Abstract. The role of microRNA (miRNA) -452-5p in lung squamous cell carcinoma (LUSC) remains unclear. Therefore, the present systematic study was performed to investigate the clinical significance and the rudimentary mechanism of the function of miR-452-5p in LUSC. The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases were utilized to confirm the expression level and clinical value of miR-452-5p in LUSC. Using online databases and bioinformatic software, gene ontology (GO), pathway and protein-protein interaction (PPI) analyses of miR-452-5p target genes were performed to examine the molecular mechanism of miR-452-5p. The association between the expression of miR-452-5p and that of its hub genes was verified using TCGA. Based on TCGA data on 387 clinical specimens, the expression of miR-452-5p in LUSC was significantly increased compared with adjacent lung tissues (7.1525±1.39063 vs. 6.0885±0.35298; P<0.001). The expression levels of miR-452-5p were significantly correlated with age (P=0.001) and tumor-node metastasis stage (P=0.028). Furthermore, the increased expression of miR-452-5p in LUSC compared with non-cancerous tissue [standard mean deviation (SMD), 0.372; 95% confidence interval (CI), 0.020-0.724; z=2.07; P=0.038] was validated by a meta-analysis of 720 clinical samples. The GO and pathway analyses revealed that miR-452-5p target genes were mainly enriched in the ‘regulation of transcription’, ‘nucleoplasm’, ‘protein binding’ and ‘cell cycle’ pathways. A total of 10 hub genes were identified by PPI analysis, and 5 hub genes (SMAD4, SMAD2, CDKN1B, YWHAE and YWHAB) were significantly enriched in the ‘cell cycle’ pathway. The expression of CDKN1B was negatively correlated with miR-452-5p (P=0.003). It was concluded that miR-452-5p may serve an essential role in the occurrence and progression of LUSC by targeting CDKN1B, which is involved in the cell cycle.

Introduction

The incidence and mortality rates of lung cancer are the highest among all types of malignant neoplasm in China and worldwide (1-3). Lung cancer can be divided into two histological categories: Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for ~80% of lung cancer cases, including lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and lung large cell carcinoma (LULC) (4,5). Although the incidence of LUSC can be prevented to some extent by regulating tobacco smoking (6), the mechanism of tumorigenesis and progression of LUSC remains poorly characterized. There has been great progress in molecular targeted therapies for LUSC (7-9). However, resistance to targeted drugs remains high, and the proportion of responsive patients is limited. Therefore, the development of novel diagnostic and therapeutic tools for LUSC is of great importance.

microRNA (miRNA) is a class of endogenous non-coding RNA molecules, which are ~22 nucleotides in length and regulate protein translation (10,11). Previous studies have demonstrated that miRNAs silence genes by inhibiting the synthesis of associated proteins or degrading the mRNA of target genes, and therefore serve an important role in the regulation of growth, proliferation, differentiation, apoptosis and development of cancer (12-14). Numerous studies have reported the role of miRNA in the progression of LUSC and its value in the diagnosis, treatment and prognosis of this disorder (15-18).
miR-452-5p (previously named miR-452) is obtained by modification of the 3' end of pre-miR-452 (19,20). Previous studies have reported that miR-452-5p expression in different types of cancer is distinct, where its expression is upregulated in urinary tract epithelial tumors, esophageal cancer and liver cancer (21-23) but downregulated in breast cancer, prostate cancer and glioma (24-26). Zhang et al (27) and He et al (28,29) reported low expression of miR-452-5p in NSCLC cells and tissues, respectively. However, these studies were limited by the quality of the LUSC samples used. Therefore, the expression level and clinical significance of miR-452-5p in LUSC remain unclear. The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) aim to provide a comprehensive understanding of cancer at the molecular level using genomic analyses, including large-scale genome sequences and microarrays for improving the diagnosis, treatment and prevention of cancer. In the present study, the expression level of miR-452-5p in LUSC was confirmed using the GEO and TCGA databases for the purpose of reducing the errors caused by sample size and study population. Gene ontology (GO) and pathway enrichment analyses of miR-452-5p target genes were also performed for clarification of the molecular mechanism of miR-452-5p in LUSC.

