# Clinical Value and Prospective Pathway Signaling of MicroRNA-375 in Lung Adenocarcinoma: A Study Based on the Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO) and Bioinformatics Analysis

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Background: Lung adenocarcinoma (LUAD) is the most frequent lung cancer. MicroRNAs (miRNAs) are believed to have fundamental roles in tumorigenesis of LUAD. Although miRNAs are broadly recognized in LUAD, the role of microRNA-375 in LUAD is still not fully elucidated.

Material/Methods: We evaluated the significance of miR-375 expression in LUAD by using analysis of a public dataset from the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) database and a literature review. Furthermore, we investigated the biological function of miR-375 by gene ontology enrichment and target prediction analysis.

Results: MiR-375 expression was significantly higher in LUAD by TCGA data compared to normal lung tissue ($p$<0.0001). In addition, a common pattern of upregulation for miR-375 in LUAD was found in our review of the literature. A total of 682 genes, both LUAD-related and miR-375-related, were obtained from the analytical integration. Critical pathways were unveiled in the network analysis of the overlaps, such as pentose and glucuronate interconversions, ascorbate and aldarate metabolism, and starch and sucrose metabolism. Furthermore, we identified covert miR-375 associated genes that might participate in LUAD by network analysis, such as FGF2 (fibroblast growth factor 2), PAX6 (paired box 6), and RHOJ. The expression of these three genes were all downregulated in LUAD. Finally, FGF2 was revealed to be negatively correlated with miR-375 in LUAD (r=−0.1821, $p$=0.0001).

Conclusions: Overall, our study provides evidence that miR-375 is essential for the progression of LUAD.

MeSH Keywords: Carcinoma, Non-Small-Cell Lung • Computational Biology • MicroRNAs

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/901460

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## Background

Lung cancer, an extremely heterogeneous disease, accounts for almost a quarter of cancer-related deaths [1]. Lung adenocarcinoma (LUAD) is the most aggressive histological type of lung cancer. The incident of LUAD has been increasing rapidly [2]. Unfortunately, LUAD mortality has not significantly decreased owing to the lack of early detection and lack of more effective therapies at earlier disease stages. Therefore, insights into the mechanisms of LUAD are considered urgent.

MicroRNAs (miRNAs or miRs), typically 18–23 nucleotides in length, have gained much attention as oncogenes or suppressors modulating gene activity at the post-transcriptional and translational levels [3]. Numerous studies have shown that aberrantly expressed miRNAs in various types of cancer are associated with cell development, cell proliferation, apoptosis, and tumorigenesis [4–7].

Growing evidence has demonstrated that downregulated miR-375 expression contributes to various types of cancers. Several studies have reported that downregulated miR-375 expression was implicated in β-cell growth and glucose regulation of insulin gene expression by directly targeting PDK1 in pancreatic carcinoma [8,9]. In addition, miR-375 has been shown to inhibit cancer cell growth in liver cancer by negatively regulating oncogene AEG-1 [10]. MiR-375 is considered as an oncomiR in prostate carcinogenesis and plays a vital role in prostate cancer progression [11].

In non-small cell lung carcinoma (NSCLC), decreased miR-375 expression level was significantly correlated with lymphatic metastasis and advanced stage disease. On the contrary, high miR-375 expression could also promote cell migration and target CLDN1 in NSCLC [12]. In a previous study, miR-375 expression levels were significantly over-expressed in LUAD and small cell lung carcinoma (SCLC), whereas they were under-expressed in lung squamous cell carcinoma (LUSC) [13]. Furthermore, studies have also found that miR-375 promotes cell growth in SCLC cell lines by directly downregulating inositol-trisphosphate 3-kinase B (ITPKB) [14]. Unfortunately, data published on miR-375 expression in LUAD are partly conflicting and highly heterogeneous.

In the present study, we sought to unveil the role of miR-375 in LUAD through modulation of miRNAs expression and identification of putative molecular targets by bioinformatics analysis and analysis based on TCGA, GEO, and literature reviews.

## Material and Methods

### TCGA data in LUAD patients

The available data on miR-375 expression and clinical information were obtained from Illumina HiSeq Level 3 isoform quantification files available at the TCGA Data Portal website (*http://tcga.cancer.gov/dataportal*; accessed June 2016). We obtained the normalized reads per million miRNA mapped (RPM) data in 517 LUAD patients and 59 normal lung samples by summing up the raw counts. The RPM data of the miR-375 identified in this study were extracted both for 450 LUAD patients and 47 normal samples. Mean values were used for a patient with more than one portion to prevent duplicates. The LIMMA package of R language was used to identify the differentially expressed genes (DEGs) between the expression profiles of 517 LUAD patients and 59 normal samples. We identified the significance of gene expression difference by using absolute log2 fold change (FC) >1. FDR (False Discovery Rate) of q-value was adjusted to 0.05.

### Selection of GEO dataset

Next, we obtained the microarray profiles of LUAD from the GEO database (Gene Expression Omnibus, *http://www.ncbi.nlm.nih.gov/geo/*). The following keywords were used in the GEO database: (lung OR pulmonary OR respiratory OR bronchi OR bronchioles OR alveoli OR pneumocytes OR "air way") AND (cancer OR carcinoma OR tumor OR neoplas* OR malignan* OR adenocarcinoma) AND (microRNA OR miRNA OR "micro RNA" OR "small temporal RNA" OR "noncoding RNA" OR ncRNA OR "small RNA"). The microarray datasets reporting miR-375 expression between LUAD and normal lung tissues was included in this study.

