Identification of high expression profiles of miR-31-5p and its vital role in lung squamous cell carcinoma: a survey based on qRT-PCR and bioinformatics analysis

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Background: The function of miR-31-5p in lung squamous cell carcinoma (LUSC) remains unclear, therefore, a systematic study was performed for the clinical significance and molecular mechanism of miR-31-5p in LUSC.

Methods: Quantitative real-time reverse transcription PCR (qRT-PCR) was utilized to test the expression level of miR-31-5p in 88 LUSC tissue samples and their matching normal tissues. Data from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) were also utilized to confirm the expression level and clinical value of miR-31-5p in LUSC. The potential target genes of miR-31-5p were predicted by several online predicted software. Gene ontology (GO), protein-protein interaction (PPI) and pathway analysis were utilized to investigate the underlying molecular mechanism of miR-31-5p in LUSC.

Results: The result from qRT-PCR found that there was significant difference of miR-31-5p between LUSC and normal tissues (P<0.001). Meanwhile, Data from TCGA also showed a higher expression of miR-31-5p in LUSC tissues than that in the normal tissues (P<0.001). on the basis of the data of GEO database, five GEO datasets indicated that the expression of miR-31-5p in LUSC tissues was significantly higher than that in normal lung tissues, include GSE51858 (P=0.025), GSE74190 (P<0.000), GSE16025 (P=0.031), GSE25508 (P=0.0.01), and GSE47525 (P=0.049). Moreover, in consideration of the meta-analysis, 1,012 clinical specimens were systematically analyzed via meta-analysis, clinical specimens were systematically analyzed via meta-analysis, and the results showed that the expression of miR-31-5p in LUSC was significantly higher than in the adjacent lung tissues (SMD =0, CI: 1.08–1.45, Z=13.30, P=0.000). In addition, result from GO and pathway analyses showed that potential target genes of miR-31-5p were significantly associated with 20 GO terms and 5 pathways, such as signal transduction, transmembrane receptor protein tyrosine kinase activity, plasma membrane and Rap1 signaling pathway. Meanwhile, we also found thatmiR-31-5p target genes were related to the Rap1 signaling pathway, Oxytocin signaling pathway and Proteoglycans in cancer. Furthermore, six hub genes were identified from PPI and three hub genes, including ADCY6, ADCY9 and EGFR, proved to coexist in the Rap1 signaling pathway, oxytocin signaling pathway and Melanogenesis simultaneously.

Conclusions: According to what has been discussed above, we speculated that miR-31-5p may play a vital role in the occurrence and development of LUSC.

Keywords: Lung squamous cell carcinoma (LUSC); miR-31-5p; quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR); target genes; molecular mechanism

Introduction

Lung and bronchus cancer is the principal cause of death and the second highest incidence rate among all malignancies worldwide. Studies show that lung cancer causes exceed one-quarter deaths from cancers in the US (1,2). Lung cancer include two major histological categories: small cell lung cancer (SCLC 15%) and non-small cell lung cancer (NSCLC 85%). NSCLC can be divided into three types, include lung large cell carcinoma (LULC), lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) (3-5). With the rapid growth of incidence of lung cancer, more and more studies have been made about its occurrence, development and metastatic mechanism in recent years, however, few studies focused on the molecular mechanism of LUSC.

By degrading mRNAs or inhibiting translation, MiRNAs could induce gene silencing (6). Researches confirm that MiRNAs play an important role in proliferation, growth, invasion and apoptosis of cancers (7-10). For example, studies identified that miR-132, miR-198 and miR-1269 may indicate the occurrence, development and prognosis of hepatocellular carcinoma through their potential molecular mechanism and their targeted genes Zhang *et al.* suggested that downregulation of miR-132 may result from the HBx expression via DNA methylation, study from Huang *et al.* reported that through targeting fibroblast growth factor receptor 1, miR-198 could decrease proliferation and depress apoptosis, Gan *et al.* reported that DACT1 may be target gene of miR-1269,and downed-regulated expression of DACT1 may result in infiltration and metastasis of tumor by inhibiting NF-κB signaling and WNT/beta-catenin signaling pathways (11-13). Some researches have also reported the important value of miRNAs in the diagnosis, therapy, and prognosis of LUSC (14-16) . Nevertheless, the role of miR-31-5p in lung squamous cell carcinoma (LUSC) was unknown (17) Therefore, additional studies are needed to clarify the molecular mechanism of miR-31-5p in LUSC.

Previous studies reported that the expression of miR-31-5p was up-regulated in pancreatic cancer, colorectal cancer and hepatocellular carcinoma (18-20), but it was down-regulated in cemento-ossifying fibroma and upper tract urothelial carcinoma (21,22). Nevertheless, only a few reports about the relationship between miR-31-5p and NSCLC have been published up to now. Chun *et al.* reported a high expression of miR-31-5p in NSCLC tissue (23), but this study was limited by the quantity of the tissue samples and different methods of data collection and analysis. In this study, we intend to detect the expression of miR-31-5p in LUSC and confirm the miR-31-5p target genes by using several online databases.

More studies were needed to understand the relationship between miR-31-5p and LUSC. In current study, we intend to detect the expression of miR-31-5p in LUSC and predict the target genes of miR-31-5p by several online databases. We will also analyze their molecular mechanisms by bioinformatics software and analyze which function and signaling pathway they have impact, expect to provide theoretical foundation for finding new therapeutic targets of LUSC.