Materials and methods

**Extraction of data on miR-452-5p expression in LUSC from TCGA and GEO.** As illustrated in Fig. 1, information regarding miR-452-5p expression and clinical characteristics of patients with LUSC was extracted from TCGA (https://tcga-data.nci.nih.gov/docs/publications/tcga/) and GEO (https://www.ncbi.nlm.nih.gov/geo/) for this retrospective study. The following strategy was used to search in GEO datasets: ‘Lung’ OR ‘pulmonary’ OR ‘respiratory’ OR ‘bronchi’ OR ‘alveoli’ AND ‘cancer’ OR ‘carcinoma’ OR ‘tumor’ OR ‘neoplas*’ OR ‘malignan*’. The resulting eligible records, including miRNA microarray and RNA-seq datasets, were reviewed. The inclusion criteria were as follows: i) Diagnosis of patient with lung squamous cell carcinoma; ii) detection of miR-452-5p expression level in tissue or blood samples and iii) availability of original expression profiling data of miR-452-5p in both cancerous and non-cancerous specimens. The exclusion criteria were as follows: i) Datasets from research on cell lines or animals; ii) cancerous or non-cancerous groups with small sample sizes (<10), and iii) poor-quality profiling expression data (0, 0.1 or 1) that accounts for >30% of the total expression data. Independent investigators (Xiaoning Gan and Tingqing Gan) reviewed the datasets that met the criteria and extracted the appropriate datasets. Discrepancies between the decisions of the two investigators were resolved by discussion among all authors. Next, the data were summarized and analyzed using Microsoft Office 2007 software package (Excel and Office programmes), SPSS (version 22.0; IBM Corp., Armonk, NY, USA) and R (version 3.3.0; https://www.r-project.org/). The primary expression data of miR-452-5p were log_2-transformed for further study.

**Prediction of miR-452-5p target genes.** The prediction was performed using online bioinformatic software, including EMBL-EBI (https://www.ebi.ac.uk/), Targetminer (https://www.EMBL-EBI...
Results

Association between miR-452-5p expression and clinicopathological features in LUSC. The data on 387 cases of LUSC (342 cancerous and 45 non-cancerous adjacent lung tissues) patients with differential miR-452-5p expression were extracted from the TCGA database. The results indicated that the expression of miR-452-5p in LUSC tissues was higher compared with adjacent lung tissues (7.1525±1.39063 vs. 6.0885±0.35298; P<0.001; Fig. 2A; Table I).

The associations between the levels of miR-452-5p expression and age, cancer location, and TNM stage were
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Table I. Association between the expression of miR-452-5p and clinicopathological parameters in LUSC as analyzed using data from The Cancer Genome Atlas.

| Clinicopathological features                  | n    | miR-452-5p expression (log₂) |
|----------------------------------------------|------|-------------------------------|
|                                              |      | Mean ± SD                     | t-test    | P-value |
| Tissue                                       |      |                               |          |         |
| Adjacent non-cancerous lung tissue           | 45   | 6.0885±0.3529                 | Z=−6.053  | <0.001  |
| LUSC                                         | 342  | 7.1525±1.39063                |           |         |
| Age (years)                                  |      |                               |          |         |
| <60                                          | 62   | 7.6847±1.39161                | 3.359     | 0.001   |
| ≥60                                          | 274  | 7.0333±1.37623                |           |         |
| Sex                                           |      |                               |          |         |
| Female                                       | 85   | 7.0867±1.30046                | -0.503    | 0.616   |
| Male                                         | 257  | 7.1742±1.42095                |           |         |
| Ethnicity                                    |      |                               |          |         |
| Caucasian descent                            | 252  | 7.1228±1.42366                | F=1.985b  | 0.139   |
| African descent                              | 24   | 7.4428±1.32204                |           |         |
| Asian descent                                | 6    | 6.1575±1.78242                |           |         |
| Recurrence                                   |      |                               |          |         |
| No                                           | 139  | 7.2663±1.29109                | -0.315    | 0.753   |
| Yes                                          | 30   | 7.3517±1.57974                |           |         |
| Cancer location                              |      |                               |          |         |
| Central lung                                 | 109  | 7.3056±1.25173                | 2.069     | 0.040   |
| Peripheral lung                              | 74   | 6.8924±1.42939                |           |         |
| Smoke                                        |      |                               |          |         |
| ≤20                                          | 29   | 7.2479±1.77796                | 0.379     | 0.705   |
| >20                                          | 258  | 7.1428±1.37157                |           |         |
| Stage                                         |      |                               |          |         |
| I-II                                         | 285  | 7.2259±1.32747                | Z=−2.191a | 0.028   |
| III-IV                                       | 54   | 6.7484±1.67113                |           |         |
| Tumor grade                                  |      |                               |          |         |
| T1-2                                         | 272  | 7.1847±1.36195                | 0.844     | 0.399   |
| T3-4                                         | 70   | 7.0273±1.50065                |           |         |
| Nodal metastasis                             |      |                               |          |         |
| No                                           | 219  | 7.2583±1.26109                | Z=−1.901a | 0.057   |
| Yes                                          | 123  | 6.9642±1.58355                |           |         |
| Metastasis                                   |      |                               |          |         |
| No                                           | 261  | 7.1327±1.35815                | -0.471    | 0.638   |
| Yes                                          | 81   | 7.2161±1.49758                |           |         |
| Tumor status                                 |      |                               |          |         |
| Tumor-free                                   | 216  | 9.2886±1.28058                | -0.682    | 0.496   |
| With tumor                                   | 57   | 9.4231±1.48230                |           |         |