### Study selection and data extraction for literature review

An electronic literature search was performed in PubMed and Web of Science (up to September 1, 2016) by using the
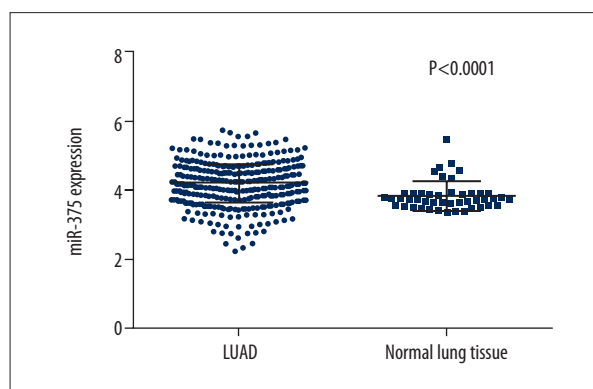


**Figure 1.** Expression of miR-375 in LUAD and normal lung tissues in TCGA dataset.

**Table 1.** Clinical covariates for the TCGA LUAD cohort.

| Clinicopathological feature | | n | miRNA-375 relevant expression($2^{-\Delta Cq}$) | | |
| --- | --- | --- | --- | --- | --- |
| | | | Mean ±SD | t | P-value |
| Tissue | LUAD | 450 | 14.0282±1.86840 | 5.542 | <0.0001 |
| | Normal lung | 46 | 12.7962±1.38416 | | |
| Age (years) | <60 | 121 | 13.9739±1.7431 | −0.156 | 0.876 |
| | ≥60 | 310 | 14.0050±1.9093 | | |
| Gender | Male | 210 | 14.1536±2.0444 | 1.317 | 0.189 |
| | Female | 240 | 13.9185±1.6963 | | |
| Tumor stage 1 | T1 | 154 | 13.8402±2.0764 | 1.834* | 0.14 |
| | T2 | 236 | 14.1677±1.8113 | | |
| | T3 | 41 | 13.6755±1.3020 | | |
| | T4 | 16 | 14.5330±1.7740 | | |
| Tumor stage 2 | T1~2 | 390 | 14.0384±1.9244 | 0.46 | 0.646 |
| | T3~4 | 57 | 13.9162±1.4849 | | |
| Nodes | Yes | 156 | 14.0911±1.7676 | 0.501 | 0.616 |
| | No | 293 | 13.9981±1.9243 | | |
| Metastasis | Yes | 159 | 13.9730±1.8354 | −0.486 | 0.627 |
| | No | 287 | 14.0630±1.8934 | | |
| Pathologic stage 1 | I | 242 | 13.9870±1.9997 | 0.357* | 0.784 |
| | II | 110 | 13.9973±1.7651 | | |
| | III | 73 | 14.1602±1.5368 | | |
| | IV | 20 | 14.3416±1.8340 | | |
| Pathologic stage 2 | I+II | 352 | 13.9903±1.9270 | −0.962 | 0.337 |
| | II+IV | 93 | 14.1992±1.5964 | | |
| Anatomic organ subdivision | L_lower | 70 | 14.0738±1.9777 | 0.985* | 0.415 |
| | L_upper | 109 | 14.0742±1.8623 | | |
| | R_lower | 85 | 14.1820±1.8741 | | |
| | R_middle | 156 | 13.8154±1.8411 | | |
| | R_upper | 19 | 14.5101±1.8248 | | |
| Location | Central | 54 | 13.8303±1.9611 | −1.03 | 0.305 |
| | Peripheral | 113 | 14.1516±1.8495 | | |

* One-way analysis of variance (ANOVA) test was performed.

following terms: (miR-375 OR miRNA-375 OR microRNA375 OR miR375 OR miRNA375 OR miR 375 OR miRNA 375 OR microRNA 375) and (lung cancer OR lung carcinoma OR lung neoplasm OR lung tumor OR lung adenocarcinoma OR LUAD OR non-small cell lung cancer OR NSCLC). Publications were considered eligible if they met the following criteria: (1) studies examining the expression of miR-375 in LUAD; and (2) normal lung tissues paired/unpaired used as healthy control group. The studies were considered ineligible based on the following criteria: (1) reviews, experimental studies, single case reports, meta-analyses, and conference abstracts; and (2) absence of healthy control groups.
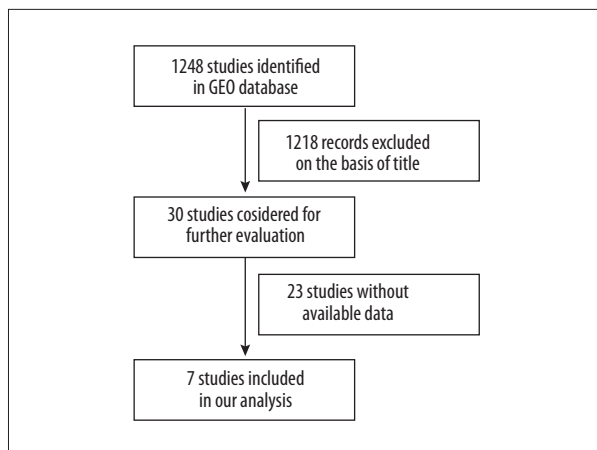
**Figure 2.** Flow chart of study selection for GEO dataset.

## Gene ontology enrichment and target prediction analysis

The targets of miR-375 were predicted through 12 programs, including miRWALK2.0 (*http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/miRretsys-self.html*). To increase the prediction accuracy, the genes were selected as targets that were overlapped in at least 5 of 12 databases (Microt4, miRWalk, mirbridge, miRanda, miRDB, miRMap, Pictar2, PITA, miRNAMap, RNAhybrid, RNA22 and Targetscan). Subsequently, we analyzed the gene overlaps integrated between DEGs in LUAD, and predicted target genes of miR-375 by bioinformatics software. Gene ontology (GO) enrichment analysis was conducted for overlapped genes by DAVID [15]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (*http://www.genome.jp/kegg/tool/search_pathway.html*) was then used to map the predicted targets by using DAVID online analysis (*https://david.ncifcrf.gov/*) [16]. STRING database (the Search Tool for the Retrieval of Interacting Genes/Proteins) was also used to predict the association between miR-375 and the target gene in the regulatory network analysis [17].

## Statistical analysis

All data are displayed as mean ± standard deviation (SD) from each group. Student's *t*-test was performed to analyze the differences between two groups, whereas one-way analysis of variance (ANOVA) test was used among more than three groups. Standardized mean difference (SMD) was applied to evaluate the association between miR-375 levels and LUAD by RevMan 5.2.0 software. We pooled the SMD across GEO datasets using the Mantel-Haenszel formula (fixed-effect model) or the DerSimonian-Laird formula (random-effect model). A fixed-effect model was adopted when the Q statistic was considered significant ($p>0.1$, or $I^2<50\%$), otherwise, a random-effect model was used. Moreover, the relationship of DEGs expression with miR-375 level was analyzed by Spearman's rank correlation. A two-sided *p*-value <0.05 was considered statistically significant.