Methods

Tissue samples

Our research gained the ethics approval from The Medical Ethics Committee of First Affiliated Hospital of Guangxi Medical University [approval number:2019 (K-E-Y-019)]. A total of 88 LUSC patients (Ages range from 23 to 82, with an average age of 56.5, include 50 males and 38 females) who had been treated in the First Affiliated Hospital of Guangxi Medical University were chosen for present study. All tissues were formalin fixed paraffin embedded (FFPE). All the clinical features were exhibited in *Table 1*.

Quantitative real-time reverse transcription PCR (qRT-PCR)

qRT-PCR was performed to detect the expression of miR-31-5p in 88 FFPE tissues as a previous study reported (24). Additionally, using NormFinder and geNorm, we found that the miR-191 and miR-103 were the most stable housekeeping miRNAs, so they were selected as the endogenous controls. The sequences of the miRNAs are as follows: miR-191(Applied Biosystems Cat. No. 4427975-000490): CAACGGAAUCCCAAAAGCAGCU; miR-103 (Applied Biosystems Cat. No. 4427975-000439):

Table 1 The relationship between miR-31-5p and clinicopathological parameters in LUSC

Clinicopathological parameters	n	Relevant expression of miR-31-5p ($2^{-\Delta Cq}$)		
		Mean ± SD	t	P value
Tissue			7.083	0.000
Para-carcinoma tissue	88	4.3008±1.73832		
LUSC	88	6.1472±1.71976		
Age (years)			0.119	0.905
<60	51	6.1659±1.67066		
≥60	37	6.1214±1.80824		
Gender			0.086	0.932
Male	50	6.1610±1.88261		
Female	38	6.1289±1.50332		
Smoke			−0.603	0.548
No	33	6.2903±2.03028		
Yes	55	6.0613±1.51732		
Tumor size (cm)			−0.915	0.590
≤3	49	6.4557±1.82857		
>3	39	5.7595±1.50688		
Lymph node metastasis			−1.140	0.257
No	46	6.3465±1.76204		
Yes	42	5.9288±1.66573		
Vascular invasion			0.651	0.651
No	66	6.0780±1.75576		
Yes	22	6.3545±1.62820		
TNM			−0.923	0.359
I–II	33	5.9265±1.60711		
III–IV	55	6.2784±1.78540		

LUSC, lung squamous cell carcinoma.

AGCAGCAUUGUACAGGGCUAUGA; and miR-31-5p (AGGCAAGAUGCUGGCAUAGCU). Applied Biosystems PCR7900 (Foster City, CA, USA) was adopted to perform the quantitative real-time polymerase chain reaction (qRT-PCR). The formula $2^{-\Delta Cq}$ was utilized to determine the expression level of miR-31-5p (25).

Information extraction of miR-31-5p in LUSC from GEO and TCGA datasets

The Gene Expression Omnibus (GEO, http: //www. ncbi.nlm.nih.gov/geo/) (26) was used for extracting the expression information of miR-31-5p in LUSC. Appropriate data were screened and selected on the basis of the criteria listed below. The inclusion criterias of this study were as follows: (I) Studies had examined the expression level of miR-31-5p in blood or tissue samples; (II) Human species that were diagnosed with lung squamous cell carcinoma; (III) Both tumor specimens and normal specimens original expression data of miR-31-5p was provided. Exclusion criterias of the current study were as follows: (I) Animal or cell line research data; (II) Tumor or normal groups with

small sample sizes (n<10). We also extracted the information from TCGA data portal (http://tcga-data.nci.nch.gov/tcga). Then, these data were summarized, calculated and analyzed using SPSS 22.0 (SPSS, IBM, USA). The primary expression data were log-transformed when they haven't been normalized.

A meta-analysis based on data in our current study, together with data from the GEO, and TCGA, was conducted in our study. In addition, receiver operating characteristic (ROC) curve was made to confirm the different expression of miR-31-5p based on each dataset. Meanwhile, summary receiver operating characteristic (sROC) curve was also applied for further confirmation of the different expression levels of miR-31-5p between LUSC tissues and the normal tissues.

Prediction of miR-31-5p potential target genes

The target genes of miR-31-5p were determined using the following 12 online bioinformatics software: miRWalk, MicroT4, RNA22, RNAhybrid, miRanda, miRBridge, miRNAMap, PICTAR2, PITA, miRDB, miRMap and Targetscan. The genes present in more than 6 prediction software packages were selected as the potential target genes of miR-31-5p. We also searched for the target genes of miR-31-5p in PubMed and EMBASE. Meanwhile, genes that were confirmed by experiments were accepted. For higher specificity, we chose the intersection elements of potential genes and the down-expression genes as the target genes of miR-31-5p.

Bioinformatics analysis of the potential target genes of miR-31-5p

The Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) was used for GO, KEGG and analysis of those potential genes. GO analysis investigates the pathways from the cell component (CC), molecular function (MF), and biological processes (BP), and KEGG was applied to determine the remarkable pathways related to those genes.

PPI network construction

The PPI network, which was based on the STRING 10.5 online software (https://string-db.org/), was utilized to point out the association of those target genes. At the same time, the hub genes (with the largest connections in the PPI network) that may play an important part in the strategic pathway of LUSC have been identified.

Statistical analyses

Statistical Package for the Social Sciences (SPSS, IBM, USA) 22.0, GraphPad Prism 5 (Inc., La Jolla, CA, USA), StataSE 12.0, and R version 3.3.0 were utilized for all of the statistical analyses. The scatter diagram was applied to show the different expressions of miR-31-5p between patients with LUSC and the controls. Meanwhile, receiver operating characteristic (ROC) and the area under the curve (AUC) were applied to confirm the differential expression of miR-31-5p in patients with LUSC and corresponding controls. Possible correlations between miR-31-5p expression and clinical features were examined using the Spearman correlation. StataSE 12.0 was used for the meta-analysis of the data and SMD <0 and 95% CI did not cross zero represented miR-31-5p had a lower expression in LUSC tissues than in normal specimens. In contrast, in this case, when the overall SMD >0 and 95% CI did not cross zero, tumor specimens were supposed to have a higher expression of miR-31-5p than normal specimens. Moreover, funnel plot and Egger's test were utilized for detecting the publication bias. In all the analyses, the P value <0.05 was considered to be statistically significant.

