Oncogenic role of miR-183-5p in lung adenocarcinoma: A comprehensive study of qPCR, in vitro experiments and bioinformatic analysis

RONG-QUAN HE1*, LI GAO2*, JIE MA1, ZU-YUN LI2, XIAO-HUA HU1 and GANG CHEN2

Departments of 1Medical Oncology and 2Pathology, First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530022, P.R. China

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Abstract. Despite the fact that previous studies have reported the aberrant expression of miR-183-5p in lung adenocarcinoma (LUAD), the oncogenic role of miR-183-5p in LUAD and its underlying mechanisms have remained elusive. Hence, we attempted to elucidate the clinicopathological significance of miR-183-5p expression in LUAD and identify the biological function of miR-183-5p in LUAD in this study. Meta-analysis of Gene Expression Omnibus (GEO) data, data mining of The Cancer Genome Atlas (TCGA) and real-time quantitative polymerase chain reaction (qPCR) were performed to evaluate the clinicopathological significance of miR-183-5p in LUAD. Then, the effect of miR-183-5p on cell growth in LUAD was assessed by in vitro experiments. Additionally, the target genes of miR-183-5p were identified via miRWalk v.2.0 and TCGA. Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Disease Ontology (DO) analysis were further carried out for the target genes. The targetability between target genes in key KEGG pathways and miR-183-5p was validated by independent samples t-test, Pearson's correlation test and immunohistochemistry results from the Human Protein Atlas (HPA). According to the results, miR-183-5p was overexpressed in LUAD and exhibited significant diagnostic value. Moreover, miR-183 expression was associated with tumor progression in the TCGA data. In vitro experiments revealed the positive influence of miR-183-5p on cell viability and proliferation as well as the negative effect of miR-183-5p on caspase-3/7 activity in LUAD, which supports the finding that target genes of miR-183-5p are mainly enriched in gene pathways containing cell adhesion molecules (CAMs) and gene pathways important in cancer. Therefore, we conclude that miR-183-5p acts as an oncogene in LUAD and participates in the pathogenesis of LUAD via the interaction networks of its target genes.

Introduction

Lung cancer (LC) ranks highly on the list of lethal cancers, with 222,500 estimated new cases and 155,870 estimated deaths in the USA in 2017. Despite improvements in diagnosis and standard therapy, the prognosis of LC patients remains poor, with a 5-year survival rate of only 18% (1). Non-small cell lung cancer (NSCLC) accounts for a large proportion of all LC cases, and lung adenocarcinoma (LUAD) is the predominant histological type of NSCLC (2-4). An early diagnosis and effective therapy of LUAD is beneficial for the improved survival of LUAD patients. Thus, an understanding of the molecular mechanisms of LUAD and a valid biomarker for LUAD are urgently needed.

MicroRNAs (miRNAs) are small non-coding RNAs that suppress the translation or initiate the degradation of target mRNAs by perfectly or imperfectly binding to their 3’-untranslated regions (5). Currently, there is mounting evidence that miRNAs have vital functions in the occurrence and progression of a wide variety of cancers, with essential roles in diverse biological processes such as the proliferation, apoptosis, differentiation, drug resistance and metastasis of cancer cells (6-9). miR-183-5p, which belongs to the miR-183 family, is located at chromosome 7q32 with a high level of homogeneity (10). Previous studies have shown that aberrantly expressed miR-183-5p is involved in the progression of a wide variety of human cancers, including epithelial ovarian cancer, breast cancer and cervical cancer (11-13). Therefore, miR-183-5p has the potential to be a promising target for the effective diagnosis and therapy of cancers.
Several studies have also focused on miR-183-5p in LUAD. miR-183-5p was reported to be overexpressed in LUAD and to correlate with the tumor progression as well as poor prognosis of LUAD (14). In a study of Zhu et al, miR-183-5p exhibited a significant ability to distinguish between non-invasive and invasive LUAD (15). An in vitro study by Zhu et al declared that miR-183-5p targets PTPN4 to promote the migratory and invasive capacity of LUAD cells (16). Despite these previous findings, there is an overall lack of evaluation of the clinico-pathological significance of miR-183-5p in LUAD, and the molecular mechanisms underlying its role remain unclear. Therefore, the present study aimed to explore miR-183-5p expression in LUAD and the diagnostic as well as prognostic significance of miR-183-5p in LUAD using the combined methods of Gene Expression Omnibus (GEO) meta-analysis, data retrieval from The Cancer Genome Atlas (TCGA) and real-time quantitative polymerase chain reaction (qPCR). We also endeavored to clarify the molecular function of miR-183-5p in LUAD using in vitro experiments and bioinformatic analysis of the target genes.

Materials and methods

Investigation of miR-183-5p expression in LUAD based on GEO data

Searching strategies and inclusion or exclusion criteria. miR-183-5p expression in LUAD and non-cancer tissue data from GEO microarray chips were acquired from GEO (http://www.ncbi.nlm.nih.gov/geo/) using the following search strategies: (miRNA OR miR) AND (lung OR pulmonary) AND (cancer OR neoplasm).

The preliminary retrieval results were first selected by scanning the title and abstract. The reserved studies after the initial selection were further screened according to the established inclusion and exclusion criteria. Studies that met the following characteristics were eligible for the meta-analysis: i) the study included LUAD and non-cancer tissue samples; ii) the study provided expression values for miR-183-5p in LUAD and non-cancer tissues; and iii) the tissue samples in the study originated from humans. Studies were excluded if i) the study included tissue samples of only either LUAD or non-cancer tissues; ii) the study contained insufficient data on miR-183 expression in the tissue samples; and iii) the tissue samples in the study were not from humans.

Data extraction. The following information was extracted from the included GEO datasets to calculate an overall standardized mean difference (SMD): GSE ID, first author, publication year, country, experimental type, sample type, platform, number (N) of cases in cancer group, mean (M) ± standard deviation (SD) of miR-183-5p expression in LUAD and non-cancer group, N of cases in the non-cancer group, and M±SD of miR-183-5p expression in the non-cancer group. To obtain the sensitivity, specificity and Youden index for the data elements, a summary receiver operating characteristic (SROC) curve was created, and a receiver operating characteristic (ROC) curve was created for each GEO dataset by SPSS v.22.0 and the expression value of miR-183 was presented in the form of M±SD. The difference of miR-183 expression in two different groups of clinico-pathological parameters was evaluated by independent samples t-test. When there were three or more groups of clinico-pathological parameters, the distribution difference of miR-183 expression was assessed by Kruskal-Wallis (K-W) test. The diagnostic significance of miR-183 in LUAD was estimated by ROC curves and the implications of AUC for diagnostic ability of miR-183 were the same as stated above. Additionally, all the LUAD patients were divided based on the average of miR-183 expression value, and Kaplan-Meier survival curves were utilized to measure the influence of miR-183 on the prognosis of LUAD patients. P<0.05 was considered significant.

