2.23; P=0.026) and PFS (HR: 1.35; 95% CI: 1.03–1.77; P=0.032)

Table 2 MiR-3940-5p Expression Associated with Clinical Characteristics (Logistic Regression)

Characteristics	Total (N)	Odds Ratio (OR)	P value
T stage (T3&T4 vs T1&T2)	518	2.139 (1.264–3.706)	0.005
N stage (N1&N2&N3 vs N0)	507	1.098 (0.760–1.589)	0.618
M stage (M1 vs M0)	374	2.601 (1.053–7.350)	0.050
Pathologic stage (Stage III & Stage IV vs Stage I & Stage II)	514	1.545 (1.008–2.383)	0.047
Primary therapy outcome (CR vs PD&SD&PR)	433	0.740 (0.477–1.142)	0.175
Gender (Male vs Female)	521	1.332 (0.943–1.882)	0.104
Race (White vs Asian & Black or African American)	455	1.791 (1.033–3.175)	0.041
Age (>65 vs ≤65)	502	0.763 (0.536–1.083)	0.130
Residual tumor (R1&R2 vs R0)	359	3.250 (1.124–11.719)	0.043
Anatomic neoplasm subdivision (Right vs Left)	506	1.034 (0.724–1.476)	0.856
Anatomic neoplasm subdivision2 (Peripheral Lung vs Central Lung)	186	1.983 (1.072–3.716)	0.030
Number_pack_years_smoked (≥40 vs <40)	354	1.470 (0.968–2.238)	0.071
Smoker (Yes vs No)	507	0.834 (0.510–1.359)	0.467

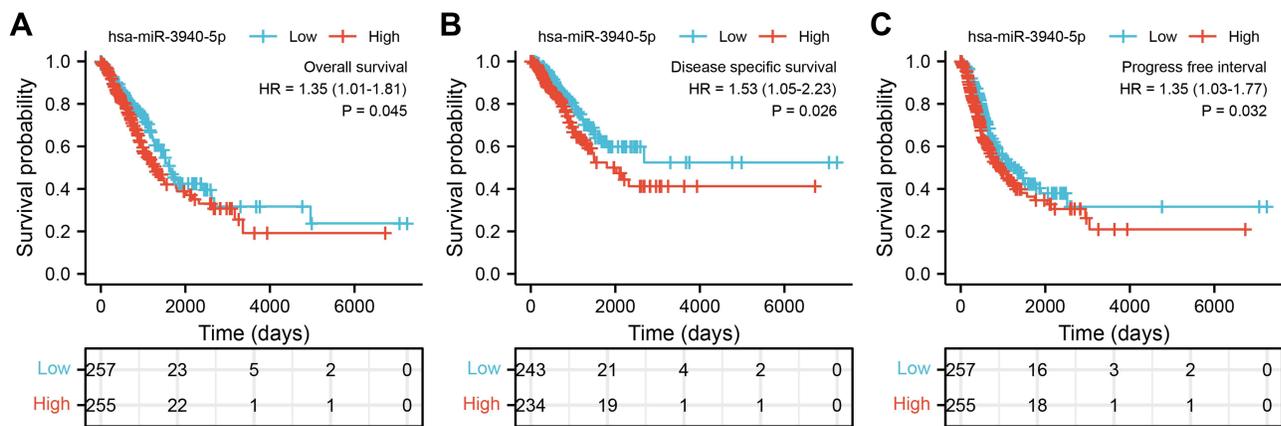


Figure 3 Relationship between miR-3940-5p expression and prognosis. (A) Overall survival, OS, (B) Disease specific survival, DSS, (C) Progress free interval, PFI.

in patients with LUAD. According to the above data, miR-3940-5P levels are associated with poor OS and high levels are associated with poor prognosis.

