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Clinical significance of miRNA-1 and its potential target gene network in lung squamous cell carcinoma

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Abstract. Previous studies demonstrated that miRNA-1 (miR-1) is downregulated in certain human cancer and serves a crucial role in the progression of cancer. However, there are only a few previous studies examining the association between miR-1 and lung squamous cell carcinoma (LUSC) and the regulatory mechanism of miR-1 in LUSC remains unclear. Therefore, the present study investigated the clinical significance and determined the potential molecular mechanism of miR-1 in LUSC. The expression of miR-1 and its clinical significance in LUSC was examined by conducting a meta-analysis of 12 studies using Stata 14, MetaDiSc1.4 and SPSS version 23. In addition, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were performed using the potential target genes of miR-1 gathered from Gene Expression Omnibus and ArrayExpress. Meta-analysis demonstrated that miR-1 was significantly downregulated in LUSC [standardized mean difference: -1.44; 95% confidence interval (CI): -2.08, -0.81], and the area under the curve was 0.9096 (Q*=0.8416) with sensitivity of 0.71 (95% CI: 0.66, 0.76) and specificity of 0.88 (95% CI: 0.86, 0.90). The pooled positive likelihood ratio and negative likelihood ratio were 4.93 (95% CI: 2.54, 9.55) and 0.24 (95% CI: 0.10, 0.54), respectively. Bioinformatics analysis demonstrated that miR-1 may be involved in the progression of LUSC via the 'cell cycle', 'p53 signaling pathway', 'Fanconi anemia pathway', 'homologous recombination', 'glycine, serine and threonine metabolism' and 'oocyte meiosis'. In summary, miR-1 was significantly downregulated in LUSC, suggesting a novel and promising non-invasive biomarker for diagnosing LUSC, and miR-1 was involved in LUSC progression via a number of significant pathways.

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Introduction

Non-small cell lung cancer (NSCLC) accounts for 85-90% lung cancer, and the most common subtypes are lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD) (1-4). LUSC typically occurs in men and is associated with smoking, accounting for ~25-30% total lung cancer cases (5-7). Therapies for LUAD are frequently ineffective for LUSC, reflecting differences between LUAD and LUSC; therefore, distinguishing LUSC from other types of lung cancer and investigating the molecular mechanism of LUSC are crucial.

MicroRNAs (miRNAs) are a class of small non-coding RNAs with lengths of 19-25 nucleotides (8-13). As post-transcriptional regulators of gene expression, these molecules are involved in the regulation of a number of biological processes, including differentiation, proliferation, migration and apoptosis (14-20). miRNAs may be used as diagnostic biomarkers and treatment targets for human cancer (21-25). The miR-1 family is comprised of miR-1-1, miR-1-2 and miR-206. In the family, miR-1-1 and miR-1-2 locate on chromosomes 20 and 18, respectively (26,27). The upregulation of miR-1 inhibits proliferation, promotes apoptosis and reverses the drug resistance of various types of cancer *in vivo* and *in vitro*, serving a role as a cancer suppressor gene (27). Previous studies demonstrated that miR-1 is downregulated in approximately all human cancer types and miR-1 serves a crucial role in the progression of cancer. Han *et al* (28) demonstrated that miR-1 is downregulated in gastric cancer, and inhibits gastric cancer cell proliferation and migration by targeting MET proto-oncogene, receptor tyrosine kinase. Wang *et al* (29) demonstrated that miR-1-3p, the mature miRNA of miR-1, suppresses the proliferation and invasion of bladder cancer cells by inhibiting C-C motif chemokine ligand 2 expression. Wang *et al* (30) observed that microRNA-1-3p is downregulated in oral squamous cell carcinoma (OSCC) tissues and cells, and serves as a suppressor of OSCC progression. There are a few previous studies that address the roles of miRNA in lung cancer progression (31-33). Meta-analyses have additionally been conducted to confirm the association between miRNA expression and prognosis in NSCLC (34).

Nevertheless, no consistent conclusion has been reached and direct evidence of the association between miR-1 and LUSC is limited. Therefore, the regulatory mechanism of miR-1 in LUSC requires further investigation.

The present study conducted a meta-analysis to evaluate the clinical significance of miR-1 in LUSC. Potential target genes of miR-1 in LUSC were obtained from the gene chip analysis of LUSC cells transfected with miR-1-3p, combined with target gene prediction and differential gene expression in The Cancer Genome Atlas (TCGA). Subsequently, a signaling pathway analysis was conducted to determine the potential molecular mechanism of miR-1 in LUSC.

Materials and methods

Collection of microarray datasets from Gene Expression Omnibus (GEO) and ArrayExpress. To examine the level of miR-1 expression in LUSC and adjacent non-cancer tissues, retrieval in GEO (http://www.ncbi.nlm.nih.gov/geo/) and ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) was performed using the following key words: ['lung' OR 'pulmonary' OR 'respiratory' OR 'bronchioles' OR 'bronchi' OR 'alveoli' OR 'pneumocytes' OR 'air way' (MeSH)] AND ('cancer' OR 'carcinoma' OR 'tumor' OR 'neoplas* OR malignan* squamous cell carcinoma'). 'Series' and 'Homo sapiens' were filtered. Studies with sample sizes ≥3 and miR-1 expression measured in LUSC and control groups were included. To identify promising miR-1 target genes, GEO and ArrayExpress were searched again using the following terms: ['lung' OR 'pulmonary' OR 'respiratory' OR 'bronchioles' OR 'bronchi' OR 'alveoli' OR 'pneumocytes' OR 'air way' (MeSH)] AND ('cancer' OR 'carcinoma' OR 'tumor' OR 'neoplas* OR malignan* squamous cell carcinoma') AND ('miR-1' OR 'miRNA-1' OR 'microRNA-1' OR 'miR-1' OR 'miRNA-1' OR 'microRNA1' OR 'miR-1' OR 'miRNA-1' OR 'microRNA-1' OR 'miR-1-3p' OR 'miRNA-1-3p' OR 'microRNA-1-3p' OR 'miR-1-1' OR 'miR-1-2' OR 'miR1-1' OR 'miR1-2'). Gene chips intervened with miR-1 in LUSC cell lines, knockdown or transfection, were included in the present analysis. Datasets are presented in Table I.

Acquisition of TCGA miRNA data. The LUSC miRNA matrix was downloaded from TCGA (http://cancergenome.nih.gov/). Data were normalized to a $\log_2$ scale. The values of miR-1-1 and miR-1-2 were included in the present study. Samples missing values for miR-1-1 or miR-1-2 were removed from the analysis. The data from TCGA were additionally included in the continuous variable meta-analysis and diagnostic meta-analysis.

Literature reviewing and selecting. PubMed (https://www.ncbi.nlm.nih.gov/pubmed), Science Direct (https://www.sciencedirect.com/), Google Scholar (https://scholar.google.com/), Ovid (https://ovidsp.ovid.com/), Wiley Online Library (https://onlinelibrary.wiley.com/), Embase (https://www.embase.com/), Web of Science (http://www.webofknowledge.com), Chong Qing VIP (http://www.cqvip.com), CNKI (http://www.cnki.net/), Wan Fang (http://www.wanfangdata.com.cn/) and China Biology Medicine Disc (http://http://www.sinomed.ac.cn/) were searched to identify all studies associated with miR-1-3p in LUSC using the following keywords: ['lung' OR 'pulmonary' OR 'respiratory' OR 'bronchioles' OR 'bronchi' OR 'alveoli' OR 'pneumocytes' OR 'air way' (MeSH)] AND ('cancer' OR 'carcinoma' OR 'tumor' OR 'neoplas* OR malignan* squamous cell carcinoma') AND ('miR-1' OR 'miRNA-1' OR 'microRNA-1' OR 'miR-1' OR 'miRNA-1' OR 'microRNA-1' OR 'miR-1' OR 'miRNA-1' OR 'microRNA-1' OR 'miR-1-3p' OR 'miRNA-1-3p' OR 'microRNA-1-3p' OR 'miR-1-1' OR 'miR-1-2' OR 'miR1-1' OR 'miR1-2'). The cut-off point for the search was July 20, 2017, so no studies later than this date were included. Subsequently, the eligible literature was independently evaluated in the present study using the same multi-step process. Eligible studies had to meet the following criteria: i) They detected miR-1 expression in human tissue or serum in LUSC and control groups; ii) the study offered sufficient data to calculate the diagnostic accuracy; and iii) the study was published in Chinese or English. The following exclusion criteria were used: i) Duplicated studies, case reports, letters, reviews and conference articles; ii) studies with unavailable data; and iii) animal studies.

Data extraction. Information was independently extracted, including the mean, standard deviation, sample size, true positive, false positive, true negative and false negative, from the included studies.