## Results

### MiR-375 expression in LUAD clinical tissues

#### *The detection of MiR-375 expression in LUAD in TCGA*

The expression levels of miR-375 were significantly upregulated in clinical LUAD specimens (14.0282±1.86840) in comparison to adjacent non-cancerous lung tissues (12.7962±1.38416; $p<0.0001$, Figure 1). For all the tested parameters, no significant differences were found in the other clinical features (Table 1).

### MiR-375 expression in LUAD based on GEO

Additionally, miR-375 expression was initially assessed in a series of LUAD and normal lung tissues based on GEO dataset (Figure 2). A total of seven GEO datasets (GSE40738, GSE47525, GSE48414, GSE51853, GSE74190, and GSE25508)

**Table 2.** Characteristics of studies based on GEO dataset.

| Study | Patients | | | Control | | | t | P |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | n | Mean | SD | n | | |
| GSE25508 | 2.2384 | 0.4558 | 5 | 2.2886 | 0.5715 | 5 | −0.154 | 0.882 |
| GSE40738 | −0.5367 | 0.1091 | 45 | −0.4921 | 0.1438 | 91 | −1.836 | 0.069 |
| GSE47525 | 10.3833 | 5.7049 | 6 | 10.4780 | 5.4595 | 14 | −0.035 | 0.972 |
| GSE48414 | −0.0060 | 1.9080 | 154 | −0.48466 | 0.4753 | 20 | 2.560 | 0.012 |
| GSE51853 | 0.8610 | 1.9923 | 76 | 0.1738 | 0.2469 | 5 | 2.708 | 0.009 |
| GSE63805 | 2.3994 | 0.4889 | 32 | 2.1065 | 0.1761 | 30 | 3.176 | 0.003 |
| GSE74190 | 6.0000 | 1.8560 | 46 | 4.3600 | 0.8690 | 44 | 4.871 | 0.000 |
| Total | SMD(95%CIs)=0.33(−0.16, 0.82), P=0.18; $I^2$=79%, P<0.0001 | | | | | | | |
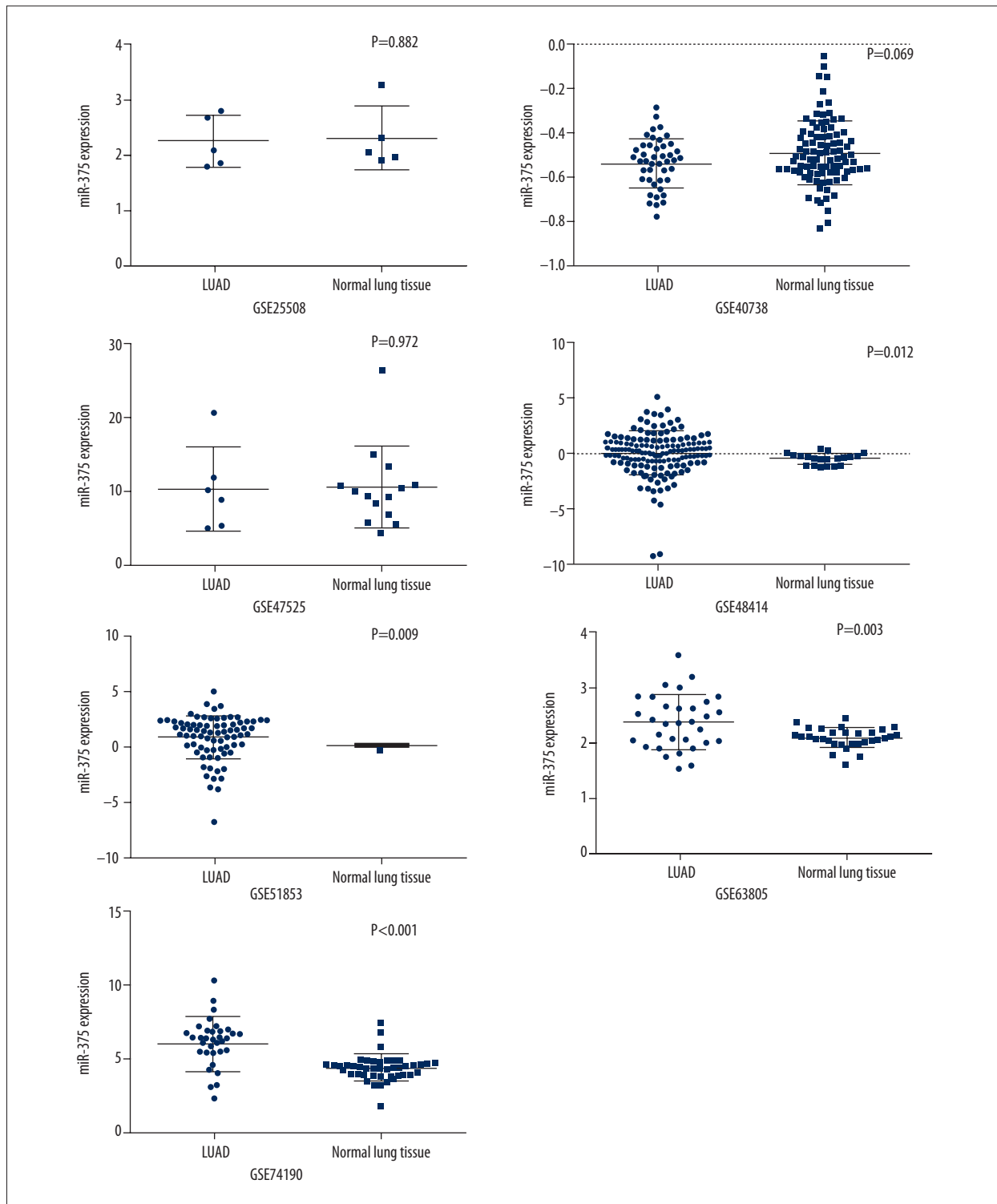
**Figure 3.** Expression of miR-375 in LUAD and normal lung tissues in GEO datasets.