Results

High expression of miR-31-5p in LUSC

The expression data of miR-31-5p in 88 LUSC tissues and the controls was detected by qRT-PCR, the result showed that the expression of miR-31-5p was significantly higher in LUSC than the normal lung tissues (P<0.001), as shown by the AUC of 0.797 (*Figure 1*).

Meanwhile, data from TCGA showed the expression of miR-31-5p in LUSC was significantly higher than the controls (P<0.001) (*Figure 2A*) and the AUC was 0.915 (CI:1.197–1.624, P<0.000) (*Figure 2B* Respect to the GEO database, five databases (GSE16025, GSE25508, GSE47525, GSE51853, GSE74190) supported the viewpoint that the up-regulated expression of miR-31-5p in LUSC patients compared to the corresponding controls (P<0.05) (*Figure 3*). Furthermore, the ROC curves, which were based on the data taken from 6 GEO datasets, were utilized for confirming the high expression levels of miR-31-5p in LUSC (shown in *Figure 3B,D,F,G,H*), reveal that five of
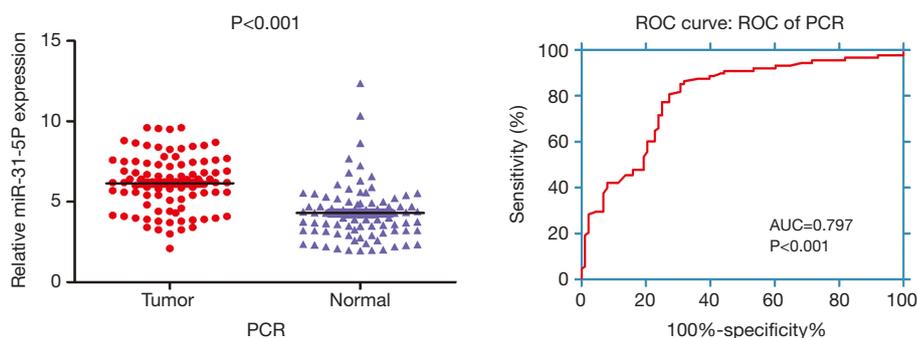
Figure 1 Expression level of miR-31-5p examined by qRT-PCR, the result showed that there was significant difference between the expression of miR-31-5P in LUSC and normal tissue (P<0.001) (AUC =0.797, P<0.001). qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; LUSC, lung squamous cell carcinoma.
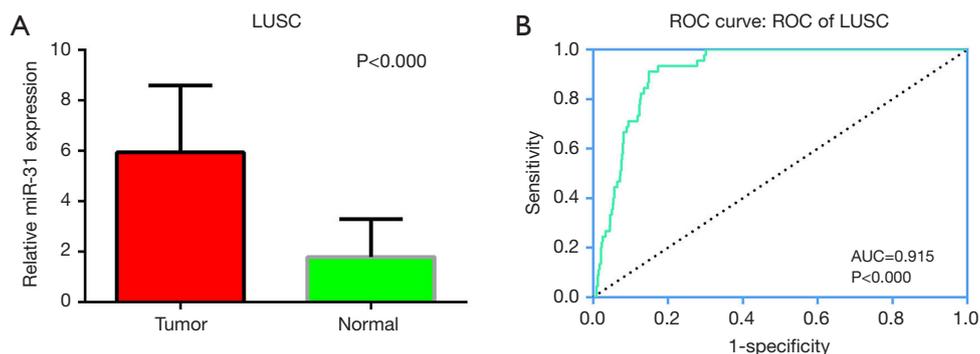

Figure 2 Up-regulated expression of miR-31-5p in LUSC. (A) Expression of miR-31-5p based on TCGA data; (B) receiver operating characteristic (ROC) curve indicated the higher expression of miR-335 in GIST (AUC =0.915, P<0.000). LUSC, lung squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

the six datasets from GEO support the high expression of miR-31-5p in LUSC, including GSE51858 (AUC =0.800, P=0.034), GSE74190 (AUC =0.796, P<0.000), GSE47525 (AUC =0.743, P=0.116), GSE16025 (AUC =0.710, P=0.035). The ROCs and AUCs also proved the higher expression of miR-31-5p in LUSC [GSE51858 (AUC =0.800, P=0.034), GSE74190 (AUC =0.796, P<0.000), GSE16025 (AUC =0.710, P=0.035), GSE25508 (AUC =0.810, P=0.023), and GSE47525 (P=0.049, AUC =0.743)] and GSE25508 (AUC =0.810, P=0.023), but the other one was without statistical significance (P>0.05, figure not shown). Meanwhile, the SROC which was based on five GEO database, TCGA and our study, also supported the up-regulated expression of miR-31-5p in LUSC (AUC=0.89) (*Figure 4*).