Validation of the clinico-pathological significance of miR-183-5p in LUAD using qPCR

Patients. A total of 101 LUAD tissues and paired non-cancer tissues (56 males and 45 females) processed with formalin fixation and paraffin embedding were collected from the First Affiliated Hospital of Guangxi Medical University (Nanning, China) during the period from January 2012 to February 2014. The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. Signed informed consents were acquired from all the LUAD patients prior to their involvement in this study.

qPCR. The extraction and normalization of RNA as well as qPCR was carried out as described in previous studies (19,20). The coding sequence of miR-183-5p as identified through TaqMan® MicroRNA Assays (cat. no. 4427975-000416; Applied Biosystems: Thermo Fisher Scientific, Inc., Grand Island, NY, USA) was 000417, UGUAAACAUCCCUCGA

GEO meta-analysis. The effect sizes of the selected studies were aggregated as the SMD with 95% confidence intervals (95% CI). Chi-square tests of Q and the I² statistic were employed for the evaluation of heterogeneity between included studies. A P<0.05 or an I²>50% was considered as significant heterogeneity, which implied that a random-effect model should be applied to pool the effect sizes. Otherwise, a fixed-effect model was utilized to pool the effect sizes when P>0.05 or I²<50% (17). Then, subgroup analysis was used to detect the source of heterogeneity based on the characteristics of the studies. The impact of a single study on the overall pooling results was evaluated by sensitivity analysis through omission of each study one at a time. Additionally, Begg's and Egger's tests were carried out to confirm whether publication bias existed in the studies.

To assess the overall diagnostic value of all the included GSE datasets as well as the diagnostic value of plasma miRNA, Meta-DiSc v.1.4 was employed to plot SROC curves based on the TP, FP, FN and TN value of all studies. Value for area under curve (AUC) value ranging from 0.5 to 1 was indicative of a diagnostic capacity from poor to superior, respectively (18).
CUGGAAG. A TaqMan® MicroRNA Reverse Transcription kit (4366596; Applied Biosystems: Thermo Fisher Scientific, Inc.) was applied to perform the RT reactions in a 10-μl volume. An Applied Biosystems PCR 7900 instrument was utilized to conduct the PCR. All the experiments including blank controls were carried out in triplicate. The difference in miR-183-5p expression between LUAD and peripheral non-cancer tissues was evaluated with the method of 2^ΔΔCT.

Statistical analysis for qPCR. The statistical analysis for qPCR data was conducted in SPSS v.22.0. miR-183-5p expression in LUAD and non-cancer tissues was compared by paired samples t-tests and the subsequent analysis of the clinicopathological significance of miR-183-5p expression in LUAD was carried out as described in the TCGA data excavation section.

Integrated meta-analysis. To achieve an overall assessment of the clinicopathological significance of miR-183-5p in LUAD, we pooled all the expression and diagnostic data of miR-183-5p from included GSE datasets, extracted TCGA data and qPCR results to conduct an integrated meta-analysis. SMD and SROC were calculated from all the pooled studies. Then, subgroup analysis, sensitivity analysis and detection of publication bias were performed to identify the source of heterogeneity, as described above.

In vitro experiment
Cell transfections and qPCR. Three human NSCLC cell lines: H460, A-549 and H1299 were acquired from American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco; Thermo Fisher Scientific, Inc., Grand Island, NY, USA) containing 10% fetal bovine serum and penicillin-streptomycin at 37°C under a humidified atmosphere of 5% CO2. Each of the in vitro experiments was performed 3 times. Before transfection, LUAD cells were plated at 96-well plates at 2.5x10^4 cells/well and maintained at 37°C for 24 h. Blank control, negative mimic control, miR-183-5p mimic, negative inhibitor control and miR-183-5p inhibitor (Ambion; Thermo Fisher Scientific, Inc., Carlsbad, CA, USA) were transfected in LUAD cell lines at a final concentration of 60 nmol/l with Lipofectamine 2000 following the manufacturer's instructions. The concentration for transfections was determined based on previous studies (21-22). miR-183-5p expression was detected with qPCR in Applied Biosystems PCR 7900 system as stated previously (23-26).

Effect of miR-183-5p on the biological behaviors of LUAD cells. The impact of miR-183-5p on the proliferation, viability and apoptosis of LUAD cells was measured by fluorometric resorufin viability assays, MTS assays and Apo-ONE Homogeneous Caspase-3/7 assays, as described in previous studies (25,26).

Statistical analysis of in vitro experiments. Statistical analysis was carried out in SPSS v.22.0. All data are expressed in the form of M±SD. Two-way analysis of variance (ANOVA) and Bonferroni post-tests were used for the comparisons among groups. We defined P<0.05 as statistically significant.

Network analysis of the target genes
Acquisition of target genes. Target genes of miR-183-5p came from two sources: TCGA data and miRWalk v.2.0. Downregulated genes in LUAD were downloaded from TCGA and subsequently processed with an edgeR software package. Genes with a false discovery rate (FDR) value <0.05 were selected as potential target genes. Apart from TCGA, miRWalk v.2.0 was also applied to the data to predict the target genes. Genes that appeared in at least four of the 12 software programs from miRWalk v.2.0 were considered as the possible target genes. Then, overlapping genes in the recorded lists of potential target genes from TCGA and miRWalk v.2.0 were regarded as the reliable target genes of miR-183-5p in LUAD.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. GO analysis and KEGG pathway analysis from the online tool Database for Annotation, Visualization and Integration Discovery (DAVID) were applied to analyze functional enrichment of the target genes as stated previously (27).

Disease Ontology (DO) analysis. The clustering of the target genes in human diseases was investigated by DO analysis, which was performed by R package: clusterProfiler. DO terms with both P and Q-value <0.05 were selected as the significant terms enriched by target genes.

Protein-protein interaction (PPI) networks. PPI networks for the key KEGG pathways of LUAD were created by Search Tool for the Retrieval of Interacting Genes (STRING)/Proteins v.10.0 with all the component genes. The relationships between proteins were established from the following four channels: i) protein interactions documented in the literature; ii) high-throughput experiments; iii) genomic analysis and prediction; and iv) studies of co-expression. Hub genes of each PPI network were confirmed by comparing the connectivity degrees of the nodes from each PPI network.