Statistical analysis. All data from the included microarray datasets were converted to a $\log_2$ scale, and the mean, standard deviation and case numbers of LUSC and control groups were calculated. Subsequently, Stata 14 (StataCorp LLC, College Station, TX, USA) was used to combine the standard mean difference (SMD) value and its 95% confidence interval (CI). The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) and 95% CI values from the accuracy data of each study were calculated using MetaDiSc 1.4 software (http://www.hrc.es/investigacion/metadisc_en.htm). The DOR value ranged between 0 and ∞, with higher values suggesting improved discriminatory performance (35-39). To assess the clinical significance of miR-1 in LUSC, the summarized receiver operating characteristic (SROC) curves were obtained using SPSS version 23 (IBM Corp., Armonk, NY, USA). An area under the curve (AUC) value near 1.0 indicates that the test has perfect discrimination, whereas, a value close to 0.5 implies poor discrimination. Potential heterogeneity among the included studies was estimated using Cochran's Q test and the I^2 index, and heterogeneity significantly existed if $P<0.05$ or $I^2>50\%$. If there was no distinct heterogeneity in the analysis, a fixed-effect model was used; otherwise, a random-effects model was employed. Heterogeneity was explained using the threshold effect analysis and meta-regression analysis. A sensitivity analysis was conducted to identify sources of heterogeneity. Finally, a funnel plot was applied to assess the publication bias among the included studies. $P<0.05$ was considered to indicate a statistically significant difference.

Acquisition of potential target genes of miR-1 in LUSC. The downregulated genes recorded in Genomic Spatial Event (GSE)56243 were cross-referenced based on two cell lines. The mRNA data were additionally downloaded at

Table I. Forest plot of studies evaluating the SMD of microRNA-1 expression between patients with lung squamous cell carcinoma and the control group (a random-effects model).

		Experimental			Control				
Author, year	Study	Mean	SD	Total	Mean	SD	Total	SMD (95% CI)	(Refs.)
Seike *et al*, 2009	GSE14936	7.06532	0.248859	3	7.384533	0.365138	21	-0.90 (-2.13, 0.34)	(48)
Raponi *et al*, 2009	GSE16025	4.811725	0.183384	61	4.9321	0.158178	10	-0.67 (-1.35, 0.01)	(49)
Ohba *et al*, 2013	GSE19945	-1.06547	2.263498	5	2.027512	0.642366	8	-2.12 (-3.55, -0.70)	(50)
Nymark *et al*, 2011	GSE25508	6.154964	0.23209	8	6.334855	0.624779	34	-0.31 (-1.09, -0.46)	(51)
Patnaik *et al*, 2017	GSE40738	-4.65092	0.333811	33	-4.45357	0.468942	56	-0.47 (-0.90, -0.03)	(52)
van Jaarsveld *et al*, 2014	GSE47525	5.378686	2.603978	5	3.84297	1.091469	14	0.97 (-0.10, 2.04)	(53)
Arima *et al*, 2014	GSE51853	-5.83195	1.588947	29	-2.78567	2.077722	5	-1.84 (-2.89, -0.79)	(54)
Jin *et al*, 2015	GSE74190	0.321576	0.861687	30	3.414288	1.172088	44	-2.92 (-3.59, -2.26)	(55)
Seike *et al*, 2009	PMID:18818206-1	0.00442	0.00416	7	0.031476	0.021179	7	-1.77 (-3.04, -0.51)	(48)
	PMID:18818206-2	0.015752	0.007522	7	0.172871	0.074251	7	-2.98 (-4.56, -1.39)	
	TCGA-1	2.861394	1.672886	444	6.550327	1.177112	45	-2.26 (-2.60, -1.92)	
	TCGA-2	2.876746	1.652493	458	6.590287	1.154916	45	-2.30 (-2.64, -1.96)	
	Total (95% CI)			1,090			296	-1.70 (-1.87, -1.52)	

SD, standard deviation; GSE, Genomic Spatial Event; PMID, PubMed ID; TCGA, The Cancer Genome Atlas; TCGA, The Cancer Genome Atlas; CI, confidence interval; SMD, standard mean difference.

the entry of LUSC in TCGA. DESeq data R package was applied to obtain the differentially expressed genes, $\log_2$ fold change >2 was considered a criterion of the differentially expressed genes (40). miRWalk version 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/custom.html) was used to predict the target genes of miR-1 based on 12 different online miRNA prediction tools: miRWalk; Microt4; miRanda; miRBridge; miRDB; miRMap; miRNAMap; PicTar; PITA; RNA22; RNAhybrid; and TargetScan (https://bioconductor.org/biocLite/). Subsequently, the downregulated genes in GSE56243, TCGA differentially expressed genes and predicted target genes of miR-1 were cross-referenced. The overlapping genes selected by VENNY diagram (https://omictools.com/venny-tool) were considered promising target genes of miR-1 in LUSC.

Bioinformatics based on the promising target genes of miR-1 in LUSC. Gene Ontology (GO) (41,42) annotation, comprising three parts (biological process, cellular component and molecular function) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (43,44) pathway analysis were performed in Database for the Annotation, Visualization and Integrated Discovery version 6.8 (https://david-d.ncifcrf.gov/) based on the promising target genes of miR-1 in LUSC. The BINGO and Enrichment Map plug-ins of Cytoscape (version 3.6.1) were used to visualize the GO annotation and KEGG pathways (45-47). Search Tool for the Retrieval of Interacting Genes/Proteins (http://www.string-db.org), an online tool, was used to construct the protein-protein interaction (PPI) network with the disconnected nodes hidden.

Results

Data selection. A search for literature, and GEO, ArrayExpress and TCGA datasets was performed to investigate the level of miRNA expression in LUSC. A total of 684 potential studies were identified following preliminary literature searches and finally a study containing two studies was deemed eligible for the present analysis following abstract screening and examination of the full text (41) (Fig. 1). A total of eight GEO datasets (GSE14936, GSE16025, GSE19945, GSE25508, GSE40738, GSE47525, GSE51853 and GSE74190) were selected (48-55) (Fig. 2), and two studies from TCGA were eligible for the present analysis. Eventually 12 studies with 1,386 cases were included. The GSE40738 samples were derived from serum, while the remaining samples were derived from tissue.

miR-1 expression in LUSC. miR-1 was upregulated in the GSE47525 study and downregulated in other previous studies (Table I; Fig. 3). A meta-analysis was performed to examine the level of miR-1 expression in LUSC. A random effects model was implied due to the high heterogeneity (I^2=90.5%), and the combined SMD was -1.44 (95% CI: -2.08, -0.81; Fig. 4A), demonstrating that miR-1 was significantly downregulated in LUSC. Publication bias was not observed in the present study (P>0.05; Fig. 4B). Sensitivity analysis was additionally performed to detect the source of heterogeneity (Fig. 4C), and the combined SMD was -1.32 (95% CI: -1.64, -1.01; I^2=95.5%) when the GSE40738 studies and TCGA data were removed

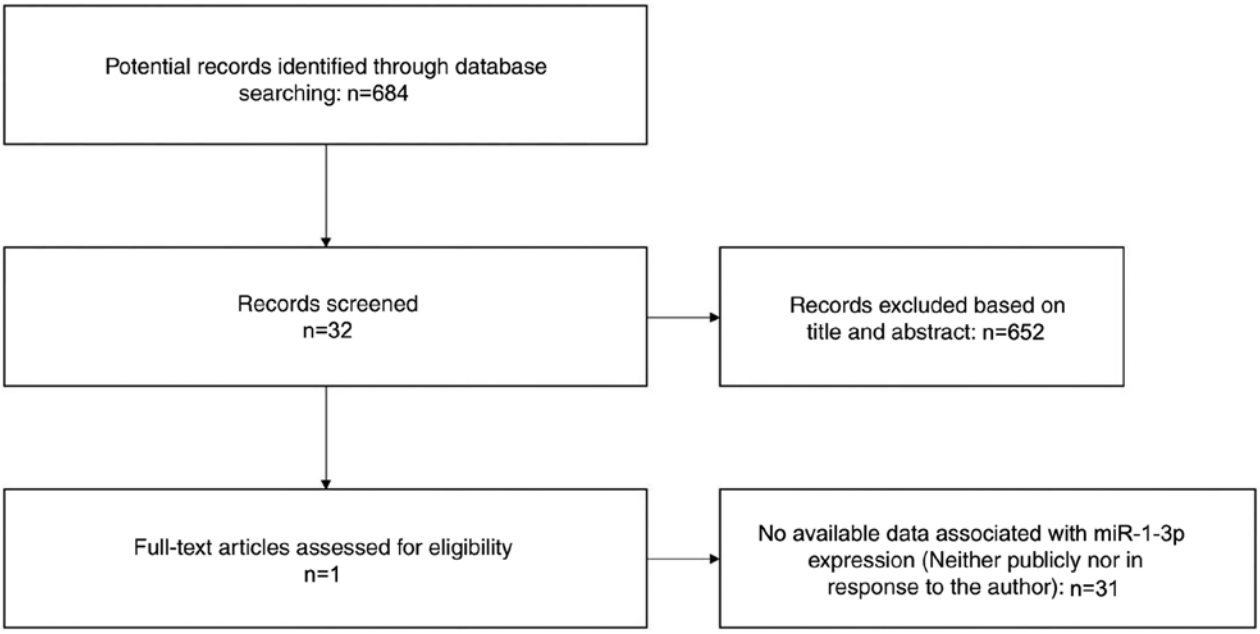

Figure 1. Flow chart of the study selection process used in the present meta-analysis. miR, microRNA.