Meta-analysis of miR-31-5p expression in LUSC

A total of 1,012 samples, including 719 tumor and 283

normal samples, of which 313 cases came from 6 GEO datasets, 523 cases were obtained from the TCGA dataset and 176 samples from our research, were sent to StataSE for meta-analysis. According to the data from the six GEO datasets, the forest plot showed heterogeneity is exist (I² =55.5%, *Figure 5*). Then, sensitivity analysis of the forest study implied that data from GSE40738 may be heterogeneous in our study (P=0.285, *Figure 6A*), and another forest plot without GSE40738 dataset was conducted, which showed no heterogeneity in those five GEO datasets (I²=0.00%, *Figure 5B*). In addition, it showed higher expression of miR-31-5p in LUSC tissues compared to normal tissues (SMD =1.132, CI: 0.797–1.467, Z=6.62, P=0.000). Furthermore, the funnel plot showed that there was no publication bias in the studies (P=0.285, *Figure 6B*).

Meanwhile, the meta-analysis result of all the data in the current study (PCR, TCGA, GEO included) showed that the expression of miR-31-5p significantly increased in
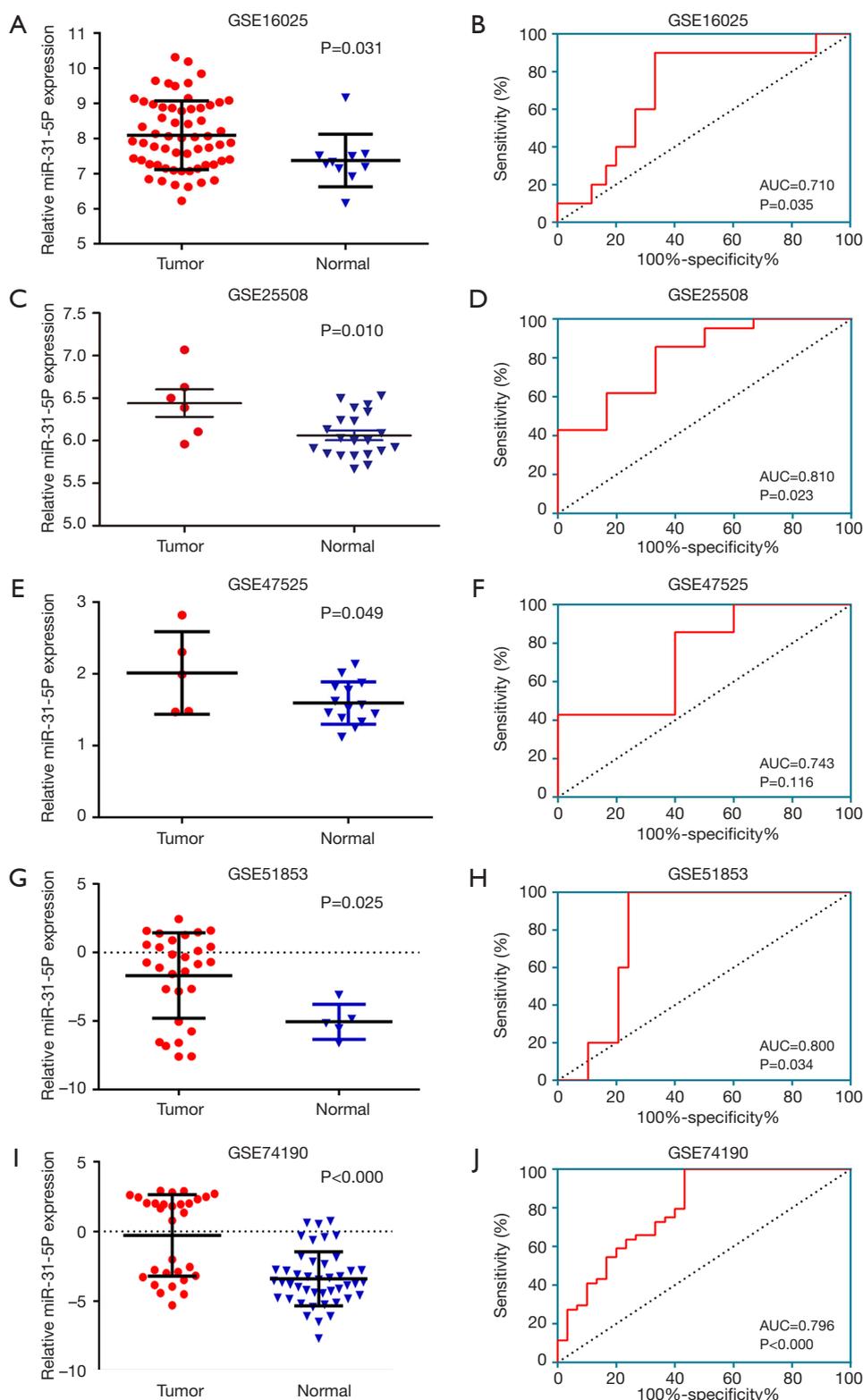
Figure 3 Relative expression levels of miR-31-5p in LUSC based on 5 GEO datasets. Expression level of miR-31-5p showed up-regulation in GSE16025 (P=0.031), GSE25508 (P=0.010), GSE47525 (P=0.049), GSE74190 (P<0.000) and GSE51853 (P=0.025). LUSC, lung squamous cell carcinoma; GEO, Gene Expression Omnibus.
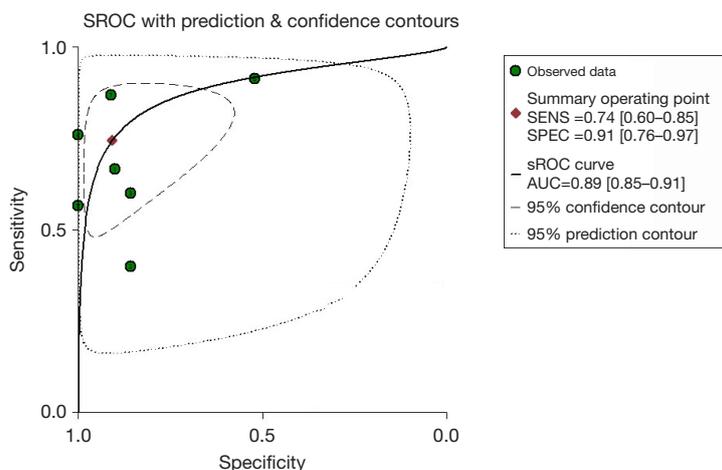
Figure 4 sROC based on data from PCR, TCGA and GEO database indicated the higher expression level of miR-31-5p in LUSC than in controls (AUC=0.91). sROC, summary receiver operating characteristic; PCR, polymerase chain reaction; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; LUSC, lung squamous cell carcinoma.

patients with LUSC than the controls (SMD =0, 95% CI: 1.08–1.45, Z=13.30, P=0.000, *Figure 7*).

