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miR-190, CDK1, MCM10 and NDC80 predict the prognosis of the patients with lung cancer

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Abstract

Lung cancer (LC), which includes small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC), is common and has a high fatality rate. This study aimed to reveal the prognostic mechanisms of LC. GSE30219 was extracted from the Gene Expression Omnibus (GEO) database, and included 293 LC samples and 14 normal lung samples. Differentially expressed genes (DEGs) were identified using the Limma package, and subjected to pathway enrichment analysis using DAVID. MicroRNAs (miRNAs) targeting the DEGs were predicted using Webgestalt. Cytoscape software was used to build a protein-protein interaction (PPI) network and to identify significant network modules. Survival analysis was conducted using Survminer and Survival packages, and validation was performed using The Cancer Genome Atlas (TCGA) dataset. The good and poor prognosis groups contained 518 DEGs. miR-190, miR-493, and miR-218 for the upregulated genes and miR-302, miR-200, and miR-26 for the downregulated genes were predicted. Three network modules (module 1, 2, and 3) were identified from the PPI network. CDK1, MCM10, and NDC80 were the core nodes of module 1, 2, and 3, respectively. In module 1, CDK1 interacted with both CCNB1 and CCNB2. Additionally, CDK1, CCNB1, CCNB2, MCM10, and NDC80 expression levels correlated with clinical survival and were identified as DEGs in both GSE30219 and the TCGA dataset. miR-190, miR-493, miR-218, miR-200, and miR-302 might act in LC by targeting the DEGs. CDK1, CCNB1, CCNB2, MCM10, and NDC80 might also influence the prognosis of LC.

***Keywords**: lung cancer, differentially expressed genes, microRNAs, enrichment analysis, protein-protein interaction network*

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Introduction

As a malignant lung tumor, lung cancer (LC) is mainly divided into small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC) (1). Patients with LC usually suffer from weight loss, coughing, chest pains, and heavy breathing (2). LC, which is the second most common tumor in women and the tumor with the highest mortality in men, affected 1.8 million people and led to 1.6 million deaths

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globally in 2012 (3). Therefore, the mechanisms of LC development and progression should be determined.

Peptidylprolyl cis/trans isomerase, NIMA-interacting 1 (*PIN1*) functions in the occurrence and metastasis of NSCLC, and thus may be a predictor for poor prognosis and a therapeutic target for the disease (4, 5). The oncoprotein cancerous inhibitor of protein phosphatase 2A (*CIP2A*) is highly expressed in NSCLC, and is related to unfavorable prognosis and tumor cell proliferation (6, 7). In addition, the overexpression of Zonula Occludens-1 (ZO-1, gene symbol *TJP1*) contributes to the good prognosis of patients with NSCLC (8). Furthermore, upregulated expression of micro RNA (miRNA) *miR-21*, and downregulated expression of *miR-143* and *miR-181a* were detected in LC tissues, indicating that these miRNAs might be potential diagnostic or prognostic factors for the patients with NSCLC (9). Although the above studies have reported some genes and miRNAs that correlated with LC, the pathogenesis of the disease remains unclear.

In 2013, Rousseaux et al. developed an approach to explore the malignant epigenetic reprogramming of LC and identified a series of candidate cancer biomarkers (10). However, they did not fully reveal the prognosis-associated genes in LC using bioinformatic analyses. In the present study, using the microarray data of Rousseaux et al. (10), the prognostic mechanisms of LC were studied through differential expression analysis, enrichment analysis, mRNA-target prediction, protein-protein interaction (PPI) network analysis, and survival analysis.

Materials and Methods

Microarray data

The gene expression profile GSE30219 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30219) was extracted from the Gene Expression Omnibus (GEO) database, which was sequenced on the platform of GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. GSE30219 contained data for 293 LC samples and 14 normal lung samples.

Ethics approval and consent to participate

This study was approved by Ethics Committee of the General Hospital of Pingmei Shenma Medical Group.