Relationship Between miR-3940-5p Target Genes and Survival of LUAD Patients

As shown in Figure 4, there were 414 target genes of miR-3940-5p, 3459 LUAD down regulated genes, and 74 common genes. The low expression of BAP1 predicted was associated with poor OS (HR: 0.72; 95% CI: 0.54–2.23; P=0.96), the low expression of BBS1 predicted was associated with poor OS (HR: 0.72; 95% CI: 0.54–0.96; P=0.027), the low expression of CCR2 predicted was associated with poor OS (HR: 0.56; 95% CI: 0.42–0.76; P<0.001), the low expression of KCNE3 predicted was associated with poor OS (HR: 0.72; 95% CI: 0.54–0.96; P=0.024), the low expression of PEBP1 predicted was associated with poor OS (HR: 0.65; 95% CI: 0.49–0.87; P=0.004), and the low expression of RABL2A predicted was associated with poor OS (HR: 0.74; 95% CI: 0.55–0.99; P=0.04) (Figure 5).

GO and KEGG Analysis of miR-3940-5p Target Genes in LUAD Patients

The common genes are involved in biological processes, including actomyosin structure organization, brown fat cell differentiation, magnesium ion homeostasis, response to stimulus, mammary gland epithelial cell proliferation, positive regulation of protein import into nucleus, translocation, hemidesmosome assembly, and lateral

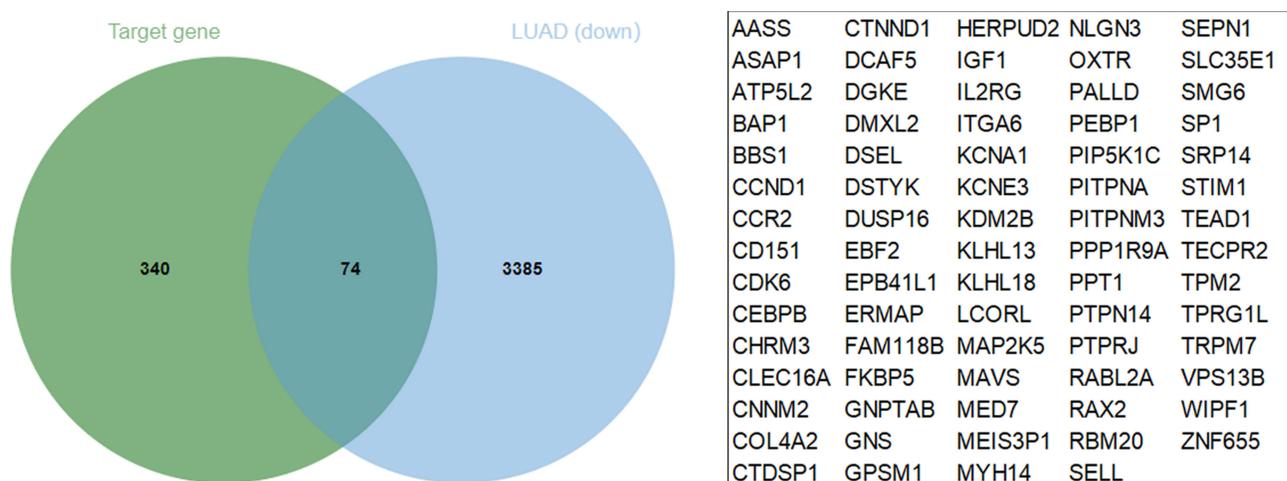


Figure 4 Venn diagram of common genes between target genes of miR-3940-5p and LUAD down regulated genes.