Verification of genes in key KEGG pathways as directly targeted by miR-183-5p. To further verify genes from key KEGG pathways as directly targeted by miR-183-5p, an independent samples t-test was conducted by SPSS v.22.0 to examine the differential expression of these genes in LUAD and non-cancer tissues from TCGA. GraphPad Prism v.5 was employed to perform a Pearson's correlation test for evaluating the correlation between miR-183 expression and expression of the genes from TCGA. Moreover, we downloaded immunohistochemistry results for these genes in LUAD and normal tissues from the Human Protein Atlas (HPA), a database integrating immunohistochemistry analysis of proteins in multiple human tissues and cancers (28), to confirm whether these genes were downregulated in LUAD tissues or not.

Results

Included GEO datasets. A total of 340 GEO datasets appeared after initial search and 84 GEO datasets were retained after scanning titles and abstracts (29-37). According to the selection criteria, a total of 13 GEO datasets with 469 LUAD tissues and 272 non-cancer tissues were eligible for the meta-analysis.
The summarization of the included studies is displayed in Tables I and II. Distribution of miR-183-5p between LUAD tissues and non-cancer tissues as well as the diagnostic value of miR-183-5p for LUAD in each GSE dataset are illustrated by scatter plots (Fig. 1) and ROC curves (Fig. 2).

**Meta-analysis of GEO datasets.** The pooled effect sizes from forest plots (Fig. 3A) indicated that miR-183-5p expression was significantly higher in LUAD tissues than in non-cancer tissues (SMD=1.24, 95% CI=0.76-1.72, I²=82.7%, P<0.001) with considerable study heterogeneity. To trace the origin of this heterogeneity, subgroup and sensitivity analyses were performed. Subgroup analysis based on sample types failed to locate the source of heterogeneity due to the insignificant 95% CI for the plasma subgroup (95% CI=-0.25-2.53) (Fig. 3B). The results of sensitivity analysis shown in Fig. 4A revealed that no study caused obvious deviation from the overall results. Moreover, Begg’s and Egger’s tests detected no publication bias (P=0.807) (Fig. 4B). With regard to the diagnostic ability of miR-183-5p for LUAD, SROC curves generated from all the included GSE datasets reported an AUC value of 0.8776 (Fig. 4C). Separating GSE datasets sampling plasma miR-183-5p from all the included GSE datasets, we obtained a SROC curve with an AUC value of 0.6813 (Fig. 4D).

**Clinicopathological significance of miR-183 expression in TCGA data.** As shown in Table III, miR-183 expression was significantly higher in LUAD tissues (P<0.001) and patients <60 years of age (P=0.012) compared with the matched control groups. Nevertheless, no significant relationships could be established between miR-183 expression and other clinicopathological variables of LUAD including sex, T stage, number of nodes, number of metastases, pathological stage, anatomic-organ subdivision and tumor location. In addition, the results from ROC curves indicated that miR-183 expression showed high diagnostic value for LUAD (AUC=0.985,

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### Table I. Basic information of all included GSE datasets.

| ID       | First author | Year of publication | Country | Experiment type | Sample type | Platform | Cancer (N) | Non-cancer (N) | Mean (SD) | Mean (SD) |
|----------|--------------|---------------------|---------|----------------|-------------|----------|------------|---------------|------------|------------|
| GSE27486 | Patnaik (29) | 2011                | USA     | Non-coding RNA profiling by array | Plasma | GPL11432 | 20          | 20            | 2.368 (0.313)         | 0.121 (0.201) |
| GSE40738 | Patnaik (30) | 2012                | USA     | Non-coding RNA profiling by array | Plasma | GPL10161 | 20          | 20            | 2.156 (0.120)         | 0.150 (0.215) |
| GSE93300 | Seike (31)   | 2013                | Japan   | Non-coding RNA profiling by array | Plasma | GPL8576  | 20          | 20            | 1.490 (0.300)         | 0.069 (0.230) |
| GSE51853 | Yochimotoa   | 2014                | Japan   | Non-coding RNA profiling by array | Plasma | GPL7331  | 20          | 20            | 1.550 (0.300)         | 0.200 (0.300) |
| GSE19945 | Yuichia      | 2015                | Japan   | Non-coding RNA profiling by array | Plasma | GPL17222 | 20          | 20            | 1.000 (0.200)         | 0.120 (0.210) |
| GSE25508 | Nymark (32)  | 2016                | Finland | Non-coding RNA profiling by array | Plasma | GPL9048  | 20          | 20            | 0.000 (0.000)         | 2.300 (0.300) |
| GSE51853 | Arima (35)   | 2016                | Japan   | Non-coding RNA profiling by array | Plasma | GPL7341  | 20          | 20            | 0.000 (0.000)         | 0.300 (0.300) |
| GSE63805 | Robles (36)  | 2017                | USA     | Non-coding RNA profiling by array | Plasma | GPL19622 | 20          | 20            | 1.000 (0.200)         | 0.200 (0.210) |
| GSE74190 | Jin (37)     | 2017                | Japan   | Non-coding RNA profiling by array | Plasma | GPL73800 | 20          | 20            | 0.000 (0.000)         | 0.200 (0.210) |
| GSE29248 | Ma (37)      | 2018                | China   | Non-coding RNA profiling by array | Plasma | GPL18248 | 20          | 20            | 0.000 (0.000)         | 0.200 (0.210) |

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### Table II. Diagnostic data of all included GSE datasets.

| Author | ID      | TP | FP | FN | TN |
|--------|---------|----|----|----|----|
| Patnaik (29) | GSE27486 | 20 | 20 | 2  | 3  |
| Patnaik (30) | GSE40738 | 20 | 18 | 15 | 38 |
| Qu* | GSE93300 | 8  | 0  | 1  | 4  |
| Seike (31) | GSE14936 | 7  | 3  | 4  | 6  |
| Yochimotoa | GSE19945 | 4  | 0  | 6  |
| Nymark (32) | GSE25508 | 7  | 1  | 3  | 9  |
| van Jaarsveld (33) | GSE47525 | 3  | 5  | 4  | 9  |
| Bjaanaes (34) | GSE48414 | 118 | 0  | 36 | 20 |
| Arima (35) | GSE51853 | 74 | 0  | 2  | 5  |
| Robles (36) | GSE63805 | 27 | 2  | 5  | 28 |
| Jin* | GSE74190 | 36 | 3  | 0  | 41 |
| Yoshimotoa | GSE77380 | 3  | 1  | 0  | 11 |
| Ma (37) | GSE29248 | 1  | 0  | 2  | 3  |

*Unpublished data. TP, true positivity; FP, false positivity; FN, false negativity; TN, true negativity.
Figure 1. miR-183-5p expression in each of the included GSE datasets. The scatter plots display the differential expression levels of miR-183-5p in LUAD and non-cancer tissues for each of the included GSE datasets. LUAD, lung adenocarcinoma.