Differential expression analysis and enrichment analysis

Using the Robust Multichip Average (RMA) algorithm (11), normalization and gene expression calculations were conducted for the original data. The top 50 LC samples with the longest or the shortest postoperative follow-up times, respectively, were classified into good and poor prognosis groups. To identify prognosis-associated genes, the differentially expressed genes (DEGs) between the good and poor prognosis groups were analyzed using the Limma (http://www.bioconductor.org/packages/release/bioc/html/limma.html) package (12) in the R software. The screening criteria were set as $|\log \text{fold change (FC)}| > 0.58$ and adjusted p-value < 0.05. Using the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) tool (13), the significant Kyoto Encyclopedia of Genes and Genomes (KEGG) (14) pathways involving the DEGs were identified.

Prediction of miRNAs targeting the DEGs

MiRNAs can regulate the transcription levels of target genes by binding with their 3' untranslated regions (15). Therefore, the dysregulated expression of the DEGs might also be caused by miRNA regulation. The web-based gene set analysis toolkit (Webgestalt, http://www.webgestalt.org) tool (16) was used to predict the miRNAs that target the upregulated and downregulated genes.

Protein-protein interaction network analysis

The DEGs were submitted to the Search Tool for the Retrieval of Interacting Genes (STRING, http://string-db.org/) database (17), and the PPIs with combined scores > 0.4 were selected. Subsequently, the PPI network was built using Cytoscape software (http://www.cytoscape.org) (18) and the clusterMaker plug-in (19) in Cytoscape was used to identify the significant network modules. The module nodes were then identified using Gene Ontology (GO) (20) functional enrichment analysis using the BinGO plug-in (21) in Cytoscape.

Survival analysis

Using the Survminer (22) and Survival (23) packages in R, survival analysis for the key genes was carried out. Based on the gene expression value, survival time, and survival state, the Survminer package could divide the samples into high expression and low expression groups. After grouping, survival analysis was conducted using the Survival package and a survival curve was drawn.

Validation using an independent dataset

The expression profile data for LC was downloaded from The Cancer Genome Atlas (TCGA) database, which included 502 LC samples and 49 normal samples. Based on the probe annotation file, the probes were mapped into gene symbols. More than one probe might correspond to the same gene symbol; therefore, their average value was calculated as the gene expression value. Differential expression analysis was then conducted using the R package, edgeR (24) (http://www.bioconductor.org/packages/release/bioc/html/edgeR.html), with the thresholds of $|logFC| > 0.58$ and adjusted p-value < 0.05. Using the VENNY tool (http://bioinfogp.cnb.csic.es/tools/venny/index.html) (25), the DEGs for GSE30219 and the TCGA dataset were compared to obtain the intersecting genes.

Results

Differential expression analysis and enrichment analysis

The top 50 LC samples with the longest (average follow-up time = 100.5 months) or the shortest (average follow-up time = 5.7 months) postoperative follow-up time were divided into good and poor prognosis groups, respectively. There were 518 DEGs (319 upregulated and 199 downregulated) in the two groups. KEGG pathway enrichment analysis suggested that the upregulated genes were significantly involved in the cell cycle. However, the downregulated genes had no significantly enriched pathways.

Prediction of miRNAs targeting the DEGs

Multiple miRNAs were predicted to target the upregulated and downregulated genes. The upregulated genes were mainly targeted by *miR-190*, *miR-493*, and *miR-218* (Table 1A). Meanwhile, the downregulated genes were mainly targeted by *miR-302*, *miR-200*, and *miR-26* (Table 1B).

PPI network analysis

For the DEGs, a PPI network (involving 110 upregulated and 39 downregulated genes) was built. Subsequently, three network modules (module 1, 2, and 3) were obtained in which the core nodes were cyclin-dependent kinase 1 (CDK1), minichromosome maintenance 10 (MCM10), and NDC80 kinetochore complex component (NDC80), respectively (Figure 1). In module 1, CDK1 could interact with both cyclin B1 (CCNB1) and cyclin B2 (CCNB2). GO functional enrichment analysis indicated that the module nodes were enriched in the M phase of the mitotic cell cycle (module 1, p-value = 4.91E-24), DNA replication (module 2, p-value = 4.44E-13), and M phase (module 3, p-value = 1.21E-13) (Table 2).

Table 1. The miRNAs targeting the up-regulated genes (A) and the down-regulated genes (B).