*Mann Whitney test was used for the comparison between 2 groups; *one-way analysis of variance was used for analysis between ≥3 groups. LUSC, lung squamous cell carcinoma; miRNA, microRNA; SD, standard deviation; TNM, tumor-node metastasis.

Statistically significant. miR-452-5p expression in patients <60 years was significantly increased compared with patients ≥60 years (P=0.001; Fig. 2B). The expression of miR-452-5p in patients with a tumor located in the central lung was significantly increased compared with patients with a tumor located in the peripheral lung (P=0.040; Fig. 2C). Additionally, the level of miR-452-5p expression in patients with TNM stages I-II LUSC was significantly increased compared with patients with stages III-IV LUSC (P=0.028; Fig. 2D). Spearman's rank correlation coefficient analysis demonstrated that the expression of miR-452-5p was negatively correlated with age (r=−0.187; P=0.001) and TNM stage (r=−0.119;
P=0.028) but was not significantly correlated with other clinical parameters.

Diagnostic value of miR-452-5p expression in LUSC. An ROC was generated to verify the predictive performance of miR-452-5p expression levels and various clinical parameters in LUSC (Fig. 3). The AUC of miR-452-5p expression for the diagnosis of LUSC was 0.778 (95% CI, 0.734-0.821; P<0.001) with a sensitivity of 65.5%, a specificity of 97.8% and a diagnostic threshold value of 6.632. The AUC of miR-452-5p expression for age was 0.640 (95% CI, 0.561-0.718; P=0.001). Furthermore, in LUSC patients with different tumor location and TNM stage, the AUC values were 0.574 (95% CI, 0.489-0.658; P=0.091) and 0.594 (95% CI, 0.507-0.681; P=0.028), respectively.

Prognostic value of miR-452-5p in LUSC. An average value of 7.1525 was selected as the miR-452-5p expression level threshold to evaluate the association between miR-452-5p expression levels and the survival of patients with LUSC. The multivariate survival curve suggested that patients with LUSC and a low miR-452-5p expression may have longer overall survival (time from randomization until mortality from any cause) compared with patients with LUSC and a high miR-452-5p expression (Fig. 4; Table II). It was not possible to determine whether miR-452-5p could be a prognostic factor in patients with LUSC [univariate hazard ratio (HR), 0.981; 95% CI, 0.648-1.487; P=0.928; multivariate HR, 1.492; 95% CI, 0.791-2.814; P=0.216].
Table II. Multivariate analysis of clinicopathological parameters for overall survival in lung squamous cell carcinoma.