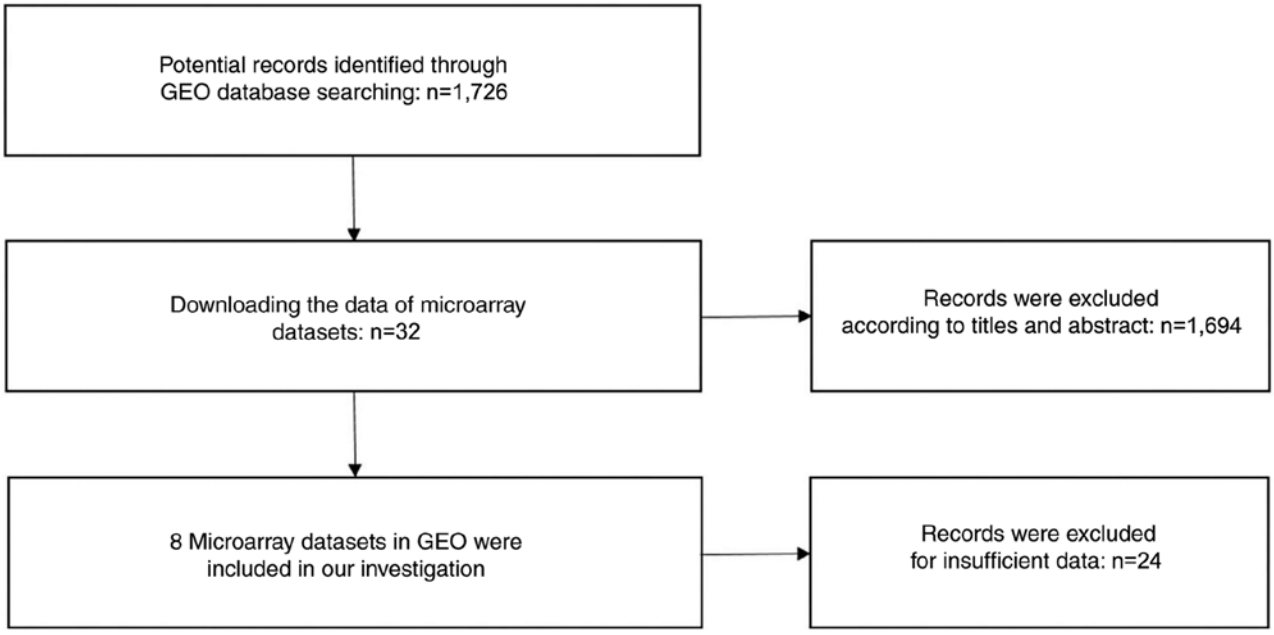

Figure 2. Flow diagram of the GEO dataset selection process used in the present meta-analysis. GEO, Gene Expression Omnibus.

(Fig. 4D). The consistent conclusions obtained confirmed that these results were statistically stable.

Clinical significance of miR-1 in LUSC. The ROC curves of miR-1 in LUSC for each study are presented in Fig. 5. It is evident that there was significant heterogeneity in these datasets, with sensitivity ranging from 0.38-1 and specificity ranging from 0.2-1. To circumvent this heterogeneity, a random effects model was adopted in the combined analyses (Fig. 6A-E). The combined sensitivity, specificity, PLR, NLR and DOR were 0.71 (95% CI: 0.66, 0.76; P<0.01), 0.88 (95% CI: 0.86, 0.90; P<0.01), 4.93 (95% CI: 2.54, 9.55; P<0.01), 0.24 (95% CI: 0.10, 0.54; P<0.01) and 28.24 (95% CI: 10.56, 75.53; P=0.0022), respectively. The SROC curve is presented in Fig. 6F, with an AUC of 0.9096 (Q*=0.8416), suggesting a good accuracy of miR-1 to distinguish patients with LUSC from control subjects.

Function analysis of miR-1-associated genes in LUSC. A total of 222 overlapping potential target genes of miR-1 in LUSC were obtained. Subsequently, GO and KEGG analyses were performed to examine the mechanism of action of miRNA in LUSC. The GO biological process annotation demonstrated that the promising target genes were primarily enriched in

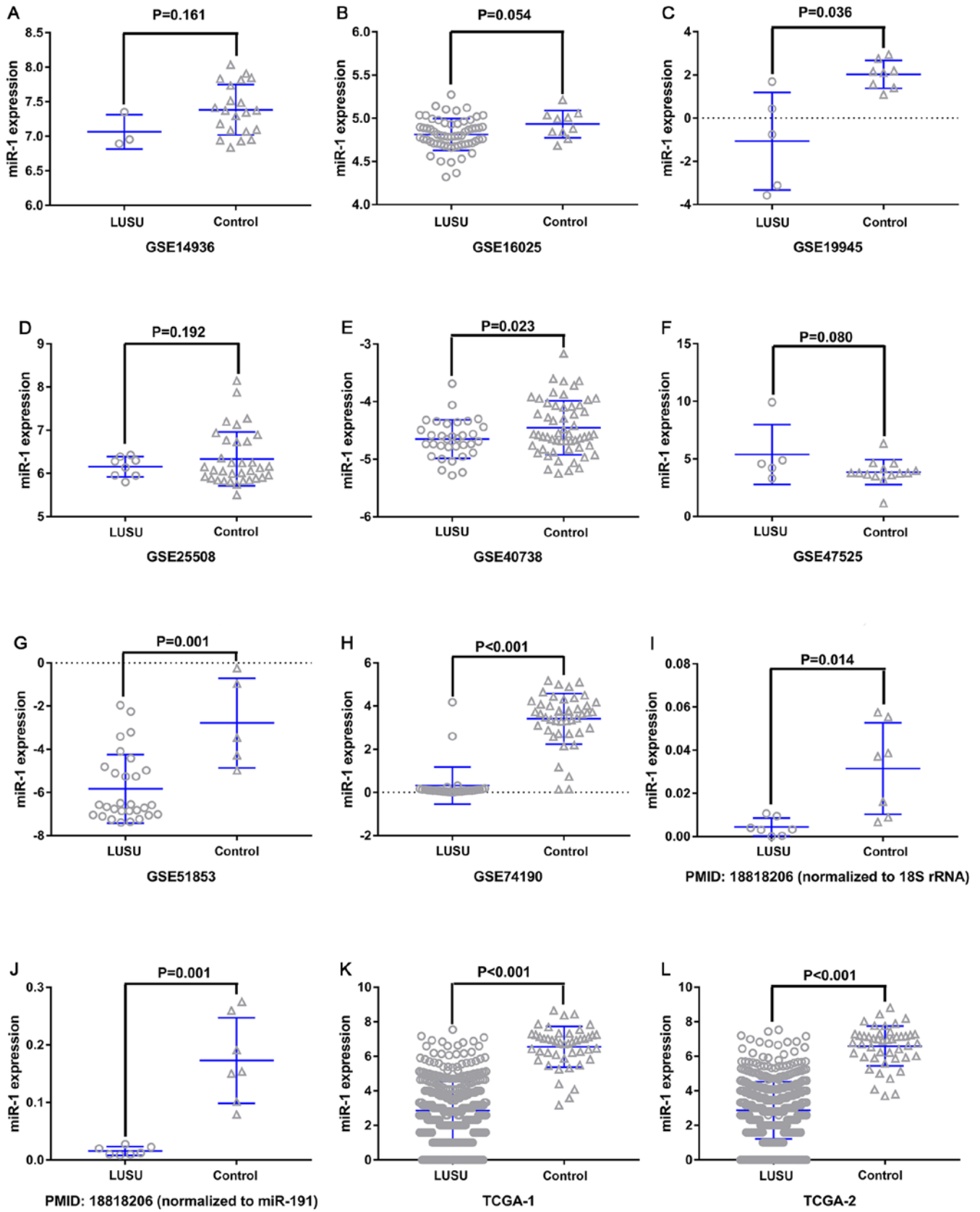

Figure 3. miR-1 expression in the LUSC and control groups. (A) GSE14936, (B) GSE16025, (C) GSE19945, (D) GSE25508, (E) GSE40738, (F) GSE47525, (G) GSE51853, (H) GSE74190, (I) PMID18818206-1, (J) PMID18818206-2, (K) TCGA-1 and (L) TCGA-2. The GSE40738 samples were derived from serum, and the remaining samples were derived from tissue. miR, microRNA; LUSC, lung squamous cell carcinoma; GSE, Genomic Spatial Event.

'DNA replication', 'cell division', 'DNA repair', 'G_1/S transition of mitotic cell cycle' and 'sister chromatid cohesion' (Table II; Fig. 7). In cellular component, the promising target genes were closely associated with 'nucleoplasm', 'chromatin',

Table II. GO analysis for the predicted target genes of microRNA-1 (only the top 10 pathways are presented).