A

MicroRNA	Gene count	Gene symbol	Adjusted p-value
hsa_ACATATC,MIR-190	6	CELF4,CHD7,ANO4,PHLPP1,STC1,NEUROD1	0.0007
hsa_ATGTACA,MIR-493	11	ZNF804A,ZNF711,ATAD2,SALL1,NCAPH,-JPH1,POU4F1,ANO4,CDH2,GAD1,MAP1B	0.0013
hsa_AAGCACA,MIR-218	12	TRIM9,RIMBP2,PCDH8,LMNB1,INHBB,ST18,PEX5L,PH-F16,SATB2,ANO4,FANCI,HOXD10	0.0013
hsa_CATGTAA,MIR-496	8	CDKN2C,CHD7,DPP10,HMP19,ASCL1,NEUROD1,KCNH8,-SATB2	0.0013
hsa_TAGCTTT,MIR-9	9	POU3F2,ELAVL2,CKAP2L,INSM1,TARDBP,PH-F16,POU4F1,HMGB2,STC1	0.0016
hsa_TGTTTAC,MIR-30A-5P,MIR-30C,MIR-30D,MIR-30B,MIR-30E-5P	14	RIMBP2,CCNE2,CHD7,NEUROD1,PHF16,SAT-B2,NKX2-2,ANO4,SCN2A,FOXG1,TTLL7,STC1,SC-N3A,RHEBL1	0.0018
hsa_TTTGCAC,MIR-19A,MIR-19B	13	DPYSL5,ZNF711,POU3F2,ZBTB10,INHBB,NEURO-D1,PRC1,CBLN2,PEX5L,POU4F1,SYT1,E2F8,RHEBL1	0.0018
hsa_CTATGCA,MIR-153	8	DPYSL5,INHBB,NEUROD1,POU4F1,DLX1,C9orf40,SYT1,-TAGLN3	0.0023
hsa_ACTGCCT,MIR-34B	8	RIMBP2,ICA1,WASF1,NEUROD1,ST18,POU4F1,INS-M1,STC1	0.0023
hsa_ATGCAGT,MIR-217	6	ZNF711,STX1A,NEUROD1,ST18,RBM38,EZH2	0.0023

B

MicroRNA	Gene count	Gene symbol	Adjusted p-value
hsa_AGCACTT,MIR-93,MIR-302A,MIR-302B,MIR-302C,MIR-302D,MIR-372,MIR-373,MIR-520E,MIR-520A,MIR-526B,MIR-520B,MIR-520C,MIR-520D	8	HLF,TBC1D8B,RAB11FIP1,ADAM9,NTN4,CCND2,S-DC1,MTUS1	0.0129
hsa_CAGTAT-T,MIR-200B,MIR-200C,MIR-429	9	TRIM2,HLF,GLI3,KCNJ2,FGFR2,DGKA,ZNF217,CIT-ED2,MTUS1	0.0129
hsa_TACTTGA,MIR-26A,MIR-26B	7	PTGS2,KCNJ2,GRHL3,HPGD,NTN4,ZNF217,CCND2	0.0143
hsa_TGGTGCT,MIR-29A,MIR-29B,MIR-29C	9	CLDN1,MUC4,ZFP36,RNF39,CCND2,SH-ROOM2,AHR,CAV2,IL1RAP	0.0150
hsa_CATTTCA,MIR-203	6	GLI3,ELL2,GRHL3,EDN1,CITED2,AHR	0.0344
hsa_TTTGCAC,MIR-19A,MIR-19B	8	LBH,HLF,ELL2,KCNJ2,ZNF217,CCND2,SDC1,IGFBP3	0.0373
hsa_AACTGGA,MIR-145	5	TRIM2,MPZL2,NEDD9,CITED2,STEAP4	0.0528
hsa_GCACTTT,MIR-17-5P,MIR-20A,MIR-106A,MIR-106B,MIR-20B,MIR-519D	8	HLF,TBC1D8B,SGMS1,RAB11FIP1,ADAM9,NT-N4,ZNF217,CCND2	0.0554
hsa_ACTGTGA,MIR-27A,MIR-27B	7	LBH,ELL2,SGMS1,LPAR6,RAB11FIP1,CACNA2D3,G-PR126	0.0554
hsa_AGCTCCT,MIR-28	3	LBH,NDRG2,SDC1	0.0800

Survival analysis

After the samples in GSE30219 were divided into high expression and low expression groups, survival analysis for *CDK1*, *CCNB1*, *CCNB2*, *MCM10*, and *NDC80* was performed. The survival curves showed that the expression in all five genes correlated with the survival of patients with LC (p-value < 0.0001) (Figure 2).