A, Univariate analysis

| Clinicopathological features | HR     | 95% CI         | P-value |
|------------------------------|--------|----------------|---------|
| Age                          | 1.163  | 0.734-1.844    | 0.521   |
| >3 vs. ≤3                    |        |                |         |
| Tumor grade                  | 1.489  | 1.016-2.182    | 0.041   |
| T3-4 vs. T1-2                |        |                |         |
| Nodal metastasis             | 1.222  | 0.886-1.686    | 0.221   |
| Yes vs. no                   |        |                |         |
| Metastasis                   | 1.343  | 0.836-2.156    | 0.222   |
| Yes vs. no                   |        |                |         |
| TNM stage                    | 1.441  | 1.001-2.075    | 0.049   |
| III-IV vs. I-II              |        |                |         |
| Cancer location              | 1.242  | 0.768-2.010    | 0.377   |
| Peripheral lung vs. central lung |        |                |         |
| miR-452-5p expression        | 0.981  | 0.648-1.487    | 0.928   |
| High vs. low                 |        |                |         |

B, Multivariate analysis

| Clinicopathological features | HR     | 95% CI         | P-value |
|------------------------------|--------|----------------|---------|
| Age                          | 1.596  | 0.535-4.760    | 0.401   |
| >3 vs. ≤3                    |        |                |         |
| Tumor grade                  | 0.999  | 0.416-2.396    | 0.998   |
| T3-4 vs. T1-2                |        |                |         |
| Nodal metastasis             | 1.307  | 0.611-2.793    | 0.490   |
| Yes vs. no                   |        |                |         |
| Metastasis                   | 1.358  | 0.301-6.126    | 0.691   |
| Yes vs. no                   |        |                |         |
| TNM stage                    | 1.358  | 0.566-3.259    | 0.494   |
| III-IV vs. I-II              |        |                |         |
| Cancer location              | 1.943  | 0.973-3.884    | 0.060   |
| Peripheral lung vs. central lung |        |                |         |
| miR-452-5p expression        | 1.492  | 0.791-2.814    | 0.216   |
| High vs. low                 |        |                |         |

HR, hazard ratio; CI, confidence interval; miRNA, microRNA; TNM, tumor-node metastasis.

Verification of miR-452-5p expression in LUSC with meta-analysis. A total of 720 cancerous and non-cancerous samples from 7 eligible GEO datasets (including 333 cases from 7 eligible GEO datasets and 387 cases from TCGA) were screened in the meta-analysis (data not shown). The miR-452-5p expression levels in LUSC of each GEO dataset are illustrated in Fig. 5 and shown in Table III. In the meta-analysis, the random-effects model was used to calculate the pooled SMD and 95% CI due to the high heterogeneity of the data ($I^2=59.3\%; P=0.016$). These results indicated that the expression of miR-452-5p in LUSC was significantly increased compared with non-cancerous specimens (SMD, 0.372; 95% CI, 0.020-0.724; z=2.07; P=0.038) (Fig. 6A). The points, which represent individual studies, were symmetrically arranged in the Begg's funnel plot (P=0.711) and the P-values calculate from the Egger's test were $>0.05$ (P=0.810), which indicates that there was no publication bias in the studies (Fig. 6B).

Potential target genes of miR-452-5p. A total of 17,094 miR-452-5p candidate target genes were screened using 14 prediction programs. A total of 249 miR-452-5p target genes were searched and subjected to GO and pathway analyses. This included 248 candidates that were verified as target
genes by ≥7 programs and 1 target gene that was validated by experiments.

**GO and KEGG pathway analyses.** The DAVID database was used to perform GO analysis of the 249 target genes of miR-452-5p (Fig. 7A). The results indicated that, at the level of biological process (BP), target gene sets of miR-452-5p were closely associated with ‘regulation of transcription’, ‘commissural neuron axon guidance’ and ‘transcription’ (P<0.01). In terms of cellular components (CC), the target genes were mainly involved in the assembly of cellular structures, including ‘nucleoplasm’, ‘nucleus’ and ‘cytoplasmic vesicle membrane’ (P<0.01). Regarding molecular function (MF), the genes were mainly enriched in protein binding, chromatin binding and zinc ion binding (P<0.01). The results from the KEGG pathway analysis indicated that the predicted miR-452-5p target gene sets were significantly enriched in signaling pathways that are involved in the regulation of pluripotency of stem cells, oocyte meiosis and cell cycle (P<0.05).

The visualization function of Cytoscape was used to graphically display the results of GO (P<0.01; Q<0.1; overlap coefficient >0.5) and KEGG (P<0.05; Q<0.5; overlap coefficient >0.5) enrichment annotation analyses, allowing a more intuitive presentation of the associations between the pathways (Fig. 7B).