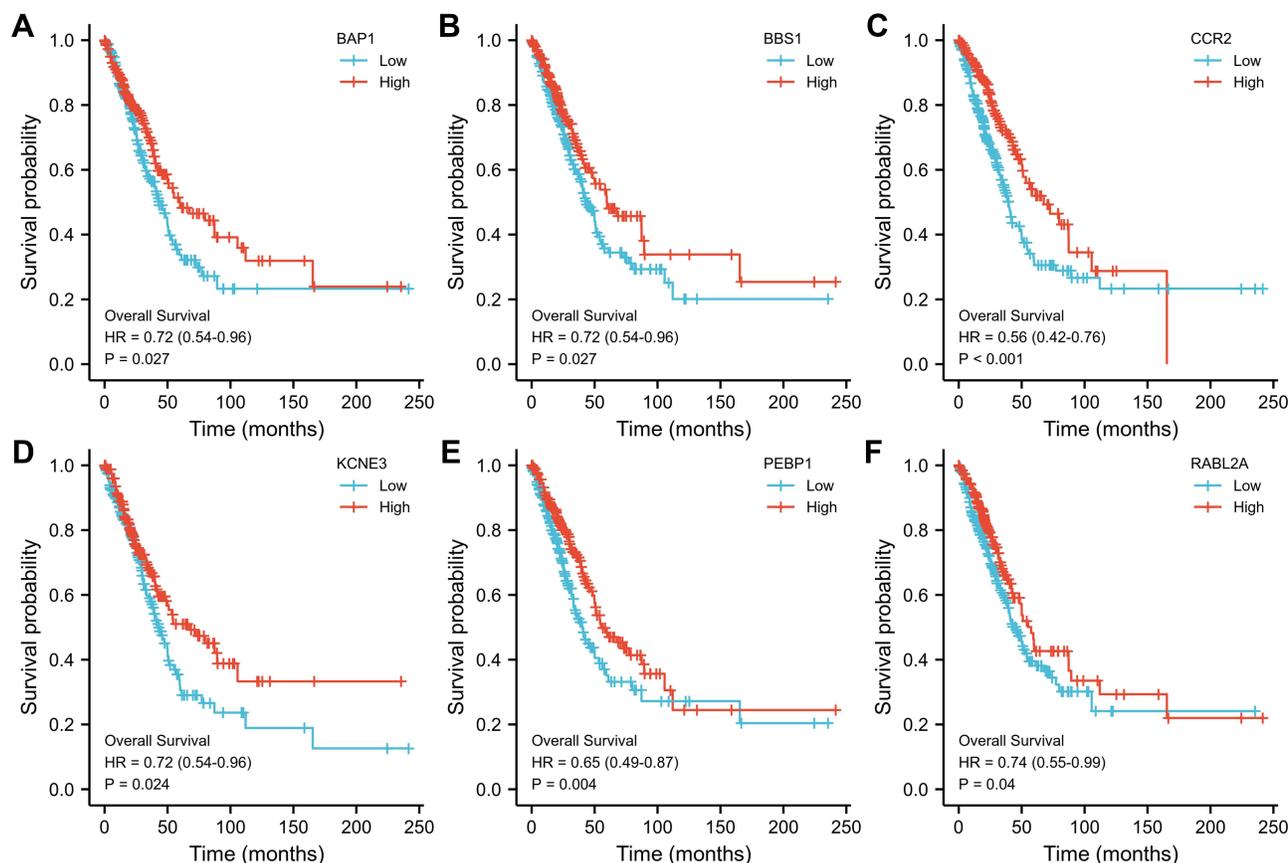


Figure 5 Relationship between expression of miR-3940-5p target genes and prognosis. (A) BAP1, (B) BBS1, (C) CCR2, (D) KCNE3, (E) PEBP1, (F) RABL2A. Overall survival, OS.

ventricle development; cellular components, including ruffle, cell junction, dendrite, growth cone, cytosol, actin filament, cell-cell adherens junction, and synaptic vesicle; molecular functions, including phosphatase activity, protein binding, actin filament binding, protein kinase binding, phosphatidylinositol transporter activity, and actin binding (Figure 6). The common genes have been found to be associated with measles, PI3K-Akt signaling pathway, and p53 pathways (Figure 7).

The Correlation Between miR-3940-5p Expression and Immune Infiltration

As shown in Figures 8 and 9, and Table 3, analysis of the relationship between miR-3940-5p and immune infiltration based on ssGESA with Spearman r showed that there was a positive correlation between miR-3940-5p expression and the expression of NK CD56dim cells ($P < 0.001$) and Th2 cells ($P < 0.001$), and a negative correlation with the expression of DC ($P = 0.003$), Eosinophils ($P < 0.001$), iDC ($P < 0.001$), Macrophages ($P = 0.021$), Mast cells ($P < 0.001$), NK CD56dim cells ($P < 0.001$), NK cells ($P < 0.001$), pDC ($P = 0.002$), Tcm ($P = 0.043$), Tem ($P = 0.045$), TFH ($P < 0.001$), and Th2 cells ($P < 0.001$).