Category	Count	P-value	Target genes
A, Biological process			
GO:0051301~cell division	17	$4.73x10^{-6}$	CDC6, KIFC1, KIF11, CENPF, AURKA, CENPE, CHEK2, etc.
GO:0006281~DNA repair	14	$4.97x10^{-6}$	EXO1, CLSPN, XRCC2, BLM, GEN1, CHEK1, RAD51, etc.
GO:0000082~G1/S transition of mitotic cell cycle	9	$2.96x10^{-5}$	CCNE2, CDC6, TYMS, DBF4, ORC6, MCM2, MCM10, etc.
GO:0007062~sister chromatid cohesion	9	$3.18x10^{-5}$	CENPN, MAD2L1, CENPA, ZWINT, CENPF, CENPE, etc.
GO:0006270~DNA replication initiation	6	$3.53x10^{-5}$	CNE2, CDC6, GINS4, ORC6, MCM2, MCM10
GO:0032508~DNA duplex unwinding	6	$1.70x10^{-4}$	GINS1, GINS2, BLM, GINS4, BRIP1, RAD54B
GO:0007067~mitotic nuclear division	12	$1.97x10^{-4}$	CENPN, CDC6, FAM64A, KIF11, TIMELESS, CENPF, etc.
GO:0000070~mitotic sister chromatid segregation	5	$2.03x10^{-4}$	KIFC1, MAD2L1, CENPA, ZWINT, ESPL1
GO:0000732~strand displacement	5	$2.38x10^{-4}$	EXO1, XRCC2, BLM, BRIP1, RAD51
B, Cellular component			
GO:0005654~nucleoplasm	59	$3.45x10^{-6}$	E2F2, CLSPN, XRCC2, DBF4, AURKA, CBX2, MCM10, etc.
GO:0000785~chromatin	8	$7.79x10^{-5}$	CENPF, CHEK1, MCM2, ASF1B, HMGA2, ESCO2, etc.
GO:0000776~kinetochore	7	$3.54x10^{-4}$	DYNC1I1, MAD2L1, ZWINT, CENPF, CENPE, CENPI, CENPH
GO:0000811~GINS complex	3	$3.97x10^{-4}$	GINS1, GINS2, GINS4
GO:0000775~chromosome, centromeric region	6	$5.09x10^{-4}$	CENPN, MKI67, CENPA, CENPF, CENPE, HELLS
GO:0000777~condensed chromosome kinetochore	7	$5.20x10^{-4}$	DYNC1I1, CENPN, MAD2L1, ZWINT, CENPE, CENPK, CENPH
GO:0031298~replication fork protection complex	3	$2.70x10^{-3}$	GINS2, GINS4, MCM10
GO:0000922~spindle pole	6	$8.77x10^{-3}$	DYNC1I1, CENPN, MAD2L1, ZWINT, CENPE, CENPK, CENPH
GO:0005634~nucleus	80	$9.28x10^{-3}$	KIFC1, FIGNL1, AURKA, CBX2, MCM10, ZIC1, GLDC, etc.
GO:0005829~cytosol	53	$1.12x10^{-2}$	TFERMT1, AURKA, GTSE1, GLDC, CCNE2, PCP4, etc.
C, Molecular function			
GO:0003682~chromatin binding	14	$8.89x10^{-4}$	EXO1, CENPF, ATAD2, CBX2, VSX1, TP73, RAD51, DLX1, etc.
GO:0005524~ATP binding	32	$1.78x10^{-3}$	KIFC1, KIF4A, XRCC2, GCLC, ATP10B, BLM, FIGNL1,etc.
GO:0043138~3'-5'; DNA helicase activity	3	$2.88x10^{-3}$	GINS1, GINS2, GINS4
GO:0003688~DNA replication origin binding	3	$7.30x10^{-3}$	ORC6, MCM2, MCM10
GO:0003777~microtubule motor activity	5	$1.55x10^{-2}$	DYNC1I1, KIFC1, KIF4A, KIF11, CENPE
GO:0030276~clathrin binding	4	$2.43x10^{-2}$	SYT1, SYT2, SYT13, SYT16
GO:0003697~single-stranded DNA binding	5	$2.55x10^{-2}$	XRCC2, BLM, NEIL3, MCM10, RAD51
GO:0004520~endodeoxyribonuclease activity	3	$2.81x10^{-2}$	XRCC2, GEN1, RAD51
GO:0005544~calcium-dependent phospholipid binding	4	$3.22x10^{-2}$	SYT1, SYT2, SYT13, SYT16
GO:0008395~steroid hydroxylase activity	3	$3.84x10^{-2}$	CYP2B6, CYP2S1, CYP2W1

GO, Gene Ontology.

Table III. KEGG pathway of validated target genes of microRNA-1.

KEGG Pathway	Count	P-value	Target genes
hsa04110: Cell cycle	12	$3.52x10^{-7}$	CCNE2, E2F2, CDC6, MAD2L1, DBF4, etc.
hsa04115: p53 signaling pathway	6	$1.47x10^{-3}$	CCNE2, SERPINB5, CHEK1, CHEK2, GTSE1, etc.
hsa03460: Fanconi anemia pathway	5	$4.29x10^{-3}$	BLM, FANCD2, BRIP1, FANCA, RAD51
hsa03440: Homologous recombination	4	$5.56x10^{-3}$	XRCC2, BLM, RAD54B, RAD51
hsa00260: Glycine, serine and threonine metabolism	4	$1.27x10^{-2}$	BHMT, PHGDH, PSAT1, GLDC
hsa04114: Oocyte meiosis	5	$4.81x10^{-2}$	CCNE2, MAD2L1, AURKA, ESPL1, CDC25C
hsa05206: MicroRNAs in cancer	8	$6.69x10^{-2}$	CCNE2, E2F2, SERPINB5, HMGA2, CDC25C, etc.

A total of eight pathways were available, four of which were significant (P<0.01). KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table IV. Top 10 genes with combined scores in the protein-protein interaction network of potential target genes of microRNA-1.

Node1	Node2	Node1 string internal ID	Co-expression	Experimentally determined interaction	Database annotated	Automated text mining	Combined score
GINS4	GINS2	1846499	0.367	0.997	0.9	0.953	0.999
DBF4	MCM2	1845796	0.164	0.922	0.9	0.875	0.999
GINS1	GINS2	1845151	0.764	0.997	0.9	0.953	0.999
PSAT1	PHGDH	1856523	0.971	0.417	0.941	0.743	0.999
MCM2	GINS2	1845676	0.859	0.898	0.9	0.484	0.999
MCM2	CDC6	1845676	0.582	0.957	0.9	0.923	0.999
MCM10	MCM2	1854261	0.769	0.934	0.9	0.842	0.999
GINS4	GINS1	1846499	0.371	0.996	0.9	0.953	0.999
CHEK1	CDC25C	1859362	0.448	0.528	0.9	0.956	0.998