Potential target genes of miR-31-5p

In total, 15,947 genes were screened base on 12 online predict softwares, and the genes present on more than 6 programs were selected to overlap with genes that were down-regulated in LUSC. Finally, 316 overlapped genes and 6 genes that were validated by the document were chosen as the target genes of miR-31-5p.

GO and KEGG pathway analyses

A total of 322 potential target genes of miR-31-5p were subjected to GO and KEGG analysis. As shown by GO analysis, these genes showed a great effect on signal transduction (GO: 0007165, P<0.001), positive regulation of endothelial cell proliferation (GO: 0001938, P<0.001) and peptidyl-tyrosine phosphorylation (GO: 0018108, P<0.001) for BP. On the level of MF, they were significantly associated with transmembrane receptor protein tyrosine kinase activity (GO: 0004714, P<0.001), Ras guanyl-nucleotide exchange factor activity (P<0.001, GO: 0005088) and growth factor binding (GO: 0005088, P<0.001) (*Table 2*). With respect to CC, the terms that most relate to the target genes were plasma membrane (GO: 0005886, P<0.001), integral component of the plasma membrane (GO: 0005887, P<0.001) and membrane raft (GO: 0045121,

P<0.001). The KEGG analysis revealed the top three signaling pathways were Rap1 signaling pathway (hsa04015, P<0.05), Oxytocin signaling pathway (hsa04921, P<0.05) and Proteoglycans in cancer (hsa05205, P<0.05) (*Table 3*).

PPI network analysis for the selection of hub genes.

The potential genes were imported to STRING for association analysis and the result showed 328 nodes and 437 edges among the network that was made up of 322 potential target genes of miR-31-5p (*Figure 8A*). Among these, the 6 hub genes of miR-31-5p, including adenylate cyclase 9 (ADCY9), adenylate cyclase 6 (ADCY6), neuromedin U receptor 1 (NMUR1), C-X-C motif chemokine ligand 12 (CXCL12), signal transducer and activator of transcription 5A (STAT5A) and epidermal growth factor receptor (EGFR) (*Figure 8B*).

Three of these hub genes, including ADCY6, ADCY9 and EGFR, appeared on the Rap1 signaling pathway, Oxytocin signaling pathway and Melanogenesis simultaneously.

Discussion

The poor diagnosis of the early stage disease and the useless treatment methods leads to poor prognosis of LUSC. Therefore, it is important to find an effective way to diagnose and treat lung cancer. In the current study, we found that the expression profile of miR-31-5p was up-
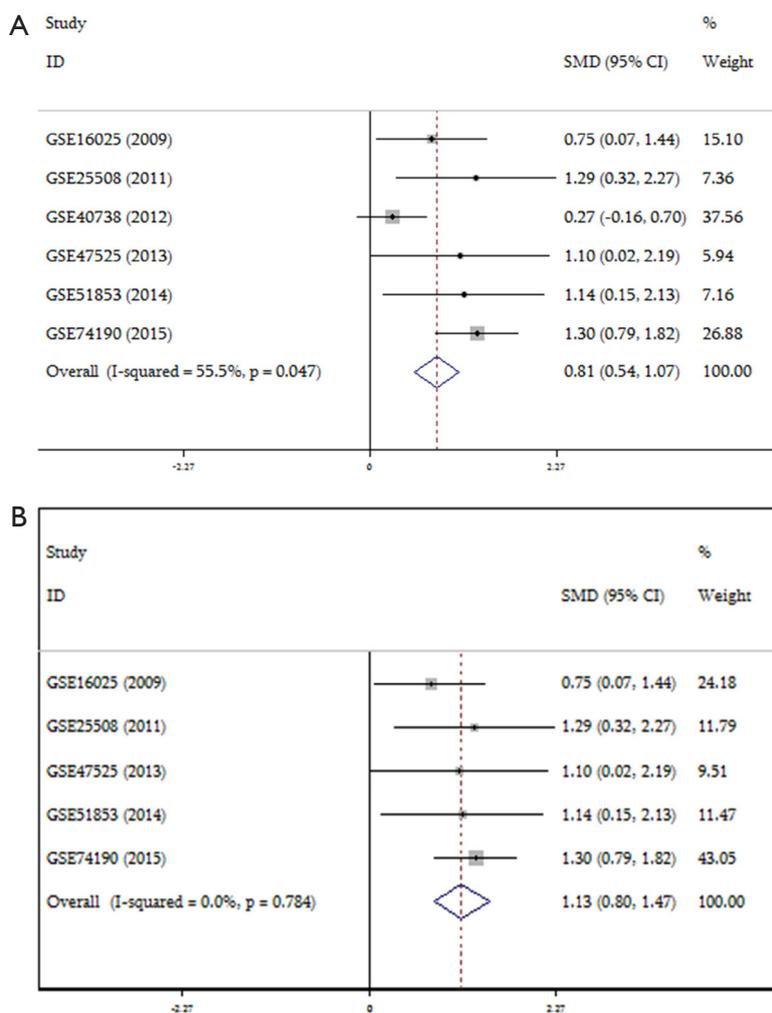
Figure 5 GSE40738 may contribute to the greater heterogeneity of this study. (A) Forest plot evaluating the differences in miR-31-5p expression in LUSC and the corresponding normal controls based on data from GEO. (B) Forest map after removing the data from GSE40738 showed that there was no heterogeneity among the GEO microarrays. LUSC, lung squamous cell carcinoma; GEO, Gene Expression Omnibus.