**PPI network analysis for the selection of hub genes.** The network consisted of 249 nodes and 118 edges. Among the 249 miR-452-5p target genes, 95 target genes were connected based on experiment, co-expression, neighborhood or other evidence summarized by STRING. The disconnected target genes (n=154) were removed from the network (Fig. 8). Finally, 10 hub genes (nodes connected with more than 6 edges) were identified from the 95 target genes. These hub genes were SMAD family member 4 (SMAD4), myocyte enhancer factor 2C (MEF2C), tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein ε (YWHAE), SMAD family member 2 (SMAD2), SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 4 (SMARCA4), tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein β (YWHAH), platelet activating factor acetylhydrolase 1b regulatory subunit 1 (PAFAH1B1),

| Dataset     | Tissue  | n | Mean ± SD       | t-test | P-value |
|-------------|---------|---|----------------|--------|---------|
| GSE40738    | Non-cancer | 72   | -0.1856±0.12812 | -0.226 | 0.822   |
| LUSC        |         | 34   | -0.1796±0.12344 |        |         |
| GSE19945    | Non-cancer | 8    | -1.3640±1.51488 | -2.213 | 0.049   |
| LUSC        |         | 5    | 0.2678±0.76523  |        |         |
| GSE16025    | Non-cancer | 10   | 2.5530±0.10050  | 0.447  | 0.656   |
| LUSC        |         | 61   | 2.5420±0.06697  |        |         |
| GSE25508    | Non-cancer | 8    | 6.2369±1.86325  | -1.085 | 0.314   |
| LUSC        |         | 8    | 6.3974±0.46815  |        |         |
| GSE74190    | Non-cancer | 14   | 2.8929±0.51361  | 0.213  | 0.840   |
| LUSC        |         | 61   | 2.7800±1.14324  |        |         |
| GSE47525    | Non-cancer | 8    | -0.7281±0.47630 | -2.062 | 0.047   |
| LUSC        |         | 29   | -0.1516±0.59057 |        |         |

*Student’s paired t-test was used to make comparisons between 2 groups; †P<0.05 vs. non-cancerous tissues. SD, standard deviation.
protein kinase cAMP-dependent type II regulatory subunit β (PRKAR2B), cyclin dependent kinase inhibitor 1B (CDKN1B) and replication protein A1 (RPA1). Among these 10 hub genes, 5 hub genes, including SMAD4, SMAD2, CDKN1B, YWHAE and YWHAB, were significantly enriched in ‘cell cycle’. 

Association between the expression of miR-452-5p and the 5 hub genes that are involved in cell cycle. The expression levels of 5 hub genes in LUSC were demonstrated with the TCGA dataset, including SMAD4, SMAD2, CDKN1B, YWHAE and YWHAB. The results indicated that the expression of CDKN1B and SMAD4 was significantly down-regulated in LUSC tissues compared with non-cancerous lung tissues (P=0.023 and P<0.001, respectively), while YWHAE expression was significantly upregulated in LUSC compared with control (P<0.001). There was no significant difference in the expression of SMAD2 or YWHAB in LUSC tissues compared with non-cancerous lung tissue (Fig. 9A; Table IV). Furthermore, Spearman’s rank correlation coefficient analysis demonstrated that miR-452-5p expression was negatively correlated with CDKN1B expression in LUSC (r=-0.163; P=0.003). However, miR-452-5p expression was not significantly correlated with the expression of SMAD4, SMAD2, YWHAE or YWHAB (Fig. 9B). In addition, in non-cancerous lung tissue, miR-452-5p was not correlated with CDKN1B expression (r=-0.013; P=0.937). These results suggested that miR-452-5p may target CDKN1B in the carcinogenesis of LUSC.