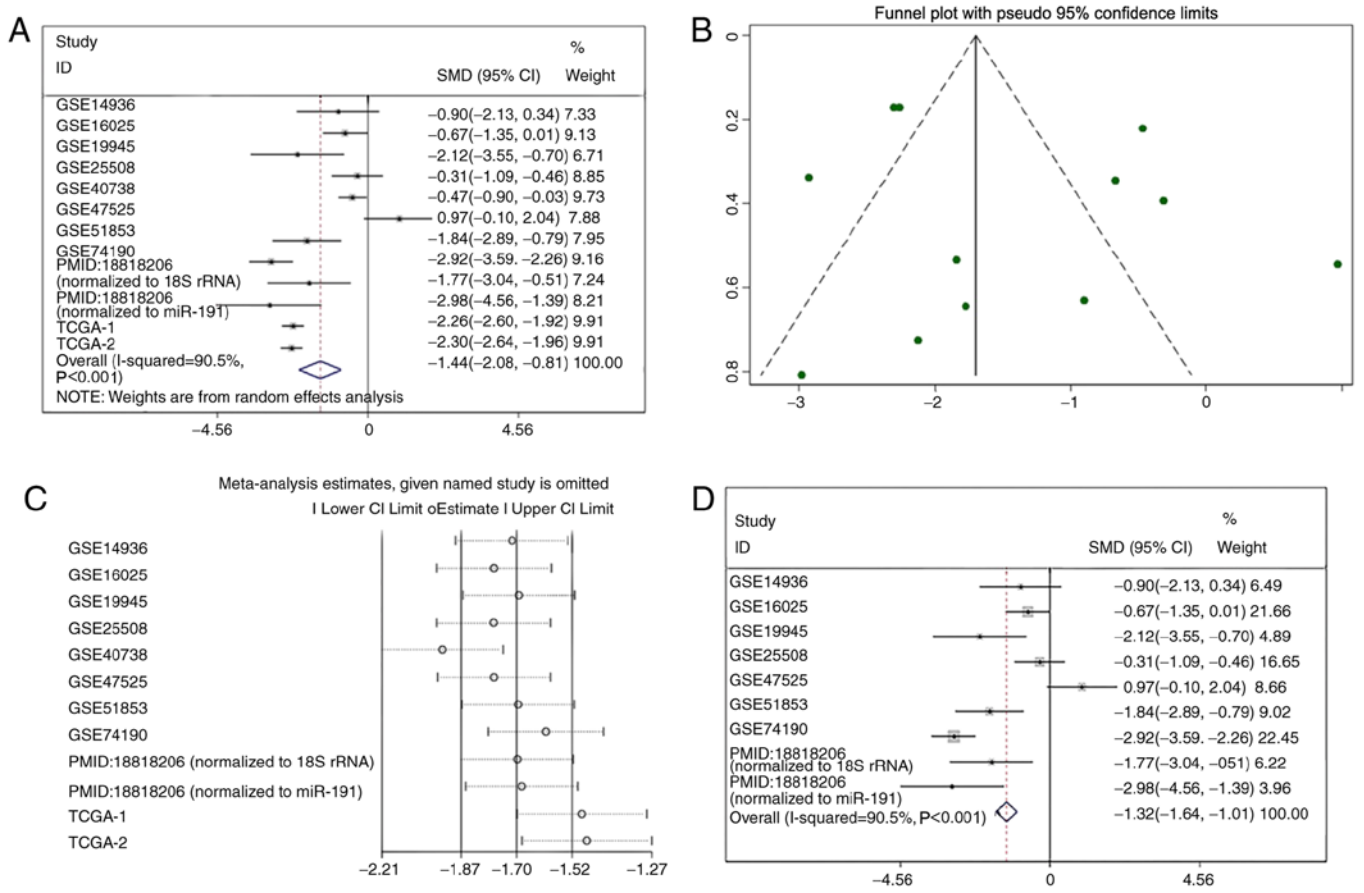

Figure 4. Summary of the included studies. (A) Forest plots of all included studies. (B) Funnel plot. (C) Sensitivity analysis. (D) Forest plots of the studies following removal of the GSE40738 and TCGA data. GSE, Genomic Spatial Event; PMID, PubMed ID; TCGA, The Cancer Genome Atlas; SMD, standard mean difference; CI, confidence interval.

'kinetochore', 'GINS complex' and 'chromosome, centromeric region' (Table II; Fig. 8). For molecular function, five of the most enriched items were 'chromatin binding', 'ATP binding', '3'-5' DNA helicase activity', 'DNA replication origin binding' and 'microtubule motor activity' (Table II; Fig. 9). In addition, the significant KEGG pathways included 'cell cycle', 'p53 signaling pathway', 'Fanconi anemia pathway', 'homologous recombination', 'glycine, serine and threonine metabolism' and 'oocyte meiosis' (Table III; Fig. 10). These results suggested that miR-1 may serve an important role in LUSC via multiple pathways. There were 222 nodes and 1,004 edges in the PPI network. In terms of the PPI network, in the current study, GINS complex subunit 4, DBF4 zinc finger, GINS complex subunit 1, phosphoserine aminotransferase 1, minichromosome maintenance complex component 2, minichromosome maintenance complex component 10, checkpoint kinase 1, GINS complex subunit 2, 3-phosphoglycerate dehydrogenase, cell division cycle 6 and cell division cycle 25C exhibited the highest degrees (Table IV; Fig. 11).

Discussion

Recently, numerous studies demonstrated that miR-1 is predominantly downregulated in multiple human tumors, including lung cancer, prostate cancer, breast cancer and sarcomas (28,56), and serves as a tumor suppressor gene involved in cancer progression. However, studies on the expression of miR-1 and its clinical significance in LUSC are rare, and the underlying mechanism of miR-1 in LUSC remains to be elucidated. The present study investigated miR-1 expression, clinical significance and the potential molecular mechanisms of miR-1 in LUSC.

To the best of the authors' knowledge, the present study is the first meta-analysis to examine the expression of miR-1 and its clinical significance in LUSC. The present meta-analysis involved 1,386 cases from 12 eligible studies. The combined SMD was -1.70 (95% CI: -1.857, 1.52) with high heterogeneity (I^2=90.5%; P=0.001), demonstrating that miR-1 was significantly downregulated in LUSC. The AUC was 0.9096 (Q*=0.8416) with a sensitivity of 0.71 (95% CI: 0.66, 0.76, P<0.01) and a specificity of 0.88 (95% CI: 0.86, 0.90, P<0.01). The pooled PLR and NLR were 4.93 (95% CI: 2.54, 9.55) and 0.24 (95% CI: 0.10, 0.54), respectively. A PLR value of 4.00 suggested that patients with LUSC possessed an ~4-fold higher probability of exhibiting downregulated miR-1 compared with control groups. An NLR value of 0.34 suggested that the probability of having LUSC was 34% when miR-1 is abnormal. In addition, the pooled DOR was 28.24 (95% CI: 10.56, 75.53). According to these results, miR-1 may better differentiate between LUSC and patients without cancer. Sensitivity analysis demonstrated that high heterogeneity may reflect the GSE40738 studies and TCGA data. To

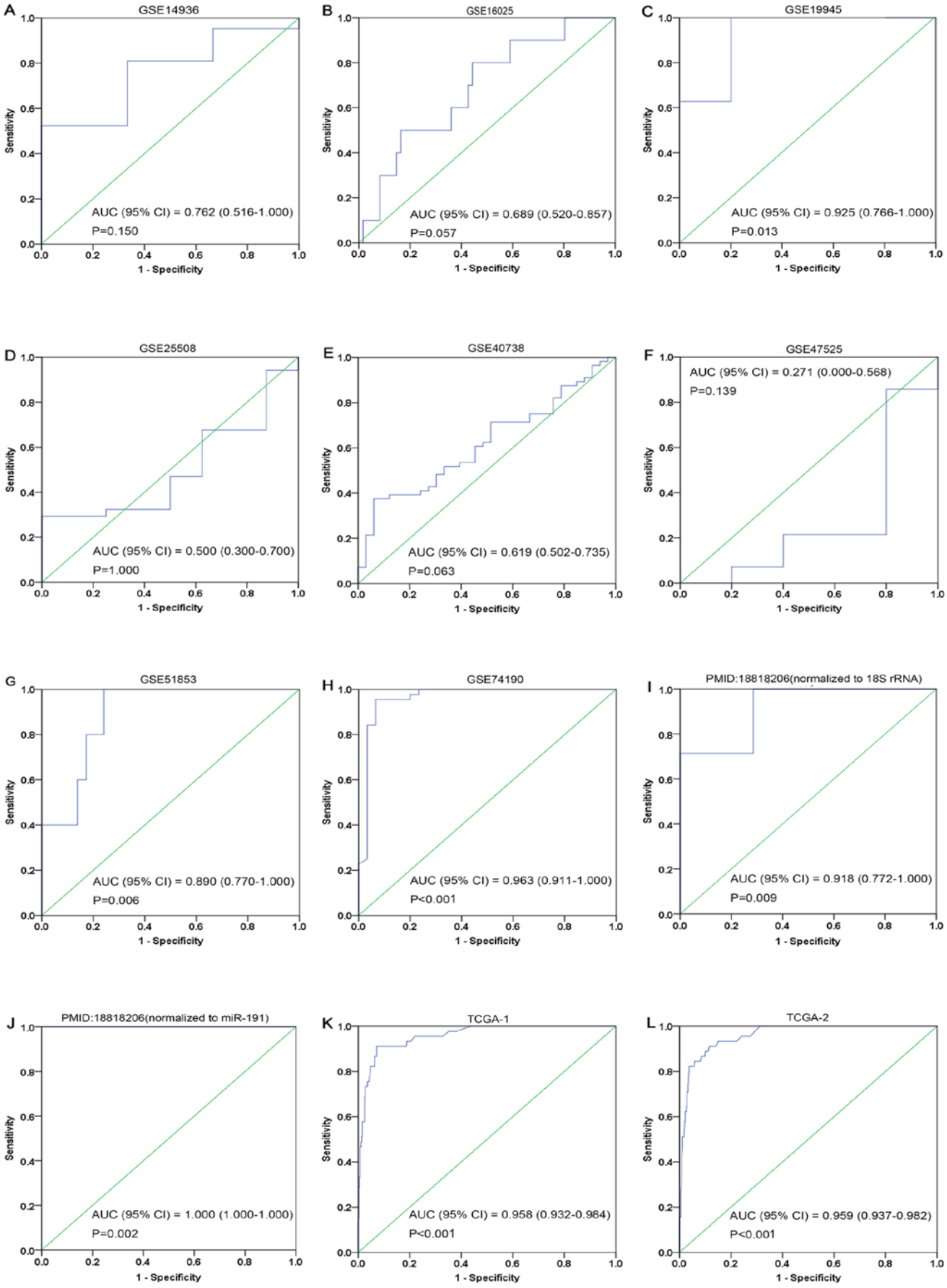

Figure 5. ROC curves of microRNA-1 in lung squamous cell carcinoma. (A) GSE14936, (B) GSE16025, (C) GSE19945, (D) GSE25508, (E) GSE40738, (F) GSE47525, (G) GSE51853, (H) GSE74190, (I) 18818206-1, (J) 18818206-2, (K) TCGA-1 and (L) TCGA-2. Blue represents a sensitive curve and green indicates the identifying line. The X-axis, presented as '1-Specificity', indicates the false positive rate. The Y-axis, presented as 'Sensitivity', indicates the true positive rate. ROC, receiver operating characteristic.