Figure 2. Diagnostic ability of miR-183-5p in LUAD for each of the included GSE datasets. A panel of ROC curves shows the diagnostic capacity of miR-183-5p for LUAD in each of the included GSE datasets. An AUC (area under the curve) value ranging from 0.5 to 1 was indicative of a diagnostic capacity from poor to superior, respectively. LUAD, lung adenocarcinoma; ROC, receiver operating characteristic; AUC, area under curve.
Figure 3. Forest plots for the GEO meta-analysis. (A) miR-183-5p presented markedly higher expression in LUAD tissues compared with non-cancer tissues (SMD=1.24, 95% CI=0.76-1.72, I²=82.7%, P<0.001), with considerable study heterogeneity. (B) Subgroup analysis was conducted to assess the influence of the sample type on the heterogeneity of the studies. The pooled SMD for the plasma and tissue subgroups was 1.14 (-0.25-2.53) and 1.24 (0.76-1.72), respectively. Significant heterogeneity still existed in subgroups (I²=94.3 and 67.8%). GEO, Gene Expression Omnibus; LUAD, lung adenocarcinoma; SMD, standardized mean difference.

Figure 4. Sensitivity analysis, funnel plot and SROC curves for the GEO meta-analysis. (A) Sensitivity analysis for the GEO meta-analysis. No study caused obvious changes to overall results. (B) Funnel plot of publication bias for the GEO meta-analysis. The symmetrical funnel plot indicated no publication bias. (C) SROC curves for all the included GSE datasets. The AUC value of the SROC curves was 0.8776, indicating the significant diagnostic value of miR-183-5p in LUAD. (D) SROC curves for GSE datasets sampling plasma miR-183-5p. The AUC value of the SROC curves was 0.6813, indicating poor diagnostic value of miR-183-5p in LUAD. SROC, summary receiver operating characteristic; GEO, Gene Expression Omnibus; LUAD, lung adenocarcinoma; AUC, area under curve.
P<0.001) (Fig. 5A). According to the log-rank test from Kaplan-Meier survival analysis, the influence of miR-183 on the prognosis of LUAD patients was not distinct (P>0.05) (data not shown).

**Table III. Relationship between miR-183 expression and clinicopathological parameters of LUAD from TCGA.**

| Clinicopathological feature | miR-183 relevant expression | N | M±SD | t | P-value |
|-----------------------------|-----------------------------|---|------|---|---------|
| Tissue                      |                             |   |      |   |         |
| Normal                      | 46                          | 10.661±0.572 | 25.544 | <0.001 |
| Lung cancer                 | 441                         | 13.180±1.072 |        |        |
| Age (years)                 |                             |   |      |   |         |
| ≤60                         | 199                         | 13.317±1.150 | 2.531  | 0.012  |
| >60                         | 232                         | 13.054±1.005 |        |        |
| Sex                         |                             |   |      |   |         |
| Female                      | 234                         | 13.166±1.082 | 0.288  | 0.774  |
| Male                        | 207                         | 13.196±1.063 |        |        |
| T stage                     |                             |   |      |   |         |
| T1+T2                       | 383                         | 13.209±1.084 | 1.433  | 0.153  |
| T3+T4                       | 58                          | 12.992±0.980 |        |        |
| Node                        |                             |   |      |   |         |
| No                          | 288                         | 13.155±1.092 | -0.711 | 0.478  |
| Yes                         | 152                         | 13.231±1.037 |        |        |
| Metastasis                  |                             |   |      |   |         |
| No                          | 278                         | 13.198±1.012 | 0.591  | 0.555  |
| Yes                         | 159                         | 13.133±1.173 |        |        |
| Pathological stage          |                             |   |      |   |         |
| I+II                        | 346                         | 13.155±1.084 | -0.69  | 0.49   |
| III+IV                      | 90                          | 13.242±1.023 |        |        |
| Anatomic-organ subdivision  |                             |   |      |   |         |
| L_lower                     | 69                          | 13.042±0.990 | 3.159  | 0.532  |
| L_upper                     | 105                         | 13.170±1.085 |        |        |
| R_lower                     | 84                          | 13.129±1.057 |        |        |
| R_middle                    | 18                          | 13.226±0.913 |        |        |
| R_upper                     | 154                         | 13.268±1.132 |        |        |
| Location                    |                             |   |      |   |         |
| Peripheral                  | 106                         | 13.264±1.031 | 0.09   | 0.928  |
| Central                     | 54                          | 13.248±1.082 |        |        |

Independent samples t-test was performed to evaluate the relationship between miR-183 expression and the clinicopathological parameters of LUAD. *Kruskal-Wallis test was performed to assess the distribution difference of miR-183 in three or more groups of clinicopathological parameters. LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; N, number; M, mean; SD, standard deviation.

**Table IV. Relationship between miR-183-5p expression and clinicopathological parameters of LUAD from qPCR data.**

| Clinical variable          | miR-183-5p relevant expression | N | M±SD | t | P-value |
|----------------------------|--------------------------------|---|------|---|---------|
| Tissue type                |                                |   |      |   |         |
| LUAD                       | 101                            | 6.579±3.737 | -2.103 | 0.038  |
| Non-cancer                 | 101                            | 5.489±3.230 |        |        |
| Age (years)                |                                |   |      |   |         |
| <60                        | 41                             | 6.556±3.613 | -0.050 | 0.960  |
| ≥60                        | 60                             | 6.594±3.849 |        |        |
| Sex                        |                                |   |      |   |         |
| Female                     | 45                             | 7.063±3.865 | -1.169 | 0.245  |
| Male                       | 56                             | 6.190±3.618 |        |        |
| Smoke                      |                                |   |      |   |         |
| No                         | 26                             | 5.648±2.473 | 1.346  | 0.186  |
| Yes                        | 18                             | 4.840±1.501 |        |        |
| Tumor size (cm)            |                                |   |      |   |         |
| ≤3                         | 53                             | 6.649±3.717 | 0.198  | 0.843  |
| >3                         | 48                             | 6.501±3.796 |        |        |
| Lymph node metastasis      |                                |   |      |   |         |
| No                         | 45                             | 5.605±2.746 | -2.513 | 0.014  |
| Yes                        | 56                             | 7.361±4.238 |        |        |
| Vascular invasion          |                                |   |      |   |         |
| No                         | 70                             | 5.949±2.867 | -2.151 | 0.038  |
| Yes                        | 31                             | 8.002±4.962 |        |        |
| TNM                        |                                |   |      |   |         |
| I-II                       | 45                             | 5.605±2.746 | -2.513 | 0.014  |
| III-IV                     | 56                             | 7.361±4.238 |        |        |
| Pathological grading       |                                |   |      |   |         |
| I                          | 17                             | 6.988±2.666 | 1.526  | 0.466  |
| II                         | 61                             | 6.305±3.767 |        |        |
| III                        | 23                             | 7.004±4.367 |        |        |
| EGFR amplification         |                                |   |      |   |         |
| No                         | 21                             | 5.191±2.162 | 0.284  | 0.778  |
| Yes                        | 12                             | 4.972±2.067 |        |        |
| EGFR protein expression    |                                |   |      |   |         |
| Low                        | 56                             | 5.336±2.275 | 0.866  | 0.393  |
| High                       | 11                             | 4.662±1.697 |        |        |
| EGFR mutation              |                                |   |      |   |         |
| Wild-type                  | 20                             | 5.275±2.152 | 0.551  | 0.585  |
| Mutation c                 | 13                             | 4.859±2.071 |        |        |