which included both LUAD patients and healthy people, were collected in our study. The expression levels of miR-375 in LUAD tissues were significantly higher than in healthy non-cancer control tissues in GSE48414, GSE51853, GSE63805,

and GSE74190 datasets (*p*=0.012, *p*=0.009, *p*=0.003, and *p*<0.0001; respectively), whereas no significant difference was found in other GEO datasets (GSE40738, GSE47525, and GSE25508). Characteristics of studies based on GEO dataset
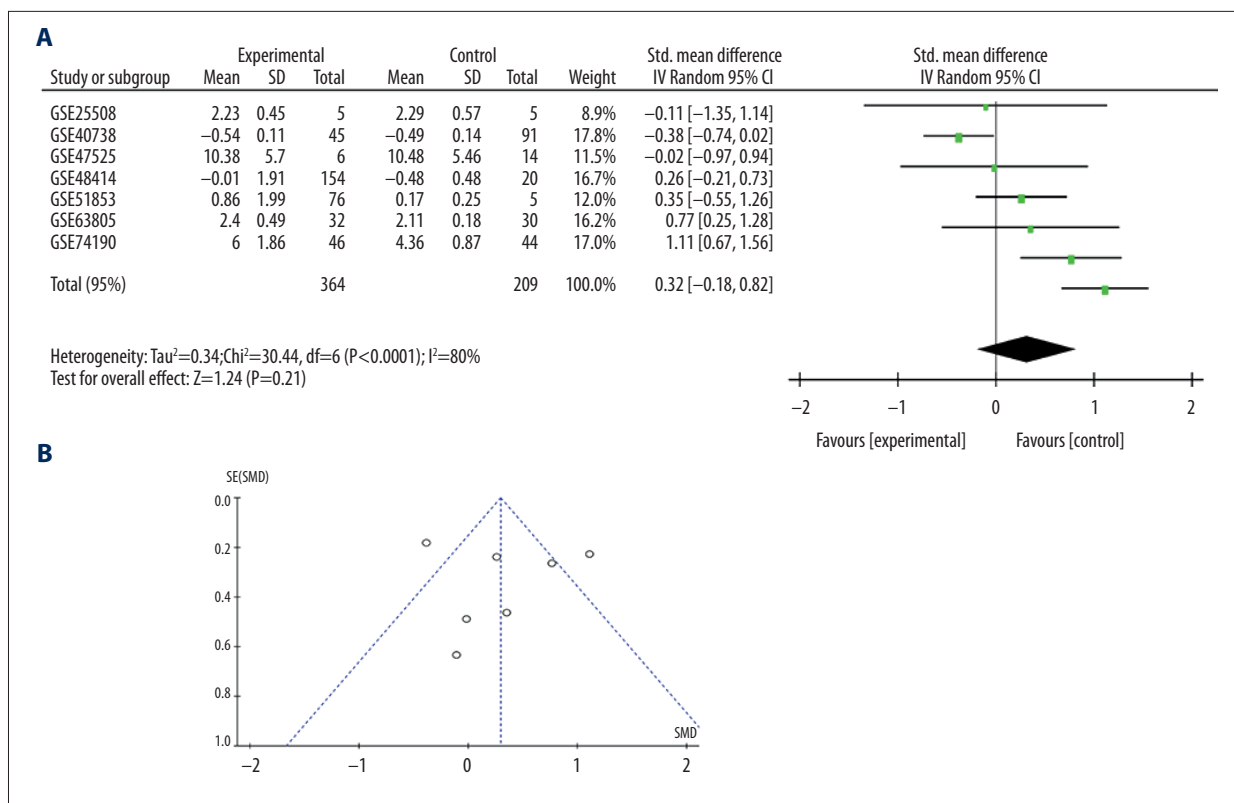
**Figure 4.** Forest plot (**A**) and funnel plot (**B**) of the combined SMD for miR-375 expression between LUAD and control group by the random effects models.
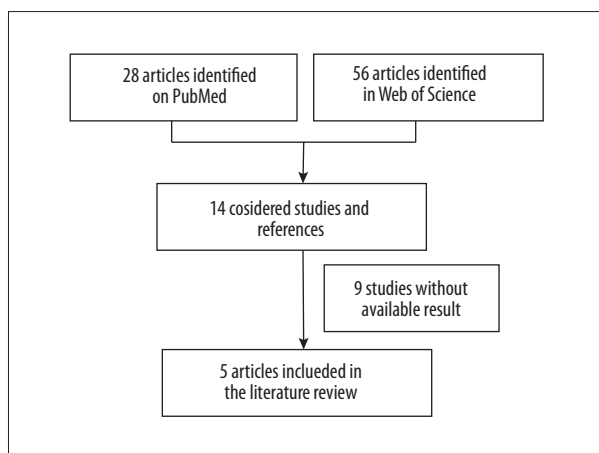


**Figure 5.** Flow chart of study selection for the literature review.

are presented in Table 2 and Figure 3. However, no significant difference was found between LUAD and control groups based on all the included GEO datasets (SMD=0.33; 95% CI, −0.16 to 0.82; *p*=0.18) with significant heterogeneity by random-effected model (*p*<0.0001, I²=79%). The results of forest plot and funnel plot are shown in Figure 4.

## Literature review of miR-375 expression profiles in LUAD versus normal lung

Next, we explored miR-375 upregulation in LUAD based on literature data. As shown in Figure 5, five studies that met the criteria for selection were selected from the literature [14,18–21]. Consistent with the result of TCGA, a common pattern of up-regulation for miR-375 in LUAD were reported across the four included studies, whereas no significant upregulation was found in one study (Table 3).

## MiR-375 prediction and bioinformatics analyses

### Data preprocessing and DEGs screening

A total of 20,531 genes were differentially compared in LUAD TCGA data. After preprocessing, 5,817 DEGs were screened out by the difference threshold (q value <0.05 and absolute $\log_2 FC$ >1), including 3,843 upregulated genes and 1,794 downregulated genes. Meanwhile, 58,976 target genes for miR-375 were identified in five up-to-date prediction algorithms. Furthermore, all the 682 miR-375 predicted target genes were sorted out by language R, which were then integrated analytically between DEGs and the predicted target genes. The flow diagram and results for DEGs screening process are depicted in Figures 6 and 7.