Validating the Expression of miR-3940-5p in Cell Lines

As shown in Figure 10, A549 expressed significantly more miR-3940-5p than Beas-2B (2.608 ± 0.253 vs 0.874 ± 0.233 , $P < 0.001$), while PC9 expressed significantly more miR-3940-5p than Beas-2B (1.823 ± 0.055 vs 0.874 ± 0.233 , $P < 0.001$). LUAD cell lines showed significantly higher levels of miR-3940-5p in contrast to normal Beas-2B cells from the human bronchial epithelium.

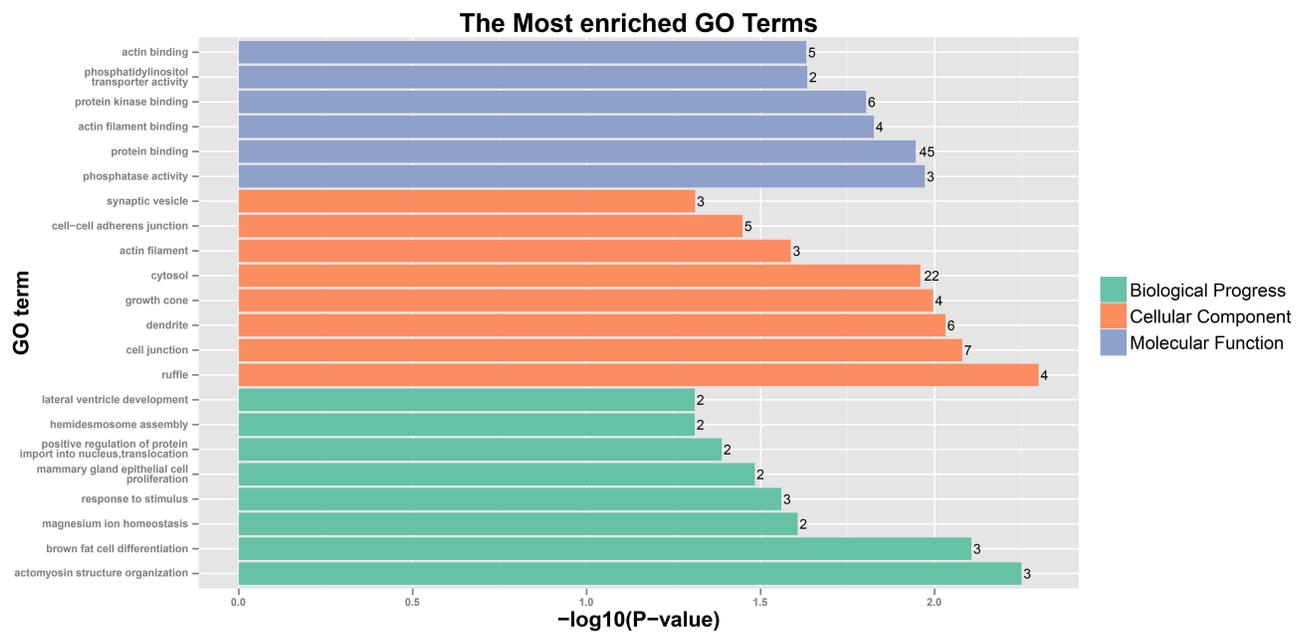


Figure 6 GO analysis of miR-3940-5p target genes.

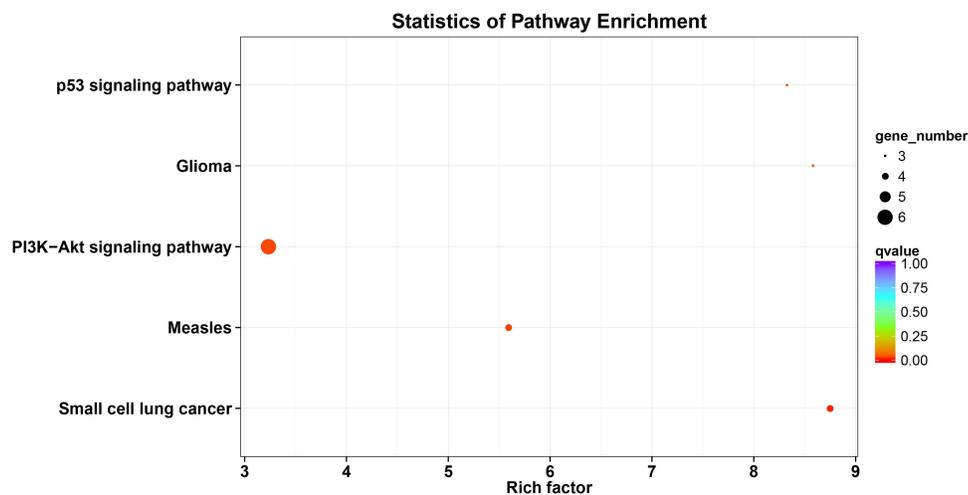


Figure 7 KEGG analysis of miR-3940-5p target genes.