Independent samples t-test was performed to evaluate the relationship between miR-183-5p expression and the clinicopathological parameters of LUAD. *Kruskal-Wallis test was performed to assess the distribution difference of miR-183-5p in three or more groups of clinicopathological parameters. LUAD, lung adenocarcinoma; qPCR, real-time quantitative polymerase chain reaction; N, number; M, mean; SD, standard deviation.

**Relationship between miR-183-5p expression and the clinicopathological features of LUAD from qPCR.** A total of 101 LUAD and paired non-cancer tissues were taken from patients enrolled in our study. General information of the collected samples is listed in Table IV. From the results of the paired samples t-test, miR-183-5p expression was obviously higher in LUAD tissues (6.579±3.737) than in non-cancer
miR-183-5p ACTS AS AN ONCOGENE IN LUAD tissues (5.489±3.230) (P=0.038) (Table IV). Analysis from independent samples t-test suggested that patients with lymph node metastasis, vascular invasion and advanced TNM stage (III-IV) exhibited higher miR-183-5p expression than patients without those features (P=0.014, 0.038, and 0.014). With respect to the diagnostic and prognostic value of miR-183-5p for LUAD, ROC curves revealed the potential diagnostic significance of miR-183-5p for LUAD (AUC=0.587, P=0.033) (Fig. 5B) while Kaplan-Meier survival analysis yielded no significant results supporting the role of miR-183-5p as an important prognostic factor for LUAD (log-rank P>0.05).

Integrated meta-analysis. The results of the integrated meta-analysis which contained a large sample with 1,011 LUAD tissues and 419 non-cancer tissues echoed with those from the GEO meta-analysis. miR-183-5p showed higher expression in LUAD tissues than in non-cancer tissues (SMD=1.27, 95% CI=0.76-1.78, I²=91.2%, P<0.001), with considerable study heterogeneity. (B) The AUC value of the SROC curves was 0.890, indicating the significant diagnostic value of miR-183-5p in LUAD; SROC, summary receiver operating characteristic; LUAD, lung adenocarcinoma; SMD, standardized mean difference; AUC, area under curve.
transfected with the miR-183-5p mimic. The influence of miR-183-5p mimic on cell viability was observed to be most significant in H460 cells, in which cell viability was clearly increased compared to that of the blank control and negative mimic control at 72 h (P<0.01) and 96 h (P<0.001) (Fig. 9C). In A549 cell lines, cell viability in the miR-183-5p mimic group was higher than in the blank control and the negative mimic control at 72 and 96 h, although without statistical significance (P>0.05) (Fig. 9D). In the H1299 cell line, there was a sharp rise in the cell viability of the miR-183-5p mimic group at 96 h (P<0.001) (Fig. 9E). In contrast, all the column diagrams in Fig. 9 reflect a downward trend in cell viability after 48 h post-transfection with miR-183-5p inhibitor. In all three tested cell lines, cell viability decreased substantially
in the miR-183-5p inhibitor group at 72 h (All P<0.01) and 96 h (All P<0.001) compared with the blank control and the negative inhibitor control (Fig. 9). For the influence of miR-183-5p on cell proliferation, MTS assays reflected almost the same effect of miR-183-5p on cell growth as the fluorometric resorufin viability assay (Fig. 10). With regard to the effect of miR-183-5p on cell apoptosis, results from Apo-ONE Homogeneous Caspase-3/7 assays showed that the increase in caspase-3/7 activity started at 48 h in the miR-183 inhibitor group and the decline of caspase-3/7 activity started at 72 h in the miR-183 mimic group. Particularly, caspase-3/7 activity was significantly enhanced (P<0.001) at 72 and 96 h in miR-183-5p inhibitor group on the basis of the blank control and negative inhibitor group (Fig. 11), which was unanimously observed in all the three tested cells. Conversely, in the miR-183-5p mimic group, caspase-3/7 activity was significantly reduced on the basis of the blank control (P<0.001) and negative mimic control (P<0.05) in all the three tested cell lines (Fig. 11). In all the three assays, miR-183-5p stimulated cell growth and suppressed cell apoptosis in a time-dependent (Figs. 9-11) and dose-dependent (data not shown) manner.

Network analysis of target genes. According to the TCGA data analysis, a total of 2,609 downregulated genes with an FDR value <0.05 were identified as potential target genes of miR-183-5p. From the predictions of miRWalk v.2.0, a total of 5,065 genes occurred in at least four of the 12 online software programs. Taking genes that were found from both analyses, we obtained 432 reliable target genes of miR-183-5p in LUAD.

The selected target genes were further refined by GO and KEGG pathway analysis in DAVID. As shown in Table V, these target genes were most significantly enriched in the following terms of biological processes (BP): positive regulation of gene expression, cell adhesion and actin filament organization. With respect to cellular component (CC), the top three terms gathered by these target genes were plasma membrane, integral component of plasma membrane and receptor complex. These target genes were also significantly clustered in terms of molecular function (MF), such as Ras guanyl-nucleotide exchange factor activity, glycosaminoglycan binding and calcium ion binding. Three GO maps (Figs. 12-14) illustrate the functional interactions of the target genes. With regard to KEGG pathway analysis, the four most significant signaling pathways for the target genes of miR-183-5p were cell adhesion molecules (CAMs) and pathways in cancer, endocytosis and axon guidance (Table VI). The interactions between component genes of the four most significant pathways are displayed in four PPI networks (Fig. 15). A total of 10 genes including CD34, sialophorin (SPN), WNT3A, FGFR2, WNT2B, PDGFB, AVPR2, VIPR1, PTGER4 and ADCYAPIR1 were selected as the hub genes based on their degrees of connectivity in corresponding PPI networks.