**Table 3.** Overview of the 5 studies selected from literature.

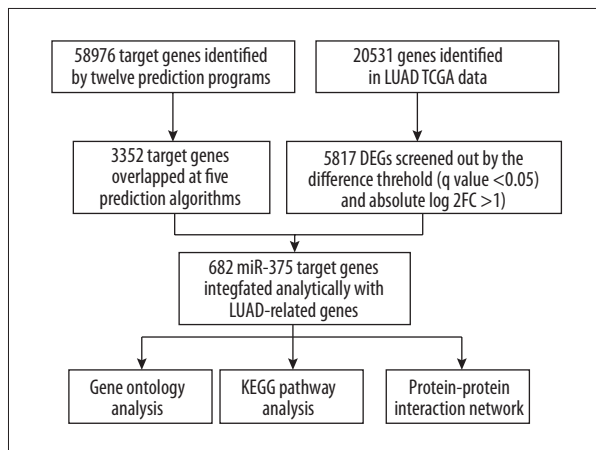| Author | Year | Country | LUAD (n) | Normal lung (n) | Result | Detection methods |
|--------|------|---------|----------|-----------------|--------|-------------------|
| Yu | 2010 | Maryland | 36 | 36 (paired) | Up-regulation | qRT-PCR |
| Hamamoto | 2013 | Japan | 54 | 54 (paired) | Up-regulation | qRT-PCR |
| Sonia | 2014 | Spain | 19 | 19 (paired) | Up-regulation | qRT-PCR |
| Jin | 2015 | China | 36 | 44 | Up-regulation | qRT-PCR |
| KIM | 2014 | Korea | 35 | 2 | NS | MicroRNA microarrays |



**Figure 6.** Flow diagram of screening for miR-375-related DEGs in LUAD.
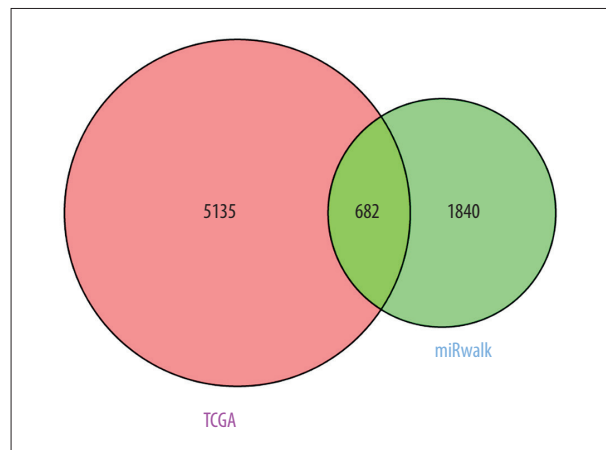


**Figure 7.** Venn diagram for the integration between DEGs and predicted target genes.

### Functional analysis of the DEGs in lung cancer

First, we identified the functional roles of 682 potential target genes in terms of biological processes in LUAD by GO enrichment analysis. The analysis revealed that numerous target genes were involved in the biological processes, such as neuron differentiation, plasma membrane part and sequence-specific DNA binding. Then, the KEGG pathways program was used to reveal the critical pathway, in which the overrepresentation of the predicted miR-375 targets was involved, linked to carcinogenesis such as pentose and glucuronate interconversions, ascorbate and aldarate metabolism, and starch and sucrose metabolism. The top ten GO processes and KEGG pathways that were most strongly enriched with respect to miR-375 in LUAD are shown in Tables 4, 5, and Figure 8.

### Protein-protein interaction network analysis

STRING was performed to construct the PPI network based on the overlapped DEGs and genes or proteins (Figure 9). In the PPI network, FGF2 (fibroblast growth factor 2), PAX6 (paired box 6), and RHOJ were revealed to exert their potential roles in LUAD by interactions with miR-375 (Table 6). The expression of

three genes were downregulated in LUAD ($log_2FC = -2.11$, FDR <0.0001; $log_2FC = -1.431$, FDR <0.0001 and $log_2FC = -1.879$, FDR <0.0001, respectively). A significant negative correlation was observed between FGF2 and miR-375 expression in LUAD patients by Spearman correlation ($r = -0.1821$, $p = 0.0001$), whereas no significant correlation was found between PAX6 and RHOJ and miR-375 ($p = 0.1221$ and $p = 0.325$, respectively; Figure 10).

## Discussion

In this study, we identified the aberrantly expressed miR-375 involved in LUAD through the comparison of miRNA expression profiles in cancerous tissues with that of normal lung tissues based on validation from TCGA and GEO datasets and published studies. Additionally, we discovered novel markers and potential targets for miR-375 that were involved in the regulation of crucial biological processes in LUAD by GO analysis and KEGG pathway annotation.

To date, there have only been several studies concerning the characteristics of miR-375 in lung cancer. Li et al. were the first to demonstrate that the expression of miR-375 was lower in

**Table 4.** Ten processes most strongly enriched by GO analysis.

| Category | Term | Count | P-value | FDR |
|---|---|---|---|---|
| **Biological processes** | | | | |
| GOTERM_BP_FAT | GO: 0030182~neuron differentiation | 52 | 1.58E-12 | 2.79E-09 |
| GOTERM_BP_FAT | GO: 0048666~neuron development | 44 | 5.76E-12 | 1.02E-08 |
| GOTERM_BP_FAT | GO: 0007409~axonogenesis | 32 | 1.39E-11 | 2.45E-08 |
| GOTERM_BP_FAT | GO: 0000902~cell morphogenesis | 44 | 2.96E-11 | 5.23E-08 |
| GOTERM_BP_FAT | GO: 0048812~neuron projection morphogenesis | 33 | 4.00E-11 | 7.06E-08 |
| GOTERM_BP_FAT | GO: 0048667~cell morphogenesis involved in neuron differentiation | 32 | 1.13E-10 | 1.99E-07 |
| GOTERM_BP_FAT | GO: 0006928~cell motion | 50 | 3.40E-10 | 5.99E-07 |
| GOTERM_BP_FAT | GO: 0000904~cell morphogenesis involved in differentiation | 34 | 3.40E-10 | 5.99E-07 |
| GOTERM_BP_FAT | GO: 0048858~cell projection morphogenesis | 34 | 3.81E-10 | 6.71E-07 |
| GOTERM_BP_FAT | GO: 0032989~cellular component morphogenesis | 44 | 9.76E-10 | 1.72E-06 |
| **Cellular components** | | | | |
| GOTERM_CC_FAT | GO: 0044459~plasma membrane part | 143 | 3.59E-12 | 4.94E-09 |
| GOTERM_CC_FAT | GO: 0005886~plasma membrane | 209 | 1.82E-11 | 2.50E-08 |
| GOTERM_CC_FAT | GO: 0031012~extracellular matrix | 38 | 6.62E-09 | 9.12E-06 |
| GOTERM_CC_FAT | GO: 0031226~intrinsic to plasma membrane | 81 | 2.56E-07 | 3.52E-04 |
| GOTERM_CC_FAT | GO: 0005578~proteinaceous extracellular matrix | 33 | 3.60E-07 | 4.95E-04 |
| GOTERM_CC_FAT | GO: 0005887~integral to plasma membrane | 77 | 1.66E-06 | 0.002279 |
| GOTERM_CC_FAT | GO: 0030054~cell junction | 41 | 9.84E-06 | 0.013541 |
| GOTERM_CC_FAT | GO: 0044421~extracellular region part | 63 | 1.28E-05 | 0.017682 |
| GOTERM_CC_FAT | GO: 0031224~intrinsic to membrane | 250 | 1.64E-05 | 0.022599 |
| GOTERM_CC_FAT | GO: 0045202~synapse | 31 | 2.48E-05 | 0.034146 |
| **Molecular function** | | | | |
| GOTERM_MF_FAT | GO: 0043565~sequence-specific DNA binding | 55 | 3.39E-09 | 5.13E-06 |
| GOTERM_MF_FAT | GO: 0015267~channel activity | 36 | 6.24E-06 | 0.00943 |
| GOTERM_MF_FAT | GO: 0022836~gated channel activity | 30 | 6.40E-06 | 0.009679 |
| GOTERM_MF_FAT | GO: 0022803~passive transmembrane transporter activity | 36 | 6.57E-06 | 0.009928 |
| GOTERM_MF_FAT | GO: 0022838~substrate specific channel activity | 35 | 7.55E-06 | 0.011411 |
| GOTERM_MF_FAT | GO: 0003700~transcription factor activity | 65 | 8.48E-06 | 0.012823 |
| GOTERM_MF_FAT | GO: 0005216~ion channel activity | 33 | 2.54E-05 | 0.038459 |
| GOTERM_MF_FAT | GO: 0008066~glutamate receptor activity | 8 | 1.25E-04 | 0.188624 |
| GOTERM_MF_FAT | GO: 0030528~transcription regulator activity | 84 | 2.57E-04 | 0.387228 |
| GOTERM_MF_FAT | GO: 0005230~extracellular ligand-gated ion channel activity | 11 | 3.51E-04 | 0.528754 |