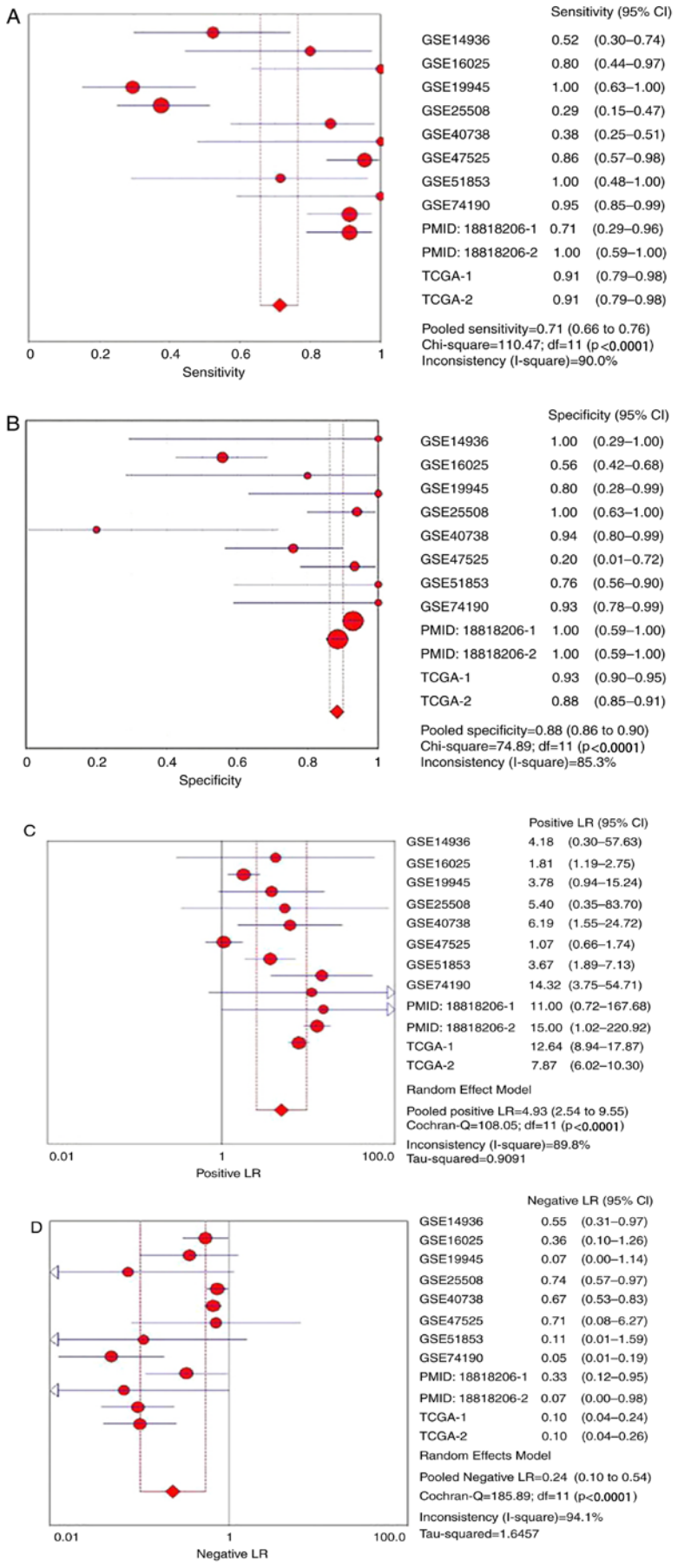

Figure 6. Forest plots of pooled miR-1 in the diagnosis of lung squamous cell carcinoma. (A) Sensitivity, (B) specificity, (C) positive LR, (D) negative LR, indicating the sensitivity and specificity of all included studies. miR, microRNA; SROC, summarized receiver operating characteristic; GSE, Genomic Spatial Event; PMID, PubMed ID; TCGA, The Cancer Genome Atlas; TCGA, The Cancer Genome Atlas; CI, confidence interval; AUC, area under the curve; LR, likelihood ratio; OR, odds ratio.

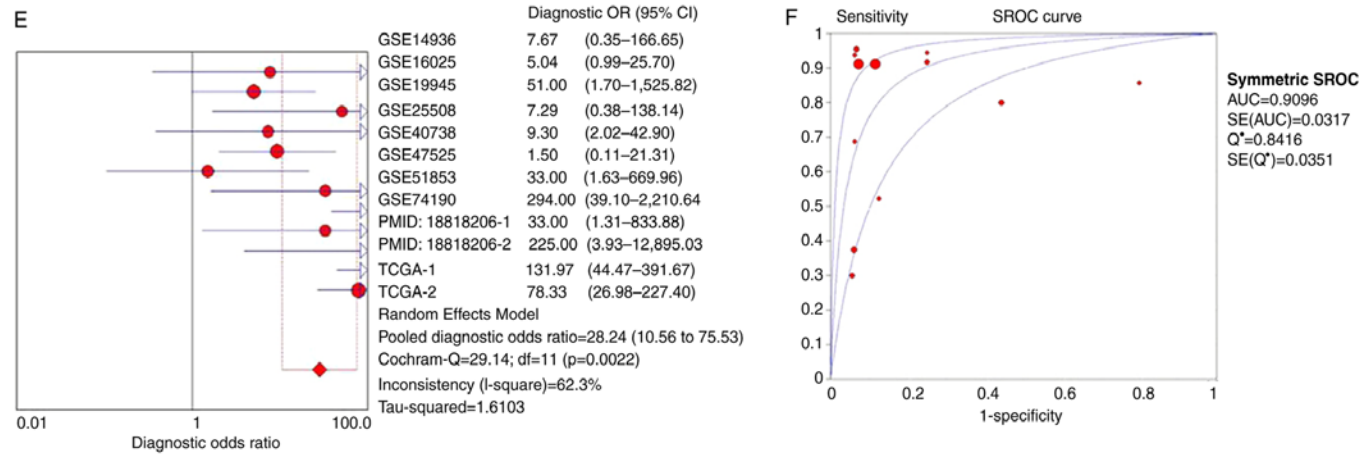

Figure 6. Continued. Forest plots of pooled miR-1 in the diagnosis of lung squamous cell carcinoma. (E) Diagnostic OR and (F) SROC graphs indicating the sensitivity and specificity of all included studies.