Validation using an independent dataset

For the TCGA dataset, a total of 15226 DEGs were obtained. After comparing the DEGs for GSE30219 and the TCGA dataset, 412 intersecting genes (including *CDK1*, *CCNB1*, *CCNB2*, *MCM10*, and *NDC80*) were identified (Figure 3).

Discussion

In this study, 518 DEGs in the good and poor prognosis groups were screened. *miR-190*, *miR-493*, and *miR-218* were the main miRNAs predicted to target the upregulated genes. Meanwhile, *miR-302*, *miR-200*, and *miR-26* were predicted to target the downregulated genes. From the PPI network for the proteins encoded by the DEGs, three network modules (module 1, 2, and 3) were identified. CDK1, MCM10, and NDC80 were the core nodes of module 1, 2, and 3, respectively. Survival analysis showed that *CDK1*, *CCNB1*, *CCNB2*, *MCM10*, and *NDC80* expression levels correlated with the survival of LC patients. In addition, 412 intersecting genes (including *CDK1*, *CCNB1*, *CCNB2*, *MCM10*, and *NDC80*) were identified between the DEGs for GSE30219 and the TCGA dataset.

Increased *miR-190* expression results in the low protein levels of forkhead box P2 (*FOXP2*); thus, *miR-190* may be a promising diagnostic

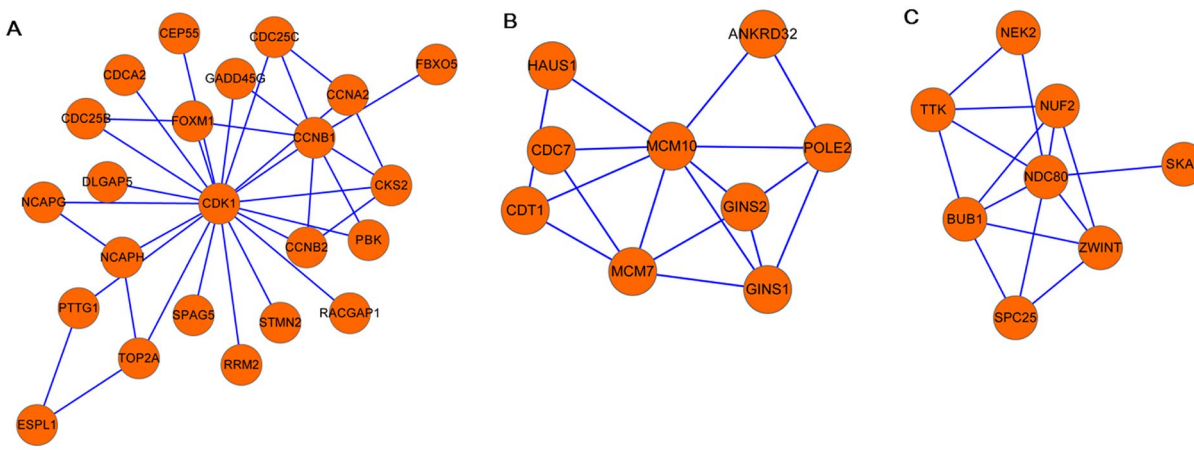

Fig. 1. Modules 1 (A), 2 (B), and 3 (C) obtained from protein-protein interaction (PPI) network.

Table 2. The Gene Ontology (GO) terms enriched for the nodes in module 1, 2 and 3.

Module	Term	Count	P-value	Genes
Module 1	M phase of mitotic cell cycle	16	4.91E-24	CDK1, DLGAP5, ESPL1, CEP55, PBK, PTTG1, CDC25C, CDC25B, CCNB1, NCAPH, CCNB2, NCAPG, SPAG5, CDCA2, FBXO5, CCNA2
Module 2	DNA replication	7	4.44E-13	CDC7, GINS1, GINS2, MCM7, POLE2, MCM10, CDT1
Module 3	M phase	8	1.21E-13	SPC25, NEK2, ZWINT, BUB1, NUF2, TTK, NDC80, SKA1