Discussion

MiRNAs have potentially important implications for regulating the development of malignancies, and their expression profile correlates well with specific clinical cancer features.^{25,26} Patients with LUAD with low miR-99a expression have a poor prognosis.²⁷ LUAD can be diagnosed using miR-210-3p as a biomarker.²⁸ There is an essential role for miR-375 in the progression of LUAD.²⁹ NSCLC is influenced by miR-139-5p, which acts as a tumor suppressor.³⁰ This emphasizes the importance of developing novel biomarkers for LUAD.

Levels of miR-3940-5p in NSCLC tumor tissues were lower than those in matched tumor-adjacent tissues.³¹ The results of the study in 90 NSCLC patients with tumor, tumor-adjacent, and normal lung parenchymal tissues, and 17 embryonic lung cDNAs showed a significant downregulation of miR-3940-5p expression in tumor and embryonic lung tissues.³² However, miR-3940-5p was highly expressed in LUAD in this study. The difference in findings may be related to the size of the sample. There was a significant association between miR-3940-5p expression and T stage ($P=0.005$),

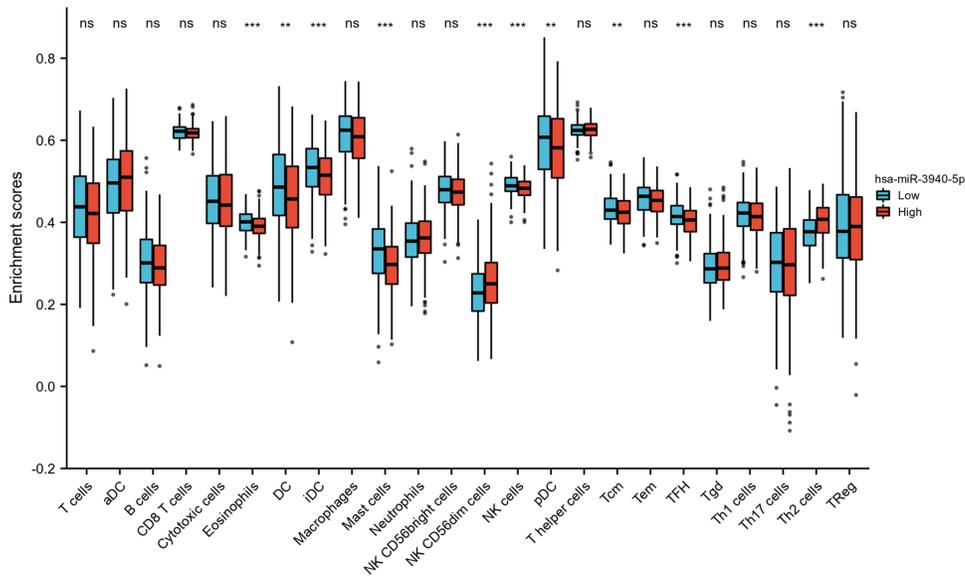


Figure 8 The expression level of miR-3940-5p was related to the immune infiltration in the tumor microenvironment (group comparison chart). Ns, $P > 0.05$; * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$.