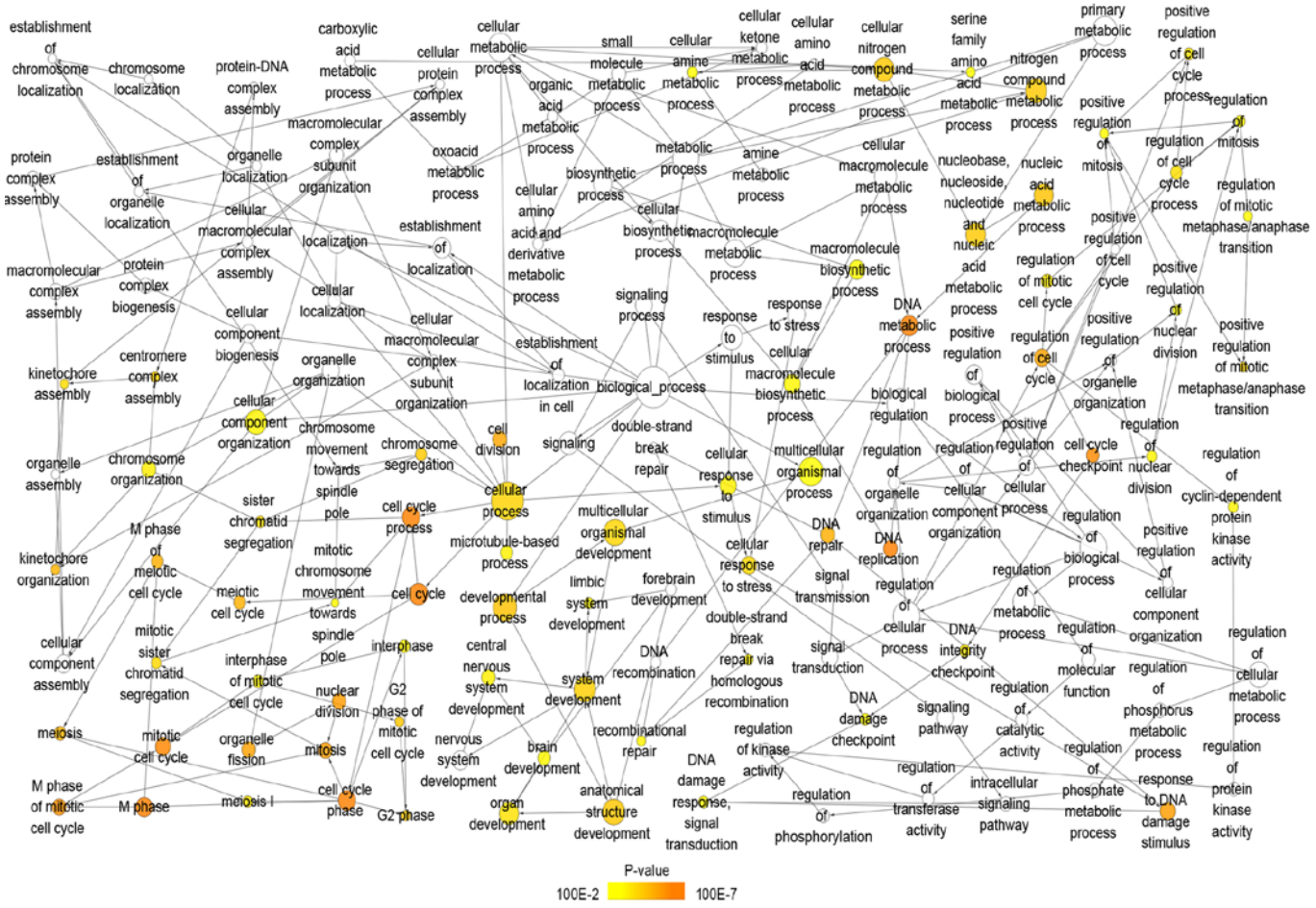

Figure 7. GO analysis of the biological process category. Nodes represent GO terms and arrows represent interactions. Orange nodes imply that the items are statistically significant (P<0.01). White nodes imply that the items only take part in connecting items but are not statistically significant. GO, Gene Ontology.

obtain more convincing conclusions, additional studies using a higher quality, larger sample size and a consistent standard procedure are required.

Accumulating evidence demonstrated that miR-1 serves an important role in the development of multiple tumors (57,58). Mataki *et al* (59) demonstrated that miR-1/133a was significantly downregulated in LUSC tissues and enhanced cancer cell invasion and migration via the regulation of Coronin1C. However, little is known of the potential molecular mechanisms of miR-1 in LUSC. Therefore, 222 validated targeting genes of miR-1 were collected and a comprehensive target genes network analysis performed. GO analysis demonstrated that miR-1 may be involved in multiple biological processes, including 'DNA replication', 'cell division', 'DNA repair' and the 'G_1/S transition

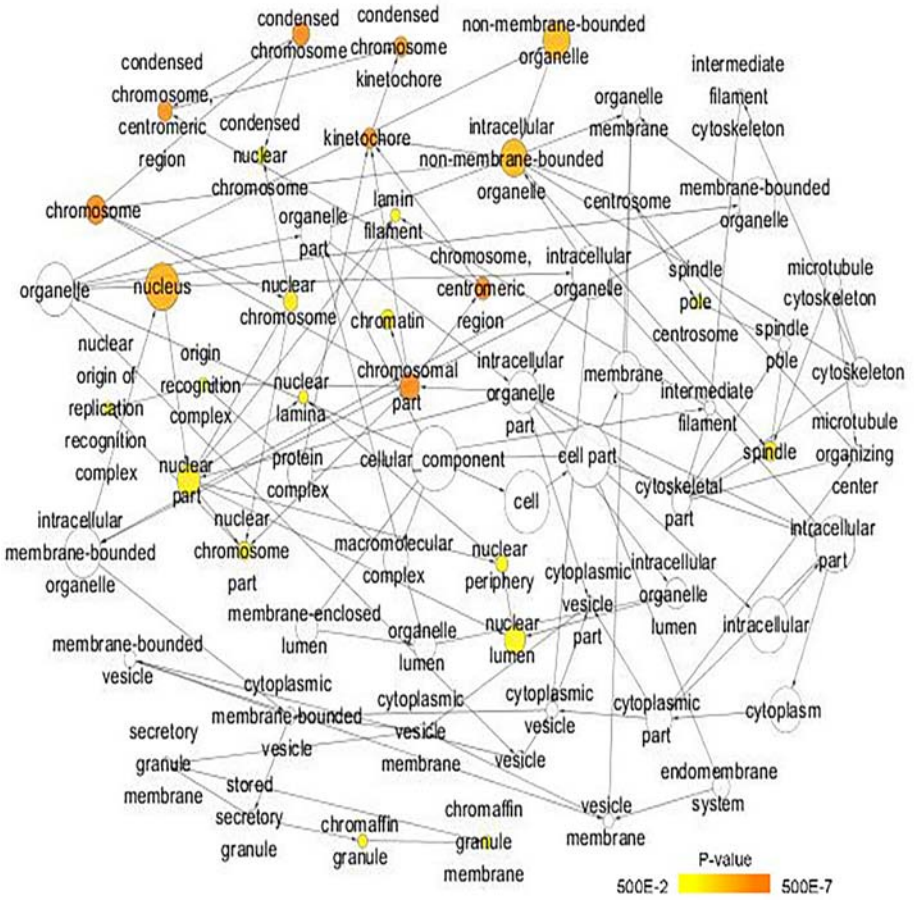

Figure 8. Gene Ontology analysis of the cellular component category. Nodes represent GO terms and arrows represent interactions. Orange nodes imply that the items are statistically significant (P<0.05). White nodes imply that the items only take part in connecting items but are not statistically significant. GO, Gene Ontology.

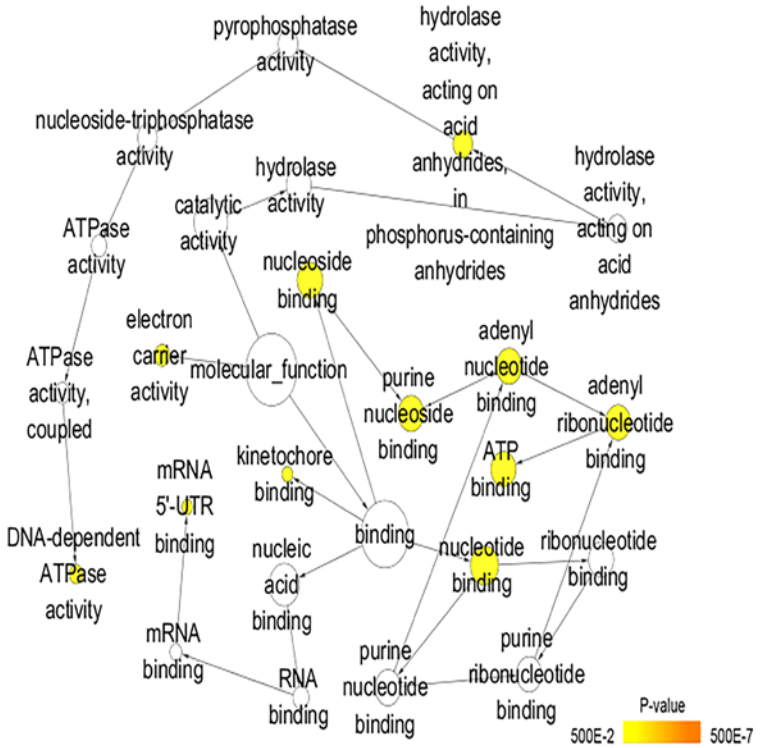

Figure 9. Gene Ontology analysis of the molecular function category. Nodes represent GO terms and arrows represent interactions. Orange nodes imply that the items are statistically significant (P<0.05). White nodes imply that the items only take part in connecting items but are not statistically significant. GO, Gene Ontology.