DO analysis by clusterProfiler revealed that the target genes were significantly associated with the following...
Table V. GO analysis of the target genes.

| Category   | ID          | Term                                           | Count | %    | P-value     |
|------------|-------------|-------------------------------------------------|-------|------|-------------|
| BP         | GO:0010628  | Positive regulation of gene expression          | 21    | 4.872| 1.66E-06   |
| BP         | GO:0007155  | Cell adhesion                                   | 27    | 6.265| 1.25E-05   |
| BP         | GO:0007015  | Actin filament organization                      | 10    | 2.320| 2.39E-04   |
| BP         | GO:0007165  | Signal transduction                             | 47    | 10.905| 9.06E-05  |
| BP         | GO:0010629  | Negative regulation of gene expression          | 12    | 2.784| 2.39E-04   |
| BP         | GO:0032870  | Cellular response to hormone stimulus           | 7     | 1.624| 4.46E-04   |
| BP         | GO:0009611  | Response to wounding                            | 8     | 1.856| 4.71E-04   |
| BP         | GO:001934   | Positive regulation of protein phosphorylation  | 11    | 2.552| 5.30E-04   |
| BP         | GO:0070374  | Positive regulation of ERK1 and ERK2 cascade     | 13    | 3.016| 5.39E-04   |
| CC         | GO:0050665  | Hydrogen peroxide biosynthetic process          | 4     | 0.928| 8.20E-04   |
| CC         | GO:0005886  | Plasma membrane                                  | 146   | 33.875| 2.38E-10  |
| CC         | GO:0005887  | Integral component of plasma membrane           | 64    | 14.849| 4.73E-08  |
| CC         | GO:0043235  | Receptor complex                                 | 15    | 3.480| 7.61E-07   |
| CC         | GO:0009986  | Cell surface                                     | 32    | 7.425| 1.20E-06   |
| CC         | GO:0005578  | Proteineucous extracellular matrix              | 19    | 4.408| 2.74E-05   |
| CC         | GO:0030054  | Cell junction                                    | 26    | 6.032| 2.97E-05   |
| CC         | GO:0016021  | Integral component of membrane                   | 151   | 35.035| 3.25E-05  |
| CC         | GO:0009897  | External side of plasma membrane                | 16    | 3.712| 7.45E-05   |
| CC         | GO:0001725  | Stress fiber                                     | 7     | 1.624| 0.001128084|
| CC         | GO:0031674  | I band                                          | 5     | 1.160| 0.001440976|
| MF         | GO:0050888  | Ras guanyl-nucleotide exchange factor activity  | 11    | 2.552| 2.01E-04   |
| MF         | GO:005539   | Glycosaminoglycan binding                       | 5     | 1.160| 5.23E-04   |
| MF         | GO:005509   | Calcium ion binding                             | 30    | 6.961| 9.67E-04   |
| MF         | GO:004714   | Transmembrane receptor protein tyrosine kinase activity | 6 | 1.392| 0.001299161|
| MF         | GO:008289   | Lipid binding                                   | 11    | 2.552| 0.001703258|
| MF         | GO:005431   | Transforming growth factor β binding            | 4     | 0.928| 0.004589053|
| MF         | GO:004896   | Cytokine receptor activity                      | 5     | 1.160| 0.007406533|
| MF         | GO:008201   | Heparin binding                                 | 10    | 2.320| 0.008250718|
| MF         | GO:005248   | Voltage-gated sodium channel activity           | 4     | 0.928| 0.008762612|
| MF         | GO:005024   | Transforming growth factor β-activated receptor activity | 3 | 0.696| 0.009159889|

There was a total of 93, 34 and 23 significant (P<0.05) GO terms of BP, CC and MF, respectively. Top ten GO terms for BP, CC and MF are displayed in the Table. GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

Figure 12. GO map for biological process (BP). The GO map for BP is composed of 27 nodes and 32 links. Each node represents a specific GO term of a BP (P<0.001) and links between nodes illustrate the interactions between GO terms of the BP. Nodes with darker color are regarded as more significant GO terms. GO, Gene Ontology.
HE et al.: miR-183-5p ACTS AS AN ONCOGENE IN LUAD

Verification of genes in key KEGG pathways as directly targeted by miR-183-5p. We conducted an independent samples t-test and Pearson’s correlation test to validate the capabilities of miR-183-5p to target all the component genes in the CAM pathway. The results from the independent samples t-test suggested that expression of all these target genes was significantly lower in 535 LUAD tissues than in 59 non-cancer tissues (all \( P<0.001 \)) (Fig. 17). Among the 11 component genes, five genes including PECAM1, CDH5, SIGLEC1, CD34 and CLDN18 showed significant correlation with miR-183-5p in LUAD (all \( P<0.05 \)) (Fig. 18). Moreover, immunostaining evidence from the HPA database supported the downregulation of CD34 (antibody HPA036722), CADM1 (antibody CAB037266), PECAM1 (antibody HPA004690), SIGLEC1
(antibody HPA053457) and SPN (antibody HPA055244) in LUAD tissues. Fig. 19 exhibits the immunostaining results of the five genes in normal and LUAD tissues. The staining intensity of the five genes was medium or strong in normal tissues, while low to non-existent in LUAD tissues.

### Table VI. KEGG pathway analysis of the target genes.

| Category            | ID      | Terms                                      | Count | %       | P-value         |
|---------------------|---------|--------------------------------------------|-------|---------|-----------------|
| KEGG_PATHWAY        | hsa04514| CAMs                                       | 11    | 2.552204176 | 0.001466545    |
| KEGG_PATHWAY        | hsa04550| Signaling pathways regulating pluripotency of stem cells | 10    | 2.320185615 | 0.004641057    |
| KEGG_PATHWAY        | hsa05200| Pathways in cancer                         | 18    | 4.176334107  | 0.0081339147   |
| KEGG_PATHWAY        | hsa04080| Neuroactive ligand-receptor interaction     | 14    | 3.248259861  | 0.010639115    |
| KEGG_PATHWAY        | hsa00380| Tryptophan metabolism                      | 5     | 1.160092807  | 0.012685346    |
| KEGG_PATHWAY        | hsa04924| Renin secretion                            | 6     | 1.392111369  | 0.015130907    |
| KEGG_PATHWAY        | hsa04670| Leukocyte transendothelial migration        | 8     | 1.856148492  | 0.018169594    |
| KEGG_PATHWAY        | hsa04360| Axon guidance                              | 8     | 1.856148492  | 0.026015978    |
| KEGG_PATHWAY        | hsa04144| Endocytosis                                 | 12    | 2.784222738  | 0.033890911    |
| KEGG_PATHWAY        | hsa04024| cAMP signaling pathway                     | 10    | 2.320185615  | 0.037334507    |
| KEGG_PATHWAY        | hsa04060| Cytokine-cytokine receptor interaction      | 11    | 2.552204176  | 0.037626628    |
| KEGG_PATHWAY        | hsa04725| Cholinergic synapse                         | 7     | 1.62412993   | 0.041290871    |
| KEGG_PATHWAY        | hsa04911| Insulin secretion                           | 6     | 1.392111369  | 0.044616235    |

KEGG, Kyoto Encyclopedia of Genes and Genomes; CAMs, cell adhesion molecules.