Discussion

The present study indicated that miR-452-5p expression in LUSC was significantly upregulated compared with non-cancerous lung tissues, and the diagnostic value of miR-452-5p expression for LUSC was also confirmed. Correlation analysis indicated that the expression of miR-452-5p correlated with clinical parameters, including age and TNM stage. Additionally, the results indicate that patients with LUSC and high expression of miR-452-5p were associated with early tumor stage but shorter survival
time. There are two potential reasons that may lead to these apparent conflicting results. Firstly, patients with relatively higher expression of miR-452-5p were more likely to succumb to disease at the early stage, while patients with relative lower expression of miR-452-5p were more likely to survive until late stages of the disease. Secondly, a number of patients with high expression of miR-452-5p were unable to accept or refused the investigation due to severe symptoms caused by the disease. These findings indicate that miR-452-5p may serve an essential role in the carcinogenesis and progression of LUSC. Furthermore, 5 hub target genes of miR-452-5p that are known to be involved in the cell cycle were predicted and screened, which may guide future studies in elucidating the mechanism of LUSC. The results also demonstrate that among these 5 hub genes, the expression of CDKN1B was negatively correlated with miR-452-5p.

miRNAs mainly perform their biological functions by complete or incomplete complementary binding with the 3'-untranslated region (UTR) of mRNAs (33-37). miR-452-5p is located at Xq28 in humans, and it can target multiple genes and thus serves a potentially important role in the biological process of carcinogenesis via a variety of mechanisms. Hu et al (24) reported that miR-452-5p expression was downregulated in breast cancer, which may lead to resistance to adriamycin by targeting the insulin analogue, growth factor receptor 1 (IGF-1R). The downregulation of miR-452-5p may also lead
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Liu et al. (26) reported that the expression of multifunctional stem cell regulatory factors, including SRY-box 2 (SOX2), was downregulated following upregulation of miR-452-5p expression in glioma cell lines. Furthermore, Liu et al. (26) confirmed that the downregulation of miR-452-5p in glioma cells and tissues was correlated with promoter methylation. Zheng et al. (23) reported high expression of miR-452-5p in hepatocellular carcinomas, suggesting that miR-452-5p could promote hepatocellular carcinoma by targeting CDKN1B (23). Zhang et al. reported that miR-452-5p inhibited the proliferation, invasion and migration of NSCLC cells via the process of epithelial-mesenchymal transition (27). He et al. (28) suggested that miR-452-5p enhanced the invasive capability of NSCLC cells by targeting BMI1 proto-oncogene, polycomb ring finger (28). However, the potential function and mechanism of miR-452-5p expression in LUSC remain unclear.

To date, to the best of our knowledge, He et al. (29) is the only group to have reported that the downregulated expression of miR-452-5p was associated with clinicopathological features in patients with NSCLC. Although the nature of the present research study was similar to the study by He et al. (29), the findings in our study are novel and provide further insights. Firstly, the present study analyzed a larger sample size using meta-analysis. The expression level of miR-452-5p in LUSC was confirmed using a total of 720 samples from TCGA and GEO, and whilst He et al. detected miR-452-5p expression in 76 LUSC samples, no association between high miR-452-5p expression and LUSC was observed. Secondly, the clinical value of miR-452-5p as a biomarker was considered in the present study, where it is indicated that miR-452-5p may...
Table IV. Relative expression of 5 hub target genes of miR-452-5p in LUSC analyzed using data from The Cancer Genome Atlas.

| Groups       | n  | Mean ± SD     | z     | P-value |
|--------------|----|---------------|-------|---------|
| CDKN1B       |    |               |       |         |
| Non-cancer   | 52 | 10.4490±0.47049 | -2.277 | 0.023   |
| LUSC         | 511| 10.3141±0.60619 |       |         |
| SMAD2        |    |               |       |         |
| Non-cancer   | 52 | 10.8716±0.18524 | -1.076 | 0.282   |
| LUSC         | 511| 10.9439±0.54278 |       |         |
| SMAD4        |    |               |       |         |
| Non-cancer   | 52 | 10.8014±0.22412 | -5.275 | <0.001  |
| LUSC         | 511| 10.4572±0.55275 |       |         |
| YWHAB        |    |               |       |         |
| Non-cancer   | 52 | 12.8296±0.16600 | -0.259 | 0.796   |
| LUSC         | 511| 12.8083±0.40408 |       |         |
| YWHAE        |    |               |       |         |
| Non-cancer   | 52 | 13.1903±0.25322 | -3.541 | <0.001  |
| LUSC         | 511| 13.4415±0.52233 |       |         |

*p<0.05 vs. non-cancerous tissue. LUSC, lung squamous cell carcinoma; SD, standard deviation; CDKN1B, cyclin dependent kinase inhibitor 1B; YWHAB, tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein β; YWHAE, tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein ε.