expressed in LUSC tissue compared to the normal tissue. We also confirmed the high miR-31-5p expression in LUSC through the ROC and sROC curve. Meanwhile, 322 potential targeted genes of miR-31-5p were predicted, and the molecular pathways like signal transduction, and Rap1 signaling pathway and transmembrane receptor protein tyrosine kinase activity, that may be involved in LUSC were discovered. Nevertheless, 6 hub genes of miR-31-5p were chosen for exploration of the molecular mechanism that involved in LUSC, among which, three genes were found to coexist in three pathways. These findings indicate that miR-31-5p play a major role in the initiation and progression of LUSC.

Studies improved that miR-31-5p closely related to the occurrence and development of tumors. Zhong *et al.* found that knockdown of miR-31-5p resulted in increasing expression of hMLH1 protein and reducing cell cycle arrest in NSCLC cells. They also reported that overexpression of miR-31-5p lead to decrease hMLH1 protein expression and induced cell cycle arrest at S phase (27). Mlcochova *et al.* (28) reported down-regulation of miR-31-5p in metastatic colorectal cancer (mCRC), and found it influenced the predicting the time to progression (TPP) in patients who received cetuximab treatment, and after transfecting several cell lines (HCT-116, DLD-1 and HT-29) in mCRC, 84 upregulated genes and 148
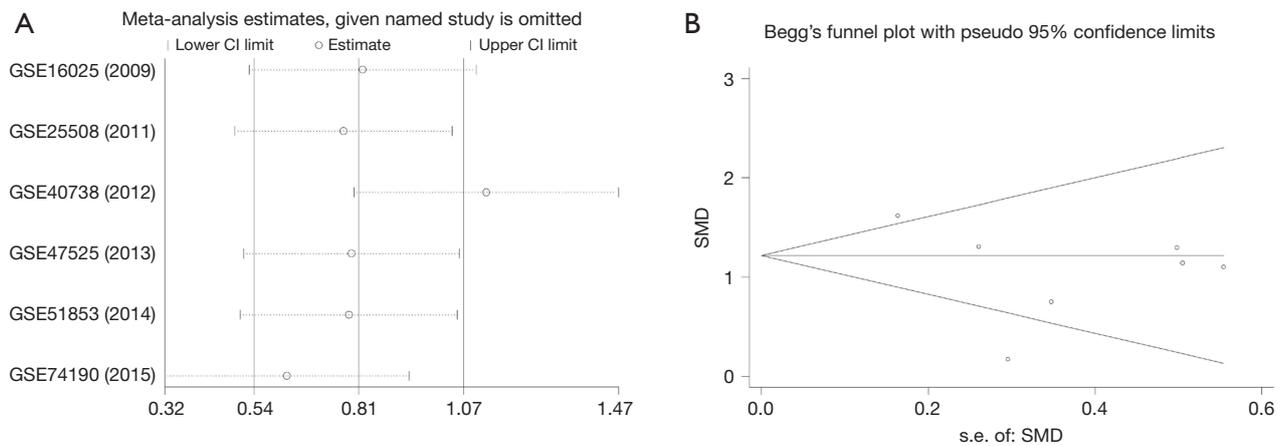
Figure 6 Find out the heterogeneous and assess for publication bias. (A) Sensitivity analysis of the current study implied that GSE40738 may be heterogeneous in our study. (B) Funnel plot showed that there was no publication bias in the studies.
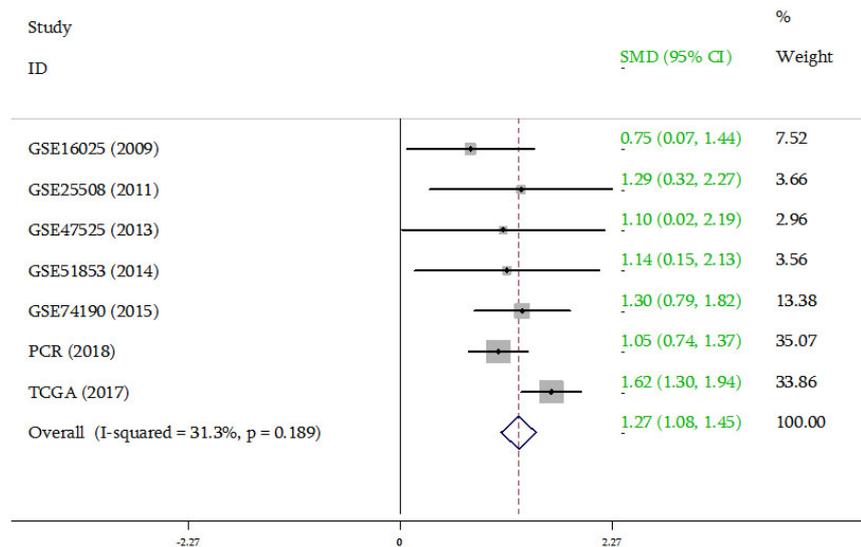

Figure 7 Forest plot based on PCR, TCGA and GEO databases showed up-regulated expression of miR-31-5p in LUSC. PCR, polymerase chain reaction; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; LUSC, lung squamous cell carcinoma.