Figure 15. PPI networks for key KEGG pathways. PPI network for (A) the CAMs pathway (11 nodes and 9 edges), (B) the pathway in cancer pathway (10 nodes and 20 edges), (C) the endocytosis pathway (18 nodes and 22 edges) and (D) the axon guidance pathway (14 nodes and 29 edges). Nodes with different colors represent different component genes. Edges connecting nodes symbolize the interactions between component genes. PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; CAMs, cell adhesion molecules.

Figure 16. Dot plot for DO analysis. The dot plot describes the assembling of target genes in human disease. The vertical and horizontal axis represent the name of disease and the proportion of genes clustered in specific human disease, respectively. As the color of the dot changes from blue to red, the significance of the term increases. The size of the dot reflects the number of enriched genes. DO, Disease Ontology.

### Discussion

In this report, we used a combined method of GEO meta-analysis, TCGA data mining, qPCR, integrated meta-analysis, in vitro experiments and bioinformatic analysis of the target genes to comprehensively investigate the clinicopathological significance of miR-183-5p in LUAD and its underlying molecular basis. The results from this report revealed that overexpressed miR-183-5p had considerable diagnostic value in LUAD and was associ-
miR-183-5p ACTS AS AN ONCOGENE IN LUAD

Further in vitro experiments and bioinformatic analysis of the target genes of miR-183-5p showed the positive effect of miR-183-5p on cell growth in LUAD and revealed the specific biological processes and gene pathways common to miR-183-5p target genes, which may provide new insights into the oncogenesis of LUAD.

GEO meta-analysis, TCGA data mining, qPCR and the integrated meta-analysis in our study concordantly reported overexpression of miR-183-5p in LUAD tissues, which was in agreement with previous studies (38,39). The diagnostic value of miR-183-5p in LUAD was also studied. We found that miR-183-5p possessed strong diagnostic capacity for distinguishing LUAD tissues from non-cancer tissues via ROC curves derived from all the included GSE datasets, TCGA data mining and the integrated meta-analysis. ROC curves from qPCR also demonstrated the diagnostic potential of miR-183-5p for LUAD, although with weak statistical significance. The difference between ROC curves from all the included GSE datasets, TCGA data mining, qPCR and integrated meta-analysis might be explained by the type and number of samples. It should be

Table VII. DO analysis of the target genes.

| ID     | Description                        | Gene ratio | Bg ratio | P-value | P-adjusted | Q-value | Gene ID                        | Count |
|--------|------------------------------------|------------|----------|---------|------------|---------|--------------------------------|-------|
| DOI:6364 | Migraine                           | 12/231     | 73/8,007 | 9.69E-07 | 0.000671237 | 0.000583196 | 477/5241/3683/6532/1636/4842/1524/3949/3952/6323/627/358 | 12    |
| DOI:1206 | Rett syndrome                       | 5/231      | 17/8,007 | 8.92E-05 | 0.030893672 | 0.026841619 | 3399/1959/3952/22854/627       | 5     |
| DOI:12326 | Chronic pulmonary heart disease     | 3/231      | 5/8,007  | 0.000227082 | 0.039342028 | 0.034181879 | 659/6532/1636                  | 3     |
| DOI:14557 | Primary pulmonary hypertension     | 3/231      | 5/8,007  | 0.000227082 | 0.039342028 | 0.034181879 | 659/6532/1636                  | 3     |

DO, Disease Ontology.

Figure 17. Expression of the component genes from the CAMs pathway in LUAD and non-cancer tissues. The scatter plots display the differential expression of the component genes from the CAMs pathway in LUAD and non-cancer tissues. All genes from the CAMs pathway were significantly downregulated in LUAD tissues (P<0.001). CAMs, cell adhesion molecules; LUAD, lung adenocarcinoma.
noted that all the non-cancer tissues from qPCR were matched with the corresponding LUAD tissues, which is not the case in the GEO meta-analysis, the TCGA data mining or the integrated meta-analysis. Now that sensitive, less non-invasive biomarker was necessary for the early detection of LUAD, we singled out GSE datasets sampling plasma miR-183-5p to assess the diagnostic capacity of plasma miR-183-5p. Nevertheless, the results indicated a poorer diagnostic ability of plasma miR-183-5p in our study compared with miR-183-5p from both tissues and plasma. We conjectured that the less significant diagnostic ability of plasma miR-183-5p might be attributed to the lower levels of miR-183-5p in blood samples than in tissues, which is related to the phenomenon that LUAD cells in lung tissues assimilating exosomes containing miRNAs from the blood as a supplement of transcribed essential miRNAs (40-42). Considering the results of all the diagnostic evaluations, we are optimistic about the prospect of miR-183-5p as a diagnostic target for LUAD.

To investigate the role of miR-183-5p in the development of LUAD, we analyzed the relationship between miR-183-5p and the clinicopathological characteristics of LUAD. Given the positive correlation between miR-183-5p overexpression and lymph node metastasis, vascular invasion and advanced TNM stage in LUAD, it can be inferred that miR-183-5p may enhance the malignant properties of LUAD. To answer the question of how miR-183-5p affects the pathogenesis of LUAD, we conducted in vitro experiments to determine the influence of miR-183-5p on cell growth in LUAD.