**Table 5.** Ten KEGG pathways most strongly enriched by target genes.

| Term | Input number | Background number | P-value | Corrected P-value |
|---|---|---|---|---|
| Pentose and glucuronate interconversions | 11 | 36 | 1.99E-06 | 0.000464663 |
| Ascorbate and aldarate metabolism | 9 | 27 | 9.55E-06 | 0.001117413 |
| Starch and sucrose metabolism | 12 | 56 | 1.56E-05 | 0.001218749 |
| Porphyrin and chlorophyll metabolism | 10 | 42 | 3.67E-05 | 0.00214613 |
| Drug metabolism – other enzymes | 10 | 46 | 7.14E-05 | 0.002817806 |
| Axon guidance | 17 | 127 | 7.23E-05 | 0.002817806 |
| Steroid hormone biosynthesis | 11 | 58 | 9.26E-05 | 0.003093956 |
| Retinol metabolism | 11 | 65 | 0.000225 | 0.006578928 |
| Protein digestion and absorption | 12 | 90 | 0.000829 | 0.021546285 |
| Drug metabolism – cytochrome P450 | 10 | 68 | 0.001135 | 0.0265701 |
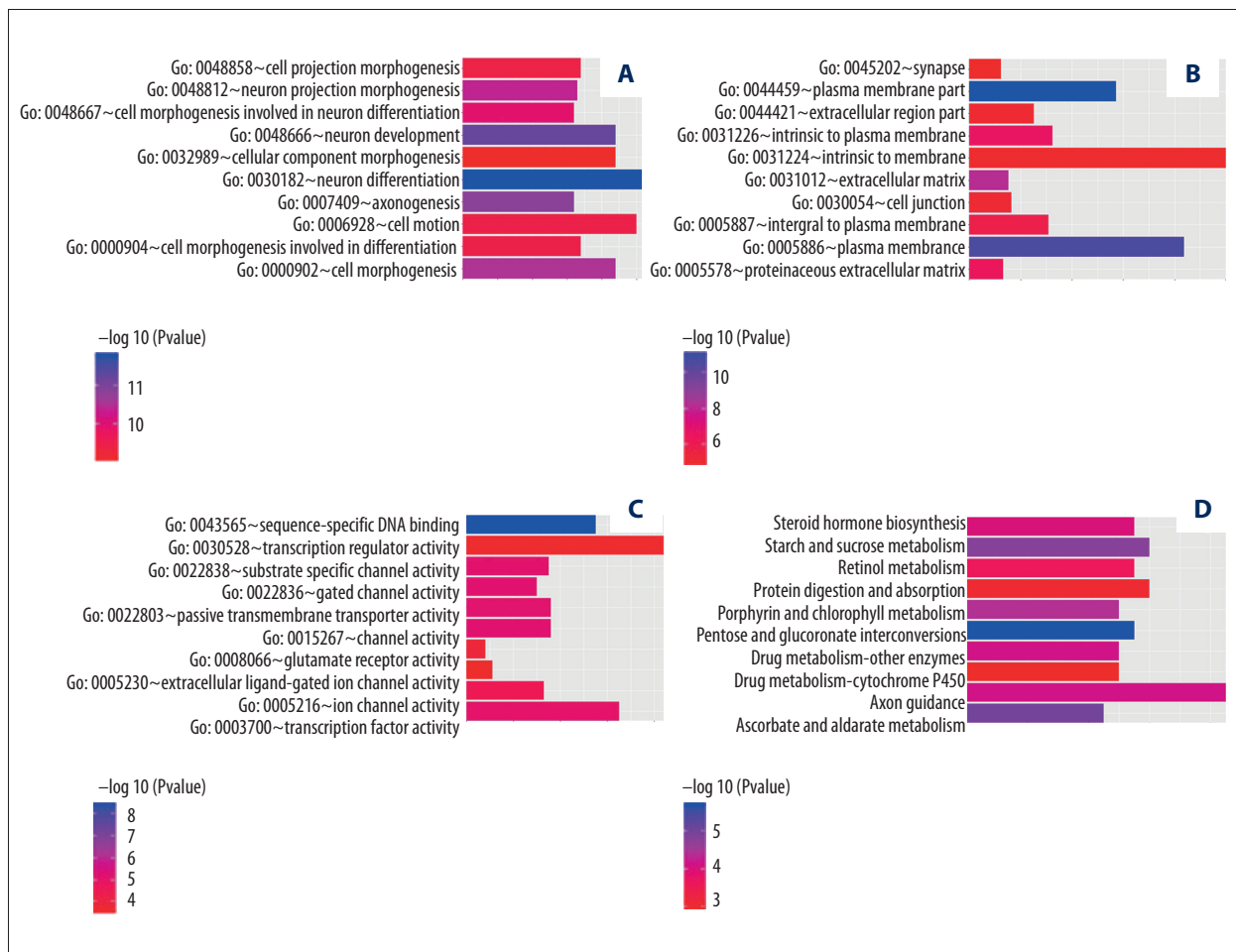