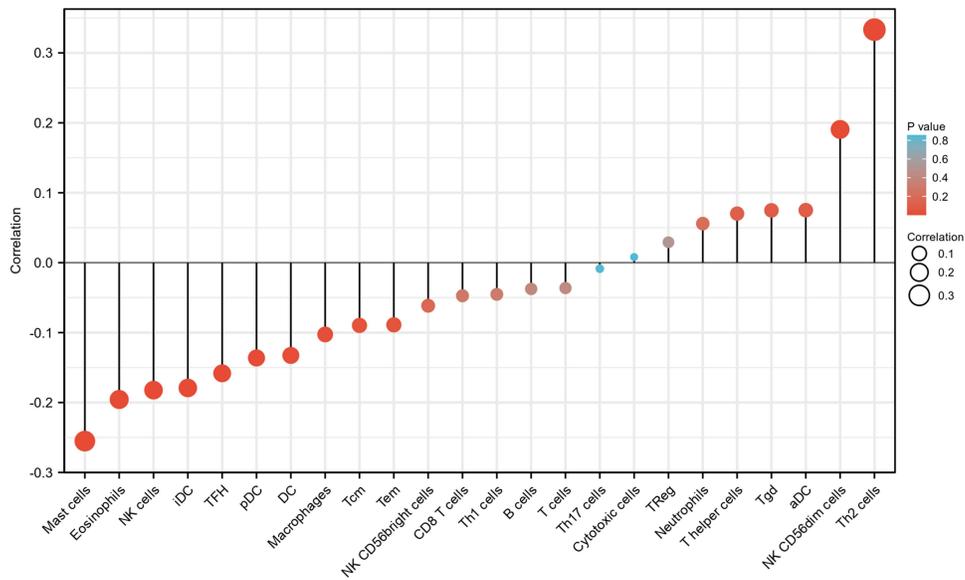


Figure 9 The expression level of miR-3940-5p was related to the immune infiltration in the tumor microenvironment (lollipop chart).

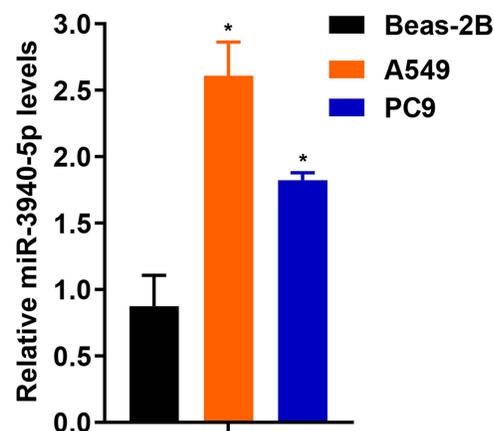
pathologic stage ($P=0.047$), race ($P=0.041$), residual tumor ($P=0.043$), and anatomic neoplasm subdivision2 ($P=0.030$). In patients with advanced T stage or clinical stage III–IV, LUAD expressed more miR-3940-5p than adjacent normal lung tissue, suggesting that miR-3940-5p may be involved in tumorigenesis and promote proliferation. There may be a high expression of miR-3940-5p in tumors with positive R1 and R2 resection margins. MiR-3940-5p expression was associated with OS (HR: 1.35; 95% CI: 1.01–1.81; $P=0.045$), DSS (HR: 1.53; 95% CI: 1.05–2.23; $P=0.026$), and PFI (HR: 1.35; 95% CI: 1.03–1.77; $P=0.032$). Poor OS was associated with low expression of miR-3940-5p target genes, including BAP1, BBS1, CCR2, KCNE3, PEBP1, and RABL2A. BAP1 promotes breast cancer cell proliferation and metastasis through deubiquitination of KLF5.³³ High BBS1 gene expression facilitates survival of malignant pleural

Table 3 MiR-3940-5p Expression Associated with Immune Cells (Spearman Method)