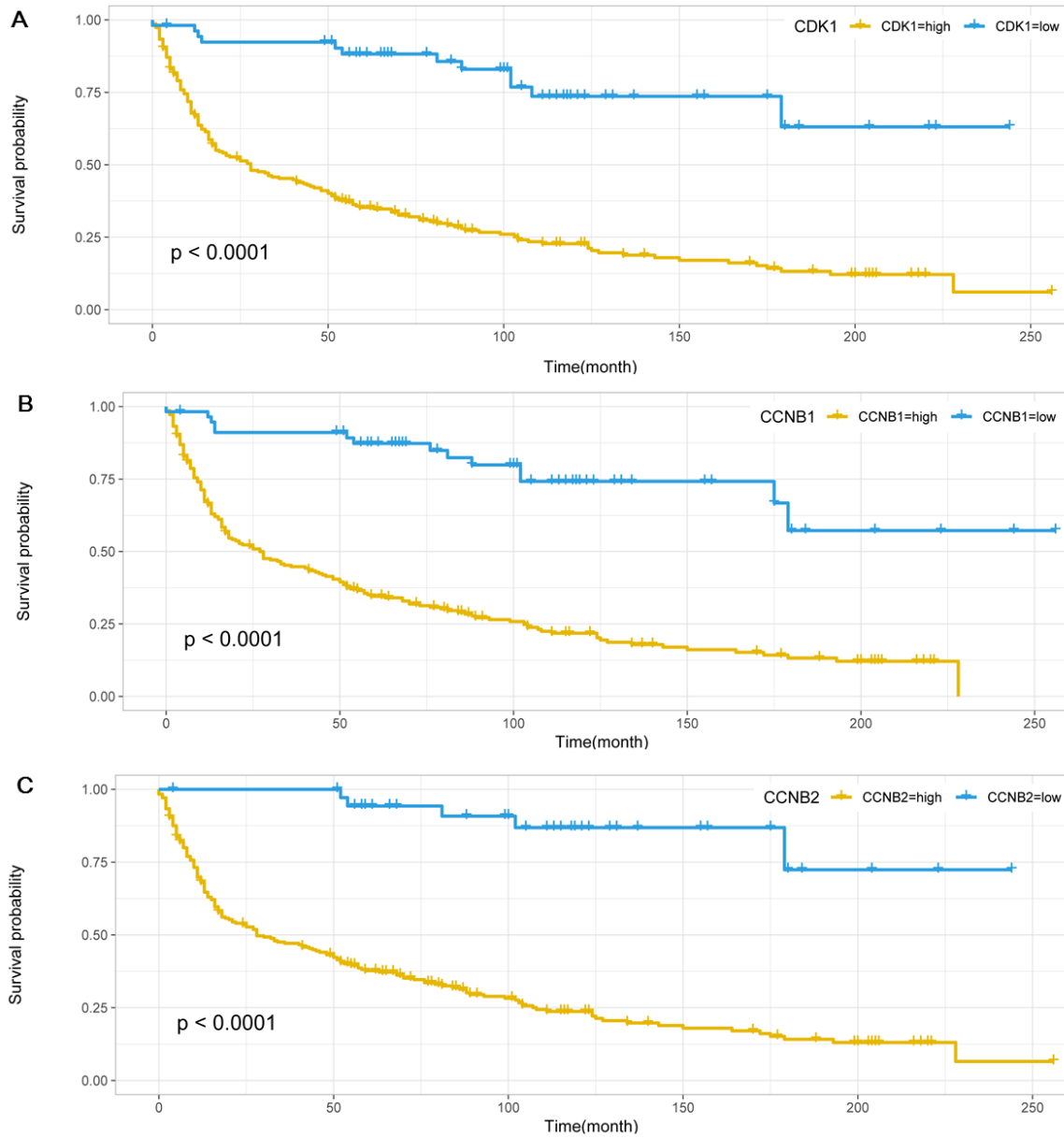

Fig. 2A-C. The survival curves for CDK1 (cyclin-dependent kinase 1) (A), CCNB1 (cyclin B1) (B), and CCNB2 (cyclin B2) (C). Yellow and blue represent the high and low expression groups, respectively.

marker for gastric cancer (26). Downregulated expression of *miR-190* inhibits the metastasis of breast cancer via inversely regulating the expression of SMAD family member 2 (*SMAD2*), which is correlated with the prognosis of patients with breast cancer (27). By targeting integrin, beta 1 (*ITGB1*), miR-493-*5p* plays a prognostic role in NSCLC and could be used to diagnose the disease (28). Four upregulated miRNAs and two downregulated miR-