**Figure 8.** Top ten GO enrichment analysis (**A–C**) and KEGG pathways (**D**). (**A**) Biological processes; (**B**) cellular components; (**C**) molecular function; (**D**) KEGG pathway.
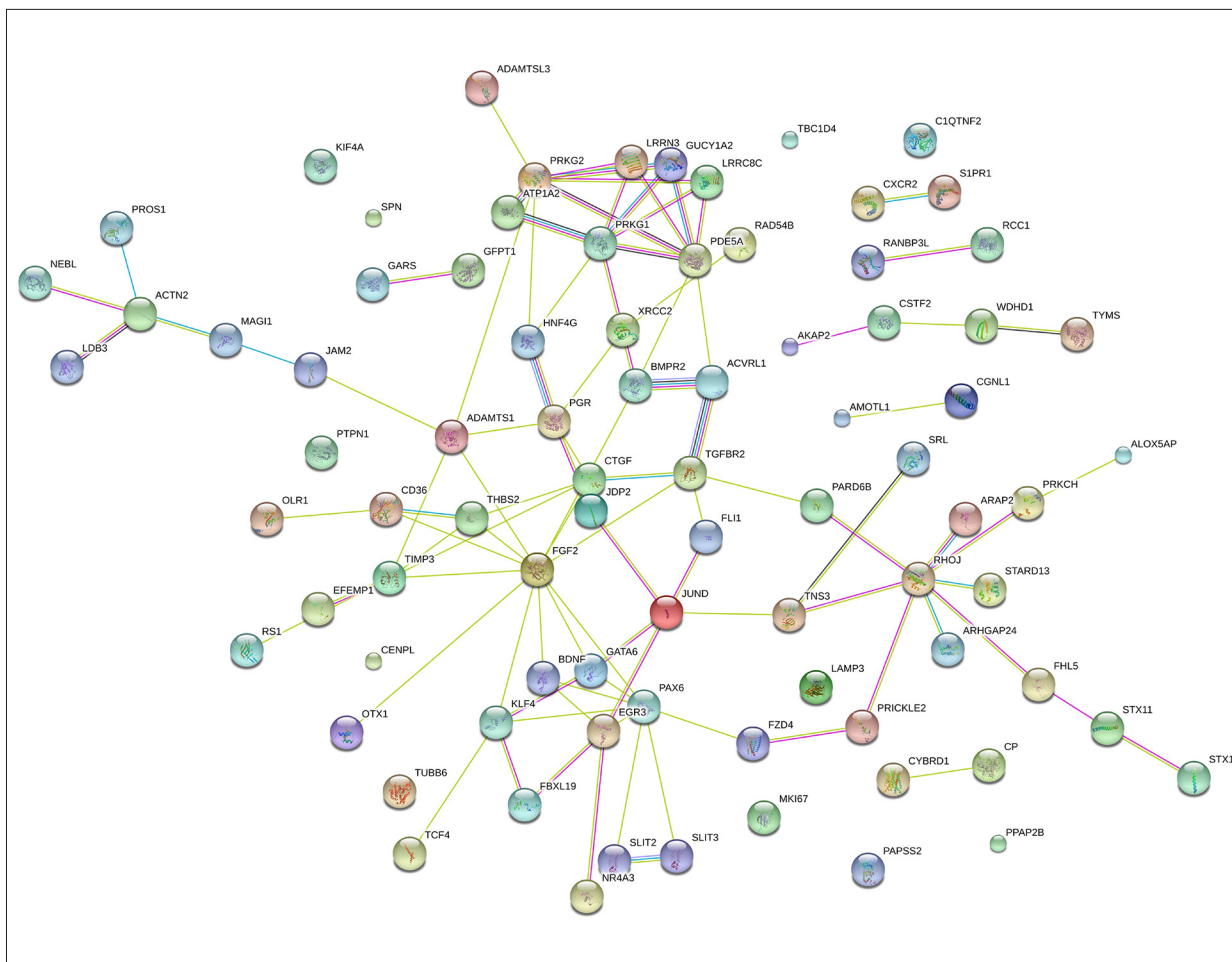
**Figure 9.** PPI network of new predicted LUAD-related genes.

**Table 6.** Top ten co-expression relationships by STRING.

| Node 1 | Node 2 | Node 1 string internal id | Homology | Coexpression | Experimentally determined interaction | Database annotated | Automated textmining | Combined score |
|---|---|---|---|---|---|---|---|---|
| TGFBR2 | SMAD7 | 1853779 | 0 | 0.072 | 0.807 | 0.9 | 0.712 | 0.994 |
| PRKG1 | PDE5A | 1856026 | 0 | 0.207 | 0.833 | 0 | 0.961 | 0.994 |
| ACVRL1 | TGFBR2 | 1857773 | 0.77 | 0.077 | 0.857 | 0.9 | 0.809 | 0.988 |
| LDB3 | ACTN2 | 1860278 | 0 | 0.215 | 0.933 | 0 | 0.789 | 0.988 |
| BMPR2 | SMAD7 | 1856145 | 0 | 0.072 | 0.573 | 0.9 | 0.728 | 0.987 |
| CENPL | CENPM | 1853309 | 0 | 0 | 0.33 | 0.9 | 0.753 | 0.982 |
| JDP2 | JUND | 1845956 | 0 | 0 | 0.874 | 0 | 0.853 | 0.98 |
| WDHD1 | GINS4 | 1854036 | 0 | 0.131 | 0.95 | 0 | 0.584 | 0.98 |
| TCF4 | TAL1 | 1853052 | 0 | 0 | 0.96 | 0 | 0.484 | 0.978 |
| FZD4 | WNT3 | 1861957 | 0 | 0 | 0.274 | 0.9 | 0.714 | 0.977 |
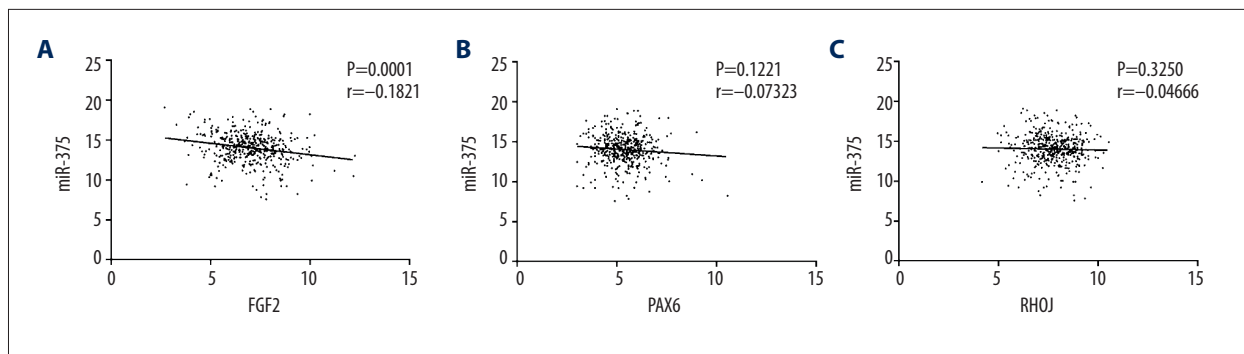
**Figure 10.** Relationship between miR-375 and target genes (**A**) FGF2; (**B**) PAX6; (**C**) RHOJ.