As suggested by fluorometric resorufin viability assay and MTS assay, the proliferative ability of LUAD cells was augmented by miR-183-5p. The promotion of tumor cell growth by miR-183-5p has also been shown in in vitro experiments with pediatric acute myeloid leukemia, tongue squamous cell carcinoma and endometrial cancer (43-46). To ascertain whether the knockdown of miR-183-5p caused apoptosis or just inhibited the proliferation of the cancer cells, we performed Apo-ONE Homogeneous Caspase-3/7 assays. Caspase-3 and -7 are effector caspses that execute cell apoptosis via cleaving relevant cellular substrates (46). The negative effect of miR-183-5p on caspase-3/7 activity indicated that the knockdown of miR-183-5p caused apoptosis of LUAD cells. Therefore, we hypothesized that miR-183-5p may accelerate the malignant progression of LUAD by
enhancing tumor growth and inhibiting tumor apoptosis. It was discovered in previous studies that miR-183-5p modulated the expression of tumor-suppressor genes such as PDCD4 and SOCS-6 to accelerate the proliferation of cancer cells (47,48). In an in vitro experiment by Yan et al, the reduced cell growth and increased cell apoptosis by miR-183-5p inhibitor was correlated with upregulated caspase-3 and downregulated anti-apoptotic protein BCL-x1 (43). These findings might provide interpretation of how miR-183-5p contributed to cell growth and suppressed cell apoptosis in LUAD.

In order to develop effective therapeutic and diagnostic targets for LUAD, it is not sufficient to merely identify the relationship between miR-183-5p expression and the malignant properties of LUAD. Therefore, we further attempted to explore the underlying molecular basis of the cell growth-promoting effects of miR-183-5p in LUAD via in silico analysis of miR-183-5p target genes. The significant terms identified from the GO analysis including positive regulation of gene expression, cell adhesion, actin filament organization, Ras guanyl-nucleotide exchange factor activity, glycosaminoglycan binding and calcium ion binding imply that target genes regulated by miR-183-5p may participate in these biological functions to cause the occurrence and development of LUAD. We can also draw conclusions from the KEGG pathway analysis. Among all the listed KEGG pathways significantly identified by analysis of the target genes of miR-183-5p, we noted that the top two pathways, CAMs and pathways in cancer, were closely associated with the formation of human cancers. CAMs are cell surface proteins that mediate the adhesion between cells and the extracellular matrix. This feature allows CAMs to stimulate the motility, invasion and angiogenesis of tumor cells (49,50). We speculate that miR-183-5p may target downstream CAMs to contribute to the progression of LUAD. Apart from CAMs and pathways in cancer, other pathways, such as endocytosis and axon guidance, were also identified as significantly enriched by analysis of the target genes of miR-183-5p. Involvement of target genes in these additional pathways may have an impact on LUAD; this awaits further study. In addition to the above results, we noted from DO analysis that the target genes were significantly assembled in chronic pulmonary heart disease and primary pulmonary hypertension, the function of target genes in chronic pulmonary heart disease and primary pulmonary hypertension may constitute part of the pathogenesis of LUAD.

Although the regulatory network of target genes was intricate, we focused on hub genes to shed light on the molecular mechanism of miR-183-5p in LUAD. Ten genes were screened out as hub genes from the four most significant KEGG pathways. However, several of the hub genes including CD34, PDGFB, FGFR2 and WNT3A were described in previous studies to relate to the tumorogenesis or poor prognosis of LUAD (51-54). Although there have been studies investigating the expression of SPN, WNT2B and PTGER4 in LUAD, all the three genes were reported to act as an oncogene in other cancers. SPN could induce cell adhesion and migration as well as suppress apoptosis when secreted by activated leukocytes (55). WNT2B controlled multiple biological events, including cell proliferation, differentiation and migration to serve as an oncogene in numerous human cancers (56-58).

PTGER4 could transduce a series of signaling pathways such as Akt, ERK1/2 and early growth response factor-1 to mediate cancer cell survival and tumor development with its overexpression in a wide variety of cancers (59). Thus, we are skeptical about the regulatory targeting relationship between miR-183-5p and these genes. Whether these genes were the direct targets of miR-183-5p needs to be verified in future study. Apart from these genes, we found evidence that VIPR1 was downregulated in LUAD and served as a tumor-suppressor gene in the study of Mlakar et al (60), which lend credence to the assumption that miR-183-5p directly targets VIPR1. In addition to the hub genes, component genes in CAMs pathway validated by Pearson's correlation test and immunostaining results from HPA were also worthy for attention. Despite that we found no literature evidence of the downregulation of expression in LUAD for all component genes in the CAMs pathway, the tumor suppressor roles of SIGLEC1 and CADM1 human cancers were explored in previous research. The study conducted by Strömvall et al confirmed that reduced expression of SIGLEC1 in metastasis-free regional lymph nodes was responsible for the inhibited antitumor immune response (61). CADM1, a membrane-spanning glycoprotein that participates in the process of attenuating cell proliferation and activating cell apoptosis, was downregulated in various cancers such as breast, prostate, pancreatic and hepatocellular cancer (62). We believe that miR-183-5p-regulated downexpression of genes such as VIPR1, SIGLEC1 and CADM1 may help explain the oncogenic function of miR-183-5p in LUAD. Future experiments are warranted to identify the interactions between miR-183-5p and these genes in LUAD.

Despite the research progress in our study, there were also limitations. In our GEO and integrated meta-analysis, great heterogeneity existed in the pooled studies, and even the subgroup analysis or sensitivity analysis failed to solve the problem, which unfortunately impacts the reliability of our results. This intractable heterogeneity may partly be attributed to the different proportion of LUAD and non-cancer tissues in the studies or the varying experimental platforms of studies. The number of LUAD tissues exceeded non-cancer tissues in GSE27486, GSE93300, GSE14936, GSE48414, GSE51853, GSE63805, GSE74190 and the TCGA data; conversely, the cases of non-cancer tissues outnumbered LUAD tissues in GSE19945, GSE40738, GSE47525 and GSE77380. The experimental platforms of studies also varied from each other. A feasible way to prevent this problem may be to enroll larger study cohorts with a balanced proportion of LUAD and non-cancer tissues in future studies. Another flaw of this study was that neither western blotting nor flow cytometry was conducted in in vitro experiments. Western blotting and flow cytometry should be conducted after knockdown or overexpression of miR-183-5p in the LC cell lines. Nevertheless, we failed to perform western blotting and flow cytometry due to the restriction of the experimental condition. This imperfect design of the in vitro experiment should be improved in future research.

In conclusion, our study confirmed that overexpression of miR-183-5p may play an oncogenic role in LUAD through involvement in the regulatory networks of its target genes. miR-183-5p is anticipated to be a novel diagnostic and therapeutic target for lung adenocarcinoma.
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Availability of data and materials

The datasets generated and analysed during the current study are available in Gene Expression omnibus (GEO; https://www.ncbi.nlm.nih.gov/pubmed) and TCGA data portal (https://portal.gdc.cancer.gov/).

Authors' contributions

RQH and LG analyzed and interpreted data and drafted the manuscript. JM, ZYL, XHH and GC provided information from database. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. Signed informed consents were acquired from all the LUAD patients prior to their involvement in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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