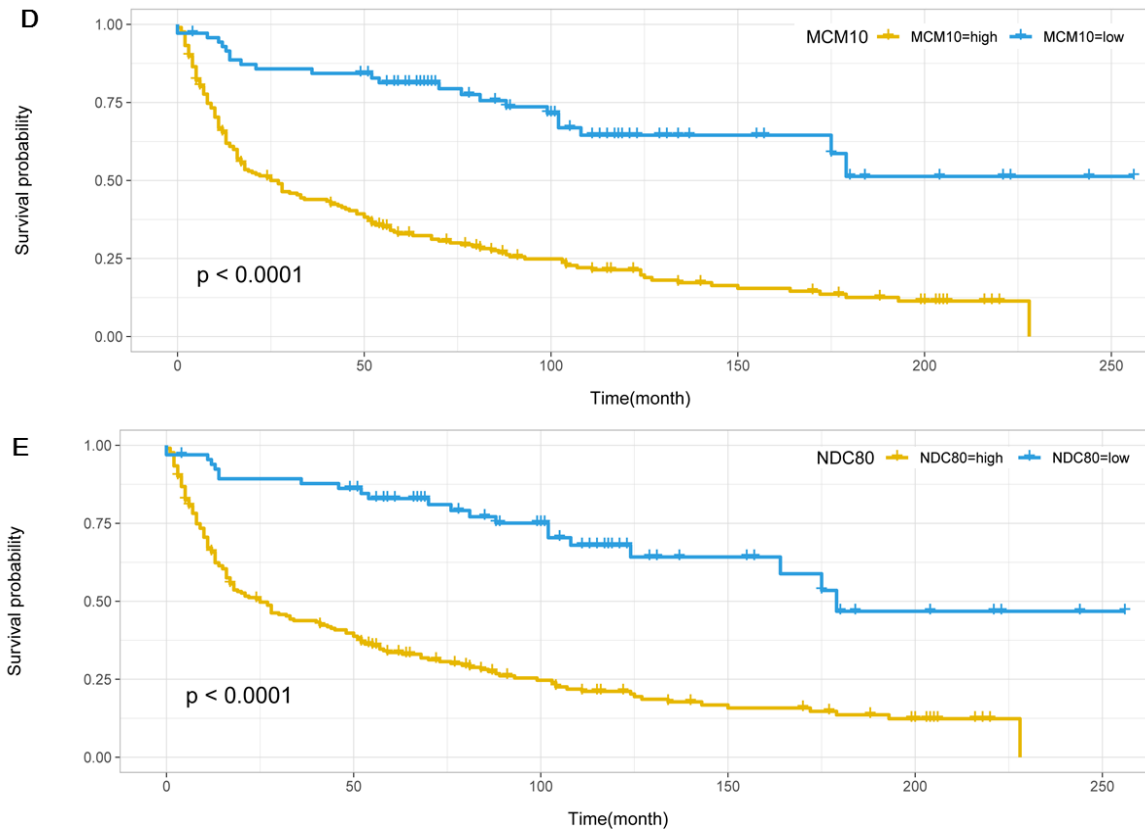

Fig. 2D-E. The survival curves for MCM10 (minichromosome maintenance 10) (D), and NDC80 (NDC80 kinetochore complex component) (E). Yellow and blue represent the high and low expression groups, respectively.

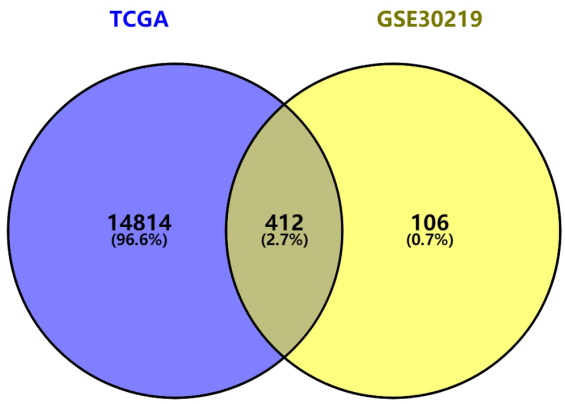

Fig. 3. Venn diagram used to identify the intersecting genes between the differentially expressed genes for GSE30219 and The Cancer Genome Atlas (TCGA) dataset.