96 paired samples of NSCLC tissue than in matched noncancerous tissue [22]. On the contrary, Yoda et al. revealed that miR-375 was overexpressed in 54 LUAD patients as compared with normal controls by qRT-PCR [19]. Additionally, another current report on miR-375 expression in LUAD was consistent with a previous study that reported miR-375 was overexpressed in microarray profiles selected LCM cancerous cell populations derived from 36 LUADs [14]. We also found that miR-375 was constantly upregulated in LUAD tissues in TCGA data and several GEO datasets. To date, the result of the expression of miR-375 level in LUAD is still controversial, and further investigation is required to elucidate the role of miR-375 in LUAD.

Recently, it has been suggested that miR-375 targets several important genes to suppress core hallmarks of cancer, such as YAP1 (yes-associated protein), IGF1R, PDK1, and AEG-1 [9,10,23,24]. In gastric cancer, miR-375 could target PDK1 and Janus kinase 2, which consequently could reduce cell viability and suppress gastric cancer cell proliferation [25,26]. Xu et al. demonstrated that the overexpression of miR-375 remarkably suppressed consequent cell invasion and metastasis by downregulating FZD8 in colorectal cancer [27]. It was reported that AEG-1 and YAP were regulated by miR-375, and inhibited proliferation and invasion of cancer cells in hepatocellular carcinoma [10,28]. MiR-375 has been revealed to act as a tumor suppressor in human cancers, whereas it functions as an oncogene in some types of cancers. It has been shown that miR-375 overexpression facilitated cell proliferation and upregulated estrogen receptor alpha through regulation of RASD1 in breast cancer [29]. Nishikawa et al. demonstrated that miR-375 was induced by ASH1/ASCL1 in lung cancer cells, which is a key transcription factor in lung cancer with neuroendocrine features. Additionally, Yoda et al. found that high miR-375 expression promoted cell invasion and metastasis in NSCLC by targeting CLDN1 [12]. Yu et al. also found that miR-375 was consistently overexpressed in LUAD, displaying higher accuracy in diagnosis of LUAD compared with LUSC [18]. Hamaoto et al. hypothesized that miR-375 could inactivate the PI3K pathway, which is more activated in SCC than in AC [19,30]. Thus,

the molecular mechanism that is related to miR-375 overexpression in LUAD has not yet been fully elucidated.

In the present study, we identified that novel candidate target genes for miR-375 were involved in the regulation of crucial biological processes in LUAD, such as FGF2, PAX6, and RHOJ. FGF2, the most potent FGF increased in NSCLC, promotes proliferation and inhibits apoptosis of lung cancer cells. FGF2 could also promote cell malignant transformation by combining with FGFR in LUAD [31]. Interestingly, our current study revealed that FGF2 mRNA was decreased in LUAD compared with normal lung tissue. Consistent with our result, both upregulation and downregulation of FGF2 have also been detected in colorectal cancers [32,33]. As far as we know, few previous studies have indicated that the downregulated pattern of FGF2 occurs in tumor tissue compared with normal tissue. FGF2 is an important proangiogenic growth factor in the promotion of development and in tumor angiogenesis [34]. Thus, further investigation is required to explore the ectopic expression of FGF2 of LUAD cells. The transcription factor PAX6 has an oncogenic role that has different signaling pathways in different tumors. A previous study showed that PAX6 was highly expressed in lung cancer tissues and lung cancer cell lines, suggesting that PAX6 promoted the cell cycle progression in lung cancer by activating the MET tyrosine kinase receptor gene and MAPK signaling [35]. On the contrary, our study demonstrated that PAX6 mRNA expression level was stronger in normal lung tissue than in lung cancer tissue. In light of previous studies, PAX6 is also recognized as a tumor suppressor in several cancers [36–38]. PAX6 suppresses cell growth, angiogenesis, and invasiveness of glioma by inhibiting vascular endothelial growth factor A and matrix metalloproteinase-2 [39,40]. In support of the presented evidence, we hypothesize that PAX6 functioned as a tumor suppressor in LUAD. Additionally, RhoJ has been revealed to be down-expressed in LUAD, which has previously been considered as a selective and effective therapeutic target in tumor tissues. Previous studies employing animal tumor models showed that the genetic deletion of host-derived RhoJ could inhibit tumor progression and metastasis by suppressing tumor angiogenesis. Recently published studies

also demonstrated that RhoJ is involved in the progression of gastric cancer by positively regulating tumor cell motility and invasiveness. Our study was the first to report the potential role of RhoJ in LUAD. Further studies are needed to validate the therapeutic role of RhoJ in LUAD.

## Conclusions

Altogether, the results presented here indicate that miR-375 exerts a vital role in the biology of LUAD. Further studies *in vitro* and *in vivo* are required on the mechanisms of pathogenesis to elucidate the potential role of miR-375-regulated molecular networks and gain insights into the mechanism underlying the involvement of miR-375 in LUAD.

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