Contribute to the diagnosis of LUSC. Furthermore, miR-452-5p may be useful in predicting the progression of LUSC due to the close association between miR-452-5p expression and TNM stage. Finally, a systematic analysis of the potential molecular mechanisms of miR-452-5p function in biological processes that underlie LUSC was performed. A combination of 14 online prediction tools were employed to screen target genes of miR-452-5p. As a result, 249 miR-452-5p target genes were selected for GO and pathway analyses, it was indicated that the target genes primarily functioned by binding with protein, chromatin or other biological molecules, which are involved in transcriptional regulation.

Additionally, in order to analyze the key protein involved in the carcinogenesis, protein-protein interaction (PPI) analysis of 249 miR-452-5p target genes was applied. Consequently, 10 hub genes including SMAD4, SMAD2, CDKN1B, YWHAB, YWHAE, MEF2C, SMARCA4, PAFAH1B1, PRKAR2B and RPA1 were identified for additional study. Of these, the hub genes involved in cell cycle, including SMAD4, SMAD2, CDKN1B, YWHAE and YWHAB, are of particular interest. The cell cycle is a critical pathway that is closely associated with the prognosis and therapy of numerous malignancies, particularly LUSC (38,39). In addition, half of the 10 hub genes were significantly enriched in ‘cell cycle’. Notably, CDKN1B has been reported to function as a tumor suppressor gene in hepatocellular carcinoma and it is targeted by miR-452-5p (23). Therefore, it was hypothesized if CDKN1B, a hub gene which is involved in cell cycle, may have an important role in molecular targeting therapy of LUSC.

In the present study, it was demonstrated that the expression of miR-452-5p was negatively associated with the levels of CDKN1B in LUSC and this association was not statistically significant in normal lung tissues. These findings provide a direction for future investigation of the molecular mechanism that underlies miR-452-5p function in LUSC. Consistent with the results in the present study, Zheng et al (23) reported that miR-452-5p may directly target and suppress the expression of CDKN1B in hepatocellular carcinoma (23). The study also indicated that miR-452-5p may have a role in LUSC. Further experimental studies that investigate the association between CDKN1B and miR-452-5p will help to elucidate the mechanisms that underlie the carcinogenesis of LUSC.

However, several limitations exist in the present study. Firstly, heterogeneity may inevitably be present in the meta-analysis of 720 samples, resulting from numerous factors, including the detection method used, region, sex, age, stage and the type of LUSC specimens (fresh frozen tissue, formalin-fixed paraffin-embedded tissue or peripheral blood specimens). Therefore, future evidence-based confirmation and subgroup analyses by large-scale clinical trials are required to investigate the source of the heterogeneity. Secondly, miRNAs regulate the function of target genes during carcinogenesis and cancer progression through different biological pathways, which form a complex regulatory network of interactions involving biological molecules. Therefore, the present study utilized bioinformatic methods to predict the rudimentary function and mechanism of target genes. GO and pathway analyses were conducted and a PPI network model of biological molecules was constructed. However, in order to determine the mechanisms of miR-452-5p involved in LUSC, further verification, through in vitro and in vivo experiments as well as clinical trials, is required to identify new targets for potential treatments of LUSC patients.

In summary, relatively high levels of miR-452-5p expression in LUSC compared with non-cancerous lung tissues were determined by meta-analysis, which utilized experimental data obtained from TCGA and GEO. In addition, high expression of miR-452-5p was closely associated with the clinical parameters of LUSC, including age, cancer location and TNM stage. Furthermore, it is speculated that miR-452-5p serves a critical role in LUSC carcinogenesis by targeting CDKN1B, which is involved in cell cycle. Taken together, these findings provide a new direction for the development of tools for early diagnosis and targeted treatment of LUSC.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors' contributions
XG, TG, RH, JL, RT, HW, HZ, JM and HQ performed the literature search, data extraction and statistical analysis, and drafted the paper. GC and XH supervised the literature search, data extraction and analysis, and reviewed the paper. All authors have read and approved the manuscript.

Ethics approval and consent to participate
The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, with informed consent signed by all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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