NAs (including *miR-218-5p*) were identified in lung adenocarcinoma (LUAD) tissues and may represent promising diagnostic and prognostic markers for patients with LUAD (29). Overexpression of *miR-200c* plays an oncogenic role in NSCLC and correlates markedly with adverse prognosis; thus, it may serve as a prognostic biomarker for patients with NSCLC (30). Exosome-derived *miR-302b* mediates the transforming growth factor beta (TGF beta RII)/ extracellular signal-regulated kinase (ERK) pathway, which can inhibit cell proliferation and migration in LC (31). Therefore, *miR-190*, *miR-493*, *miR-218*, *miR-200*, and *miR-302* might affect the prognosis of LC by regulating the expression of the DEGs.

CDK1 and MAD2 mitotic arrest deficient-like 1 (*MAD2L1*) can lead to high risk of recurrence and poor prognosis of patients with LUAD; therefore, they are negatively correlated with prognosis and may be promising therapeutic targets for the disease (32). *CCNB1* and *CDK1* levels are higher in LC compared with those in normal tissues, and their expression levels vary among different types of LC (33, 34). *CCNB1* and cyclin A (*CCNA*) have dysregulated expression in NSCLC, and *CCNB1* may serve as a potential prognostic factor for patients with NSCLC (35). *CCNB2* is overexpressed in LUAD cells, which independently predicts poor survival of patients with LUAD (36). CDK1 was predicted to interact with both CCNB1 and CCNB2 in module 1, suggesting that CDK1 might affect the prognosis of LC via interactions with CCNB1 and CCNB2.

Minichromosome maintenance (MCM) proteins are involved in multiple cancers. In particular, *MCM5* and *MCM7* are independent prognostic markers for patients with LC (37, 38). *MCM4* is highly expressed in NSCLC and is related to non-adenocarcinoma histology, indicating that it might represent a therapeutic target in NSCLC (39). *MCM2*, *MCM3*, *MCM7*, *MCM10*, polo-like kinase 1 (*PLK1*), and S-phase kinase associated protein 2 (*SKP2*) are involved in cancer-associated pathways and may coordinately facilitate cell cycle-associated processes in tumors (40). Cell division associated 1 (*CDCA1*) and *NDC80* (also called *KNTC2*) are simultaneously upregulated in LC, which is a critical feature of tumor cell growth and survival and provides a valuable therapeutic strategy for the disease (41). Therefore, our results, and those of previous studies, demonstrated that *MCM10* and *NDC80* might also be related to LC prognosis.

Although multiple bioinformatic methods were used to explore the prognostic mechanisms of LC, this study had several limitations. The lack of experimental validation was the most prominent limitation. Therefore, further experimental research is needed to support these results.

In conclusion, 518 DEGs were screened from the good and poor prognosis groups. MicroRNAs *miR-190*, *miR-493*, *miR-218*, *miR-200*, and *miR-302* might correlate with the prognosis of LC by regulating the expression levels of certain DEGs. Furthermore, *CDK1*, *CCNB1*, *CCNB2*, *MCM10*, and *NDC80* might be associated with the prognosis of LC.

Abbreviations

Lung cancer (LC)
Small-cell lung carcinoma (SCLC)
Non-small-cell lung carcinoma (NSCLC)
Gene Expression Omnibus (GEO)
Differentially expressed genes (DEGs)
Protein-protein interaction (PPI)
Robust Multichip Average (RMA)
Gene Ontology (GO)
Kyoto Encyclopedia of Genes and Genomes (KEGG)
Cyclin-dependent kinase 1 (CDK1)
Minichromosome maintenance 10 (MCM10)
NDC80 kinetochore complex component (NDC80)
Cyclin B1 (CCNB1)
Cyclin B2 (CCNB2)

Conflict of interst

The authors declare that they have no potential conflict of interest. This study was not supported by any external funding

Authors' contributions

L-WG conceived and designed the research, participated in the acquisition of data, and drafted the manuscript.

G-LW carried out the analysis and interpretation of data, and participated in the statistical analysis.

L-WG and G-LW conceived the study, partici-

pated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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