Gene name	Cell Type	Correlation Coefficient (Spearman)	P value (Spearman)
hsa-miR-3940-5p	aDC	0.075	0.091
hsa-miR-3940-5p	B cells	-0.038	0.399
hsa-miR-3940-5p	CD8 T cells	-0.047	0.288
hsa-miR-3940-5p	Cytotoxic cells	0.008	0.857
hsa-miR-3940-5p	DC	-0.133	0.003
hsa-miR-3940-5p	Eosinophils	-0.196	<0.001
hsa-miR-3940-5p	iDC	-0.179	<0.001
hsa-miR-3940-5p	Macrophages	-0.103	0.021
hsa-miR-3940-5p	Mast cells	-0.255	<0.001
hsa-miR-3940-5p	Neutrophils	0.056	0.209
hsa-miR-3940-5p	NK CD56bright cells	-0.062	0.166
hsa-miR-3940-5p	NK CD56dim cells	0.191	<0.001
hsa-miR-3940-5p	NK cells	-0.182	<0.001
hsa-miR-3940-5p	pDC	-0.136	0.002
hsa-miR-3940-5p	T cells	-0.036	0.415
hsa-miR-3940-5p	T helper cells	0.07	0.114
hsa-miR-3940-5p	Tcm	-0.09	0.043
hsa-miR-3940-5p	Tem	-0.089	0.045
hsa-miR-3940-5p	TFH	-0.158	<0.001
hsa-miR-3940-5p	Tgd	0.075	0.092
hsa-miR-3940-5p	Th1 cells	-0.045	0.309
hsa-miR-3940-5p	Th17 cells	-0.009	0.847
hsa-miR-3940-5p	Th2 cells	0.333	<0.001
hsa-miR-3940-5p	TReg	0.029	0.512

mesothelioma.³⁴ Crosstalk between macrophages and cancer cells via CCR2 and CX3CR1 is the underlying mechanism driving lung cancer.³⁵ RIPK4 overexpression promotes pancreatic cancer cell migration and invasion through PEBP1 degradation-induced activation of the RAF1/MEK/ERK pathway.³⁶ The RABL2A-CCDC34 axis plays an important role in mediating p38/MAPK and JNK/MAPK signaling, leading to acquired sorafenib resistance in hepatocellular carcinoma.³⁷ The specific mechanisms by which miR3940-5p mediates LUAD development through the regulation of BAP1, BBS1, CCR2, KCNE3, PEBP1, and RABL2A need to be further investigated.

There are studies on the regulatory pathway of miR-3940-5p in some solid tumors. LncRNA-SNHG14/miRNA-3940-5p/NAP12 mRNA may be potential for CRC diagnosis and prognosis.⁸ miR-3940-5p negatively regulates CUL7 and activates NF- κ B to promote gliomagenesis in human gliomas.³⁸ miR-3940-5p targets CCND1 and USP28 to inhibit

**Figure 10** Expression of miR-3940-5p in A549, PC9, and Beas-2B. *P<0.05.

proliferation and induce apoptosis in NSCLC cells.³¹ In this study, miR-3940-5p target genes participate in several pathways, including measles, PI3K-Akt signaling pathway, and p53 signaling pathway.

It is important to understand the immune infiltration in LUAD to develop immunotherapy for it. The present study found miR-3940-5p expression to be associated with infiltration of NK CD56dim cells, Th2 cells, DC, Eosinophils, iDC, Macrophages, Mast cells, NK CD56dim cells, NK cells, pDC, Tcm, Tem, TFH, and Th2 cells. In other words, miR-3940-5p promotes the function of NK CD56dim cells and Th2 cells, and inhibits the function of DC, Eosinophils, iDC, Macrophages, Mast cells, NK CD56dim cells, NK cells, pDC, Tcm, Tem, TFH, and Th2 cells.

Its limitations lie in its reliance on database data and bioinformatics analysis as well as some experimental validation. More biological experiments will be carried out to further explore the function of miR-3940-5p in LUAD. Circulating tumor cells (CTCs) are an independent prognostic indicator of PFS and OS.³⁹ In future studies, the role of miR-3940-5p can be compared with the role of circulating tumor cells and other prognostic factors in LUAD.

Conclusion

MiR-3940-5p showed high expression in LUAD tissues and cell lines. High expression of MiR-3940-5p was associated with poor prognosis in LUAD patients. Poor OS in LUAD was associated with low expression of miR-3940-5p target genes, including BAP1, BBS1, CCR2, KCNE3, PEBP1, and RABL2A. Several pathways may play a role in the development of LUAD, including measles, PI3K-Akt signaling pathway, and p53 signaling pathway. Immune infiltration was associated with miR-3940-5p expression. MiR-3940-5p may provide insight into LUAD patient prognosis.

Data Sharing Statement

The data used to support the findings of this study are included within the article.

Ethics Approval

The Medical Ethics Committee of Jiangmen Central Hospital reviewed this study, and the ethics requirement was waived (Approval No: [2021]01).

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Disclosure

The authors declare that they have no conflicts of interest relevant to this study.

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