Table 2 The top five KEGG pathways enriched in lung large cell carcinoma

Category	Term	Count	P value
KEGG_PATHWAY	Rap1 signaling pathway	14	1.10×10^{-4}
KEGG_PATHWAY	Oxytocin signaling pathway	11	5.80×10^{-4}
KEGG_PATHWAY	Proteoglycans in cancer	12	1.00×10^{-3}
KEGG_PATHWAY	Melanogenesis	8	1.00×10^{-3}
KEGG_PATHWAY	Tight junction	8	2.20×10^{-3}

downregulated genes came to light (P<0.01) and Slattery *et al.* also reported the correlation between colorectal tumor molecular phenotype and miR-31-5p, and they found that miR-31-5p is significantly related to the KRAS and BRAF mutations, which are associated with survival of patients with the colorectal tumor molecular phenotype (29). Capri *et al.* reported that miR-31-5p had four seed sequences within the 30 UTR of GLT1 (30). ZHANG G validated another target gene (SP1) of miR-31-5p and suggested that up-regulated expression of miR-31-5p came into play as a

Table 3 The top 20 GO terms enriched in Lung large cell carcinoma

Category	Terms	Counts	P value
GOTERM_BP_DIRECT	Signal transduction	45	$4.10×10^{-7}$
GOTERM_BP_DIRECT	Positive regulation of endothelial cell proliferation	9	$2.10×10^{-5}$
GOTERM_BP_DIRECT	Peptidyl-tyrosine phosphorylation	12	$6.10×10^{-5}$
GOTERM_BP_DIRECT	Positive regulation of GTPase activity	24	$1.00×10^{-4}$
GOTERM_BP_DIRECT	Positive regulation of cell migration	12	$3.10×10^{-4}$
GOTERM_BP_DIRECT	Activation of phospholipase C activity	5	$9.00×10^{-4}$
GOTERM_BP_DIRECT	Transmembrane receptor protein tyrosine kinase signaling pathway	8	$1.20×10^{-3}$
GOTERM_BP_DIRECT	Intracellular signal transduction	17	$1.50×10^{-3}$
GOTERM_BP_DIRECT	Adenylate cyclase-activating G-protein coupled receptor signaling pathway	6	$1.50×10^{-3}$
GOTERM_BP_DIRECT	Establishment of endothelial barrier	4	$2.30×10^{-3}$
GOTERM_MF_DIRECT	Transmembrane receptor protein tyrosine kinase activity	7	$3.40×10^{-5}$
GOTERM_MF_DIRECT	Ras guanyl-nucleotide exchange factor activity	9	$6.30×10^{-4}$
GOTERM_MF_DIRECT	Growth factor binding	5	$9.40×10^{-4}$
GOTERM_MF_DIRECT	Rho guanyl-nucleotide exchange factor activity	7	$1.70×10^{-3}$
GOTERM_MF_DIRECT	Steroid hormone receptor activity	6	$2.20×10^{-3}$
GOTERM_CC_DIRECT	Plasma membrane	113	$1.10×10^{-8}$
GOTERM_CC_DIRECT	Integral component of plasma membrane	54	$2.00×10^{-8}$
GOTERM_CC_DIRECT	Membrane raft	17	$3.90×10^{-7}$
GOTERM_CC_DIRECT	Cell surface	24	$8.20×10^{-4}$
GOTERM_CC_DIRECT	Focal adhesion	17	$9.20×10^{-4}$

GO, gene ontology.

suppressor in hepatocellular carcinoma (18). Accumulating evidence shows that miR-31-5p is expressed differently in different cancers, which affects the prognosis, diagnosis and treatment of cancers, until some molecular mechanisms have been confirmed.

In the current study, we focus on finding the potential signal pathways that are related to miR-31-5p in LUSC. Our results, 25 potential pathways that supposedly correlate with miR-31-5p are displayed in the *Tables 1-3*. In addition, the protein interaction network based on those potential target genes also has been conducted, and we then selected the top six genes for further study. This study intended to find the function of the six potential genes of miR-31-5p, to better understand the molecular mechanism in LUSC. Here, we would like to discuss the possible mechanisms of the genes that were verified by previous studies.

C-X-C motif chemokine ligand 12 (CXCL12) was

proven by a number of studies to be connected with tumor growth, invasion, angiogenesis, and metastasis in different types of cancer, such as epithelial ovarian carcinoma, breast cancer, clear cell renal cell carcinoma, prostate cancer and pancreatic cancer. As a result, researchers identified the connection between CXCL12 and diagnosis, prognosis and treatment of cancers in patients (31-34). Wang *et al.* reported that up-regulation of CXCL12 expression enhances lung cancer progression through the CXCR4/JAK2 pathway (35). Another study showed CXCL12 expression was connected with a reduced overall survival in lung cancer patients (36). Wang *et al.* suggested that through activating cancer stem cell, CXCL12 could influenced initiation and progression of lung cancer (37). However, the role that CXCL12 played in LUSC remains unclear.