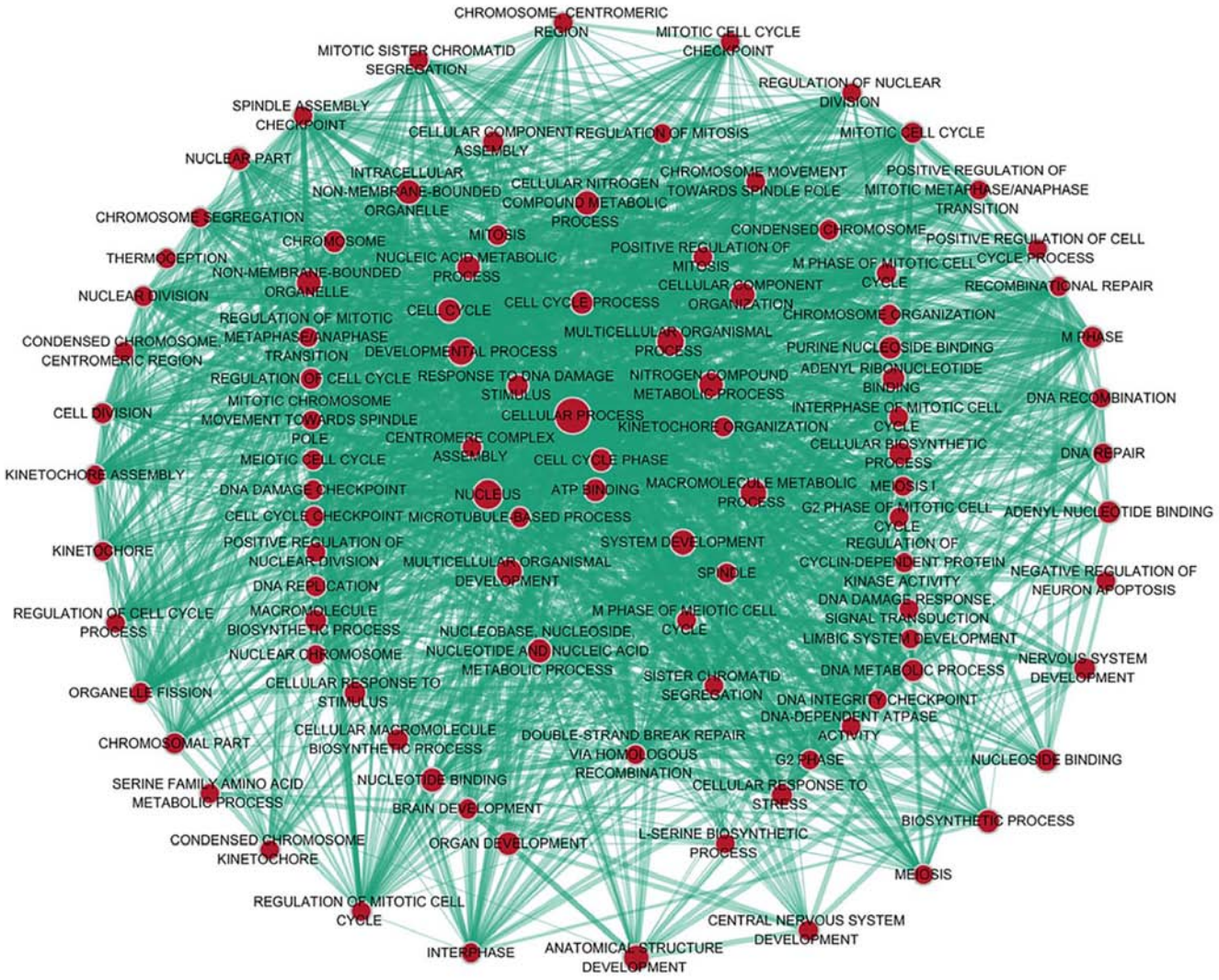

Figure 10. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses. Nodes represent proteins and edges represent interactions.

of the mitotic cell cycle'. KEGG pathway analysis identified that miR-1 may serve a pivotal role in LUSC via different pathways, including 'cell cycle', 'p53 signaling pathway', 'Fanconi anemia pathway', 'homologous recombination', 'glycine, serine and threonine metabolism' and 'oocyte meiosis'. The p53 gene, a key tumor suppressor located on chromosome 17p13, is the most frequently mutated gene in cancer and is involved in in a variety of biological processes to prevent tumorigenesis through the transcriptional regulation of downstream target genes (60). A previous study demonstrated that p53 is associated with cell cycle arrest, apoptosis and drug resistance in lung cancer cells (61). Zhang *et al* (62) demonstrated that GluA2 induces apoptosis in non-small cell lung cancer A549 cells through the p53 signaling pathway; the p53 signaling pathway was a significant pathway (Table III; P=0.001) that ranked second in the biological process of LUSC. The p53 pathway may serve an important role in radio sensitivity in non-small cell lung cancer H460 cells via the upregulation of phosphatase and tensin homolog expression level (63). A number of previous studies (64-66) demonstrated that miRNAs may be a key effector of p53 tumor-suppressor function; mediating the biological effects of p53 and inactivating this molecule may contribute to specific cancer types (67), suggesting that miRNAs may serve a vital role in the p53 gene signaling pathway. Therefore, it is hypothesized that miR-1 may be involved in the progression of LUSC through the p53 signaling pathway.

There are a number of limitations to the present study. The study size included in this meta-analysis was relatively small. The prognostic value of miR-1 was not discussed in this analysis, reflecting that there are no studies regarding the association between miR-1 and prognosis and clinic pathological parameters in LUSC. Only studies reported in Chinese and English were included in this meta-analysis, which may result in the omission of eligible studies due to language criteria. Furthermore, among the 12 studies included in the present study, only one was derived from serum, and the remaining 11 studies used samples derived from tissue. For a better understanding of the role of miR-1, large cohort and independent studies are required to examine the clinical significance of miRNA and its potential mechanism in LUSC.

In conclusion, the present study demonstrated that miR-1 is significantly downregulated in LUSC and may be involved in cancer progression via multiple, crucial pathways. Therefore, miR-1 may be used as a screening tool for LUSC in the future. Large-scale studies are required to further investigate the clinical significance of miR-1.

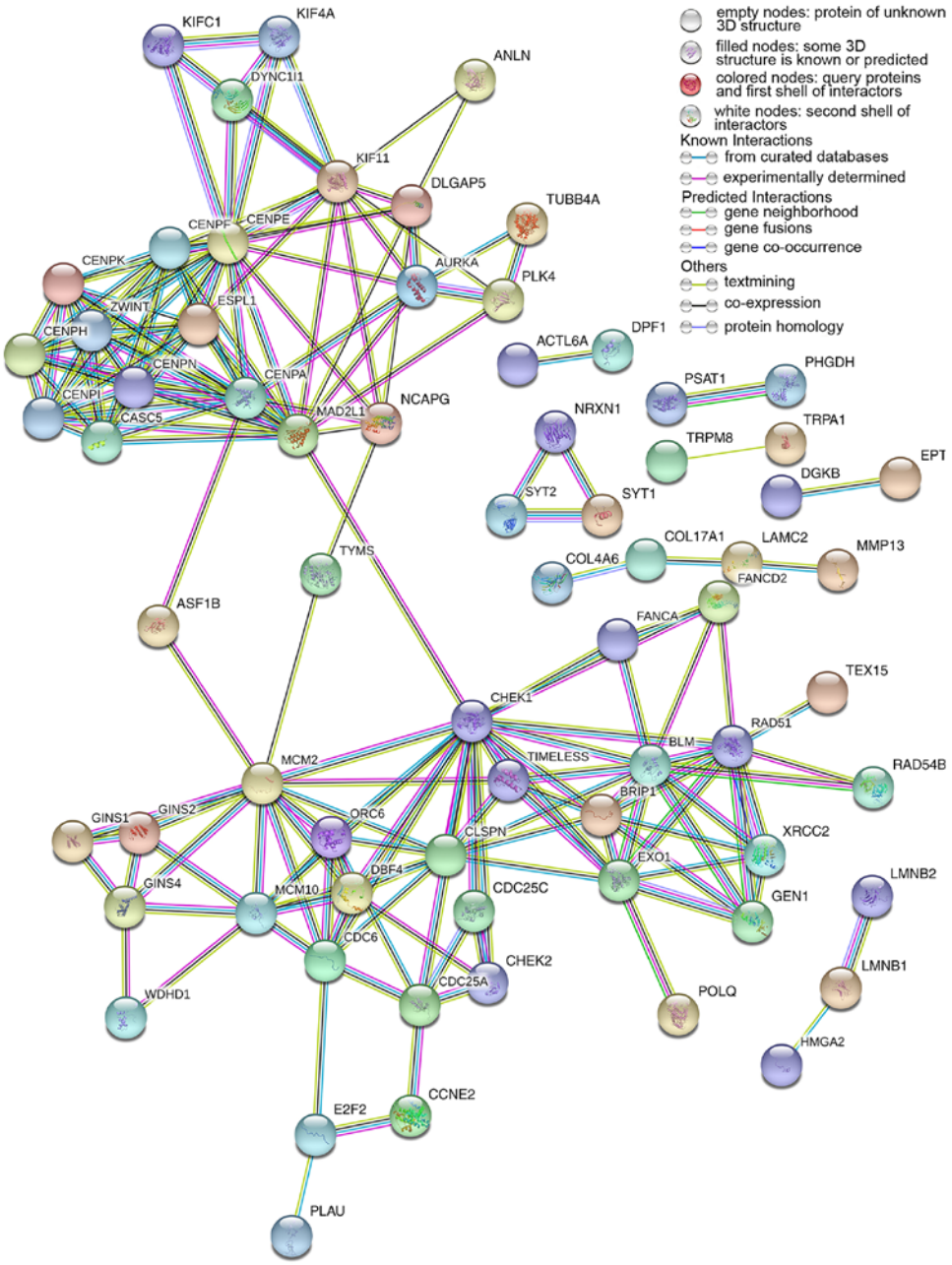

Figure 11. Protein interactions of correlative genes for miR-1; the protein-to-protein network analysis was performed using Search Tool for the Retrieval of Interacting Genes/Proteins version 10.5. miR, microRNA.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XL, MQ and JH contributed to the design of the study, data collection, analysis and drafting of the manuscript. JM and XH contributed to the design of the study, interpretation of the data and drafting of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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