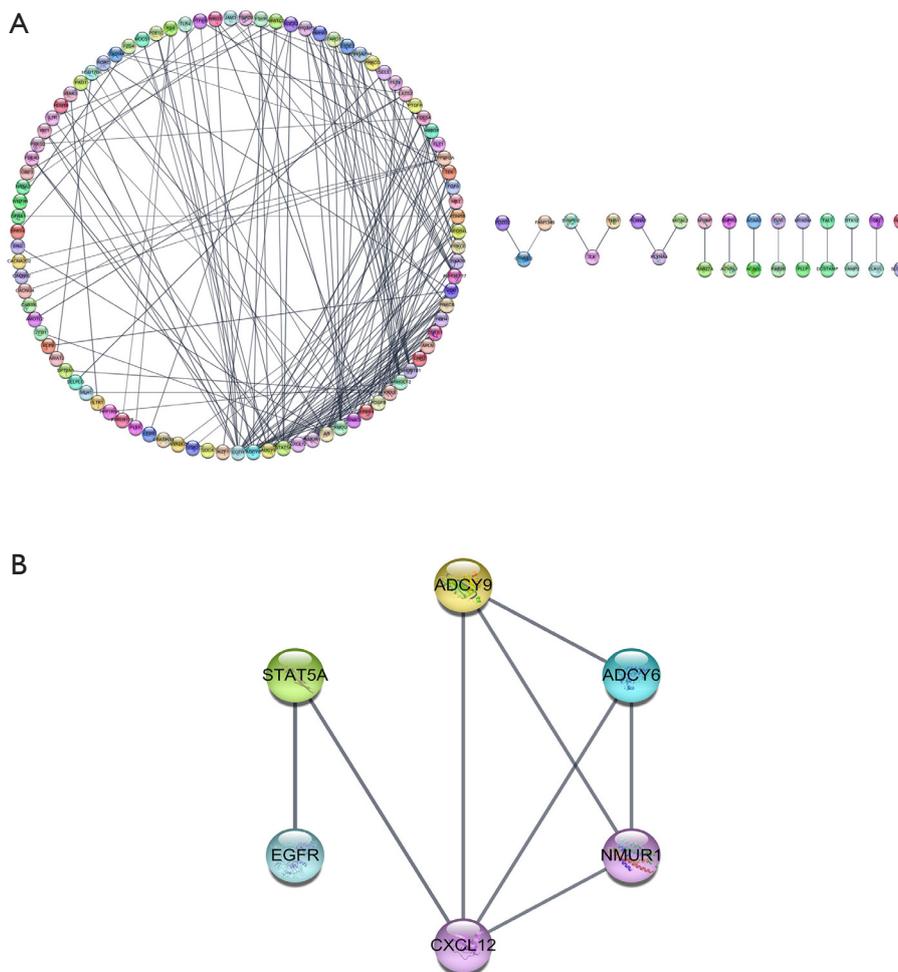STAT5A, an encoded member of the STAT family,

Figure 8 Point out the association of the target genes of miR-31-5p and identified the hub genes. (A) PPI network of 322 potential target genes of miR-31-5p. (B) The top six hub genes with the largest connections in the PPI network. PPI, protein-protein interaction.

was identified to be a downstream target of miR-1469. The miR-1469 can bind the 3'-untranslated region of the Stat5a directly, and the expression of mRNA and protein of Stat5a was reduced. Finally, it was proved that by targeting STAT5A, the miR-1469 can lead to apoptosis of lung cancer cells (38). Pastuszak-Lewandoska D also found the expression of STAT5A is down-regulated in non-small cell lung cancer (NSCLC), but the mechanism of how it works in LUSC has not been explored (39).

Epidermal growth factor receptor (EGFR), one of the most popular research genes, has been confirmed to have an important function in various types of cancers (40-42). Because EGFR mutation was found in lung squamous cell carcinoma , drugs for lung cancer with EGFR mutation are in clinical trial and show a promising future as a target for

lung cancer patients (43). Liu *et al.* also found that EGFR-tyrosine kinase inhibitors (EGFR-TKIs) could make a difference for LUSC patients with EGFR mutation, which predicts a better outcome for the patient who is given EGFR-TKIs (44,45). Meanwhile, Du *et al.* suggested that EGFR was one of the cross-talk genes that was associated with the 12 pathways that were connected to LUSC (46). On the basis of clinical data analysis, there was significantly correlation between EGFR and the survival of LUSC patients. In conclusion, EGFR plays a significant role in the treatment and survival of patients in LUSC, but the relationship between miR-31-5p and EGFR remains unexplored.

However, there were also quite a few limitations in this study. First, the total 882 samples from GEO, TCGA and

the First Affiliated Hospital of Guangxi Medical University underwent different methods of detection, were from different countries and regions, represented different ages of patients and different stages of tumors and a number of other factors, which means additional subgroup analyses within a large sample are needed to search for the source the heterogeneity. Second, miRNAs come into play through different pathways and molecular mechanisms, therefore several bioinformatics methods were used to evaluate the biomolecular function of the targeted genes of miR-31-5p, including GO and KEGG pathway analysis. Beyond that, a PPI network model was used to visually display the connection of those targeted genes. Nevertheless, to confirm the treatment target of LUSC, more verification experiments are indispensable.

In conclusion, higher expression levels of miR-31-5p in LUSC patients than normal were confirmed by meta-analysis based on the data from GEO, TCGA and our experiment. Furthermore, the diagnostic value of miR-31-5p was verified in the current study. In addition, our research proposed that miR-31-5p may play a major role in the carcinogenesis of LUSC by targeting the hub genes we identified. Moreover, the underlying pathways reveal the molecular mechanism of miR-31-5p in LUSC. To conclude, it could be hypothesized that miR-31-5p serves as a molecular biomarker for LUSC in the clinical setting. However, more studies are needed to confirm the actual function of miR-31-5p in diagnosis, prognosis and therapy of LUSC.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.04.21). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all patients. Our research gained the ethics approval from The Medical Ethics Committee of First Affiliated Hospital of Guangxi Medical University [approval number:2019 (K-E-Y-019)].

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